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研究簡報

菱角紋枯病菌侵害過程之掃描式電子顯微鏡觀察

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臺中縣霧峰鄉臺灣省農業藥物毒物試驗所農葯應用系

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菱（*Trapa taiwanensis* Nakai）係水生植物，主要產地臺南縣官田鄉，近年來有白絹病菌[1]及紋枯病菌[2]（*Rhizoctonia solani*）為害菱葉片、葉柄及莖部，並漸趨嚴重，本病次為白絹病已成菱角生產之限制因素，在菱葉片上最初產生圓型水浸狀病斑或不規則小型黑褐色斑，病斑逐漸擴大而相癒合，使部份或整個葉片腐爛，且在腐爛葉片上形成菌核，初呈白色後變褐，菱紋枯病及白絹病最早為蔡及吳[1,2]於1981年報導。*Rhizoctonia solani* 於菜豆及紅蘿蔔幼苗胚軸上[3,4]以侵入墊（infection cushion）或葉狀附著器（lobate appressorium）侵入組織，本文利用掃描式電子顯微鏡觀察菱紋枯病菌侵染菱葉片及葉片上形成菌核之過程。

田間採集病葉經分離後，長出之菌絲移入馬鈴薯葡萄糖瓊脂（potato dextrose agar）試管培養於室溫（約26-28℃）一個月後，取出成熟菌核於菱葉片上，並亦採自菱葉片上之菌核，兩者病原性結果頗一致，菱葉片採自臺南縣官田鄉取回種於溫室塑膠水缸中數週之菱健株，接種後之葉片置於濾紙保溼培養皿中，於26℃之恆溫箱中每隔4小時取樣一次，取樣時分兩種大小，一種為5×6㎜，另一種爲1×6㎜之細條，至培養皿中長出褐色菌核止，樣品以2.5%戊二醛（PH＝7.0，溶於磷酸緩衝液中）於室溫固定4至6小時後經系列酒精脫水及乾燥處理後鍍上金膜，置於日立掃描式電子顯微鏡（Hitachi S-410）中，以加速電壓15KV觀察及照像。

菱紋枯病菌菌核在菱葉片上6-8小時開始發芽，發芽後6-12小時可見單一菌絲直接穿透上表皮侵入組織（圖1）或由氣孔侵入（圖2），菌絲亦可長出葉狀附著器（圖3）或形成侵入墊（圖4），利用侵入菌絲（infection hyphae）侵入寄主組織（圖5），侵入菌絲達寄主組織途徑，可由氣孔下氣室（圖6），或直接經表皮細胞侵入柵狀組織加害（圖7），34-40小時菌絲破壞柵狀組織，使褐變面積擴大，逐漸變黑褐色，48小時後菌絲侵害海綿組織細胞及維管束細胞（圖8）並以直接穿透細胞壁的方式擴展，54小時後菌絲直接穿透下表皮組織曝露大氣中（圖9）。

若將菌核接種於葉片下表皮時，菌核發芽後可由單一菌絲、葉狀附著器或侵入墊穿透表皮組織，進入組織內，蔓延極速，72小時後可見菌絲在上表皮下組織蔓延並由氣孔伸出一或數支菌絲者（圖10）。

接種菌核約72小時後葉片開始腐爛，待葉片組織破壞殆盡，於濾紙幾乎乾燥之情況下，

136-140小時後於葉片上或其附近之濾紙上開始有菌絲纏繞（圖11）、癒合（圖12），約148小時時，有初級白色菌核形成，直徑約1.35㎜（圖13），154小時時，白色菌核增大約1.5㎜，168小時後菌核仍白色增大至2.1㎜，約188小時時菌核大小不變但轉深褐色，外表似絨毛狀，菌核球形（圖14），盆栽菱角接種時病徵進展稍慢外大致相同，於後期如同田間，在葉面能形成菌核。

紋枯病菌在水稻、菜豆、胡蘿蔔等寄主上多利用侵入墊或葉狀附著器直接侵入寄主[4,5,6,7]，在菱葉片上除侵入墊或葉狀附著器經由氣孔或直接侵入寄主外，亦可利用單一菌絲侵入，菜豆[5]上侵入墊形成乃由多數菌絲連續分枝聚合而成，在胡蘿蔔[5]則可由單一菌絲分枝聚合形成侵入墊，而菱角葉片上則兩種情形均可見到，菜豆及胡蘿蔔[5]形成葉狀附著器一般經由氣孔侵入寄主組織，而在菱角葉片上葉狀附著器可經由氣孔或直接穿透表皮組織侵入寄主，菌絲侵入組織後，由氣室細胞而貫穿下表皮，或經由柵狀組織而擴展，為害海綿組織及維管束組織，菌絲亦可在葉片上面擴展而為害相疊之其他健全葉片組織，待葉片組織破壞殆盡時，於其上面菌絲互相纏繞聚集，逐漸成團而形成菌核。

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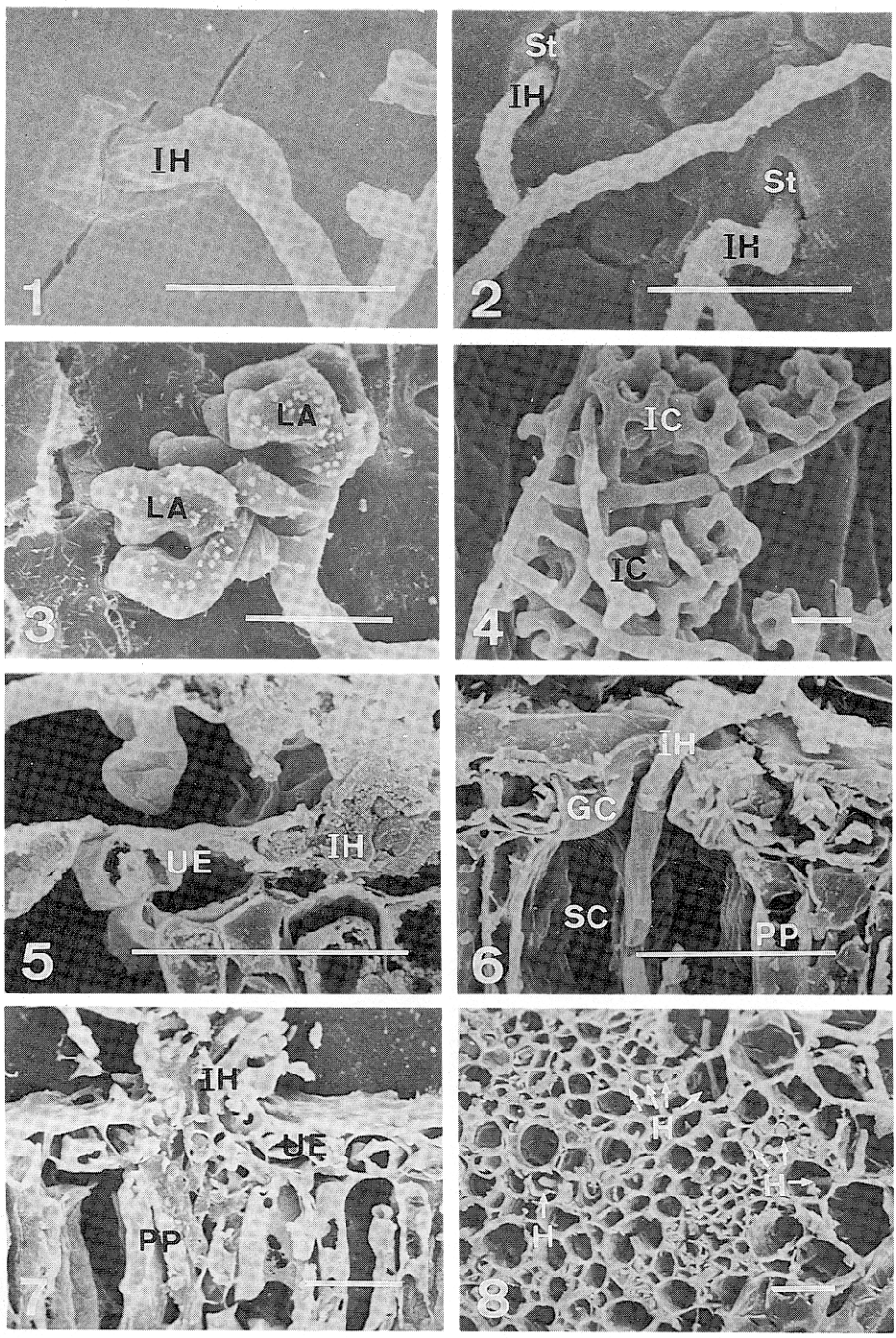
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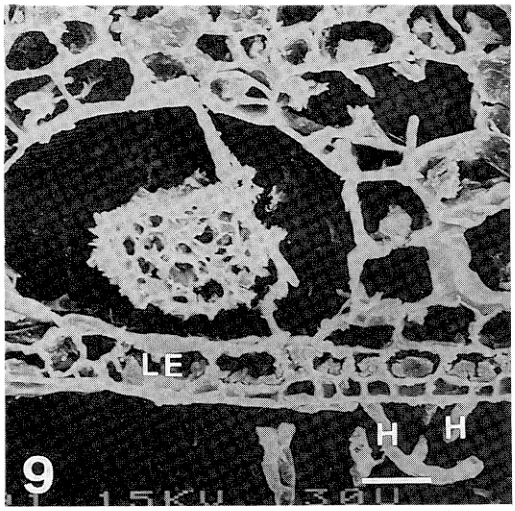
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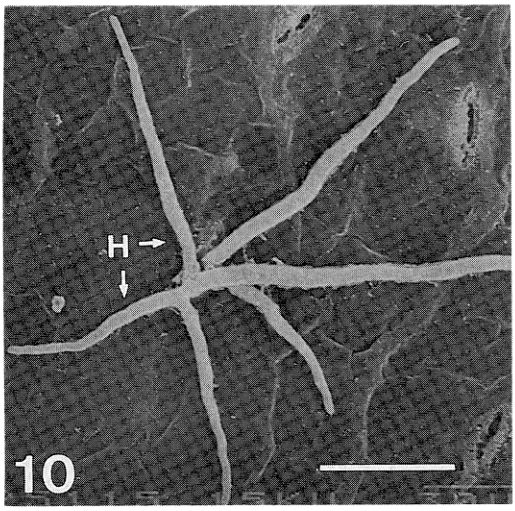
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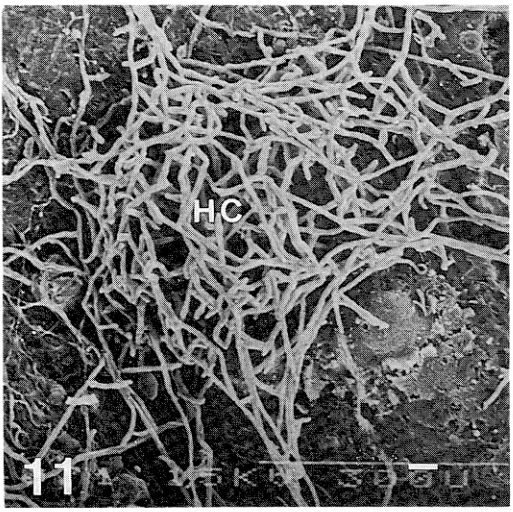
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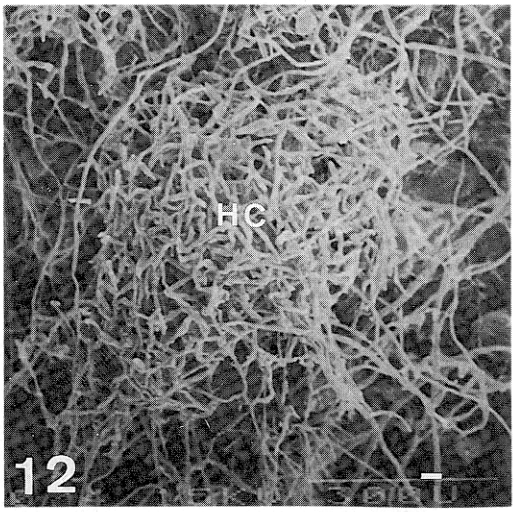
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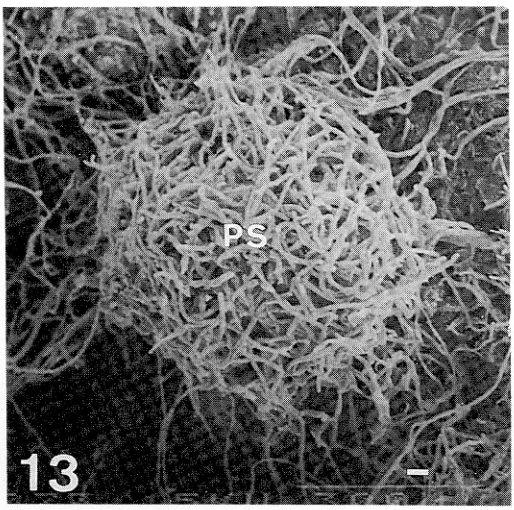
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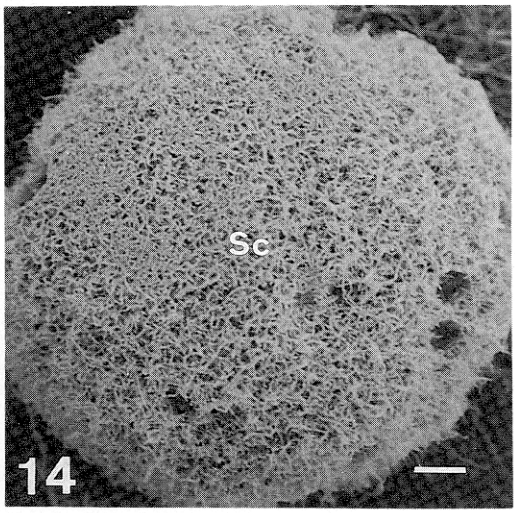
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圖版說明 (explanation of plates): 長度尺均爲30μm但圖14爲300μm(bar presents 30μm except 300μm in Fig. 14). GC (guard cell), 保衛細胞; H (hyphae), 菌絲; HC (hyphal cluster), 菌絲纏繞癒合; IC (infection cushion), 侵入墊; IH (infection hyphae), 侵入菌絲; LA (lobate appressorium), 葉狀附着器; LE (lower epidermis), 下表皮; PP (palisade parenchyma), 柵狀組織; PS (primary sclerotium), 初級菌核; SC (stomatal cavity), 氣孔下氣室 Sc (sclerotia), 菌核; ST (stomata), 氣孔; UE (upper epidermis), 上表皮; VB (vascular bundle), 維管束

圖1：單一菌絲直接穿透上表皮組織

Fig. 1: Single infection hypha (IH) penetrated into host tissues via epidermal cells.

圖2：單一菌絲由氣孔侵入葉片組織

Fig. 2: Single infection hyphae (IH) penetrated into leaf tissues via stomatal (St) opening.

圖3：菌絲表皮上長出葉狀附著器侵入寄主組織

Fig. 3: Lobate appressorium (LA) formed on epidermal cells to penetrate into host tissues.

圖4：菌絲在表皮上成侵入墊侵入寄主組織

Fig. 4: Infection cushion (IC) formed on epidermal cells to penetrate into host tissues.

圖5：由葉狀附著器或形成之侵入菌絲侵入寄主組織

Fig. 5: Infection hyphae (IH) formed from lobate appressorium (LA) penetrating into host tissues via upper epidermal (UE) cell.

圖6：侵入菌絲由氣孔下氣室侵入柵狀組織

Fig. 6: Infection hyphae (IH) penetrating into palisade parenchyma (PP) through stomatal cavity (SC).

圖7：侵入菌絲直接經上表皮細胞後侵害柵狀組織

Fig. 7: Infection hyphae (IH) penetrating into upper epidermal cells and ramifying in palisade parenchymas (PP).

圖8：接種48小時後菌絲侵害維管束細胞

Fig. 8: Hyphae (H) penetrating into vascular bundles (VB) 48 hrs after inoculation.

圖9：接種54小時後菌絲直接穿透下表皮組織曝露大氣中

Fig. 9: At 54 hrs after inoculation, hyphae (H) pass through lower epidermal (LE) cells directly and expose in open air.

圖10：菌核由下表皮接種後72小時，可見菌絲由氣孔伸出一或數支菌絲

Fig. 10: At 72 hrs after inoculation hyphae protruding from stomata in the upper epidermis (UE), while the sclerotia were put on lower epidermis (LE).

圖11：菌絲癒合

Fig. 11: Hyphae tangle each other to form hyphal cluster (HC).

圖12：菌絲纏繞

Fig. 12: Hyphae tangle each other compactly.

圖13：形成菌核

Fig. 13: A primary sclerotium (PS) formed.

圖14：形成成熟菌核

Fig. 14: A mature sclerotium (Sc).

Pathway on Infection of *Rhizoctonia solani* Kühn Induces Leaf Blight of *Trapa taiwanensis* Nakai as Observed by Scanning Electron Microscope

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ABSTRACT

Lii, Y. H., Chang, C. W., and Leu, L. S. 1989. **Pathway on infection of Rhizoctonia solani Kühn induces leaf blight of *Trapa taiwanensis* Nakai as observed by scanning electron microscope.** Plant Prot. Bull. 31: 211–216.

Rhizoctonia solani Kühn AG-1 induces leaf blight on water caltrop or so-called Shinghara-nut (*Trapa taiwanensis* Nakai). Sclerotia of *R. solani* germinated 6-8 hr after inoculating on the upper leaf surface. Single hyphae penetrated directly through epidermis or indirectly through stomatal opening 6-12 hr after germination. Mycelium also differentiated into lobate appressorium or infection cushion. The infection hyphae directly devastated or passed through stomatal cavities and then destroyed palisade parenchyma. The palisade parenchyma was destroyed 34-40 hr later and the discolored tissues expanded and turned to black brown eventually. Spongy parenchyma and vascular bundles were invaded after 48 hr. Hyphae ramified in the tissues by penetrating through cell walls. The hyphae were detected on the surface of lower epidermis in 54 hr. The tissues including lower epidermis start to decay 54 hr later and decayed completely within 72 hr. As paved filter paper drying up, hyphae tangled each other on the decayed leaves or nearby filter paper. White sphaearical sclerotia of 1.25 mm formed 148 hr later, then enlarged to 1.5mm at 154 hr and retained 2.1mm in a diameter at 168 hr. The sclerotia then turned to dark brown with velvety appearance 188 hr later. If inoculation was performed on the lower surface, penetration pattern was as same as those on the upper surface except penetrating through stomata. The hyphae would appear on the outside of the upper surface through stomata at 72 hr after the inoculation. Same tendency was observed on those inoculated plants maintaining in water bucket kept in a glass house.

(Key words: *Trapa taiwanensis, Rhizoctonia solani,* infection, sclerotial formation)