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HYPHAL INTERACTION MECHANISM BETWEEN SOME FUNGAL BIOCONTROL AGENTS AND SOME CUCURBITACEOUS DAMPING-OFF PATHOGENS

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ABSTRACT: Light microscope was used to dual cultures, containing the tested fungal biocontrol agents: namely; *Trichoderma harzianum*, *T. viride*, *T. konningii* and *Gliocladium roseum* and some cucurbitaceous damping-off pathogens: namely; *Rhizoctonia solani*, *Pythium ultimum*, *Fusarium solani* and *Alternaria alternata*. Microscopic examinations indicated that growth of the mycoparasitic fungal mycelium was characterized by the formation of highly active, thin, dense and well-branched hyphae compared to those of the tested pathogens, where hyphae were thicker and less branched. This behavior was common in most of the tested mycoparasitic isolate/pathogen interactions.

After colonization, short infection hyphae were mostly formed by the mycoparasitic isolate, grew toward host hyphae and penetrate them directly with or without the formation of prepenetration structures. However, in most of the examined combinationss, mycoparasitic hyphae grew toward the hyphae of the pathogen, then parallel and in complete contact with them, twisted in most cases around host hyphae for establishment before the formation of penetration structures. Many distinguished structures were formed from tips of the attacking hyphae such as appressorium, foot, pincer, hook or hyphopodium-like structures before penetrating the host hypha. After penetration and lysis of the host hyphae, conidiophores of the mycoparasitic isolates were observed protruded from the attacked host hypha.

Hyphal interaction between the tested isolates of *T. harzianum* and *G. roseum* and isolates of *R. solani* and *P. ultimum* were also

studied using scanning electron microscope. Examinations results were almost similar to those of light microscope. Growth of attacking hyphae toward host hyphae, formation of appressorium-like structures and direct penetration without the formation of pre-penetration structures were also recorded.

Key words: Biocontrol agents, hyphal interaction, mycoparasitism, damping-off, *Trichoderma*, *Gliocladium*.

INTRODUCTION

The process of mycoparasitism always starts with hyphal colonization by *T. harzianum*, the biocontrol agent at the surface of host tissue (El-Farnawany and Shama, 1996). They reported a complete surface colonization of *R. solani* hyphae and on *S. sclerotiorum* (El-Farnawany, 2006a).

The biocontrol fungal hyphae were found to establish close contact with the host by coiling around the hyphae (Weindling, 1932; Liu and Baker, 1980; Chet *et al.*, 1981; Elad *et al.*, 1981; Tu and Vaartaja, 1981; Elad *et al.*, 1983 and 1987; Benhamou and Chen *et al.*, 1993). Coiling of the antagonist *T. harzianum* around the hyphae of *R. solani* was an early event preceeding hyphal damage (Benhamou and Chen *et al*, 1993). Attachment between the antagonist and *R. solani* is mediated by the binding of carbohydrates in *Trichoderma* cell wall to locations on the target fungus (Harman *et al.*, 2004).

Formation of appressoria-like structures by the fungal antagonist when coming in touch with those of the target fungal host was recorded in *T. harzianum*/*R. solani* interaction (Chet *et al.*, 1981) and between *G. virens* and *R. solani* (Tu and Vaartaja, 1981) to occur directly before penetration of the target fungal host by the hyphae of the antagonist (Weindling, 1932), after which subsequent dissolution of the host cytoplasm occurred. More than one of hyphal branches of *T. harzianum* were found to penetrate directly *R. solani* or *S. sclerotiorum* cells through the same site (El-Farnawany and Shama, 1996 and El-Farnawany, 2006a). Antagonism might occur by pre-contact antibiosis (Berry and Deacon, 1992).

Therefore, the present work was performed to investigate some possible mechanisms of hyphal interaction between some fungal biocontrol agents and the target damping-off pathogens.

MATERIALS AND METHODS

Origin of the Tested Pathogens (TP)

The tested pathogens; *Rhizoctonia solani* (RS), *Pythium ultimum* (PU), *Fusarium solani* (FS) and *Alternaria alternate* (AA) isolated by the authors from infected seeds of cucumber (*Cucumis sativus*), squash (*Cucurbita pepo*), watermelon (*Citrullus lanatus*) and sweetmelon (*Cucumis melo*), (Abou-Shaala, 2008).

Origin of the Fungal Biocontrol Agents (FBAs)

The fungal biocontrol agents (FBAs) used throughout this investigation were *Trichoderma harzianum* (TH), *T. viidi* (TV), *T. koningii* (TK) and *Gliocladium roseum* (GR). These isolates were kindly obtained from Mycological Center, Assiut University, Egypt.

Hyphal Interaction in Dual Culture

According to the technique described by Chet *et al.* (1981) and Elad *et al.* (1983), dual fungal isolates from each of fungal bioagents and the tested fungal host were grown on a cellophane membrane apart between each other in Petri dishes with potato dextrose agar (PDA) medium. Due to its slow growth rate, the cultures were kept in the dark at 25°C. After 4-6 days, pieces of cellophane membrane from the contact zone were cut and mounted on a slide, stained with cotton blue and examined using light field phase-contract microscope. The hyphal interactions were photographed using light microscope built in digital camera and also by scanning electron microscope.

RESULTS AND DISCUSSION

Results of light and electron microscopic examinations are shown in Figs 1, 2, 3 and 4. Microscopic investigation of hyphal interaction between the tested antagonistic fungi and the target fungal host showed that there were no specific mechanism characteristics any of the tested FBA/TP interaction. Most of the characteristics modes of mycoparasitism were similar to all the tested interactions.

It was, generally evident that the main characteristic feature of the early stages of the tested fungal biocontrol agents (FBAs) and the target pathogen (TP) were the

formation of dense mycelium of the tested FBA with fine active hyphae surrounding the hyphae of the target pathogen. This phenomenon was recorded in all the tested FBA/TP interaction trials. Colonization of TP hyphae mostly occurred by the FBA hyphae Fig. 1A-TH/RS and 4A-Th/RS. However, development of FBA conidiophores Fig. 1C-TK/FS or FBA spores Fig. 1B-TV/PU was also detected.

After colonization of the TP by the FBA, the FBA hyphae started to attack the hyphae of the target pathogen by growing toward them (Fig. 1A - TH/RS), then parallel to the hyphae close but not in contact to it (Fig. 1F-TV/PU). However, in some cases the FBA attacking hyphae grew in complete contact on the surface of the TP hyphae (Fig. 1I-GR/RS; 1D-TK/FS; 1E-TH/RS; 4A-TH/RS and 4D-GR/PU).

The collected data indicate that the attacking FBA hyphae, in many cases, twisted around the TP hyphae in order to fix and fasten itself on it before penetration (Fig. 1G-TH/AA; 1H-TA/RS; 1I- GR/RS). Penetration of the TP hyphae by FBA may take place directly (Fig. 4C TH/PU) or through the formation of short branches with fine ends (Fig. 2E TH/RS; 2A-TH/RS; 2B-TH/RS; 2C-GR/RS and 2D – GR/AA).

Many FBA hyphae prepeneration and penetration structures were detected throughout the present investigation:

(a) A Flattened hyphae pressing structure similar to the appressorium-like structures exhibited close to TP hyphae (Fig. 3A-TH/FS; 4A-TH/RS; 4B-TH/RS; 4D and E-GR/PU).

(b) A structure formed at the lower extremely of hyphae similar to the foot stood at the penetration site on TP hyphae (Foot-like structures) (Fig. 3B-TV/FS).

(c) A structure of exhibited form FBA hyphae with a pair of branches similar to levers of the pincer (Pincer-like structures) (Fig. 3C-TV/PU). When the FBA hyphae came closer to the TP, a pincer movement was exhibited by these PLS.

(D) The mother hyphae may directly come close to TP hyphae and penetrate it (Fig. 2A-TH/RS; 2BTH/RS; 2C-GR/RS and 2D-GR/AA).

(E) Formation of hyphopodium-like structures inside the TP hyphae (Fig. 3D-TA/RS).

(F) Formation of hook-like structures (Fig. 3E-TV/PU).

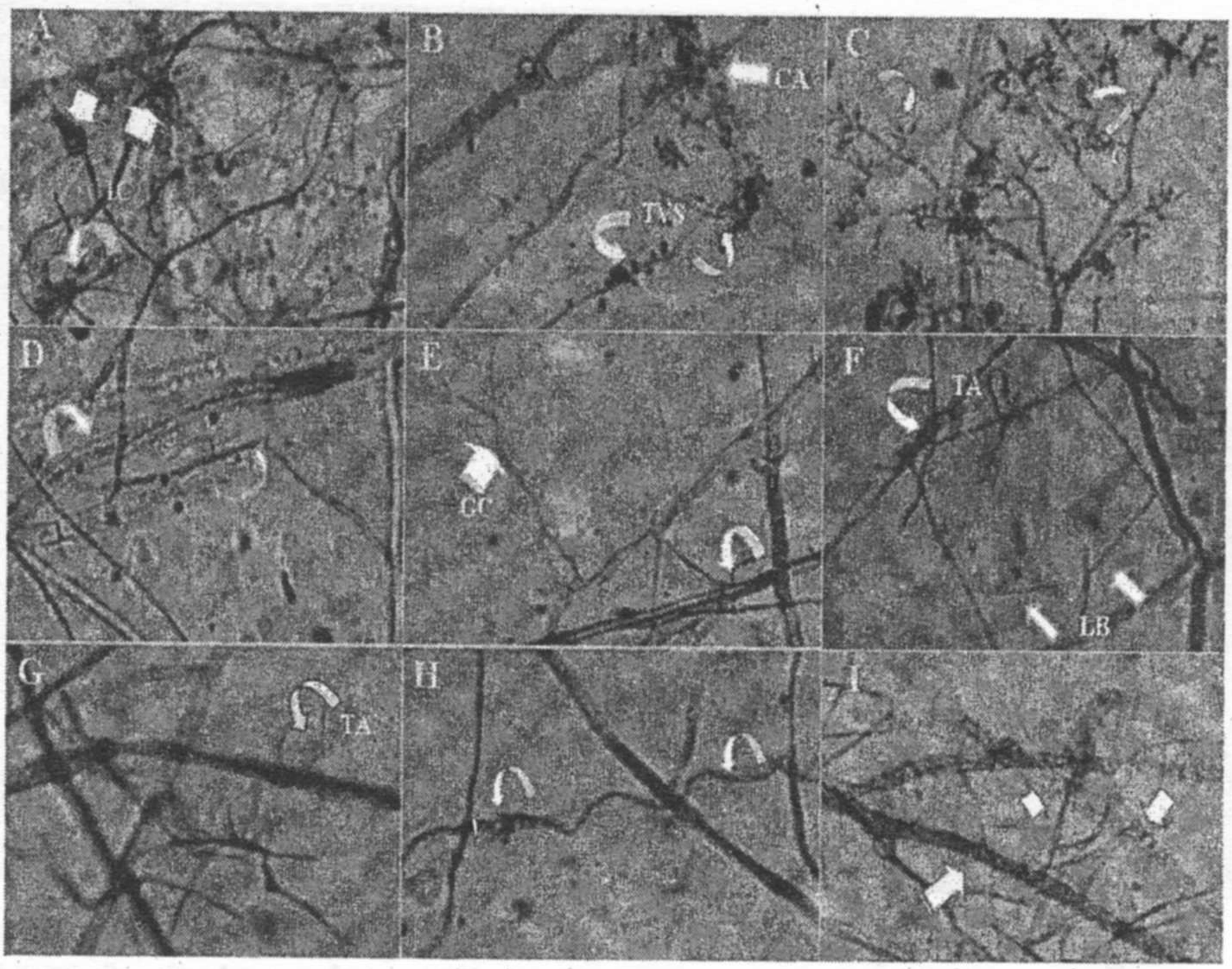

Fig. 1. Light microscopic observation showing first stage of mycoparasitism; colonization of the target pathogens by FBA hyphae (A), conidiophorea (B) conidiophore (D), growth of FBA hyphae parallel and in close contact with TP hyphae (D, E &F) and twisting around the TP hyphae (G, H & I). Where: TH = *T. harzianum*, TV = *T. viridi*, TK = *T. koningii*, DP = direct penetration, FT = fine tip, H = hostoria, GC = grow on contact, SB = short branches,VSB = very short branches, ALS = appressorium-like structure, CA = coiling around, *T. viride* spores, LB = lateral branches, TA = twisted around, C = colonizing, IC = infection cushion and C = conidiophores.

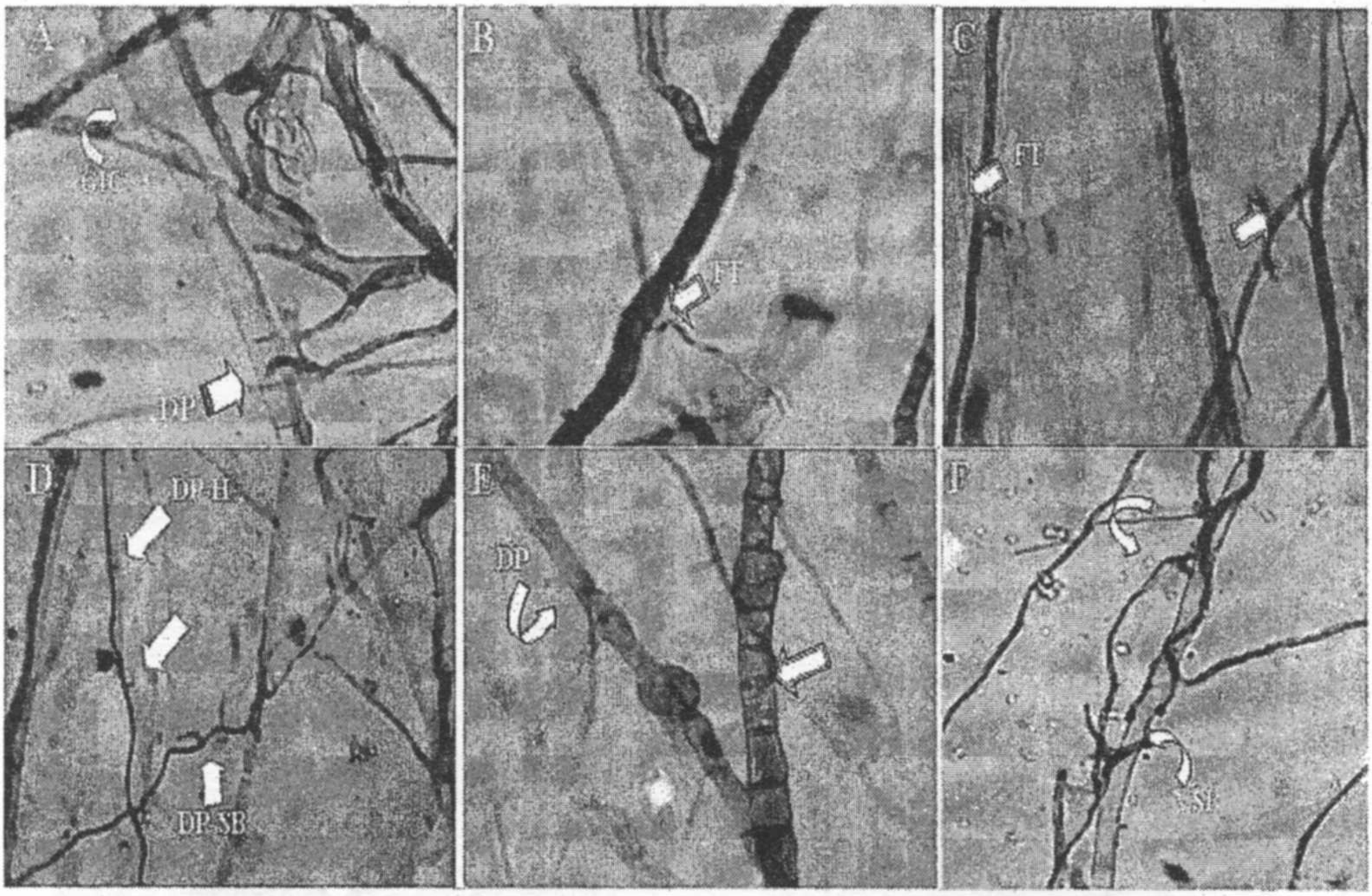

Fig. 2. Light microscopic observation showing direct penetration of TP hyphae by FBA hyphae (A, B, C, & D), or through the formation of node-like structure (E) or short branches with fine ends (F).

Where: TH = T. harzianum, TV = T. viridi, TK = T. koningii, DP = direct penetration, FT = fine tip, H = hostoria, GC = grow on contact, SB = short branches,VSB = very short branches, ALS = appressorium-like structure, CA = coiling around, T. viride spores, LB = lateral branches, TA = twisted around, C = colonizing, IC = infection cushion and C = conidiophores.

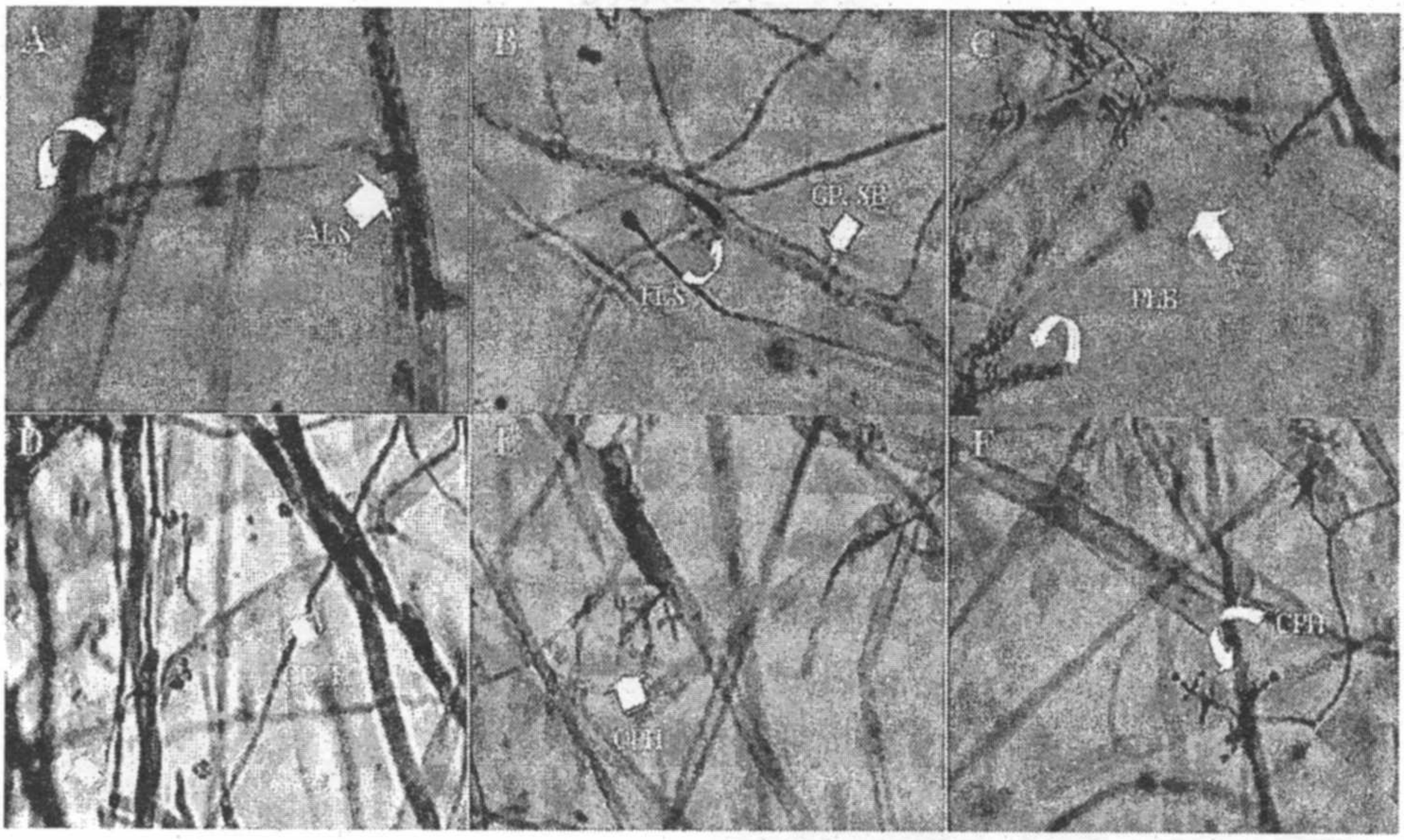

Fig. 3. Light microscopic observation showing penetration of TP hyphae by FBA hyphae through special hyphal structures: appresoria-like structure (A), foot-like structure (B), pincer-like structure (C), hock-like structure (D) and protrusion of conidiophores from TP attacked hyphae (E & F).

Where: TH = *T. harzianum*, TV = *T. viridi*, TK = *T. koningii*, DP = direct penetration, FT = fine tip, H = hostoria, GC = grow on contact, SB = short branches,VSB = very short branches, ALS = appressorium-like structure, CA = coiling around, *T. viride* spores, LB = lateral branches, TA = twisted around, C = colonizing, IC = infection cushion and C = conidiophores. Where: TH = *T. harzianum*, TV = *T. viridi*, TK = *T. koningii*, DP = direct penetration, FT = fine tip, H = hostoria, GC = grow on contact, SB = short branches,VSB = very short branches, ALS = appressorium-like structure, CA = coiling around, *T. viride* spores, LB = lateral branches, TA = twisted around, C = colonizing, IC = infection cushion and C = conidiophores.

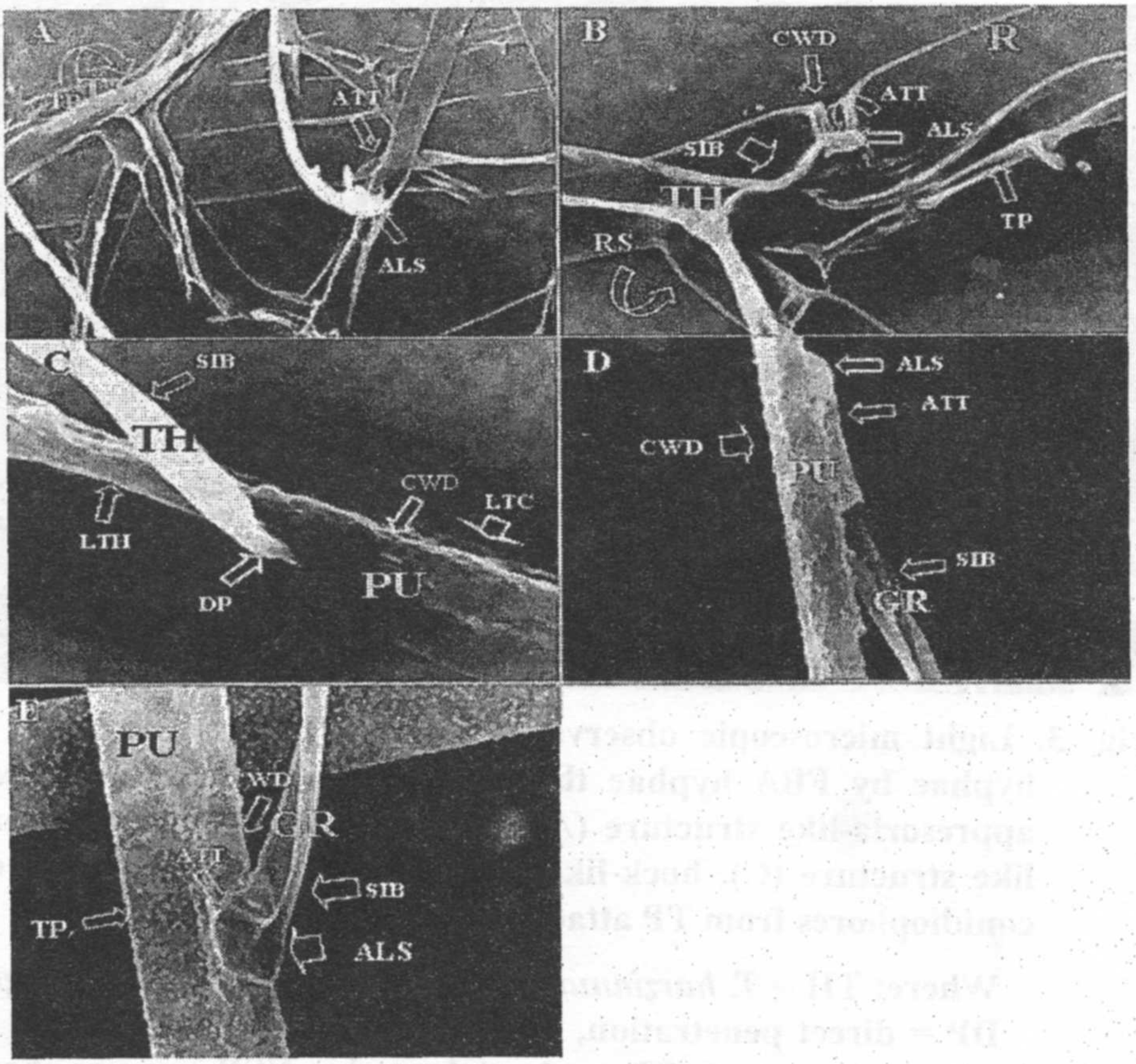

Fig. 4 .SEM observation showing interation between The FBAs *T. harzianum* (TH) and *G. roseum* (GR) and the target pathogens *R. solani* (RS) and *P. ultimum* (P), where :

A. TH/RS – colonization and formation of prepenetration attacking structures.

B. TH/RS – penetration through short branches ending with the formation of appressorium.

C. TH/PU – direct penetration.

D. GR – growth in contact with TP hyphae and the formation of ALS.

E. GR/PU – attacking GR hyphae with the formation of ALS.

Where : PU = *P. ultimum P. ultimum*, RS = *R. solani*, GR = *G. roseum*, TH = *T. harzianum*, ATT = attachment of the hyphae of target pathogen, ALS = appresorrium-like structure, CWD = cell wall degredation, SIB = short infection branches, TP = target pathogen, LTC = lysis target cells, LTH = loss of target hyphal pathogen and DP = direct penetration.

After the establishment of the mycoparasite in the TP, FBA conidiophores with conidia could be detected protruding from the attacked TP hyphae (3-F – TH/PU).

The present findings on the role of antagonist/pathogen hyphal interactions confirmed the suggested some mechanisms of biological control. It was found that, colonization of hyphal by FBA, generally, starts with growing of the antagonistic hyphae toward the target pathogen. Recognition of the host fungus by the antagonist was thought to be the result of remote sensing, which is at least partially due to the sequential expression of cell-wall degrading enzymes (Harman *et al.*, 2004). According to our finding, once the antagonist came into contact, it attaches the host and can coil around it along the host hyphae, coiling around host mycelium, forming intensive hyphal growth. These observations were similar to those described by many authors in *T. harzianum* / *R. solani* and *F. solani* hyphal interactions (Liu and Baker, 1980; Elad *et al.*, 1981& 1987); *Trichoderma* sp. / *Sclerotiorum rolfsii* (Elad *et al.*, 1983); *Trichoderma* sp. / *S. sclerotiorum* (Whipps, 1987); *T. viride* / *R. solani* (El-Farnawany and Shama, 1996); *G. roseum* / *R. solani* and *F. solani* (El-Farnawany and Shama, 1996; Allen, 2003 and El-Farnawany, 2006a). Inbar *et al.* (1996) believed that attachment between the antagonist and the host fungus is mediated by the binding of carbohydrates in the Trichoderma cell wall to lectins on the target fungus.

After attachment to the target pathogen, it was observed the formation of short infection hyphae, penetration and lysis of hyphal cell contents of the host fungus were observed. This was also recorded by Schirmböck *et al.* (1994) and Lorito *et al.* (1998). They pointed out that, several fungistatic cell wall degrading enzymes and probably also peptailbol antibiotics involved in penetration and subsequent parasitism. Penetration was observed to occur through the formation of appressoria-like structures. These findings confirmed the findings of many of the previously mentioned authors. Direct penetration was also occurred with the formation of appressoria. This was also reported by Itamar and Jane (2000). Other infection structures were also observed, i.e. nodes, foot-like

structures and pincer-like structures. Appressoria and haustoria-like structures were recorded by El-Farnawany and Shama (1996) and Metcalf and Wilson (2001). Colonization of spores along with the formation of short coiled around them were also recorded (El-Farnawany, 2006b).

Antagonist /host pathogen interaction obtained in the present research offers an approach to deep understanding of this relation which might help in finding out new safe control measures in the near future. The author believed that, *in vitro* antagonism studies and microscopic examinations should be considered in the evolution of *Trichoderma* species, especially when more than one species are used as potential biocontrol agents. An understanding of the compatibility between species or isolates of *Trichoderma* under various cultural conditions will provide informations on the use of multiple species of *Trichoderma* as biocontrol agents against a particular plant pathogen.

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ميكانيكية التداخل الهيفى بين بعض فطريات المقاومة الحيوية والفطريات المسببة لمرض موت البادرات فى القرعيات

إبراهيم السمرة - مصطفى عامر - ماهر الفرنوانى- فرج أبو شعاله

قسم النبات الزراعى- كلية الزراعة (سابا باشا)- جامعة الإسكندرية

استخدام الميكروسكوب الضوئى فى فحص مزارع ثنائية تجمع فطريات المكافحة البيولوجية المختبرة وهى تريكوديرما هارزيانم (ت. ه)، تريكوديرما فيردى (ت. ف)، تريكوديرما كوننجاى (ت.ك) و جليوكلاديوم روزيم (ج.ر) والمسببات المرضية لأمراض

موت البادرات فى القرعيات وتشمل ريزوكتونيا سولانى (ر.ن)، بيثيام ألتمم (ب.أ)، فيوزاريوم سولانى (ف. س) وألترناريا ألترناتا (أ.أ)

أوضح الفحص الميكروسكوبى الضوئى عديد من خصائص عملية التطفل الفطرى على المسببات المرضية حيث لوحظ نشاط هيفى متزايد من فطريات المكافحة البيولوجية (ف.م. ب) حول هيفات وتراكيب المسببات المرضية . ولقد تميزت هيفات فطريات المكافحة البيولوجية (ف٠م٠ب) بسرعة النمو ودقة الهيفات وكثافة تفريعها منتهية باستعمار هيفات الفطر المسبب والتى قد بدت أقل كثافة فى النمو وذات هيفات أكثر سمكاً. ولقد أتضح إشترك جميع فطريات المكافحة البيولوجية المستخدمة فى تلك الخصائص.

بعد تمكن فطريات المكافحة البيولوجية من الالتفاف واستعمار المسبب المرضى، إما لاختراقها مباشرة أو على الأغلب بالالتفاف الجيد حول هيفات العائل ثم أصبح نموها موازياً وملاصقاً لهيفات الفطر المرضى لمسافة غير محددة قبل تكوين تراكيب ما قبل الاختراق. ولقد أظهر الفحص بالميكروسكوب الضوئى وجود عديد من أنواع تلك التراكيب، فهى تختلف مابين تراكيب مشابهة لأعضاء الالتصاق المعروفة و تراكيب شبيهه بالقدم، أو تراكيب كلابية أو خطافية. ويستخدم ف.م.ب تلك التركيب فى تثبيت نفسه على العائل تمهيداً لإختراقه٠ ولقد لوحظ تكوين هذه التراكيب من أغلب ف.م.ب المختبرة دون تمييز وذلك قبل الاختراق مباشرة. وبعد اختراق الفطر وتحلل مكونات الخلايا يبدأ الفطر فى الخروج من هيفات المسبب المرضى المصابة مكوناً حوامل كونيدية تحمل الجراثيم.

وتم الفحص بالميكروسكوب الإلكترونى الماسح لمزارع ثنائية من ف.م.ب مثل ت. هارزيانم و ج. روزيم من ناحية والفطريات الممرضة ر. سولانى و ب. ألتيميم من ناحية أخرى. وأكدت النتائج المتحصل عليها تلك المتحصل عليها من الفحص بالميكروسكوب الضوئى، ولقد لوحظ أن نمو هيفات ف.م.ب تتوجه إلى هيفات العائل، وتكون تراكيب شبيهة بعضو الالتصاق، والإخترق مباشرة دون تكوين أى تراكيب تمهيدية.