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Host Penetration and Infection by the Anastomosis Groups of *Rhizoctonia solani* Kühn Isolated from Potatoes*

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Abstract: The modes of penetration and infection of the isolates belonging various anastomosis groups (AG-2 type 1, AG-2 type 2, AG-3, AG-4 and AG-5) of *Rhizoctonia solani* Kühn isolated from potatoes were investigated on the underground stems of the host. During the process of penetration, infection cushions were observed in all isolates in addition to the formation of lobate appressoria by AG-3, AG-5 and direct hyphal penetration of AG-4.

Numerous fine infection pegs formed from infection cushions grew between or into epidermal cells following the penetration of cuticula. A similar mode of penetration was also occurred by the infection peg produced from every lobe of appressorium except that the infection peg enlarged immediately after passing through the cuticula. Further invasion of tissues beneath the epidermal layer continued inter- and intracellular manner.

Patatesten İzole Edilen *Rhizoctonia solani* Kühn'nin Anastomosis Gruplarının Konukçuya Penetrasyonu ve Enfeksiyonu

Özet: *Rhizoctonia solani* Kühn'nin patatesten izole edilen anastomosis gruplarına (AG-2 tip 1, AG-2 tip 2, AG-3, AG-4 ve AG-5) ait izolatların konukçunun gövdesine penetrasyon ve enfeksiyon yolları incelenmiştir. Penetrasyon esnasında bütün izolatların enfeksiyon yastığı oluşturduğu, ayrıca AG-3 ve AG-5'e ait izolatlarda loblu apresorium oluşumu, AG-4'e ait izolatta ise direkt hif penetrasyonu gözlenmiştir.

Enfeksiyon yastıklarından oluşan çok sayıda enfeksiyon çivisi kütikuladan penetrasyon yaptıktan sonra epidermis hücreleri arasında veya içinde gelişmiştir. Apresoriumdan oluşan enfeksiyon çivisi de benzer şekilde penetrasyon yapmış ancak enfeksiyon çivisi kutikuladan geçtikten hemen sonra genişlemiştir. Epidermal tabakanın altındaki dokuların istilası hücreler içi ve hücreler arası olarak devam etmiştir.

Introduction

Rhizoctonia solani Kühn (teleomorph *Thanatephorus cucumeris* (Frank) Donk) which is represented by several anastomosis groups (AGs) with different cultural characteristics and host specifications is distributed to all over the world (1). The agent is also present in potato (*Solanum tuberosum* L.) growing areas of Erzurum-Turkey (2).

The penetration of different anastomosis groups and various isolates of *R. solani* to host plants other than potatoes occurs by producing some infection structures. As a matter of fact, infection cushions solely play role in penetrating to beans (3), cottons (4), also together with lobate appressoria facilitate penetration to beans besides radish (5) and rice plants (6). On the other hand, direct hyphal penetration is accompanied to entry achieved by infection cushions in sugar beets (7) and barley (8). Following the penetration of cuticula and cell walls by infection pegs, the growth of hyphae into cells and intercellular spaces was also described on the same hosts (3, 5).

The objective of this study was to observe and determine the formation of fungal structures aiding penetration and development in stem tissues of potato by the anastomosis groups of *R. solani* isolated from potatoes in Erzurum-Turkey.

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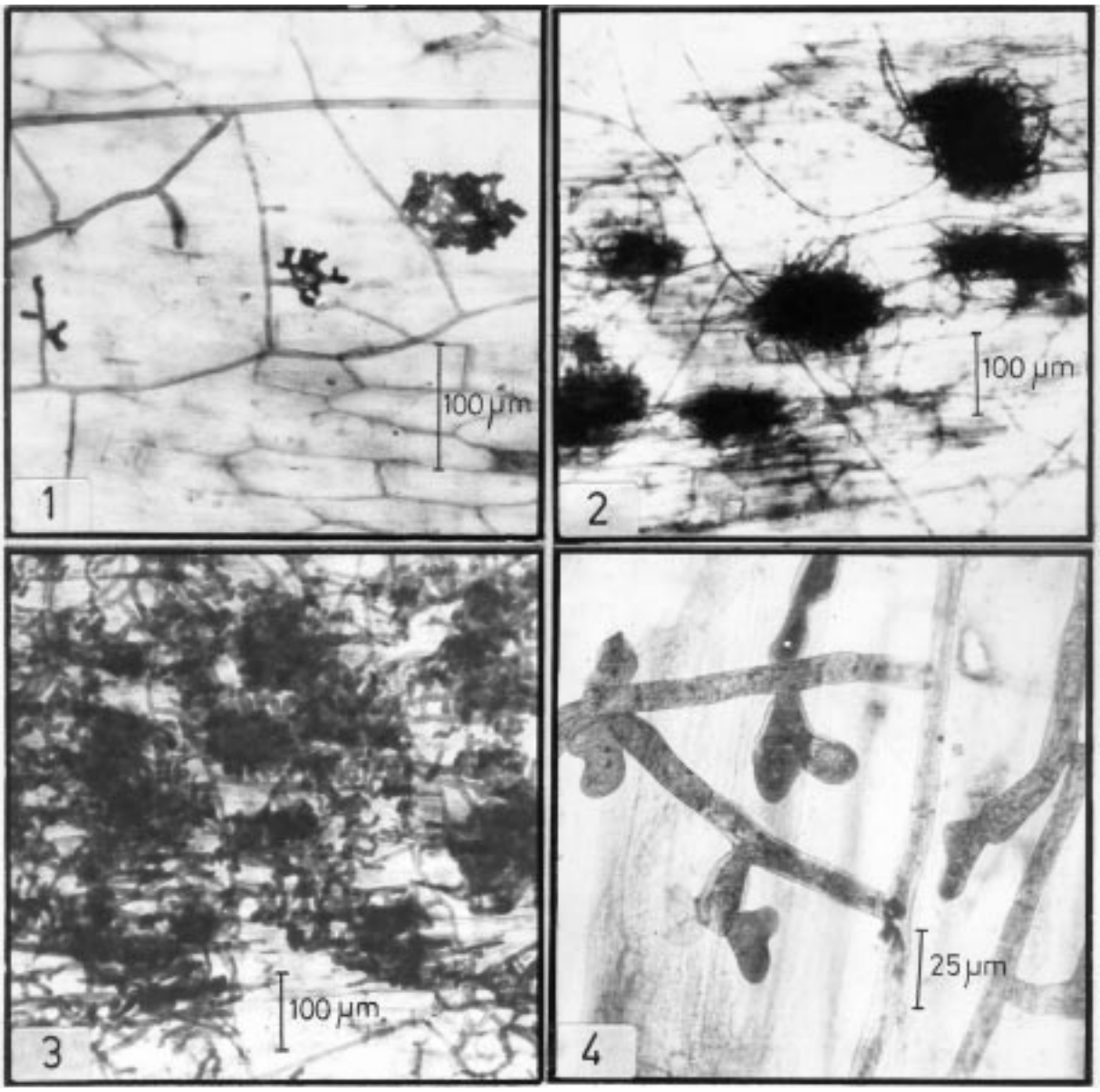


Figures 1-4. Prepenetration development of *Rhizoctonia solani* on potato stem. 1, Different stages in development of infection cushions. 2, Tightly compact infection cushions formed sparsely. 3, Loose infection cushions formed close to each other. 4, Lobate appressoria.

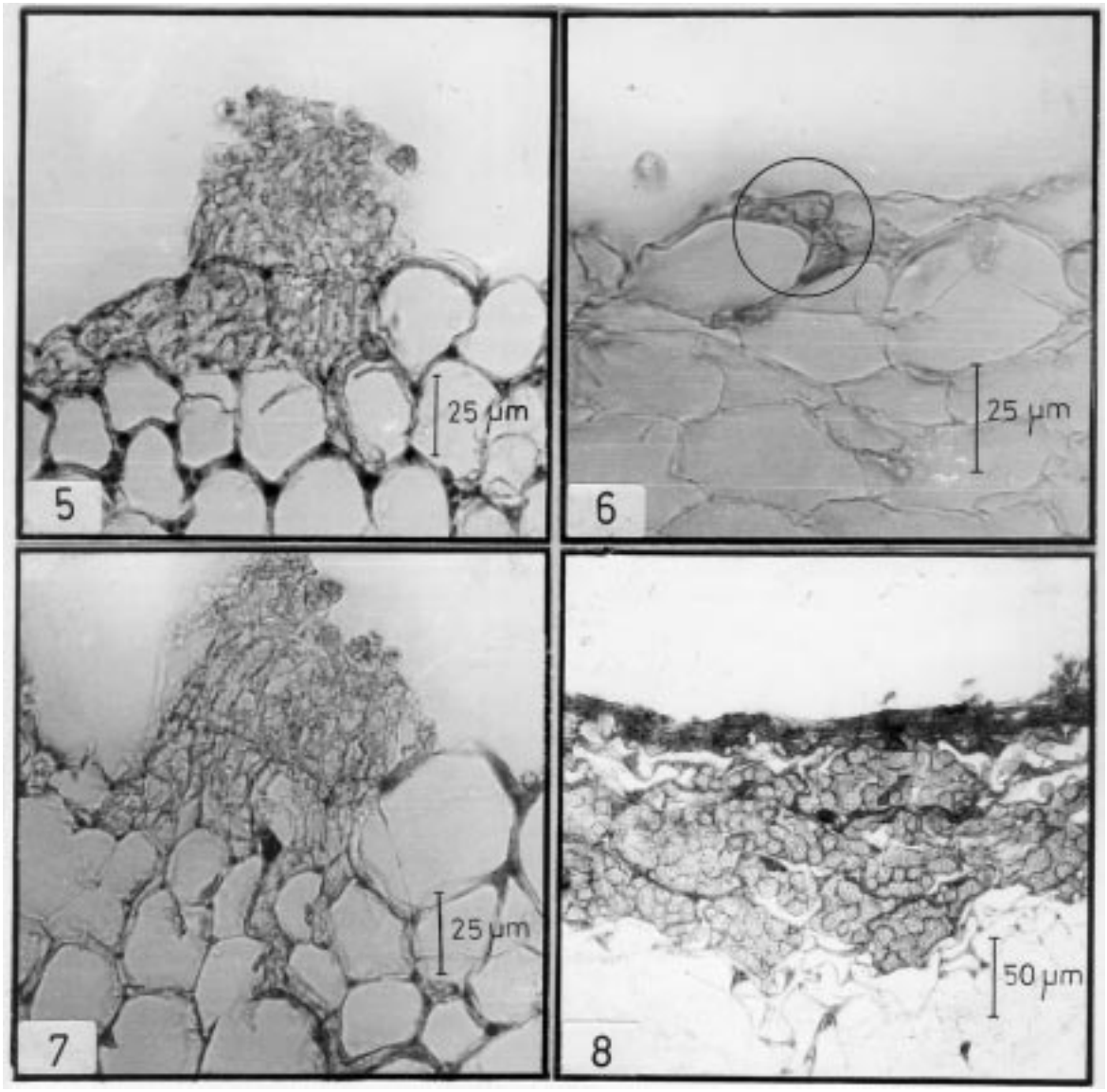
Materials and Methods

Isolates and potato cultivars

Studies on the modes of penetration to underground stems by *R. solani* were conducted on two potato cultivars Bintje and Resy by using an isolate belonging to each anastomosis group of *R. solani* (AM2-90 from AG-2 type 1, AS5-89 from AG-2 type 2, EL3-89 from AG-3, OL3-88 from AG-4 and OL3-89 from AG-5) obtained from potatoes in Erzurum Region (2).

Prepenetration studies

Potato tubers disinfected in 2% formaldehyde for 5 min. were sown into pots containing sterile sand and soil (2:1) mixture. After ten days, the young plants were removed, washed free of soil and laid on microscope slides. Each slide was then placed in a petri dish containing moistened filter paper (5). For inoculation, mycelial discs (5 mm in diameter) taken from every anastomosis group which was cultured on potato



Figures 5-8. Penetration and postpenetration activities of *Rhizoctonia solani* on potato stem. 5. The penetration of cuticula and epidermal cells by infection pegs produced from infection cushion. 6. The enlargement of infection peg of lobate appressorium after cuticular penetration. 7. The invasion of tissues beneath the epidermal layer. 8. Monilioid cells in cortical tissues.

dextrose agar were placed singly on stem of each plant and they were incubated at 25°C in darkness. Then 3-4 hour intervals, tissue pieces (0.5 x 1 cm^2) peeled from epidermis were immersed in 0.1% (w/v) trypan blue in 50% (v/v) acetic acid from 10 minutes and rinsed in distilled water to remove excess stain (4) and examined under light microscope.

Postpenetration studies

Two cm long segments were excised at different times from the stems used for the prepenetration studies or from those raised in soil which infested at sowing with the pathogen as described by Papavizas and Ayers (9). Then the stems were fixed in formaldehyde + acetic acid + ethyl alcohol (FAA) solution. After dehydrating and embedding in paraffin, transverse sections were taken.

Sections stained with safranin and fast green were observed under light microscope.

Results

Histological observations during the penetration of *R. solani* revealed that all isolates of AG-2 type 1, AG-2 type 2, AG-3, AG-4 and AG-5 produced infection cushions in both potato cultivars (cv. Bintje and Resy). Lobate appressoria together with infection cushions occurred in cv. Bintje by AG-3 and AG-5, but only by AG-5 in cv. Resy. However, direct hyphal entry was observed by AG-4 in both cultivars.

Prepenetration processes

Longitudinal axis of the stems, primary and secondary branches formed from the main hyphae 6-8 hours after inoculation. These branches elongated or formed the infection cushions and/or lobate appressoria. Primary and secondary hyphae did not elongate further and formed short, swollen, usually irregular clusters of side branches leading to formation of infection cushions (Fig. 1). The infection cushions of AG-2 type 1, AG-2 type 2 and AG-4 isolates from the several branches of the parent hyphae or even from a single branch of one hyphae formed separately from each other (Fig. 2). However, in isolates of AG-3 and AG-5 the aggregation of side branches of the same or different hyphae produced loose infection cushions. But, they formed very close to each other, densely covering the surface of the stem (Fig. 3). On the other hand, lobate appressoria were formed by swelling of primary and secondary hyphae and formation of lobes at their apex (Fig. 4). Lobate appressoria were not aggregated into clumps and thus distinguishable from the infection structures.

Penetration processes

Numerous fine infection pegs produced in the base of infection cushions penetrated into epidermal cells or in spaces between them following the penetration of cuticula (Fig. 5). A similar mode of penetration also occurred by the infection peg produced from each lobe of appressorium, unless the infection pegs enlarged after passing through cuticula (Fig. 6). As a result of penetration by means of the infection cushions, lobate appressorium or directly by hyphae, epidermal layer was invaded by the hyphae of *R. solani.*

Postpenetration processes

Further invasion of inter- and intracellular tissues continued under the epidermal layer (Fig. 7). The tissues beneath the infection cushions collapsed and typical stem lesions were produced. In cortical tissues of stems infected by the isolate of AG-3, monilioid cells were observed 90 days after inoculation (Fig. 8).

Discussion

R. solani was a common agent of diseases in many plant species. Details of penetration and infection by this fungus were well documented by various studies. Infection structures and penetration depended upon the isolate, anastomosis groups, plant species and plant part on which they formed (5, 10, 11). The infection cushions were produced by all isolates of anastomosis groups in both potato cultivars although lobate appressoria were formed by AG-5 and by AG-3 depending on the cultivar. Direct hyphal penetration which was detected rarely (7, 8) was observed by the isolate of AG-4 both in Bintje and Resy. From the results presented above it could be concluded that the penetration of *R. solani* to potato stems occurred mostly by producing infection cushions and secondly by lobate appressoria as observed in other plant species (3, 5, 6). The developmental steps in the morphogenesis of infection cushions and lobate appressoria were similar to those described by previous studies. However, infection cushions by AG-3 and AG-5 did not have a tightly compacted structure and formed sparsely as was in isolates of AG-2 type 1, AG-2 type 2 and AG-4. They were loosely formed, being very close to each other and covering the surface of stems. Dense growth of infection cushions indicated high virulence of AG-3 and virulence of AG-5 which was also reported to be virulent on potatoes besides AG-3 (12). There were also positive correlation between the density of infection structures and disease severity (6). Therefore, the increase in the number of infection structures in a given area may mean increases in the virulence of the isolate or anastomosis group of *R. solani* to host plants.

Penetration from cushions observed as the formation of numerous fine infection pegs was most commonly described by other studies (3, 4, 5). However, Dodman et al. (5) found that penetration of radish hypocotyls took place in the center of cushions by a single well developed infection peg. Moreover, the penetration facilitated by an infection peg produced from each lobe of appressorium was similar to those occurred from infection cushions except for the enlargement of infection pegs in subcuticular position. These result confirm previous reports by Christou (3), Dodman et al. (5) and Armentrout and Downer (4). Beneath the epidermal layer inter- and intracellular development continued and the collapse of tissues in infected areas produced the lesions.

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