

洋香瓜囊叢枝內生菌根菌與根瘤線蟲之相互關係

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摘要

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為探討囊叢枝內生菌根菌 (VAM fungus) *Glomus clarum* 與根瘤線蟲 *Meloidogyne incognita* 在洋香瓜根系內之相互關係及對其生長之影響，進行二者之單獨及混合接種，以菌根之形成率、根瘤線蟲為害洋香瓜根部所形成之根瘤數及其繁殖數、及對洋香瓜生長之影響，來明瞭兩者在宿主植物內之相互影響關係。測定根瘤線蟲最初感染源濃度 (Initial inoculum density)，顯示每盆 (株) 接種5隻二齡線蟲即可感染成功，在根部產生根瘤；接種 50隻者8星期後即可造成根部之傷害，每株罹患根瘤數平均為 221個；每株植物接種 100隻線蟲，即可產生 580個根瘤。於二者相互關係接種試驗，即採用每株接種 50隻二齡線蟲。因囊叢枝內生菌根菌完成感染根部之時間比線蟲侵染所需之時間為長，故內生菌根菌之處理採先行接種2星期，再進行線蟲混合接種或單獨接種。處理計有：(一)單獨接種囊叢枝內生菌根菌 (M)，(二)囊叢枝內生菌根菌再接種根瘤線蟲 (M+N)，(三)單獨接種根瘤線蟲 (N)，(四)對照不接種菌根菌及根瘤線蟲 (Ck)。於根瘤線蟲接種後8星期調查，試驗結果顯：示囊叢枝內生菌根菌處理者 (M) 可促進植株地上部及地下部之生長，其每株平均鮮重分別為 63.3公克及 8.14公克，菌根形成率 73.3%；對照植株 (Ck) 分別為 47.6公克及 6.98公克；而根瘤線蟲處理者 (N) 則植株與根系生長均受抑制，每株平均鮮重分別為 41.9公克及 6.82公克，根部罹根瘤數 103個。若有菌根菌存在時，可減輕根瘤線蟲之為害，囊叢枝內生菌根菌與根瘤線蟲之混合接種處理者 (M+N)，植株地上部與地下部之每株平均鮮重分別為 49.5公克及 7.40公克，根部罹根瘤數 65個，菌根形成率 78%。根瘤線蟲之存在不影響囊叢枝內生菌根菌感染洋香瓜根部形成菌根，然而囊叢枝內生菌根菌之存在，可能因侵染點之佔據，減低線蟲之侵染，使得洋香瓜根系之根瘤數目減少，進而影響根瘤線蟲之繁殖。應用囊叢枝內生菌根菌防治作物根瘤線蟲，應預先培育囊叢枝內生菌根苗，再移植於本田，即使有根瘤線蟲存在，也可減輕其為害。

關鍵詞：洋香瓜、根瘤線蟲、*Meloidogyne incognita*、囊叢枝內生菌根菌、*Glomus clarum*

緒言

囊叢枝內生菌根菌 (Vesicular-arbuscular mycorrhizal fungus) 與根瘤線蟲 (Root-knot nematode) 同為作物根部棲息之微生物，但對作物根部則有相反之影響，後者侵染根部會破壞根部組織引起細胞膨大形成根瘤，影響根部發育及功能，阻礙植株生長；而前者感染根部與根部發生密切關係形成共生體，稱之為囊叢枝內生菌根 (Vesicular-arbuscular mycorrhizae, VAM)，是內生菌根之一種，能促進根系發育，增加根部對礦物元素之吸收，尤其是對磷、鉀素，促使囊叢枝內生菌根植株生長旺盛^(2,3,4,9,10,11,20)。

囊叢枝內生菌根菌與土傳病原微生物之相互關係，近20幾年來引起許多學者之注意，自 1972 Fox 等氏⁽¹⁸⁾ 發表

囊叢枝內生菌根菌與包囊線蟲 (*Heterodera solanacearum*) 之相互關係，相繼有各種相關之研究報告^(1,8,12,13,14,15,16)。除為害葡萄之根瘤線蟲 (*Meloidogyne arenaria*) 例子，內生菌根菌增加病害嚴重程度外⁽⁵⁾，大多數的報告均指出囊叢枝內生菌根菌對線蟲病害有抑制效果，又多偏重於固著性內寄生線蟲^(6,29,30)，而囊叢枝內生菌根菌與外寄生性線蟲相互作用之研究則較少。

由於根瘤線蟲是以二齡幼蟲侵入作物根部，並固著在一定部位取食，經多次脫皮後而成肥大雌蟲。在取食過程消耗寄主養分，以及成長時造成根系受傷，常引起寄主地上部生長不良與營養缺乏症狀⁽⁷⁾，但是囊叢枝內生菌根菌可增進根系生長並幫助宿主吸收養分，因此可補償宿主受此種線蟲之為害。Roncadori 等氏⁽²⁸⁾ 報導棉花接種囊叢枝

內生菌根菌則可減輕根瘤線蟲對棉花之為害，其生長僅減少10%，而未接種內生菌根菌之棉花生長則減少達30%。在其他作物胡瓜、燕麥等，內生菌根植株受害程度均較非內生菌根者為輕⁽¹⁶⁾。

潛移性內寄生線蟲在幼蟲侵入根後，可在根系內取食，並不固著於一定部位，而線蟲在外形上亦無甚大改變，仍保持線形。由於在根系內移動造成根系受損嚴重，影響根的功能，亦提供土壤病原侵染的途徑，因此其為害程度較其他線蟲嚴重，如根腐線蟲、穿孔線蟲等即屬於此類線蟲⁽²²⁾。O'Bannon及Nemec等氏⁽²⁷⁾報告，粗皮檸檬接種囊叢枝內生菌根菌，受穿孔線蟲之為害較輕微，植株較非內生菌根者高大，然而線蟲之繁殖在內生菌根與非內生菌根植株上並無差異。Hussey等氏⁽²²⁾報告內生菌根植株受根腐線蟲 (*Pratylenchus brachyurus*) 為害亦較非內生菌根植株輕微，然而線蟲之繁殖不因接種囊叢枝內生菌根菌而受影響。

洋香瓜為本省重要經濟作物之一，亦是根瘤線蟲之適當寄主，洋香瓜栽培常遭遇根瘤線蟲為害，根部產生纍纍根瘤。阻礙地上部枝蔓之生長，影響產量與品質至鉅，尤見於砂質壤土連作田。鑑於囊叢枝內生菌根菌與洋香瓜有良好之親和性，易感染形成囊叢枝內生菌根，產生有益效應。而囊叢枝內生菌根菌及根瘤線蟲二者同為棲息根部之微生物，實必要探討二者同時存在根部時之相互關係，究明囊叢枝內生菌根菌對病原根瘤線蟲侵染根部之影響，以及如何應用囊叢枝內生菌根菌，達到減輕根瘤線蟲之為害，是為本研究之目的。

材料與方法

供試植株與接種源

供試洋香瓜品種為蜜世界，購自農友種苗公司，種子經10% Clorox表面消毒，經無菌水漂洗後，預先催芽，才行播種。囊叢枝內生菌根菌 *Glomus clarum* 則分離自大豆根圈土壤，經純化培養保存在盆栽百喜草，並大量繁殖於玉米根系，收集玉米根圈土壤，依篩網傾注法分離厚膜孢子供盆栽接種試驗用之接種源⁽¹⁹⁾。根瘤線蟲 *M. incognita* 分離自洋香瓜根瘤線蟲之單一卵塊，經純化後，培養繁殖保存於盆栽甘薯根部，試驗時再自罹病甘薯根部，挑取成熟卵塊，經孵化，挑取二齡幼蟲供作接種源。

洋香瓜囊叢枝內生菌根形成率及土壤厚膜孢子密度之計算

囊叢枝內生菌根菌在根系內感染形成囊叢枝內生菌根之評估，係將洋香瓜根系剪成長約1.5公分的根段，在90-98% (w/v) KOH溶液中煮約1分鐘，冷卻後水洗，再以0.5% Trypan blue染液 (Lactic acid 286 ml, Glycerin 354 ml, Distilled water 230 ml, Trypan blue 0.5 g) 染色4小時，經自

來水漂洗後，在顯微鏡下，調查根段內 Vesicles 及 Arbuscules 之產生情形，以評估菌根之形成率⁽²¹⁾。土壤中厚膜孢子數量之調查，係取100公克陰乾土壤，加水1公升，攪拌均勻後，通過孔徑0.25 mm的篩網，收集過濾液，再經孔徑0.074 mm的篩網，以自來水清洗留在銅網上的孢子，再以50 ml的蒸餾水將厚膜孢子洗下，置於小燒杯內，取定量之孢子懸浮液，在解剖顯微鏡下，計算孢子數。

洋香瓜感染根瘤數及土中二齡幼蟲之調查

定期採集洋香瓜根部，清洗後調查根系罹病形成根瘤之數目，並經透化染色，調查根瘤線蟲侵染根部組織之變化，並依改良式柏門氏漏斗法，調查土壤中二齡幼蟲數。

根瘤線蟲引起洋香瓜生長不良之最適接種量測定

以陶土素燒盆裝消毒過之砂質壤土，移植萌芽後10天之洋香瓜幼苗。並分別接種根瘤線蟲 (*M. incognita*) 二齡幼蟲0, 5, 10, 25, 50, 100及200隻/株等七處理。每栽盆視同一重複，每處理五重複，接種後之植株置於玻璃溫室中 (25 ± 3)，按一般栽培法管理之，定期調查植株生長。於處理8週後，採集根部調查罹患根瘤數，以決定影響洋香瓜生長不良之最適感染源數量，以提供根瘤線蟲與囊叢枝內生菌根菌相互接種試驗之接種量。

囊叢枝內生菌根菌與根瘤線蟲之相互關係

供試囊叢枝內生菌根菌之厚膜孢子係取自繁殖於玉米之根圈土壤；根瘤線蟲二齡幼蟲係由罹根瘤之甘薯根系，挑取卵塊經孵化後挑取而得。陶土栽盆裝消毒過砂質壤土，移植萌芽後10天洋香瓜苗，囊叢枝內生菌根菌處理者 (M) 係先行接種每栽盆200個厚膜孢子。14天後再進行根瘤線蟲混合接種 (M+N) 及根瘤線蟲單獨接種 (N)，線蟲之接種量是依試驗3之結果，並另設對照組 (Ck) 不進行任何接種，僅注入不含孢子及線蟲之無菌水。以上每處理五重複，置於玻璃溫室 (25 ± 3)，按一般栽培法管理之，每星期供應10 ml Hoagland營養液，於處理8週後調查植株生長、內生菌根形成率、根瘤線蟲為害率、土壤中囊叢枝內生菌根菌和根瘤線蟲之數量變化、以及囊叢枝內生菌根對根瘤線蟲侵害之影響。取部份洋香瓜根系依石腊切片法進行根瘤病組織切片及利用掃描電子顯微鏡觀察⁽¹⁷⁾，瞭解囊叢枝內生菌根菌與根瘤線蟲之相互關係。

結 果

根瘤線蟲引起洋香瓜生長不良之最適接種量

接種8週後調查根瘤線蟲 *M. incognita* 不同接種量對洋香瓜生長之影響，結果顯示以每株洋香瓜接種5隻二齡線蟲即可造成感染，在根部產生根瘤 (Root-knot)，而每株洋

香瓜接種50隻線蟲即顯著地造成根部傷害影響根群發育，每株罹患根瘤數平均為221個，根部鮮重平均為14.4公克，若洋香瓜接種100隻線蟲，即可產生580個根瘤數，根部鮮重平均為13.6公克，而對照植株不接種線蟲者，根部平均鮮重為19.3公克（表一，圖一）。於是有關囊叢枝內生菌根菌與根瘤線蟲相互關係之接種試驗，根瘤線蟲之接種數量即採用每株洋香瓜接種50隻二齡線蟲。

囊叢枝內生菌根菌與根瘤線蟲之相互關係及對洋香瓜生長之影響

於根瘤線蟲接種後8星期，調查結果顯示囊叢枝內生菌根菌處理之植株（M），因根部形成囊叢枝內生菌根，明顯促進植株生長，其地上部及地下根部平均鮮重分別為63.3公克及8.14公克，對照植株（CK）則分別為47.6公克及6.98公克。根瘤線蟲之處理（N），分別為41.9公克及6.82公克，植株生長明顯受到抑制。但若有囊叢枝內生菌根菌存在時，即可減輕根瘤線蟲之為害，囊叢枝內生菌根菌與根瘤線蟲混合接種處理之植株（M+N），其地上部及地下部平均鮮重分別為49.5公克及7.40公克（表二，圖二）。

表一、根瘤線蟲不同接種量對洋香瓜生長之影響

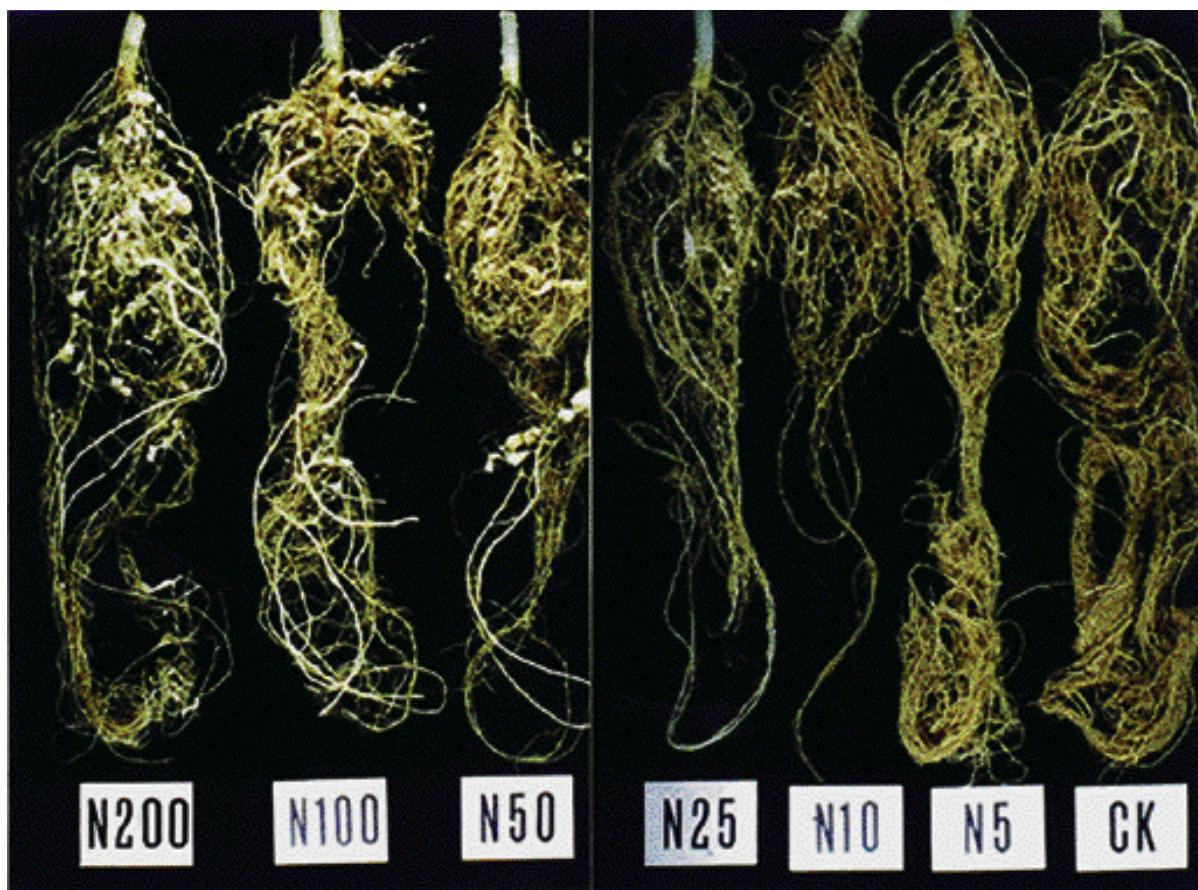
Table 1. Effect of inoculum density of *Meloidogyne incognita* on the growth of muskmelon in greenhouse

| Inoculum density (no. of larvae/plant) | Fresh weight ¹ (g/plant) | No. of root-knot per plant ¹ | No. of 2nd larvae per 100ml soil ¹ |
|-------------------------------------------|----------------------------------------|--------------------------------------------|--------------------------------------------------|
| 0 | 19.3 a ² | 0 a | 0 a |
| 5 | 18.2 ab | 22 a | 23 b |
| 10 | 16.8 ab | 159 a | 44 c |
| 25 | 16.3 ab | 202 ab | 75 e |
| 50 | 14.4 bc | 221 ab | 60 d |
| 100 | 13.6 c | 580 c | 78 e |
| 200 | 13.9 c | 346 bc | 85 e |

¹. Each value is the mean of 5 replicates.

². Column followed by the same letter was not different significantly ($p=0.05$) according to Duncan's multiple range test.

依組織透化染色法鏡檢根部，調查囊叢枝內生菌根菌與根瘤線蟲之相互關係，結果顯示根瘤線蟲不影響囊叢枝內生菌根之形成，二種微生物混合接種之植株（M+N），其菌根形成率達78%，而單獨接種囊叢枝內生菌根菌之植株



圖一、根瘤線蟲不同接種量對洋香瓜根系發育之影響。

Fig. 1. Effect of inoculum density of *Meloidogyne incognita* (the 2nd stage of larvae) on the development of root systems of muskmelon. (Ck=check, N5=5 larvae per plant).

表二、囊叢枝內生菌根菌與根瘤線蟲之相互關係及對洋香瓜生長之影響

Table 2. Effect of interaction between VA-mycorrhizal fungus and root-knot nematode on the growth of muskmelon in greenhouse

| Treatment | Ground part of plant | | Fresh wt. of root (g/plant) | Mycorrhiza infection (%) | No. of root-knot per plant | No. of 2nd larvae per 100ml soil |
|---------------------------------|----------------------------------|------------------------|--------------------------------|-----------------------------|-------------------------------|-------------------------------------|
| | Length (cm) | Fresh wt. (g/plant) | | | | |
| <i>Glomus clarum</i> | 80.5 ¹ a ² | 63.3 a | 8.14 a | 73.3 a | 0 | 0 |
| <i>Meloidogyne incognita</i> | 71.2 a | 41.9 b | 6.82 a | 0 | 103 a | 66 a |
| <i>G. clarum + M. incognita</i> | 77.4 a | 49.5 ab | 7.40 a | 78.0 a | 65 b | 53 a |
| Check | 70.0 a | 47.6 ab | 6.98 a | 0 | 0 | 0 |

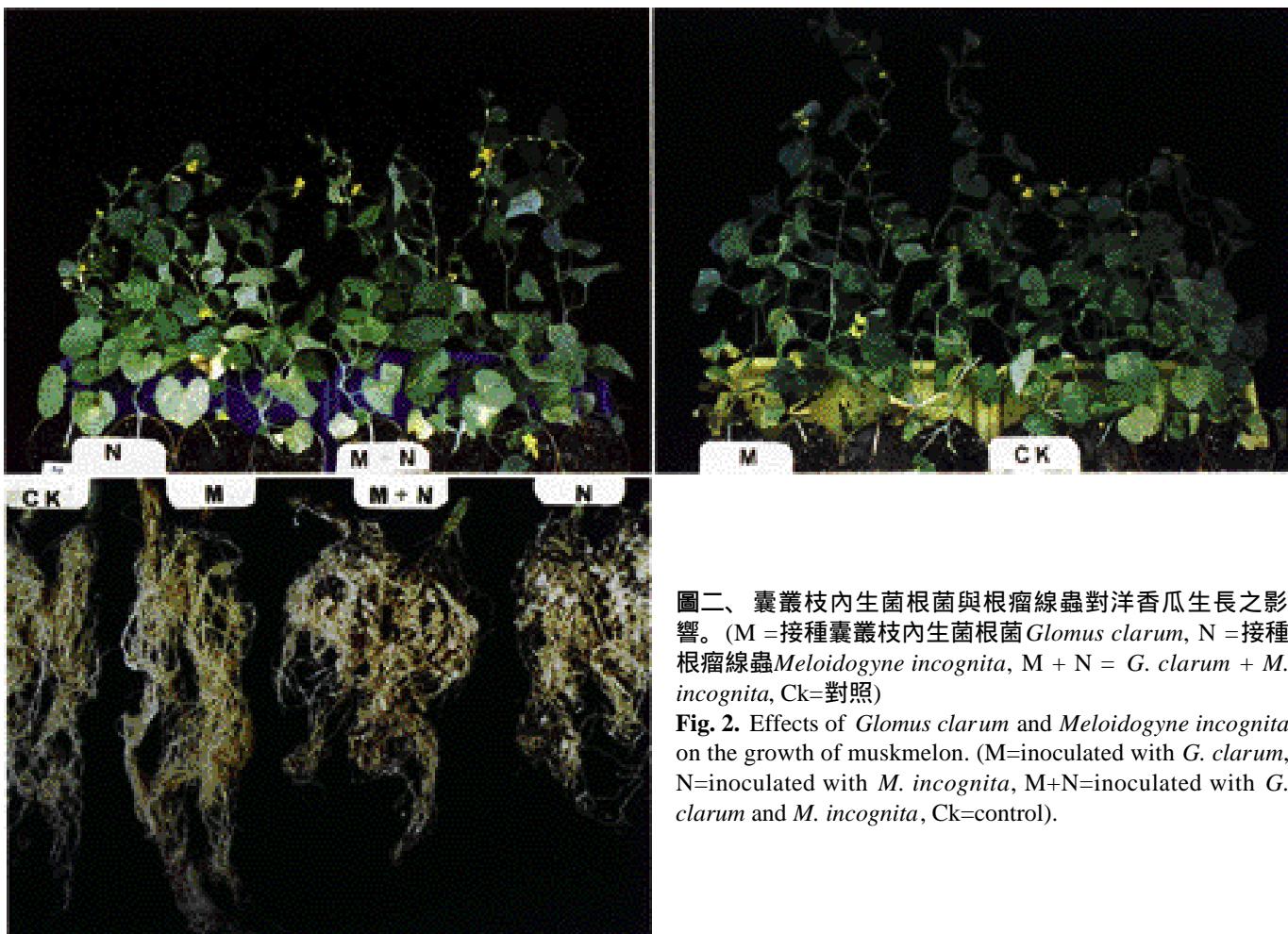
¹. Each value is the mean of 5 replicates.². Column followed by the same letter was not different significantly ($p=0.05$) according to Duncan's multiple range test.圖二、囊叢枝內生菌根菌與根瘤線蟲對洋香瓜生長之影響。(M = 接種囊叢枝內生菌根菌 *Glomus clarum*, N = 接種根瘤線蟲 *Meloidogyne incognita*, M+N = *G. clarum + M. incognita*, CK = 對照)

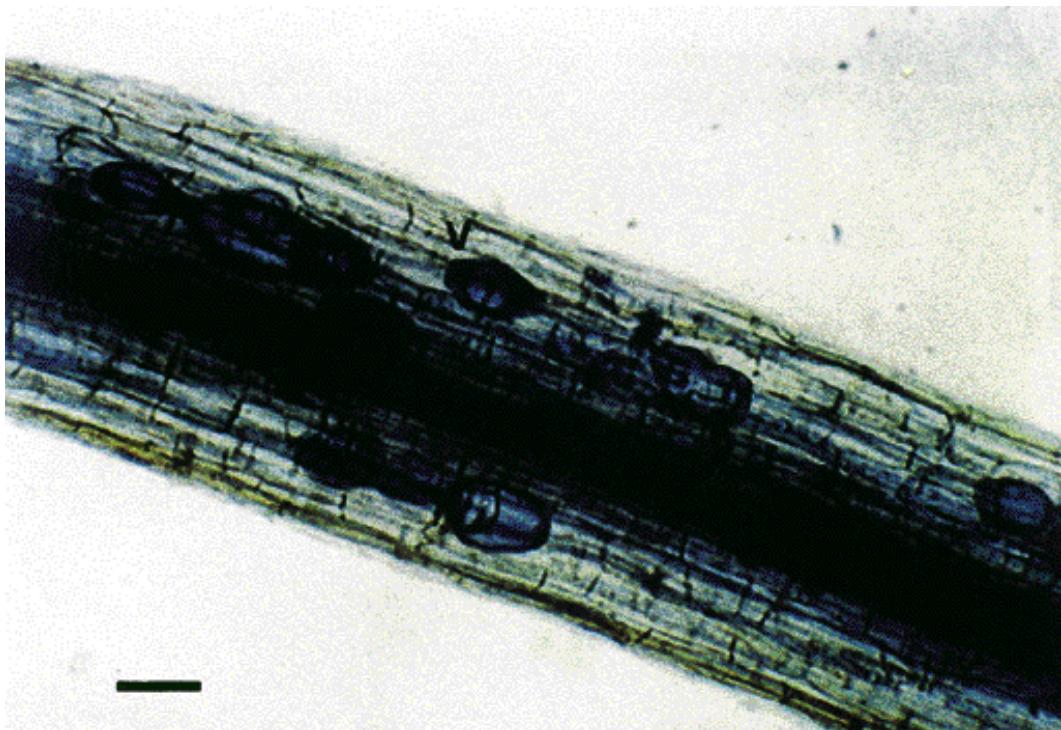
Fig. 2. Effects of *Glomus clarum* and *Meloidogyne incognita* on the growth of muskmelon. (M=inoculated with *G. clarum*, N=inoculated with *M. incognita*, M+N=inoculated with *G. clarum* and *M. incognita*, CK=control).

(M)，其菌根形成率達73% (表二)，惟產生根瘤的部位未發現菌根形成，形成囊叢枝內生菌根之部位也不產生根瘤(圖三)；依石腊切片法製作根瘤病組織切片，及掃描電子顯微鏡觀察根部組織，未發現二種微生物可同時存在同一根組織內(圖四,五)，可見此二種微生物在根組織內有彼此相剋之現象。根部若形成囊叢枝內生菌根時，可減少根瘤線蟲之侵染並形成根瘤。囊叢枝內生菌根菌與根瘤線蟲混合接種者(M+N)與根瘤線蟲單獨接種者(N)比較，每

株洋香瓜平均根瘤數分別為65與103個，土壤中的二齡線蟲數，每100 ml土中分別有53與66隻(表二)。

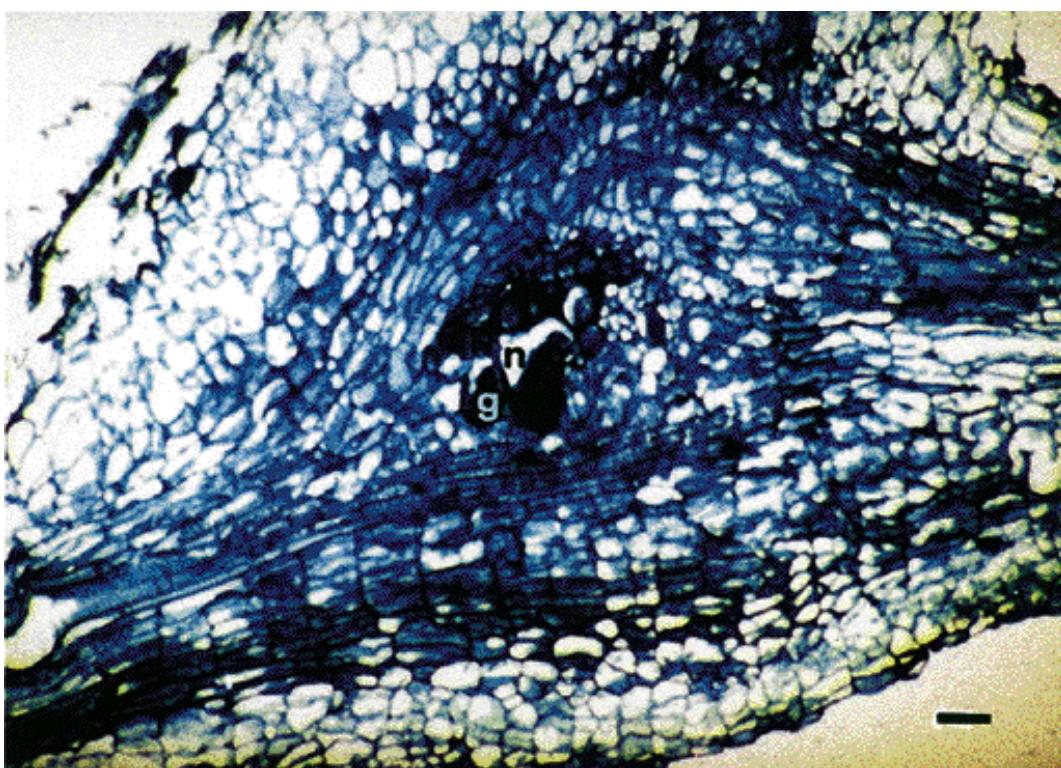
討 論

內生菌根植物與土傳病害之關係，近20年來引起許多學者研究之興趣⁽²⁶⁾，在這些研究報告中，大多數均顯示作物接種囊叢枝內生菌根菌後，病害嚴重程度較未接種植



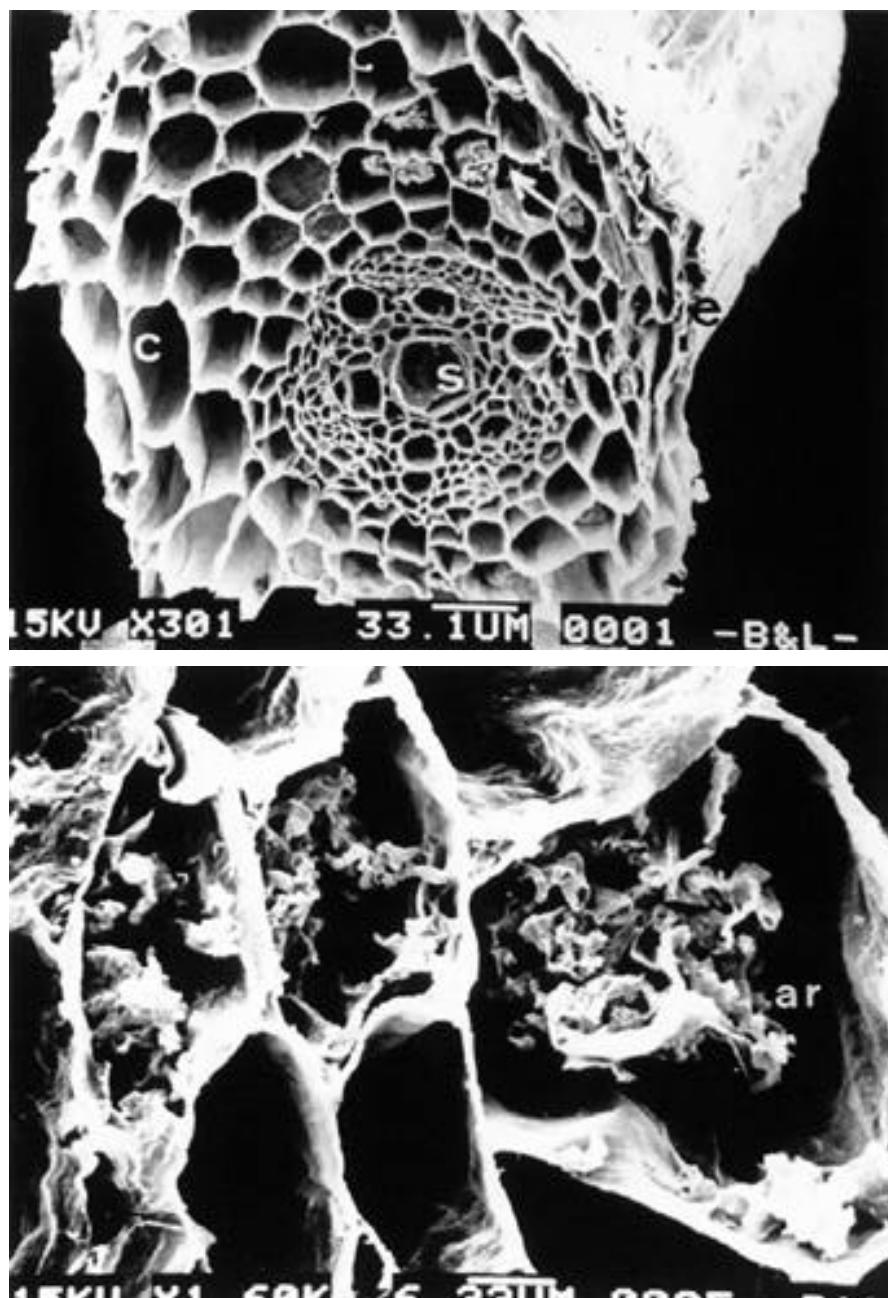
圖三、洋香瓜根系形成囊叢枝內生菌根（可免受根瘤線蟲之侵染）。(V=囊狀體)

Fig. 3. VA mycorrhizal fungus occupied the root tissue of muskmelon (preventing infection by root-knot nematode). (V=Vesicles) (Bar=75 μ m)



圖四、根瘤線蟲侵染洋香瓜根部引起之根瘤組織切片。n-線蟲體遺留之空洞，g-巨型細胞。

Fig. 4. Longitude section of root-knot cells induced by root-knot nematode in muskmelon root. (n: the hole of nematode left, g: giant cells). (Bar=50 μ m)



圖五、洋香瓜根部形成囊叢枝內生菌根(可免受根瘤線蟲侵染)之掃描電顯觀察。(ar=叢枝菌體,c=細胞壁,s=中心柱)
Fig. 5. The formation of VA mycorrhizal in muskmelon root tissue (free from infection by root-knot nematode) through SEM observation. (ar=arbuscule, c=cell, s=stele)

株為輕，尤其是囊叢枝內生菌根與寄生性線蟲病害之關係。Kellam等氏⁽²⁴⁾報導囊叢枝內生菌根菌接種在大豆上，可抑制根瘤之形成，非內生菌根植株根系則產生較多的根瘤，但囊叢枝內生菌根與非內生菌根植株之線蟲數量並無顯著性差異。本試驗研究也有類似的結果，囊叢枝內生菌根洋香瓜植株受根瘤線蟲為害之程度較為輕微，在根部產生較少根瘤，而單獨接種根瘤線蟲之植株產生較多根瘤，因此阻礙根群發育與植株生長，但是囊叢枝內生菌根不影響根瘤線蟲之發育與根瘤之形成，惟當根部感染囊

叢枝內生菌根菌形成囊叢枝內生菌根時，這一部位的根段，就可減少根瘤線蟲侵害機會。由組織透化及切片觀察，形成菌根的部位從未發現根瘤線蟲侵入，其對根瘤線蟲具有相剋之現象，可能是由於感染點(Infestation site)之先行佔據而減少根瘤線蟲侵入機會，Jalali及Linderman^(23,25)亦有相同之報告。

本試驗結果顯示，洋香瓜幼苗先行接種囊叢枝內生菌根菌，使之感染成功形成囊叢枝內生菌根達到70%以上之形成率，即可減少根部70%部位被線蟲侵害的機會。囊叢

枝內生菌根菌與根瘤線蟲之拮抗機制，就不如外生菌根植物 (Ectomycorrhizal plant) 根系有明顯之形態改變，因其根系外包覆一層菌絲鞘 (Fungal mantle)，提供寄主一種機械式的保護，阻礙病原微生物之侵染⁽²⁶⁾，至於抗生物質之直接作用機制則沒有明顯有力証據。囊叢枝內生菌根之作用機制，目前還是以作物生理生化營養之觀點，及增強根系發育，補償（或減輕）病原微生物所造成之傷害，獲得較多學者支持。

本試驗結果啟示吾人，囊叢枝內生菌根菌可藉菌根苗先行培育，達到實際應用之目的。在溫網室人工控制環境下，洋香瓜等園藝作物種苗先行接種囊叢枝內生菌根菌，培育成菌根苗，當移植於本田或設施栽培，即可把囊叢枝內生菌根菌藉種苗帶到本田。隨著植株生長，囊叢枝內生菌根發揮其有益效應，促進根系發育，增強對根瘤線蟲之抵抗能力，或補償（減輕）根瘤線蟲造成根部之傷害，達到應用囊叢枝內生菌根菌防治或減輕根瘤線蟲為害之目的。

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ABSTRACT

Cheng, Y. H.^{1,3}, Chuang, M. F.¹, Tsay, T. T.² 2001. Interaction of vesicular-arbuscular mycorrhizal fungus with root-knot nematode in muskmelon. *Plant Pathol. Bull.* 10:19-26. (¹ Chiayi Agricultural Experiment Station, TARI, Chiayi, 600, Taiwan, R.O.C., ² Department of Plant Pathology, Chung-hsing University, Taichung, 400, Taiwan, R.O.C., ³ Corresponding author, E-mail:cyh@dns.caes.gov.tw; Fax:05-2773630)

Glomus clarum and *Meloidogyne incognita* were separated or mixed to inoculate the root systems of muskmelon for study of the effect of interaction between VAM fungus and nematodes on the host development. The interaction effects were determined and evaluated by the degree of formation of VA mycorrhiza and root knots in root systems, and the growth of the host plant. To find the minimal inoculum density of nematodes for inducing disease symptoms, seedling root systems were inoculated with 0, 5, 10, 25, 50, 100 or 200 larvae of the 2nd stage nematodes in each pot. Results showed that 5 larvae per seedling inoculation could induce formation of root knots in the host. Whereas 50 and 100 larvae per seedling, causing serious damage to root systems, induced 221 and 580 root knots, respectively, in average after inoculation for 8 weeks. VA mycorrhiza was formed in the seedling root of muskmelon after inoculation with chlamydospores of *G. clarum* for 2 weeks. When host growth and disease incidence of the seedlings pre-inoculated with *G. clarum* for 2 weeks and then inoculated with 50 larvae of *M. incognita* per plant were studied, and compared with those inoculated with *G. clarum* or *M. incognita* individually. Results showed that *G. clarum* individual stimulated both top and root growth of muskmelon whereas *M. incognita* inhibited the top growth. After 8 weeks inoculation, the average fresh weight of ground parts of muskmelon seedlings treated with *G. clarum* plus nematode (M+N), *G. clarum* (M), nematode(N), and controls(CK) were measured as 49.5, 63.3, 41.9, and 47.6g, respectively. Whereas the root systems of the four treatments were as 7.40, 8.14, 6.82, and 6.98g, respectively. This indicated that inoculation of *G. clarum* in muskmelon root could decrease the host damage caused by *M. incognita*. Results also showed that nematodes were unable to attack the mycorrhizal occupation parts of root systems. The number of root knot produced in the treatments of inoculated with *G. clarum* plus nematode (M+N) and individual nematode (N) were 65 and 103 per plant, respectively. Meanwhile, the percentage of formation of mycorrhizal root in the treatments of *G. clarum* plus nematode (M+N) and *G. clarum* (M) were 78% and 73.3%, respectively. This indicated that nematode was also unable to interfere the formation of mycorrhizal roots in this study.

Key words : muskmelon, root-knot nematode, vesicular-arbuscular mycorrhizal fungus, *Glomus clarum*, *Meloidogyne incognita*