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Histochemical Aspects of Penetration and Vascular Connection of Broomrape Haustoria in the Host Root, and the Possible Implication of Phenylpropanoids

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ABSTRACT

Penetration of haustorial cells of Broomrape (*Orobanche* spp.) was studied in the root tissues of pea (*Pisum sativum* L.). It was similar in a number of other hosts (faba bean, tomato, Indian cress, dill, & chamomile). The vascular xylem of the broomrape primary and secondary haustoria eventually connected with phloem conducting elements of the host root. By use of an epifluorescent microscope, boundary features of the penetration and connection of the secondary to the primary root (in healthy pea plants) were compared histochemically with those of the haustoria and the host root cells (in infected pea plants). The relative deposition of cellulose in the cortical and phloem cells did not show differences between healthy and broomrape-infected pea plants. Both cases also showed similar trends in the accumulation of lignin and other phenylpropanoids at the attachment zones, but were markedly more intense in the haustorial connection, probably reinforcing the barriers between the host root cells and the haustoria. No mechanical damage or rupture was observed in the root cells of both healthy and infected plants. Thus accumulations of phenylpropanoids along the connection zones of haustoria within the host may be concomitants of sealing and recovery from cell penetration. The appearance of such compounds at the boundaries of normal connections between the main and lateral roots shows that lignin and allied phenylpropanoids are probably induced in the infected host cells.

Key Words: *Orobanche;* Pea plant; *Pisum sativum* L.; Parasitism; Phenylpropanoid

INTRODUCTION

Broomrape (*Orobanche* sp. Orobanchaceae) is a parasitic plant that attacks and causes yield loss of many important dicotyledonous crops throughout the world (Parker, 1986), by removing carbohydrates and water (Schaffer *et al.,* 1991). At the start of the process of parasitism, haustorial cells of *Orobanche* penetrate the host root tissues, eventually connecting the parasite to the vascular system of the host. This phase is a critical step in parasitism (Lonser *et al.,* 1994). The anatomy of the haustoria in contact with the host root cells has been studied by many workers (e.g. Kuijt, 1991). In such studies, a link was found between the phloem of the host and broomrape (Parker & Riches, 1993), confirmed by the scanning electron microscope (Dorr & Kollmann, 1995) showing that primary haustoria of *Orobanche crenata* parasitizing the roots of *Vicia narbonensis* formed an uninterrupted system connecting both partners. The sieve elements of *Orobanche crenata* and *Vicia narbonensis* show continuous sieve pores derived from interspecific plasmodesmata (Dorr, 1996).

Several studies refer to the possible involvement of phenolics and lignin in resisting haustorial penetration within the host tissue (e.g. Jorrin *et al.,* 1996).

Phenylpropanoids and derivatives such as coumarins, lignin, suberin, cutin, and tannin represent structural material for plant stability (Heldt, 1997) and can be involved in the resistance of the host as defence compounds against broomrape (Jorrin *et al.,* 1996). The penetration of the host roots was reported by Parker and Riches (1993) to take place by enzymatic action, and to result in the separation of the host cells, rather than their destruction or intracellular penetration. Joel and Losner (1994b) described a multi-step process of penetration, beginning with the secretion of an adhesive substance that facilitated the internal anchoring of the parasite to the host. Westwood *et al.* (1998) also indicated that the parasite (*Orobanche* spp.) grows between rather than through the host cortical cells, and forms haustoria which connect the parasite to the host xylem and phloem tissue. Accordingly, reinforcement of the host cells might provide further resistance to the penetration of haustoria.

The present work is a histochemical study using epifluorescent microscopy to follow the entry path of broomrape haustoria during penetration through the roots of many different hosts. Possible changes in phenylpropanoids were also tracked by matching host responses to infestation with those of healthy uninfected plants.

MATERIALS AND METHODS

Broomrape-infected hosts at similar ages were collected during their juvenile phase from Nubaria (Alexandria-desert road, Egypt) and El-Aiat (Giza, Egypt). These hosts included faba bean (*Vicia faba*), pea (*Pisum sativum*), tomato (*Solanum lycopersicum)*, Indian cress (*Tropaeolum majus*), dill (*Anethum graveolens*), and chamomile (*Camomilla recutita*).

Toludine blue o, Phloroglucinol, Sudan III, and Sudan IV were obtained from Sigma-Aldrich Co. (USA), Safranin T from Fluka Chemie (Switzerland), and Light green SF from BDH Chemicals Ltd. (UK).

Collected samples were washed, cut into segments (1-2 cm length), then fixed in FAA (5 mL 40% formaldehyde, 5 mL galcial acetic acid, 90 mL 70% ethyl alcohol) for not less than 48 h following Johansen (1940). Samples were taken at the middle region of the lateral roots of the hosts and at their basal junction with the primary root, where the broomrape outgrowth was attached to the secondary roots. Broomrape was at the vegetative stage when the parasite stalks acquire their characteristic pigmentation (Hassan, 1973). Serial sections were taken at constant thickness, using a hand microtome. Sections were then preserved in 50% aqueous ethanol, ready for direct examination or staining.

Preparation of the stains, their filtration by micromembranes, and the staining of sections for epifluorescence examination was as described in detail by El-Awadi (2001). Double stain was used with safranin and light green. Stained and unstained sections were examined using a Nikon Optophoto-2 (Nikon, Japan) microscope (Wilson & Peterson, 1983). The microscope is equipped with epifluorescent filter blocks for ultraviolet, blue, and green light.

Photomicrographs were prepared using attachment microflex (FX-35Dx) camera system (Nikon, Japan) and Kodak Gold 100 and 400 color print films (Brammall & Higgins, 1988 a, b).

RESULTS

Sections prepared from the connecting regions between broomrape and various hosts were subjected to different light sources and stains, and examined by epifluorescent microscope. The entry path of broomrape haustoria through the host root cells and the connection of their vascular elements were followed. The results were similar in all the different hosts, and therefore the connection of primary and secondary haustoria of boomrape to the lateral roots of pea was taken as a model and studied in detail.

Fig. 1 shows penetration of broomrape haustoria through the lateral roots of pea at the connection point. The ultraviolet (UV-2A) illumination of unstained sections shows the xylem tissue of the primary haustorium in the core of the section surrounded by a mass of undifferentiated cells. Penetration of the primary haustorium throughout the host cortex is also evident, as is the connection of the haustorial xylem (differentiated cells) to the phloem conducting elements of the host. The xylem of both parasite and host showed a whitish autofluorescence under UV illumination. This is an indication of the occurrence of lignin and/or other phenolics in their structures.

The connection of secondary haustoria to the lateral roots of pea is shown in Fig. 2 and 3. The autofluorescence of unstained sections under ultraviolet (UV-2) light indicated that secondary haustorial tissues of broomrape possessed lignin and/or other phenolics in their structures. Blue illumination light (Fig. 2A) induced the bright yellowish autofluorescence of lignin and/or other phenolics in both broomrape secondary haustoria and pea cells. The yellowish autofluorescence was mixed with quenching tissues which indicated that secondary haustoria also attacked the conductive tissues of pea lateral root via phloem structures (Fig. 2B). The penetration of secondary haustoria and their connection to the host lateral-root phloem was further clarified under visible light, using double stain (Fig. 3A), phloroglucinol-HCl (Fig. 3B) and toludine blue o (Fig. 3C).

Transverse sections of the roots of both healthy and infected pea plants were examined under ultraviolet light and differently stained as mentioned previously. Comparison was carried out to show the difference in lignins and other phenylpropanoids between healthy and infected plants. Fig. 4 and 5 show the results obtained with double stain and Toludine blue O, respectively, in sections taken at similar connecting regions between the main and lateral roots of healthy and infected plants of the same age and grown in the same field.

It is evident from Fig. 4A which double stained, under visible light showed the red colour characteristic of lignin in the outer layer and xylem vessels of the main and lateral roots of healthy plants. In infected plants, a deeper colour reaction was observed in the same regions (Fig. 4B). No differences were observed in both cases between the cortex and phloem layers, which showed similar densities of the green colour of cellulose (Fig. 4 A, B).

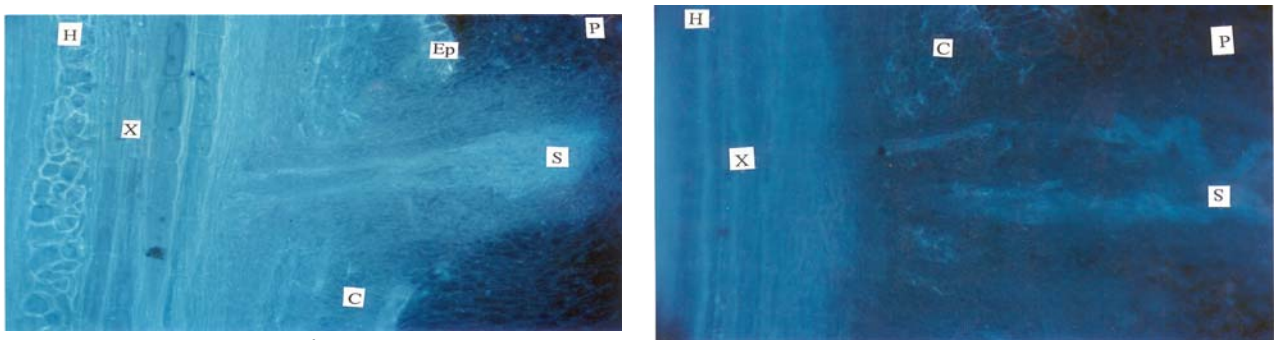
With toludine blue O, the xylem tissue of roots showed blue-green and purple colour reactions which further indicated the occurrence of lignin and other phenylpropanoid materials in both healthy and infected plants. However, the colour was more intense in infected (Fig. 5B) as opposed to healthy plants (Fig. 5A). The pink colour reactions of pectin and cellulose material in the cortex and other parenchymatous cells of phloem and xylem were similar in roots of both healthy and infected plants (Fig. 5 A & B, respectively).

DISCUSSION

A number of strategies have been developed by plants

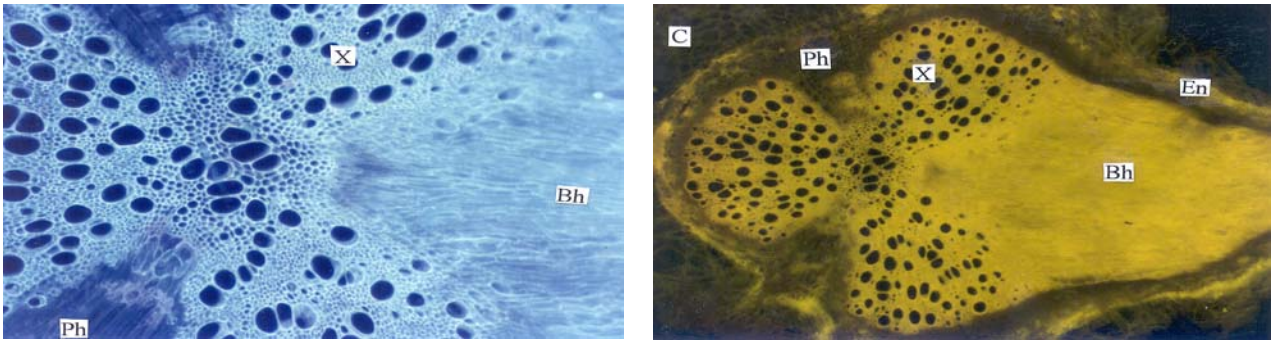
to deter invasion or limit the spreading of an invading organism. Changes in the composition and properties of the cell walls of the host, and enhanced biosynthesis of secondary metabolites, represent general defensive mechanisms against parasitic angiosperms (Orcutt & Nilsen, 2000). Understanding the role of natural products (secondary metabolites) is vital to exert sustainable control over plant diseases, particularly phenylpropanoids and allied

Fig. 1. Section at the connecting region between broomrape haustoria (T.S) (A) and pea lateral root (L.S) (B)



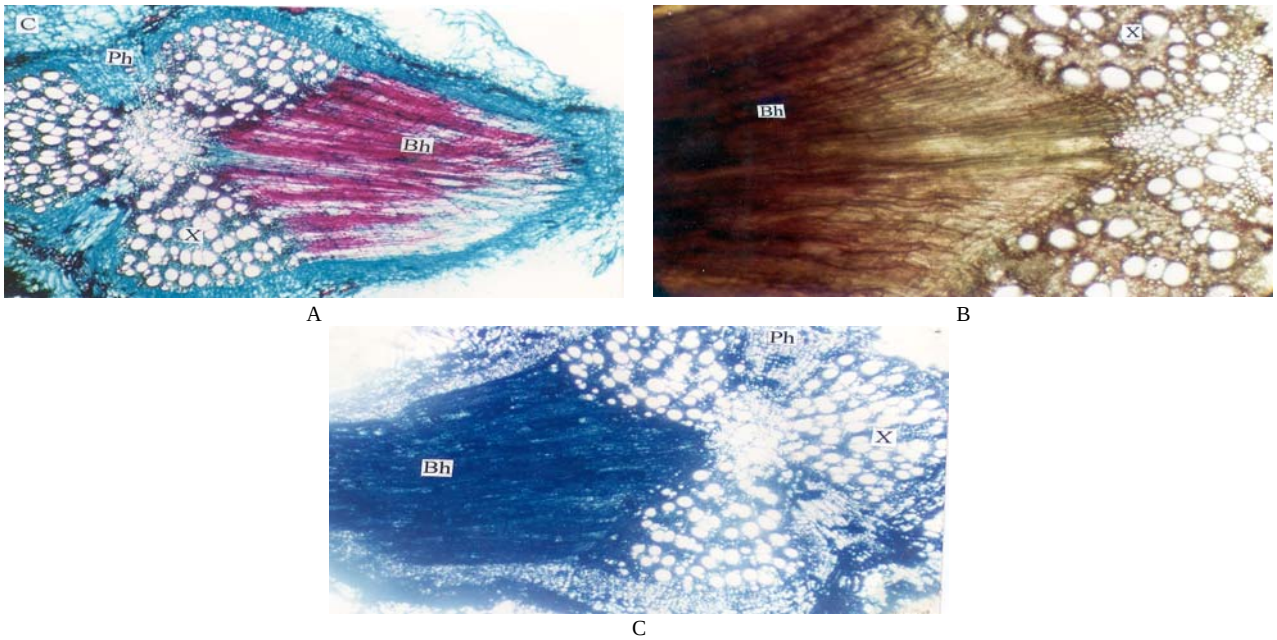
A B

Fig. 2. Unstained transverse section at the connecting region between broomrape haustoria and pea lateral root under: A: ultraviolet (UV-2A) light. B: blue (B-2A) light



A B

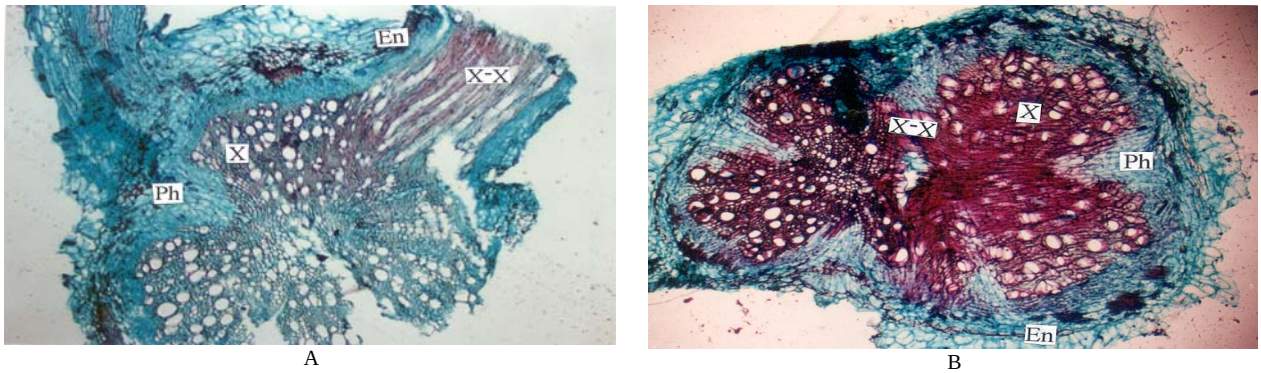
Fig. 3. Transverse section at the connecting region between broomrape haustoria and pea lateral root under visible light: A: stained with double stain (safranin and light green). B: stained with floroglucinol-HCl. C: stained with toludine blue O



A B

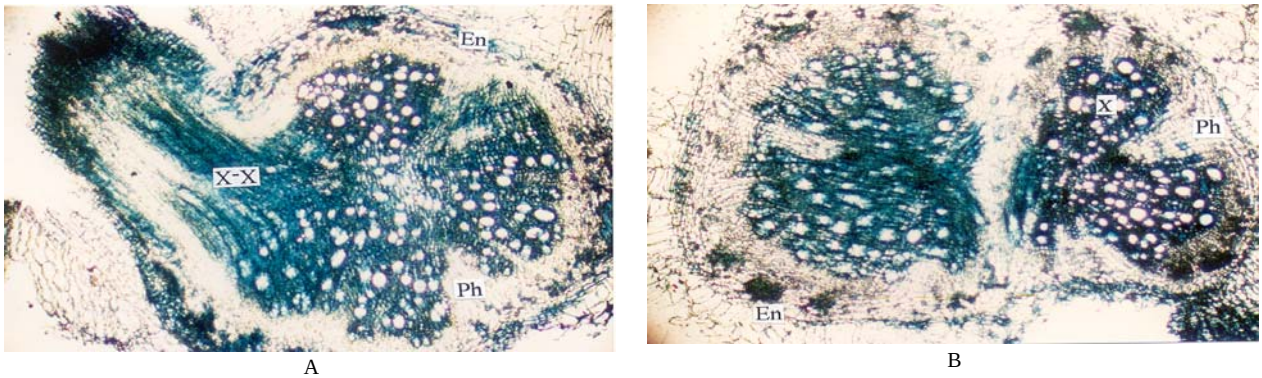
C

Fig. 4. Double stained (safranin and light green) transverse section at the connecting region between the main and lateral root of pea under visible light: A: Healthy area; B: Infested with broomrape



A B

Fig. 5. Toludine blue O-stained transverse section at the connecting region between the main and lateral root of pea under visible light: A: Healthy area; B: Infested with broomrape



A B

phenolic compounds (Croteau *et al.,* 2000).

Our results showed the similarities in behaviour between the connection of broomrape primary and secondary haustoria with the root vascular elements in a relatively wide variety of hosts. The vascular tissue of the haustoria of broomrape appeared as a xylem core at the central part of the tubercle surrounded by a mass of undifferentiated cells. Some of these cells penetrated the outer layers of the host lateral roots after attachment of the primary as well as the secondary haustoria. These cells penetrated the cortical cells of the host root just like hyphal growth of fungi. Progressive growth of broomrape cells took place until the xylem of the parasitic haustoria connected to the phloem-conducting elements of the host (Fig. 1). These results are supported by those of other workers who reported connection of broomrape haustoria to the phloem elements of the host (Dorr, 1996; Dorr & Kollmann, 1995), but disagree with some others that suggest a xylem-xylem connection between broomrape and its hosts (Brog-ter, 1998). Our results coincide with the generality (Kuijt, 1991) that the interface of the connection would be a strongly developed xylem-xylem bridge in semiparasites and a well-differentiated phloem bridge in holoparasites. Intensive lignification and/or allied phenylpropanoid accumulation was obvious in the walls of the host cells at the points of attachment between the host cells and the parasite haustoria throughout their entry path within the root cortex and the sites of vascular connection. Furthermore, a comparison was done between the general framework of the anatomical and histochemical features in the boundaries of penetration and connection of the secondary to the primary root in healthy plants, and that of haustoria and host cells in infected plants. It was clear that both cases are similar in the accumulation of lignin and other phenylpropanoids at the attachment zones, but such reinforcement was markedly more intense in case of haustorial connection. The relative deposition of cellulose in the cortical and phloem cells did not show differences between healthy and infected plants. Lignified and/or suberized cells were also considered to form physical barriers by strengthening the cell walls in a different host-parasite system (*Lycopersicon esculentum* parasitized by *Cuscuta reflexa*), in addition to a defence involving enhanced concentrations of soluble phenolic compounds and peroxidases (Sahm *et al.,* 1995). Lignification has been associated before with resistance after penetration of the host by *Orobanche* (Dorr *et al.,* 1994): resistant sunflower root cells in contact with *Orobanche cumana* develop lignin impregnated layers (Antonova, 1994). Similarly,

one of the mechanisms that cause resistance against *Striga gesnerioides* in cowpea is the development of lignified cells around the parasite-penetration sites (Moore *et al.*, 1995). Understanding the molecular mechanisms behind the development of lignified cells and the enhanced formation of defence metabolites (such as phenylpropanoids & others) in response to parasite attack may provide an excellent window into possible general defensive mechanisms against parasitic angiosperms (Orcutt & Nilsen, 2000).

Neither in the penetration of secondary roots of healthy peas nor of broomrape haustoria in cases of infection, was mechanical damage or rupture observed in the root cells. Parker and Riches (1993) suggested that haustorial extension in the host roots may occur by enzymatic action, resulting in separation of the host cells, rather than destruction or intracellular penetration. Joel and Losner (1994a) also concluded that the parasite appeared to grow between, rather than through the cortical cells of the host. However, such responses may be similar in cases of infection and wounding (Zhou & Thornburg, 1999). Consequently, the accumulation of defensive compounds seen here along the connection zones of the haustoria within the host may be concomitants of sealing and recovery from cell penetration. The appearance of such compounds at the boundaries of connection between the main and lateral roots of a healthy host show that compounds such as lignin and allied phenylpropanoids can be induced normally in host cells. In the presence of broomrape infection it might be tentatively suggested that a signal is perceived and translated to the host genome, resulting in an upregulation of the genes encoding these defensive metabolites. Hormones, receptors and intermediate signaling molecules are no doubt of prime significance in this response.

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