

# 疫病菌誘導蛋白 elicitins 之研究現況

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## 摘要

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Elicitins 是在疫病菌 (*Phytophthora*) 及腐霉菌 (*Pythium*) 所發現的誘導蛋白，根據蛋白質結構及類緣分析結果，可將其區分為三群，大多數目前已被發現之 elicitins 屬於第一群，特性主要包括：(一) 分子量大約為 10 kDa，一般含 98 個胺基酸，且胺基酸序列在不同菌種間有相當程度之保守性，(二) 對煙草引發過敏性反應，及(三) 能夠誘導煙草產生系統性抗病反應。在煙草引發過敏性反應與系統性誘導抗病時，elicitin 所啟動之訊息傳導機制與其他病原微生物所援用者有相似之處，但 elicitin 與煙草之交互作用顯然比其他系統還要複雜。此外，近來之研究發現 elicitin 具有固醇載體活性，由於 *Phytophthora* 與 *Pythium* 均不具有合成固醇之能力，其是否利用 elicitin 自植物細胞膜獲取固醇以供生長與繁殖之需，仍待深入探討，固醇載體活性是否為 elicitin 展現誘導活性之必要條件也待進一步釐清。

關鍵詞：疫病菌、誘導蛋白、過敏性反應、系統性誘導抗病、腐霉菌

## 緒言

植物與病原微生物之交互作用是近年來極受重視的研究領域，在探討這一個問題時，一般可將寄主植物與病原之交互作用分成兩類：親和性交互作用 (compatible interaction) 與非親和性交互作用 (incompatible interaction)<sup>(34)</sup>；病原微生物進行植物侵染時，若能克服植物防禦機制所展現的重重難關，成功感染植物、並造成病害，則兩者間所發生之作用稱為親和性交互作用。相對的，若病原微生物侵害植物時，植物啟動其防禦機制，並有效遏止病原微生物的擴散與侵害，則兩者間所發生之作用稱為非親和性交互作用。非親和性交互作用發生時，植物經常會表現過敏性反應 (hypersensitive response, HR)，此時病原侵入點附近之植物細胞會快速死亡，以致病原菌無法擴散<sup>(13,18)</sup>。過敏性反應之發生常使植物對於後續入侵之病原微生物產生系統性抗病力，此即所謂系統性誘導抗病 (systemic acquired resistance, SAR)<sup>(49,50)</sup>。過敏性反應與系統性誘導抗病是病原微生物所產生的誘導性物質 (elicitor)，作用於植物之後所引發之一連串錯綜複雜反應的整體呈現。因此，分析病原菌所分泌之物質、了解植物如何辨識這些物質，並探討這些物質對於植物之作用，將有助於我們更進一步了解病原菌與植物間之交互作用情形。

疫病菌 (*Phytophthora* De Bary) 與腐霉菌 (*Pythium* Pringsh.) 同屬於卵菌綱 (Oomycota)、露菌目 (Peronosporales)、腐霉菌科 (Pythiaceae)，其中包括多種重要植物病原真菌。Elicitin 是在疫病菌及少數腐霉菌之培養菌液中所發現的一類分泌性蛋白質。由於近年來的研究顯示，其會在煙草引發過敏性反應與系統性誘導抗病<sup>(30,47,59)</sup>，這一類蛋白質一直被視為疫病菌所分泌之誘導性物質。本篇報告針對近年來關於 elicitins 之研究情形作一簡要的介紹，以提供大家做參考。

## ELICITINS 在 PYTHIACEAE 之存在情形

根據 Bonnet 等人的研究結果<sup>(5,7)</sup>，在所有 *Phytophthora* 測試菌種中，僅一部分 *P. parasitica* Dastur (= *Phytophthora nicotianae* Breda de Haan) 分離株 (*P. parasitica* Dastur var. *nicotianae* (Breda de Haan) Tucker) 能夠危害煙草，自其他寄主分離到的 *Phytophthora parasitica* 及其他 *Phytophthora* spp. 僅會在煙草葉片造成壞疽病斑 (necrotic lesion)，並誘發植物產生抗病反應。為了探討為何 *Phytophthora* 會在煙草葉片造成壞疽病斑，並誘發抗病反應，Bonnet 等人<sup>(8)</sup> 首先嘗試自 *Phytophthora cryptogea* Peth. et Laff. 培養液純化具誘導活性之蛋白質。之後的研究發現，其他 *Phytophthora* 也會分泌這一類蛋白質，其特

性主要包括：(一) 分子量大約為 10 kDa，(二) 對煙草引發過敏性反應，導致葉片壞疽，及 (三) 能夠誘導煙草對後續接種之病原菌產生系統性抗病 (詳見下文)，故通稱為 elicitins<sup>(2,30,47)</sup>。

Elicitins 目前只在 *Phytophthora* 與 *Pythium* 被發現 (表一)。在已經測試之 *Phytophthora* 屬中，包括 *P. cactorum* (Leb. et Cohn.) Sch., *P. capsici* Leon., *P. cinnamomi* Rands, *P. citricola* Sawada, *P. citrophthora* (R. E. Smith et E. H. Smith) Leon., *P. cryptogea*, *P. drechsleri* Tucker, *P. infestans* (Mont.) de Bary, *P. megasperma* Drechsler, *P. palmivora* (Butler) Butler, *P. parasitica*, 及 *P. sojae* Kaufmann et Gerdemann，除了分離自煙草之 *P. parasitica* 菌株外，其他均能產生 elicitins<sup>(26,44)</sup>；而在 *Pythium* 屬中，僅少數與 *Phytophthora* 有近緣關係之種類確定會產生 elicitin，包括 *Py. marsipium* Drechsler, *Py. oedoehilum* Drechsler, *Py. oligandrum* Drechsler, 與 *Py. vexans* de Bary<sup>(19,35,42)</sup>。此外，在 *Py. aphanidermatum* (Edson) Fitzpatrick 所發現之分泌性蛋白質 PaE1，其胺基酸序列也與 *P. infestans* 所產生之 elicitins (INF1, INF2A 及 INF2B) 有相當程度之相似性<sup>(58)</sup>。

## ELICITIN 之生化特性

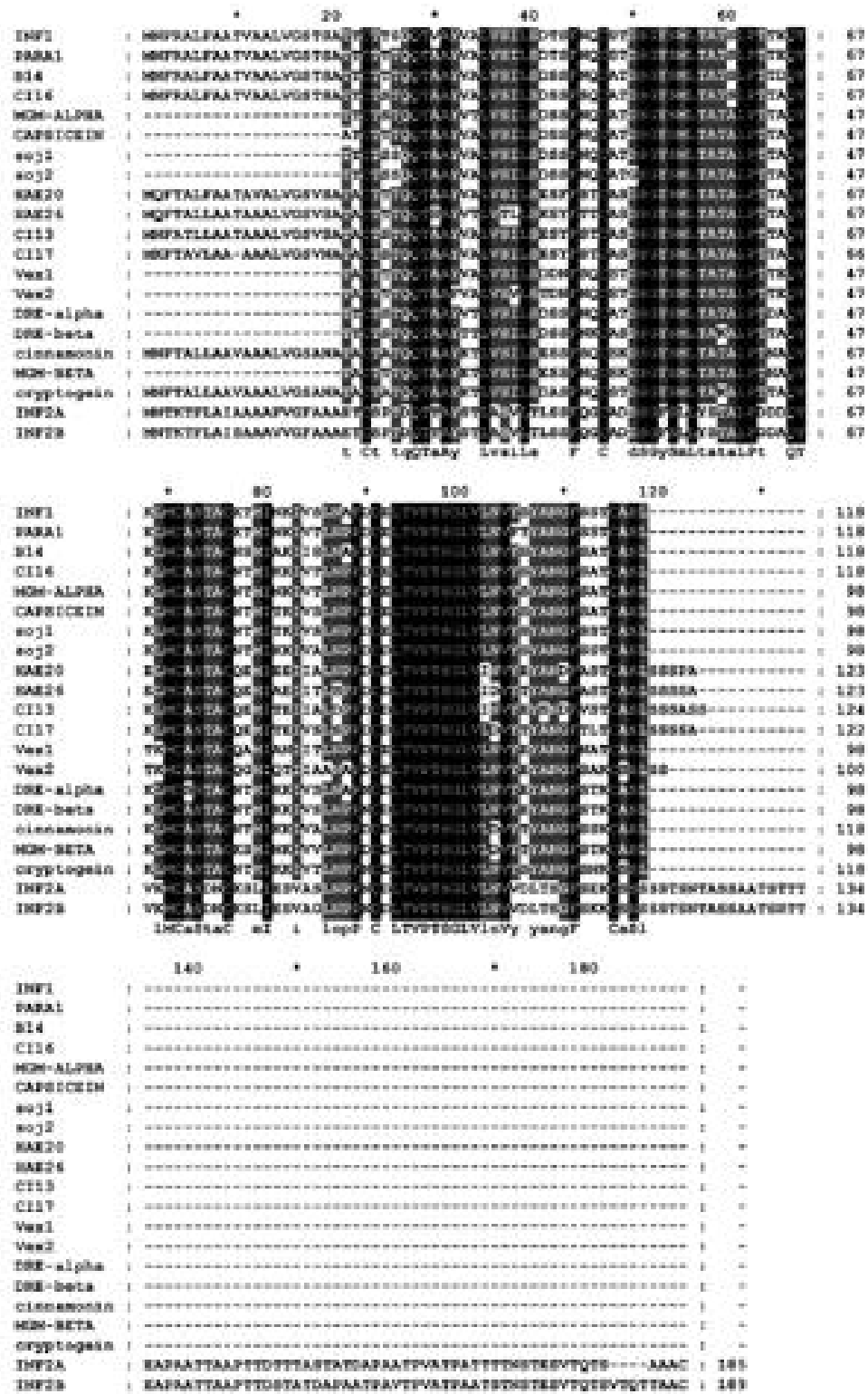
不同疫病菌與腐霉菌所分泌之 elicitin 的結構不盡相同，引發煙草產生過敏性反應的能力也互有差異。根據蛋白質結構及類緣分析結果，可將 elicitins 區分為三群<sup>(45)</sup>。第一群包含大多數目前已被發現之 elicitins (圖一)，其一般含 98 個胺基酸 (其中 6 個為 cystein)，且胺基酸序列在不同菌種間有相當程度之保守性。根據蛋白質特性還可進一步將這一群 elicitins 分成二個類型：型與型。pI 值低於 5 者屬於型，其第 13 個胺基酸一般為纈胺酸 (valine)，典型代表為 *P. parasitica* 所分泌之 parasiticein<sup>(24,48)</sup>。pI 值高於 7.5 者屬於型，其第 13 個胺基酸一般為 lysine，典型代表為 *P. cryptogea* 所分泌之 cryptogein<sup>(41,54)</sup>。一般而言，型 elicitin 引發煙草產生過敏性反應之能力較強<sup>(40)</sup>。第二群 elicitins 為在 *P. cryptogea* 及 *P. cinnamomi* 之 elicitin gene cluster 所發現的 hyperacidic elicitins，其比第一群 elicitins 在 C 端還多了 5 個胺基酸<sup>(16,41)</sup>。第三群為 elicitin-like proteins，目前包括 *P. infestans* 所產生之 INF2A 與 INF2B<sup>(25)</sup>，其與第一群 elicitins 間呈現

表一、疫病菌和腐霉菌的 elicitins 及 elicitin—擬似蛋白質

Table 1. Elicitins and elicitin-like proteins identified in *Phytophthora* and *Pythium*

Fungal species	Elicitins	Class <sup>1</sup>	References
<i>Phytophthora cactorum</i>	cactorein	I	(44)
<i>Phytophthora capsici</i>	capsicein	I	(2, 47)
<i>Phytophthora cinnamomi</i>	-cinnamomin	I	(2, 21)
	-cinnamomin	I	(16, 44)
	cinnamomin-CI13	II	(16)
	cinnamomin-CI17	II	(16)
<i>Phytophthora citricola</i>			(26)
<i>Phytophthora citrophthora</i>	citrophthorin	I	(44)
<i>Phytophthora cryptogea</i>	B14	I	(41, 47)
	cryptogein	I	(44)
	HAE20	II	(41)
	HAE16	II	(41)
<i>Phytophthora drechsleri</i>	-drechslerin	I	(20, 44)
	-drechslerin	I	(20, 44)
<i>Phytophthora infestans</i>	infestin (INF1)	I	(28)
	INF2A	III	(25)
	INF2B	III	(25)
<i>Phytophthora megasperma</i>	-megaspermin	I	(22)
	-megaspermin	I	(22)
<i>Phytophthora palmivora</i>			(26)
<i>Phytophthora parasitica</i>	parasiticein	I	(24, 48)
<i>Phytophthora sojae</i>	sojein	I	(1)
<i>Pythium aphanidermatum</i>	PaE1		(58)
<i>Pythium marsipium</i>	marsipin	I	(42)
<i>Pythium oedoehilum</i>	oedoehilin	I	(42)
<i>Pythium oligandrum</i>	oligandrin	I	(35)
<i>Pythium vexans</i>	Vex1	I	(19)
	Vex2	I	(19)

<sup>1</sup> Elicitins are classified according to Ponchet *et al.* (1999).



圖一、Elicitin 胺基酸序列並列分析。

**Fig. 1.** Multiple sequence alignment of elicitins from *Phytophthora* and *Pythium*. B14, HAE20, HAE26, and cryptogein, *Phytophthora cryptogea* elicitins<sup>(41,47)</sup>; capsicein, *Phytophthora capsici* elicitin<sup>(47)</sup>; cinnamomin, -CI13 and -CI17, *Phytophthora cinnamomi* elicitins<sup>(16)</sup>; DRE-alpha and DRE-beta, *Phytophthora drechsleri* elicitins<sup>(20)</sup>; INF1, INF2A, and INF2B, *Phytophthora infestans* elicitins<sup>(25,28)</sup>; MGA-alpha and MGA-beta, *Phytophthora megasperma* elicitins<sup>(22)</sup>; PARA1, *Phytophthora parasitica* elicitin<sup>(24)</sup>; soj1 and soj2, *Phytophthora sojae* elicitins<sup>(1)</sup>; Vex1 and Vex2, *Pythium vexans* elicitins<sup>(19)</sup>. Sequences were aligned using PILEUP in the GCG software package. Amino acid residues that were conserved in all the elicitins were shaded in black, while those conserved in most of the sequences were shaded in gray.

相當顯著之保守性，但在 C 端還額外多了將近 70 個胺基酸；INF2A 與 INF2B 之功能及其在 *P. infestans* 與植物交互作用時可能扮演之角色目前都還不清楚。

## ELICITIN 引發過敏性反應與系統性誘導抗病之機制

Elicitins 只能在部分植物引發過敏性反應，包括茄科的 *Nicotiana* spp. 與十字花科蘿蔔及蕪菁之特定栽培種 (27,48)。關於 elicitin 在煙草所引發之過敏性作用，目前以 cryptogein 與 INF1 (分別由 *P. cryptogea* 與 *P. infestans* 所分泌之 elicitin) 之相關研究較多。以 cryptogein 處理煙草時，cryptogein 可以在植物組織中移動，不僅造成乙烯和 capidiol (一種 phytoalexin) 在葉片迅速累積、葉綠體及細胞快速崩解，葉片也會產生壞疽病斑 (14,39,60)。以 cryptogein 處理煙草之細胞懸浮液時，處理後不久即可觀察到活性氧產生 (10,51)、胞內鈣離子濃度升高 (53)、蛋白質磷酸化 (57)、細胞膜去極化 (46)、細胞培養液之 pH 值及鉀離子濃度升高等現象 (4,57)。此外，煙草基因之表現也會受到影響 (30,31,52)。

Cryptogein 可誘導煙草表現與 SAR 相關之 pathogenesis-related proteins，顯示 cryptogein 在煙草所啟動之抗病反應與 SAR 發生之機制有其相似之處 (30)。當以 cryptogein 或 capsicein 處理水楊酸水解酵素 (salicylate hydrolase) 之轉基因煙草 (NahG tobacco) 時，煙草仍會呈現壞疽，但植物不再能展現系統性誘導抗病現象，顯示水楊酸可能在 elicitin 所引起之系統性抗病反應中扮演著相當重要的角色，但與 elicitin 所引發之過敏性反應並無直接關係 (31)。此外，以 parasiticein (型) 和 cryptogein (型) 分別處理煙草細胞懸浮液時發現，不僅細胞培養液之 pH 值升高，植物防禦基因 phenylalanine ammonia lyase (PAL) 之表現量增加，salicylic acid-induced protein kinase (SIPK) 及 wound-induced protein kinase (WIPK) 也會被活化 (61,62,63)。因此 elicitin 引起抗病反應時，其訊息傳導的路徑和水楊酸誘導抗病反應之路徑應有相通的地方。事實上 Bonnet 等人 (6) 的接種試驗也顯示，由 elicitin 在煙草所誘導之抗病反應不僅對 *P. parasitica* 有效，對菌核病菌 (*Sclerotinia sclerotiorum*) 也有抑制效果。此外，將 cryptogein 基因轉殖到煙草後，cryptogein 之表現不但會抑制 *P. parasitica* var. *nicotianae* 之感染，也會強化轉基因煙草對於其他病原菌，包括白粉病菌 (*Erysiphe cichoracearum*)、煙草根腐病菌 (*Thielaviopsis basicola*) 及灰黴病菌 (*Botrytis cinerea*) 之抗性 (32)。

## ELICITIN 是 AVIRULENT FACTOR 嗎？

根據 'gene-for-gene theory' (17)，植物抗病反應是病原微生物之 avirulence gene (*Avr*) 與植物之 resistance gene (*R*)

的基因產物交互作用所造成之結果。由於 Bonnet 等人 (9) 的研究結果顯示 *P. parasitica* 產生 elicitin 之能力相當程度的反映了其對於煙草之致病力，而且 elicitin 引發過敏性反應與系統性誘導抗病所涉及之機制也與其他病原微生物所啟動者十分相似，曾有學者認為在煙草與疫病菌之交互作用中，elicitin 所扮演之角色或可類比於 avirulent factor (26,59)。Kamoun 等人 (29) 以抑制基因表現 (gene silencing) 之方式抑制 *P. infestans* 表現其 elicitin 基因 (*inf1*)，再將其接種於煙草 (*N. benthamiana*) 時發現，原本無法感染煙草之菌株，於失去表現 INF1 之能力後，變成可以感染 *N. benthamiana*，顯示 INF1 是決定 *P. infestans* 能否感染 *N. benthamiana* 的重要因子之一。不過，在其他例子情況卻不是這麼單純。以 *P. parasitica* 而言，會分泌 parasiticein 者，由於會引發煙草產生過敏性反應，一般均無法成功感染煙草，可感染煙草之菌株則一般都不會產生 parasiticein，但許多研究顯示，有些煙草分離株仍可產生 parasiticein (9,12,26,48)。此外，當以單元抗體檢測 elicitin 之表現情形時也發現，在以 *P. parasitica* 接種蕃茄，或以 *P. capsici* 接種蕃茄及辣椒後一至二天，即可在接種植物偵測到 elicitins，顯示在與寄主植物建立親和性交互作用時，疫病菌也會產生 elicitins (15)。因此，elicitin 除了誘導煙草產生過敏性反應與系統性抗病外，可能也參與其他作用，與一般所認知之 avirulent factor 不盡相同。

## ELICITIN 具有固醇載體活性

Mikes 等人 (38) 之研究顯示，cryptogein 可與脫氫麥角固醇 (dehydroergosterol, DHE) 結合，並將其轉移到人工磷脂膜 (phospholipid membrane)，因此可能是一類新的固醇載體蛋白 (sterol carrier proteins) (3,37)。Vauthrin 等人 (56) 也發現 cryptogein 可自煙草細胞懸浮液或純化之原生質膜獲取固醇、促使 DHE 在微團 (micelles) 之間作轉移，並能將 DHE 自微團轉移至煙草原生質膜。由於 *Phytophthora* 與 *Pythium* 均不具合成固醇之能力，這類真菌是否必須利用 elicitin 自植物細胞膜取得固醇，以提供生長及繁殖所需，仍需要進一步研究才能獲得確切結論。此外，如前文所述，elicitin 是分泌性蛋白質，能夠引發煙草產生過敏性反應與系統性誘導抗病，其誘導活性是否必須涉及固醇載體活性也是一個十分重要有趣的問題，值得深入探討 (3,11)。

## 結 論

近年來以核糖體及粒線體核酸序列進行類緣分析的結果都顯示，卵菌 (oomycetes) 與金黃藻 (golden-brown algae) 及異鞭毛藻 (heterokont algae) 之類緣關係較近，而與一般絲狀真菌之關係較遠 (33,43,55)，elicitin 之相關研究基本上也反映了這一個現象 (45)。Elicitin 目前只在 *Phytophthora*

與 *Pythium* 被發現，而且結構相當獨特，與其他目前已被發現之真菌蛋白質間無明顯相似性。Elicitin 可在煙草引發過敏性反應與系統性抗病，但其真正扮演之角色可能不僅侷限於一般為“ avirulent gene ”所設定之範疇。一般在探討 *Avr* gene 與 *R* gene 之作用時主要均著眼於病原生理小種 (race) 與寄主植物栽培種 (cultivar) 這一個層次之交互作用，但 INF1 (*P. infestans*) 之實驗顯示，除了 cultivar-specific *Avr* genes 外，也可能存在 species-specific *Avr* genes，其經由與植物 *R* gene 之作用而決定病原菌能否感染特定種類之寄主，這一個現象是否也發生於其他植物病原菌值得進一步探討<sup>(23,36)</sup>。另一方面，*P. parasitica* 與煙草之交互作用也顯得相當複雜，很難以一般所熟悉之‘ gene-for-gene theory ’解釋；在親和性交互作用發生時，有些 *P. parasitica* 分離株仍然會分泌 parasiticein，顯示除了誘導活性，elicitin 可能還參與其他作用，近來在 elicitin 所發現之固醇載體活性無疑為這一個可能性提供了最令人興奮的答案<sup>(37,38)</sup>。*Phytophthora* 與 *Pythium* 均缺乏自行合成固醇的能力，故可能利用 elicitin 自植物獲取固醇，以提供生長與繁殖之需。在這種情形下，*P. parasitica* 為因應自身所需而分泌 elicitin 時，其實並不利於此菌與煙草建立親和性交互作用，藉由深入探討其感染煙草後 elicitin 之表現情形，應可釐清 *P. parasitica* 與植物交互作用時所涉及之調控機制。

## 引用文獻

1. Becker, J., Nagel, S., and Tenhaken, R. 2000. Cloning, expression and characterization of protein elicitors from the soybean pathogenic fungus *Phytophthora sojae*. J. Phytopathol. 148:161-167.
2. Billard, V., Bruneteau, M., Bonnet, P., Ricci, P., Pernolet, J.C., Huet, J.C., Vergne, A., Richard, G., and Michel, G. 1988. Chromatographic purification and characterization of elicitors of necrosis on tobacco produced by incompatible *Phytophthora* species. J. Chromatogr. 44:87-94.
3. Boissy, G., O'Donohue, M., Gaudemer, O., Perez, V., Pernolet, J.C., and Brunie, S. 1999. The 2.1 angstrom structure of an elicitin-ergosterol complex: a recent addition of the sterol carrier protein family. Protein Sci. 8:1191-1199.
4. Blein, J.P., Milat, M.L., and Ricci, P. 1991. Responses of cultured tobacco cells to cryptogein, a proteinaceous elicitor from *Phytophthora cryptogea*. Plant Physiol. 95:486-491.
5. Bonnet, P. 1985. Réactions différentielles du tabac à 9 espèces de *Phytophthora*. Agronomie 5:801-808.
6. Bonnet, P., Boudon, E., Ponchet, M., Blein, J.P., and Ricci, P. 1996. Acquired resistance triggered by elicitors in tobacco and other plants. Eur. J. Plant Pathol. 102:181-192.
7. Bonnet, P., Poupet, A., Abad, P., Venard, P., and Cardin, L. 1986. Induction de nécroses foliaires, de protéines et de résistance dans les interaction tabac-*Phytophthora*. Agronomie 6:829-837.
8. Bonnet, P., Poupet, A., Bruneteau, M. 1985. Toxicité vis-à-vis du tabac des fractions purifiées d'un filtrate de culture de *Phytophthora cryptogea* Pethyb. & Laff. Agronomie 5:275-282.
9. Bonnet, P., Lacout, I., Venard, P., and Ricci, P. 1994. Diversity in pathogenicity to tobacco and in elicitin production among isolates of *Phytophthora parasitica*. J. Phytopathol. 141:25-37.
10. Bottin, A., Vermési, C., Pontier, D., Esquerre-Tagayé, M.T., Blein, J.P., Rusterucci, C., and Ricci, P. 1994. Different responses of tobacco cells to elicitors from two *Phytophthora* species. Plant Physiol. Biochem. 32:373-378.
11. Bourque, S., Binet, M.N., Ponchet, M., Pugin, A., and Lebrun-Garcia, A. 1999. Characterization of the cryptogein binding sites on plant plasma membranes. J. Biol. Chem. 274:34699-34705.
12. Colas, V., Lacourt, I., Ricci, P., Vanlerberghe-Masutte, F., Venard, P., Poupet, A., and Panabières, F. 1998. Diversity of virulence in *Phytophthora parasitica* on tobacco, as reflected by nuclear RFLPs. Phytopathology 88:205-212.
13. Dang, J.L., Dietrich, R.A., and Richberg, M.H. 1996. Death don't have no mercy: cell death programs in plant-microbe interactions. Plant Cell 8:1793-1807.
14. Devergne, J.C., Bonnet, P., Panabières, F., Blein, J.P., and Ricci, P. 1992. Migration of the fungal protein cryptogein within tobacco plants. Plant Physiol. 99:843-847.
15. Devergne J.C., Fort, M.A., Bonnet, P., Ricci, P., Vergnet, C., Delaunay, T., Grosclaude, J. 1994. Immunodetection of elicitors from *Phytophthora* spp. using monoclonal antibodies. Plant Pathol. 43:885-896.
16. Duclos, J., Fauconnier, A., Coelho, A.C., Bollen, A., Cravador, A., and Godfroid, E. 1998. Identification of an elicitin gene cluster in *Phytophthora cinnamomi*. J. Seq. Mapp. 9:231-237.
17. Flor, H.H. 1971. Current status of the gene-for-gene concept. Annu. Rev. Phytopathol. 9:275-296.
18. Goodman, R.N., and Novacky, A.J. 1994. The hypersensitive reaction in plants to pathogens: A resistance phenomenon. APS Press, St. Paul, MN.
19. Huet, J.C., Le Caer, J.P., Nespoulous, C., and Pernolet, J.C. 1995. The relationships between the toxicity and the primary and secondary structures of elicitin-like protein elicitors secreted by the phytopathogenic fungus *Pythium vexans*. MPMI 8:302-310.
20. Huet, J.C., Nespoulous, C., and Pernolet, J.C. 1992. Structure of elicitin isoforms secreted by *Phytophthora drechsleri*. Phytochemistry 31:1471-1476.
21. Huet, J.C., and Pernolet, J.C. 1989. Amino acid sequence of cinnamomin, a new member of elicitin family, and its comparison to cryptogein and capsicein. FEBS Lett. 257:302-306.
22. Huet, J.C., and Pernolet J.C. 1993. Sequences of acidic and basic elicitin isoforms secreted by *Phytophthora megasperma megasperma*. Phytochemistry 33:797-805.

23. Kamoun, S., Huitema, E., and Vleeshouwers, V.G.A.A. 1999. Resistance to oomycetes: a general role for the hypersensitive response? *Trends Plant Sci.* 4:196-200.
24. Kamoun, S., Klucher, K.M., Coffey, M.D., and Tyler B.M. 1993. A gene encoding a host-specific elicitor protein of *Phytophthora parasitica*. *MPMI* 6:573-581.
25. Kamoun, S., Lindqvist, H., and Covers, F. 1997. A novel class of elicitor-like genes from *Phytophthora infestans*. *MPMI* 10:1028-1030.
26. Kamoun, S., Young, M., Forster, H., Coffey, M.D., and Tyler, B.M. 1994. Potential role of elicitors in the interaction between *Phytophthora* species and tobacco. *Appl. Environ. Microbiol.* 60:1593-1598.
27. Kamoun, S., Young, M., Glascock, C., and Tyler, B.M. 1993. Extracellular protein elicitors from *Phytophthora*: Host-specificity and induction of resistance to fungal and bacteria phytopathogens. *MPMI* 6:15-25.
28. Kamoun, S., van West, P., ds Jong, A.J., de Groot, K.E., Vleeshouwers, V.G.A.A., and Govers, F. 1997. A gene encoding a protein elicitor of *Phytophthora infestans* is down-regulated during infection of potato. *MPMI* 10:13-20.
29. Kamoun, S., van West, P., Vleeshouwers, V.G.A.A., de Groot, K.E., and Govers, F. 1998. Resistance of *Nicotiana benthamiana* to *Phytophthora infestans* is mediated by the recognition of the elicitor protein INF1. *Plant Cell* 10:1412-1425.
30. Keller, H., Blein, J.P., Bonnet, P., Panahières, F., and Ricci, P. 1996. Physiological and molecular characteristics of elicitor-induced systemic acquired resistance in tobacco. *Plant Physiol.* 110:365-376.
31. Keller, H., Bonnet, P., Galiana, E., Pruvost, L., Friedrich, L., Ryals, J., and Ricci, P. 1996. Salicylic acid mediates elicitor-induced systemic acquired resistance but not necrosis in tobacco. *MPMI* 9:696-703.
32. Keller, H., Pamboukdjian, N., Ponchet, M., Poupet, A., Delon, R., Verrier, J.L., Roby, D., and Ricci, P. 1999. Pathogen-induced elicitor production in transgenic tobacco generates a hypersensitive response and nonspecific disease resistance. *Plant Cell* 11:223-235.
33. Kumar, S., and Rzhetsky, A. 1996. Evolutionary relationships of eukaryotic kingdoms. *J. Mol. Evol.* 42:183-193.
34. Lamb, C.J., Lawton, M.A., Dron, M., and Dixon, R.A. 1989. Signals and transduction mechanisms for activation of plant defenses against microbial attack. *Cell* 56:215-224.
35. Lascombe, M.B., Milat, M.L., Blein, J.P., Panahières, F., Ponchet, M., and Prange, T. 2000. Crystallization and preliminary x-ray studies of oligandrin, a sterol-carrier elicitor from *Pythium oligandrum*. *Acta Cryst. D* 56:1498-1500.
36. Laugé, R., and De Wit, P.J.G.M. 1998. Fungal avirulence genes: structure and possible functions. *Fungal Genet. Biol.* 24:285-297.
37. Mikes, V., Milat, M.L., Ponchet, M., Panahières, F., Ricci, P., and Blein, J.P. 1998. Elicitors excreted by *Phytophthora* are a new class of sterol carrier proteins. *Biochem. Biophys. Res. Comm.* 245:133-139.
38. Mikes, V., Milat, M.L., Ponchet, M., Ricci, P., and Blein, J.P. 1997. The fungal elicitor cryptogein is a sterol carrier protein. *FEBS Lett.* 416:190-192.
39. Milat, M.L., Ducruet, J.M., Ricci, P., Marty, F., and Blein, J.P. 1991. Physiological and structural changes in tobacco leaves treated with cryptogein, a proteinaceous elicitor from *Phytophthora cryptogea*. *Phytopathology* 81:1364-1368.
40. Nespoulous, C., Huet, J.C., and Pernollet, J. C. 1992. Structure-function relationships of and elicitors signal proteins involved in the plant—*Phytophthora* interactions. *Planta* 186:551-557.
41. Panahières, F., Marais, A., Le Berre, J.Y., Penot, I., Fournier, D., and Ricci, P. 1995. Characterization of a gene cluster of *Phytophthora cryptogea*, which codes for elicitors, proteins inducing a hypersensitive-like response in tobacco. *MPMI* 8:996-1003.
42. Panahières, F., Ponchet, M., Allasia, V., Cardin, L., and Ricci, P. 1997. Characterization of border species among Pythiaceae: several *Pythium* isolates produce elicitors, typical proteins from *Phytophthora* spp. *Mycol. Res.* 101:1459-1468.
43. Paquin, B., Laforesst, M.-J., Forget, L., Roewer, I., Wang, Z., Longcore, J., Lang, B.F. 1997. The fungal mitochondrial genome project: evolution of fungal mitochondrial genomes and their gene expression. *Curr. Genet.* 31:380-395.
44. Pernollet, J.C., Sallantin, M., Salle-Tourne, M., and Huet, J.C. 1993. Elicitor isoforms from seven *Phytophthora* species: comparison of their physico-chemical properties and toxicity to tobacco and other plant species. *Physiol. Mol. Plant Pathol.* 42:53-67.
45. Ponchet, M., Panahières, F., Milat, M.L., Mikes, V., Montillet, L.M., Suty, L., Triantaphylides, C., Tirilly, Y., and Blein, J.P. 1999. Are elicitors cryptograms in plant-Oomycete communications? *Cell. Mol. Life Sci.* 56:1020-1047.
46. Pugin, A., Frachisse, J.M., Tavernier, E., Bligny, R., Gout, E., Douce, R., and Guern, J. 1997. Early events induced by the elicitor cryptogein in tobacco cells: involvement of a plasma membrane NADPH oxidase and activation of glycolysis and pentose phosphate pathway. *Plant Cell* 9:2077-2091.
47. Ricci, P., Bonnet, P., Huet, J.C., Sallantin, M., Beauvais-Cante, F., Bruneteau, M., Billard, V., Michel, G., and Pernollet, J.C. 1989. Structure and activity of proteins from pathogenic fungi *Phytophthora* eliciting necrosis and acquired resistance in tobacco. *Eur. J. Biochem.* 183:555-563.
48. Ricci, P., Trentin, F., Bonnet, P., Venrad, P., Mouton-Rerronnet, F., and Bruneteau, M. 1992. Differential production of parasiticein, an elicitor of necrosis and resistance in tobacco, by isolates of *Phytophthora parasitica*. *Plant Pathol.* 41:298-307.
49. Ross, F.A. 1961. Systemic acquired resistance induced by localized virus infections in plants. *Virology* 14:340-358.
50. Ryals, J.A., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H.Y., and Hunt, M.D. 1996. Systemic acquired resistance. *Plant Cell* 8:1809-1819.

51. Sasabe, M., Takeuchi, K., Kamoun, S., Ichinose, Y., Govers, F., Toyoda, K., Shiraishi, T., and Yamada, T. 2000. Independent pathways leading to apoptotic cell death, oxidative burst and defense gene expression in response to elicitor in tobacco cell suspension culture. *Eur. J. Biochem.* 267:5005-5013.
52. Suty, L., Blein, J.P., Ricci, P., and Pugin, A. 1995. Early changes in gene expression in tobacco cells elicited with cryptogein. *MPMI* 8:644-651.
53. Tavernier, E., Wendehenne, D., Blein, J.P., and Pugin, A. 1995. Involvement of free calcium in action of cryptogein, a proteinaceous elicitor of hypersensitive reaction in tobacco cells. *Plant Physiol.* 109:1025-1031.
54. Tercé-Laforgue, T., Huet, J.C., and Pernollet, J.C. 1992. Biosynthesis and secretion of cryptogein, a protein elicitor secreted by *Phytophthora cryptogea*. *Plant Physiol.* 98:936-941.
55. Van de Peer, Y., and De Wachter, 1997. Evolutionary relationships among the eukaryotic crown taxa taking into account site-to-site rate variation in 18S rRNA. *J. Mol. Evol.* 45:619-630.
56. Vauthrin, S., Mikes, V., Milat, M.L., Ponchet, M., Maume, B., Osman, H., and Blein, J.P. 1999. Elicitins trap and transfer sterols from micelles, liposomes and plant plasma membranes. *Biochim. Biophys. Acta* 1419:335-342.
57. Viard, M.P., Martin, F., Pugin, A., Ricci, P., and Blein, J.P. 1994. Protein phosphorylation is induced in tobacco cells by the elicitor cryptogein. *Plant Physiol.* 104:1245-1249.
58. Wang, H.W., Chen, S.F., and Liou, R.F. 1999. Characterization of an elicitor-like protein from *Pythium aphanidermatum*. *Plant Pathol. Bull.* 8:175. (Abs.)
59. Yu, L. M. 1995. Elicitins from *Phytophthora* and basic resistance in tobacco. *Proc. Natl. Acad. Sci. USA* 92:4088-4094.
60. Zanetti, A., Beauvais, F., Huet, J.C., and Pernollet, J.C. 1992. Movement of elicitors, necrosis-inducing proteins secreted by *Phytophthora* spp., in tobacco. *Planta* 187:163-170.
61. Zhang, S., Du, H., and Klessig, D.F. 1998. Activation of the tobacco SIP kinase by both a cell wall-derived carbohydrate elicitor and purified proteinaceous elicitors from *Phytophthora* spp. *Plant Cell* 10:435-449.
62. Zhang, S., and Klessig, D.F. 1997. Salicylic acid activates a 48-kD MAP kinase in tobacco. *Plant Cell* 9:809-824.
63. Zhang, S., Liu, Y., and Klessig, D.F. 2000. Multiple levels of tobacco WIPK activation during the induction of cell death by fungal elicitors. *Plant J.* 23:339-347.

### ABSTRACT

Liou, R.F. 2001. Roles of elicitors in the biology of *Phytophthora*. Plant Pathol. Bull. 10:97-104. (Department of Plant Pathology, National Taiwan University, Taipei, Taiwan; E-mail: rliou@ccms.ntu.edu.tw; Fax: +886-2-23620271)

Elicitins are structurally related proteins produced by species of *Phytophthora* and *Pythium* (oomycetes). Based on the overall structure of the proteins as well as the phylogenetic analysis, elicitors may be divided into three classes. Class I encompasses most of the elicitor proteins identified thus far, with polypeptides of 98 amino acid residues and able to induce hypersensitive response as well as systemic acquired resistance in tobacco. Members of class II include elicitor sequences from *P. cryptogea* or *P. cinnamomi*, which encode hyperacidic elicitor proteins with extra short hydrophilic C-terminal tails. Class III contains two elicitor-like proteins, INF2A and INF2B of *P. infestans*, which encode the conserved 98-amino acid elicitor signature, followed by a C-terminal domain of about 70 amino acid residues. However, it is not clear whether INF2 can elicit defense response in plants similar to the class I elicitors. In addition to their role as elicitors of plant defense response, elicitors also exhibited a sterol-binding activity. Therefore, it might act as shuttles trapping the sterols from the host plants. This property opens new perspectives concerning the role of elicitors in plant-*Phytophthora* interactions.

Key words: elicitor, hypersensitive response, *Phytophthora*, *Pythium*, systemic acquired resistance.