The response of two legume crops (hyacinth bean and kidney bean) to the parasitism of field dodder (*Cuscuta campestris)*

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Abstract: Microscopic examinations and chemical studies were performed to study the response of two legume crops, hyacinth bean (*Lablab purpureus* (L.) Sweet and kidney bean (*phaseolus vulgaris* L.) to the filed dodder (FD) (*Cuscuta campestris* Yuncker) parasitism.

Hyacinth bean, showing no effective resistance mechanisms, was found to be a highly susceptible host to FD. However , kidney bean, displaying resistant reactions towards the parasitism of FD, was found to be an incompatible host.

The possible reasons for the resistance of kidney bean to FD were anatomical (hypersensitivity) and chemical (high contents of phenolic acids and lignin) stimulated defence mechanisms , which developed during the actual intrusion of FD haustorial cells inside its tissues.

Keywords: Filed dodder, Hyacinth bean, hypersensitivity, incompatible host, kidney bean , lignin , phenolic acids, susceptible host.

Introduction:

Field dodder (FD) (*Cuscuta campestris* Yuncker , family Cuscutaceae) is an obligate parasite attacking the shoot system of numerous species of dicotylednous plants, specially legume crops. Dawson *et al.* (1994) reported the genus *Cuscuta* as a serious problem in forage legumes principally alfalfa *(Medicago sativa)*, clover *(Trifolium spp.)* and lespedeza (*Lespedezia* spp.). However , *Cuscuta* species may grow poorly or not at all on some hosts. These are known as incompatible hosts. Several factors may cause incompatibility e.g. hypersensitivity as in tomato, hyperplasia as in okra and hypertrophy as in potato (Farah, 2007).

The objectives of the present work were to study through anatomical and chemical techniques, the response of two legume crops (hyacinth bean: *Lablab purpureus* (L.) Sweet , and kidney bean: *Phaseolus vulgaris* L.) to FD (*Cuscuta campestris* Yuncker) parasitism, and to identify the anatomical and chemical mechanisms involved in their response.

Materials and Methods:

Two legume crops, hyacinth bean and kidney bean were raised in towenty 25 cm diameter plastic pots filled with 1:1 mixture of sand and peatmoss, at the rate of 10 pots per each crop. In one half of the pots, the soil was thoroughly mixed with FD seeds at the rate of 150 seeds per pot. The second half of the pots were left free from FD seeds, as untreated control.

Ten legume seeds per pot were sown in 2004/2005. Ten days after emergence, the seedlings were thinned to four per pot.

The pots were placed in a greenhouse (28^0 C / 23^0C day and night temperatures) at the king Faisal University Farm, AL-Ahsa ($25^0$22' N Latitude, $49^0$34' E Longitude), Saudi Arabia.

After one week from the beginning of parasitism, samples (one plant / pot) of infected and non infected plants of the two legume crops were taken for anatomical studies at the Botany laboratory, College of Agricultural and food Sciences, King Faisal University , Saudi Arabia. The samples were fixed in F.A.A (Formaldehyde, Acetic acid , Alcohol) in the ratio of 1:1:18 ; dehydrated in gradual ethanol solutions ranging from 15% to 100% then treated in mixtures of absolute ethanol (99.9%) and xylene in three different ratios, 3;1 , 1:1 , 1:3 , respectively (Berlynand and Miksche , 1976).

The samples were infiltrated with xylene and embedded in paraffin wax. Sections (15-20 µm thick) were prepared using Bright 5040 type rotary microtome. They were stained with safranin and light green stains and mounted in Canada balsam. The stained sections were examined microscopically using an Olympus 1-12 Binocular Microscope. Microscopic photographs (micrographs) were taken under 10 x and 40 x objective lenses using Leitz laborlux 12 microscope supplemented with Leitz Vario-Orthomat (Camera system) for automatic photography.

The total phenolic acids and lignin contents were determined for the two crops (hyacinth bean and kidney bean) at the Biochemistry laboratory, Department of Animal Production, College of Veterinary Medicine, King Faisal University, Saudi Arabia. Samples of the shoot system of the infected and non-infected crops were allowed to dry at room temperature for 10 days. The air dried samples were ground into powder and used for determination of total phenolic acids contents according to Basden and Dalvi (1983), and determination of lignin according to Van Soest and Win (1967).

Results and Discussion:

Hyacinth bean was found to be a highly susceptible host, while kidney bean was found to be a resistant host to FD. Figure 1 depicts the susceptibility of hyacinth bean. A clear haustorial connections were established between the vascular tissues of the host and the parasite (Fig.2). Moreover , the presence of a conspicuous xylem – ylem bridge between the two partners (Fig.2) revealed the higher susceptibility of hyacinth bean. Thus, in the current study, it was evaluated as a true host. This result is in agreement with that of Kuijt (1969) who stated that true hosts are only those in which hyphal tracheary connections are complete. The formation of effective connections between FD searching hyphae and hyacinth bean vascular tissues was of paramount importance for successful development of the parasite. In this respect, Joel *et al.* (1996) reported that haustorial cells must differentiate as soon as the parasite intrusive cells contact the host conductive tissues. However, Fineran (1987) claimed the existence of an extreme apoplastic pathway suitable for transport between the haustorium and the host tissues.

It was noticed, during the present study, that the steps of field dodder parasitism in hyacinth bean were as follows:

The attachment of FD shoot to the host surface; the formation of superficial protuberances at the contact surface of the parasite with the host; the formation of several haustoria and the penetration of the functional ones across the host tissues; the formation of searching hyphae; the differentiation of the searching hyphae reaching hyacinth bean conductive tissues into xylem and phloem cells. All these steps are crucial for the survival of the parasite. Failure of the parasite to perform one or more of these steps may lead to the failure of the desired functional and dynamic host-parasite union.

In the present study, the best example illustrating the failure of the host-parasite union, is the one manifested by kidney bean and FD (Fig.3). The resistance of kidney bean to the parasitism of FD was attributed to hypersensitive reactions, i.e. the damage of the epidermal and / or cortical cells of kidney bean surrounding the FD haustorium , thus isolating the latter from reaching the host vascular tissues (Fig.4). The hypersensitivity of kidney bean was an stimulated defence mechanism which developed during the actual intrusion of FD haustrial cells inside the incompatible host (kidney bean) tissues. This finding was in harmony with that of Arnaud *et al*. (1996) in the case of *Phaseolus vulgaris* and *Cuscuta reflexa.*

Phenolic acids and lignin comprised a second type of defence mechanism against FD parasitism. They are chemical barriers used by certain incompatible hosts to resist the attack of parasitic weeds (Farah,2000). Phenolic compounds probably constitue one of the widely spread and diverse group of secondary plant metabolites, and their role includes excretion of waste products and defence against invading pathogens (Abercrombie *et al.*,1978). Lignin , on the other hand, is formed of phenylpropanoid units that encrust the cellulose of the cell walls of the higher plants thus contributing to their mechanical strength and rigidity (Walker, 1975). The results of the current study, indicated a rise in both phenolic acids and lignin contents with FD infestation in hyacinth bean and kidney bean. The FD infected plants of kidney bean and hyacinth bean, showed 116.2% and 7.4% ; and 48.0% and 1.5% increase over the control in phenolic acids and lignin contents, respectively (Table1). Thus, these chemicals were found to be much higher in the resistant crop (kidney bean) as compared to the highly susceptible crop (hyacinth bean). The increase in the levels of both chemicals in the infected kidney bean may be attributed to the fact that these chemicals were stimulated as part of the defence reactions of this crop against the penetration of FD haustorium into its tissues. These results are in line with those of Arnaud *et al.*(1996) in *Cusuta reflexa* and *Phaseolus vulgaris;* Antonove and terBorg (1996) in *Orobanche cumaua* and *Helianthus annuus*, and Goldwasser *et al*. (1999) in *Orobanche aegyptiaca* and *Vicia atropurpurea* , who attributed the resistance of the host plants to a number of factors including phenolic compounds and lignin. On the other hand, susceptible crops can also respond to the invasion of the parasite. This was demonstrated by the limited increase in the levels of both phenolic acids and lignin contents in the infected plants of hyacinth bean. Similarly , Joel *et al.* (1996) claimed that the susceptible host plants can also sense the invasion of the parasite , but lacking the appropriate genes, they do not display any effective resistance mechanism.

In conclusion, hypersensitivity (a stimulated anatomical defence mechanism) and the higher contents of phenolic acids and lignin (stimulated chemical defence mechanisms) were assumed to isolate the FD haustorium from the incompatible host (kidney bean) vascular tissues , by blocking its intrusion. The susceptibility of hyacinth bean may be attributed to lacking such defence mechanisms.

Fig.1: On the highly susceptible host (hyacinth bean) field dodder is characterized by luxuriant and prolific growth, with many functional coils , thick stem, yellow or yellowish orange colour, and high capacity to produce flowers and fruits.

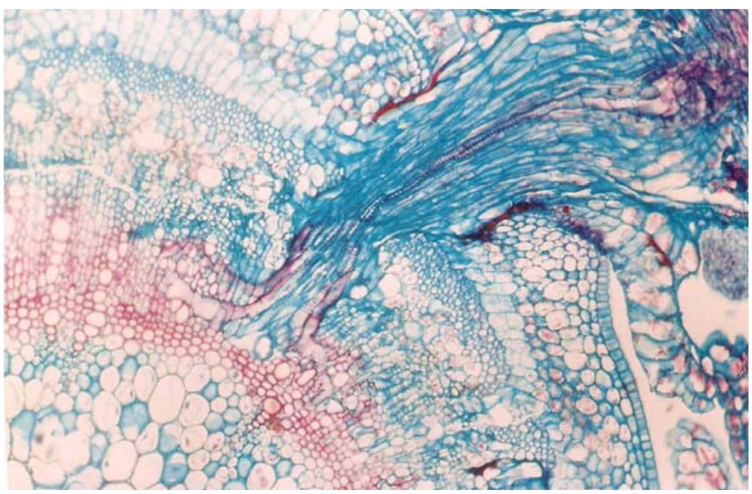

Fig.2: Establishment of connections between filed dodder's searching hyphae and the host (hyacinth bean) vascular tissues , and formation of clear xylem– xylem bridge between the two partners (100x).

Fig.3: Field dodder on the incompatible host (Kidney bean) looks pale green, inadequately nourished, with thin stem and limited number of coils.

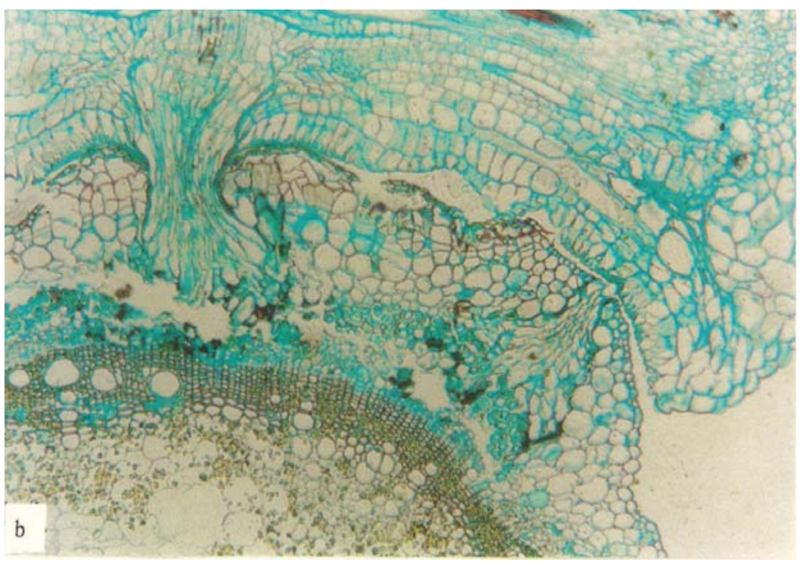

Fig.4: Isolation of field dodder's haustoria form reaching the vascular tissues of kidney bean,(an example of hypersensitive reaction)(100x).

Table 1. Total phenolic acids and lignin contents in kidney bean and hyacinth bean

Legume crop	Treatment	Phenolic acids content (%)	Lignin content (%)
Kidney bean	Untreated plants*	3.7	4.6
	Treated plants**	8.0	6.8
Hyacinth bean	Untreated plants	2.7	6.5
	Treated plants	2.9	6.6

*Untreated plants ≡ plants free from field dodder.

**Treated plants ≡ plants infected with field dodder.

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