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Occurrence of pathogenesis-related (b) and similar proteins in different plant species

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Abstract

The main properties of 'pathogenesis-related' proteins induced in *Nicotiana* species during hypersensitive reactions to different pathogens, as well as by chemical or physical treatments, are listed. These properties are compared with those of similar protein compounds occurring in other plant species in similar circumstances. The plants include cucumber, cowpea, *Gomphrena globosa,* kidney bean, *Gynura aurantiaca,* tomato, potato, citron and celery. Similarities with other proteins normally occurring in plants, such as proteinase inhibitors, are considered. Analogies and differences with proteins induced in plants by environmental stresses, and with the ' antiviral factors' and the 'inhibitor of viral replication' occurring in *Nicotiana* species are briefly discussed.

Additional keywords: pathogenesis; stress; resistance.

Introduction

In 1970 Gianinazzi et al. and Van Loon and Van Kammen independently observed that in tobacco plants *(Nicotiana tabacum)* responding hypersensitively to infection with tobacco mosaic virus (TMV), particular changes in the pattern of the soluble leaf proteins occurred. Several proteins, absent from healthy plants, were found to be formed at the onset of hypersensitivity. Working with two different tobacco varieties, 'Xanthinc' (Gianinazzi et al., 1970) and 'Samsun NN' (Van Loon and Van Kammen, 1970), they obtained comparable results confirmed later by other authors (Rohloff and Lerch, 1977; Antoniw and Pierpoint, 1978; Coutts, 1978; Fraser, 1981). About 30 papers have now been published on these 'new' proteins of tobacco plants. They were named 'b-proteins' by Gianinazzi et al. (1970) and 'pathogenesis-related proteins' or PRs by Antoniw et al. (1980). Their characteristics have been widely investigated and their biological function hypothesized (Gianinazzi, 1982; Van Loon, 1982) though not established.

The problem arises whether this phenomenon is peculiar to tobacco plants or is a common feature of most plant species. The first difficulty to overcome is to establish whether new proteins found in different plants as a result of hypersensitive reactions are 'similar' to those found in tobacco.

I propose to consider here as similar to PR (b) proteins of tobacco those found in other plant species in analogous circumstances, and having analogous chemical and biological properties.

Properties of PR (b) and similar proteins

Main properties of PR (b) proteins of Nicotiana species

Seven different PRs have been identified in 10 *Nicotiana* species and at least 30 *N. tabacum* varieties (cf. Gianinazzi, 1983). In spite of this increasing number, the main properties of PRs substantially remain those of the first four discovered (Table 1). Six additional more slowly migrating ones were described by Van Loon (1982) but have not been fully characterized.

Occurrence of similar proteins in other plant species

Cowpea (Vigna sinensis L.). Two distinct protein components, named α and β, are induced by hypersensitive viral infection in cowpea leaves (Coutts, 1978; Coutts and Wagih, 1981). The main properties of these proteins are given in Table 1.

Table 1. Properties of PR (b) and similar proteins of different plant species.

Property	Plant species						
	Nicotiana spp.	cowpea	cucumber	*G. globosa*	kidney bean	*G. aurantiaca*	common properties
preferentially extracted at acidic pH	+	+	+	+	+		+
set of compounds	+	+	−	+	+	+	+
charge isomers	+			+	+		+
low mol. wt (10 000-20 000)	+	+	+		+	+	+
acidic character	+		+		+		+
resistant to proteolytic digestion	+				+		+
containing carbohydrates	−	−	+				
induced during hypersensitive response to different pathogens	+	+	+	+	+		+
induced by chemicals or plasmolysis	+	+	+		+	+	+
pattern variability among varieties	+		+				+
References	a	b	c	d	e	f	

References: a) cf.: Gianinazzi, 1982, 1983; Van Loon, 1982; b) Coutts, 1978; Coutts and Wagih, 1981; Wagih and Coutts, 1981, 1982a; c) Tas and Peters, 1977; Andebrhan et al., 1980; Wagih and Coutts, 1981, 1982a, 1982b; Gessler and Kuc, 1982; Wagih et al., 1983; d) Pennazio and Redolfi, 1980; Pennazio, 1981; Redolfi et al., 1982; Redolfi and Pennazio, unpublished; e) Redolfi and Cantisani, 1981; Redolfi, 1983, unpublished; f) Conejero and Semancik, 1977; Conejero et al., 1979; Conejero, 1981, 1982.

Cucumber (Cucumis sativus L.). Hypersensitive infections by viruses, bacteria or fungi induce in leaves of cucumber a protein component, named E or γ, whose properties are given in Table 1 (Ziemiecki and Wood, 1975; Tas and Peters, 1977; Andebrhan et al., 1980; Gessler and Kuc, 1982). In most varieties a single component appears, but at least in one case two proteins are formed (Wagih and Coutts, 1982b).

It is interesting that the new proteins were not found in extracts from protoplasts prepared from cowpea and cucumber leaves in which they were present in considerable amounts. Such observations suggest that the new proteins are localized either in the free space between the cell wall and the plasmalemma, or bound to cell walls or to the plasma membrane (Wagih and Coutts, 1981).

Attempts to demonstrate *de novo* synthesis of the new proteins of cowpea and cucumber by incorporation of radio-labelled precursors did not provide unequivocal results (Coutts and Wagih, 1981; Wagih et al., 1983).

Gomphrena globosa L. Five proteins, named p_0 to p_4, are induced by hypersensitive viral infections in *Gomphrena globosa* leaves (Pennazio and Redolfi, 1980; Pennazio, 1981). Among the properties reported in Table 1 it is noteworthy that at least two of these proteins are charge isomers (P. Redolfi and S. Pennazio, unpublished results). A further observation has been made when working with this plant. Tomato bushy stunt virus normally gives a strong hypersensitive reaction in *G. globosa* but when inoculated plants are kept at 28°C under continuous illumination a systemic reaction ensues, only later followed by tissue necrosis. The temporary suppression of hypersensitivity and the systemic spread of the virus are accompanied by a delay in the appearance of the new protein compounds (P. Redolfi and S. Pennazio, unpublished results).

Bean (Phaseolus vulgaris L.). Hypersensitive infections of bean by viruses or fungi are accompanied by the accumulation of several new protein compounds (Abu-Jawdah, 1981; Redolfi and Cantisani, 1981). In 'Saxa' bean plants, the major change in the soluble protein pattern following hypersensitive viral infection is the appearance of four proteins, named PS (initials of *Phaseolus* and 'Saxa') 1 to 4, whose properties are reported in Table 1. One of these proteins behaves differently from the others in some respects: a) it is detected in healthy mature leaves of flowering plants (Redolfi, 1983) but hypersensitive infections dramatically increase its concentration; b) it is induced during systemic infections though later than in hypersensitive ones; c) it is detected in virus-free tissues adjacent to the inoculated ones (P. Redolfi, unpublished results). A mol. wt of 14 000 has been determined for all the four PS proteins, which must therefore be considered as charge isomers (P. Redolfi, unpublished results).

Five other bean varieties have been tested for soluble protein changes under hypersensitive conditions. In all cases the four PS proteins are induced (P. Redolfi, unpublished results). In two further varieties three new proteins are induced by viral or fungal infection (Abu-Jawdah, 1982). Direct comparisons between extracts from all these different varieties must be performed in order to ascertain if the same protein components are involved.

Gynura aurantiaca DC. A strong stimulation of two host-coded proteins, named CEV-P_1 (mol. wt = 13 700) and CEV-P_2 (mol. wt = 18 000), has been reported in

Gynura aurantiaca systemically infected by citrus exocortis viroid (CEV) (Conejero and Semancik, 1977). A protein stimulated in the same host-viroid combination has recently been reported (Camacho Henriquez and Sänger, 1982). This protein probably coincides with one of those previously reported.

Some of the properties of these proteins (Table 1) are reminiscent of those of PRs, but a real comparison is difficult due to differences in approach. In fact, the *G. aurantiaca* results refer to a systemic and not to a hypersensitive infection. Furthermore, only electrophoresis in the presence of SDS has been carried out, precluding a comparison with other PRs. However, the stimulation of these proteins in old plants or by Ag^+ treatment, as well as by viroid infection, indicates that they are not linked to viroid replication but part of a host response that can be elicited by different events.

Other species. Similar observations have been made in a few other plant species. Conejero et al. (1979) reported CEV-induced proteins in tomato *(Lycopersicon esculentum* M.), potato *(Solanum tuberosum* L.) and citron *(Citrus medica* L.). As in *G. aurantiaca,* two proteins having similar mol. wts (12 000 and 16 300 for potato and tomato; 13 700 and 18 000 for citron) were always induced. Camacho Henriquez and Sänger (1982) recently investigated the alterations induced in phenol-soluble protein patterns of several plant species infected by viroids. Polypeptides of similar mol. wt were found to accumulate in potato (14 000), tomato (14 000), citron (13 000) and celery *(Apium graveolens* L.) (12 000). However, only SDS-PAGE has been carried out.

A new protein with a mol. wt ranging between 55 000 and 70 000 has been reported to be induced in tomato by a hypersensitive fungal infection (De Wit and Bakker, 1980). This protein seems to differ considerably from those induced in tomato by viroids.

General remarks on PR (b) and similar proteins

It appears from Table 1 that the new proteins occurring in different plant species share several distinctive properties, giving rise to a list of properties to check when dealing with new proteins from plants. Some further characters of tobacco PRs have not been clearly verified in other species, and conflicting data have sometimes been reported. Some of these questions are now briefly discussed.

Occurrence of new proteins in healthy plants. For tobacco plants it has been clearly established that PRs do not normally occur in healthy plants (Van Loon, 1976; Gianinazzi et al., 1977; Pierpoint et al., 1981). It has also been found that they are induced by a number of non-pathogenic agents and may appear when particular physiological conditions such as senescence or flowering are involved (Fraser, 1981). This leads us to consider here all those proteins whose amount increases dramatically when particular agents or factors act on the plant, including those that are present in trace amounts in control leaves. Sometimes, these proteins seem to be truly absent from healthy control leaves, as with cowpea (Coutts, 1978), but traces are found in control leaves in the case of cucumber (Tas and Peters, 1977; Wagih and Coutts, 1982a). *G. globosa* and bean are in an intermediate category where traces of some of these proteins are occasionally present in control leaves, depending also on factors such as aging and growing conditions (Pennazio, 1981; Redolfi et al., 1982; Redolfi, 1983).

Presence of new proteins in tissues distant from infection points. Some of the PRs were consistently found, although in low amount, in the younger upper leaves of infected tobacco (Van Loon and Van Kammen, 1970) and tomato plants (De Wit and Bakker, 1980), but in other cases such as cowpea (Coutts and Wagih, 1981) and bean (P. Redolfi, unpublished results) small amounts were found only in nearby tissues, such as the half leaf adjacent to the inoculated one. In cucumber plants, several different authors have failed to find any new protein at a distance from necrotized tissues (Tas and Peters, 1977; Andebrhan et al., 1980; Gessler and Kuc, 1982).

The presence of PRs in tissues outside the infected area has been attributed to the spread of a hypothetical chemical inducer and not to the translocation of the proteins themselves (Van Loon and Antoniw, 1982). It may then be that the mobility and/or activity of such inducer(s), if existing, vary greatly from species to species.

Presence of carbohydrates. The presence of a carbohydrate moiety in PRs from tobacco has been excluded (Van Loon and Ritter, 1978). A positive Schiff staining reaction has also been excluded for the new proteins from cowpea (Coutts and Wagih, 1981), but it has been reported for tomato (De Wit and Bakker, 1980) and cucumber (Tas and Peters, 1977; Andebrhan et al., 1980). In the latter case, however, Gessler and Kuc (1982) reported carbohydrates to be absent from a new protein of mol. wt = 16 000, lower than the value of 22 000 found by previous authors. This should point our attention to a possible loss of the carbohydrate moiety during the extraction and purification procedures.

Comparison with other classes of protein compounds

The possible role of proteins induced in a number of plant species by hypersensitive infections is presently unclear. No biological function of these protens has been unambiguously demonstrated although hypotheses have been formulated, sometimes supported by numerous circumstantial evidences (Gianinazzi, 1982).

A better understanding of this problem may come from a comparison to other protein compounds present or induced in plant cells.

Proteinase inhibitors. Inhibitors of proteolytic enzymes are present in almost all cells. In plants, they are usually concentrated in storage tissues, particularly in those of *Leguminosae* and *Solanaceae,* but they can also be induced by mechanical injury of the leaf. When some of the properties of proteinase inhibitors are considered, a surprising similarity to PRs becomes evident (Table 2). Nevertheless, it has been clearly

Table 2. Properties of proteinase inhibitors.

high stability to extreme pHs
resistant to proteolytic digestion
not containing carbohydrates
low mol. wt, most frequently ranging from 8 000 to 20 000
frequently families of closely related isoinhibitors

demonstrated that tobacco PRs are not inhibitors of proteolytic enzymes (Pierpoint et al., 1981). However, such proteins have, as a consequence of their chemical structure, a highly specific ability to interact with other proteins (here proteolytic enzymes), interfering with their biological activity.

Shock proteins. The pattern of protein synthesis in different plant species has been found to change rapidly and dramatically when the temperature is raised from 20-25 to 40°C. Upon polyacrylamide gel electrophoresis in the presence of SDS, ten new protein bands were identified in seedlings of soybean (Key et al., 1981; Altschuler and Mascarenhas, 1982) and maize (Cooper and Ho, 1983; Altschuler and Mascarenhas, 1982), and in tobacco and cowpea leaves (Dawson and Grantham, 1981). The mol. wts of these 'heat shock proteins' range from 15-18 000 to 87-100 000. The heat shock response appears to be very rapid but transient, as the synthesis of the new proteins generally occurs during the first hour of incubation and declines 4 to 6 hours later.

Another group of new proteins, called 'osmotic shock proteins', has been described in protoplasts and hypertonic cells isolated from leaves of *N. sylvestris*. Five new proteins have been identified whose mol. wts range from 20 000 to 70 000 (Fleck et al., 1982). The synthesis of a single new polypeptide has also been reported in pea root meristem cells as a consequence of nutrient stress (Webster, 1980).

It is difficult to compare these shock proteins, generally detected by measuring the actual rate of protein synthesis, with PRs, which are generally detected as a result of net accumulation. The greater number of heat shock proteins and the wider range of their mol.wts could be the consequence of the different experimental methods involved. A distinctive feature seems to be the very rapid and transient response to shock, particularly to heat, when compared to accumulation over several days of PRs and similar proteins induced either by pathogens or by chemicals.

Although partial overlapping between the effects of different stresses cannot be excluded, it is evident that stresses induce specific changes in protein synthesis. It has been proposed that the new proteins will enable the plant to cope with the stress. For example, in soybean seedlings heat shock treatments protect from death when cells are subsequently subjected to severe temperature stress (Altschuler and Mascarenhas, 1982).

Protein compounds involved in resistance mechanisms. Since 1962 Sela and coworkers have extensively studied the occurrence and properties of a so called 'antiviral factor' (AVF) (cf. Sela, 1981) induced by hypersensitive viral infections in *Nicotiana* species. More recently, an 'inhibitor of viral replication' (IVR) was found to be released into the medium from protoplasts of *N. tabacum* 'Samsun NN' following TMV infection (Loebenstein and Gera, 1980; Gera and Loebenstein, 1983).

It is not intended here to critically examine these reports, but simply to recall some of the properties of AVF and IVR as a first comparison with PRs and similar proteins (Table 3). Analogies (host-coding, induction) and differences (chemical properties, biological activity) are evident and lead to a first conclusion: we are certainly dealing with different compounds but possibly with related biological mechanisms.

The crucial point seems to be the antiviral activity shown by both AVF and IVR but never demonstrated for PRs. This draws our attention to the possibility that PRs and AVF/IVR could act at different steps of a process going from recognition to inhibition of the pathogen. The impressive similarities recently reported between AVF and in-

Table 3. Comparative properties of PR (b) and similar proteins, 'antiviral factor' (AVF) and 'inhibitor of viral replication' (IVR).

Properties	PRs	AVF	IVR
host-coded	+	+	+
occurrence	several species and families	*Nicotiana* (*N* gene)	*N. tabacum* 'Samsun NN' (protoplasts)
induced by hypersensitive infection	+	+	+ (protoplasts)
induced by other agents	+	+	?
antiviral activity	−	+	+
chemical nature	proteins	phosphoglyco-protein	proteinaceous material
stable at acidic pHs	+	+	+
molecular weight	15 000	22 000	26 000 and 57 000

terferon (Devash et al., 1982; Orchansky et al., 1982; Gat-Edelbaum et al., 1983) make AVF and perhaps IVR good candidates for the last step of a specific resistance mechanism in *Nicotiana* plants, although evidence for direct involvement in virus localisation and acquired resistance is still lacking, particularly for IVR which is formed in protoplasts. On the other hand, the occurrence of PRs or similar proteins in different plant species leads one to consider them to be involved in intermediate steps of some general mechanism in higher plants.

Concluding remarks

The induction of large amounts of soluble leaf proteins not present, or present in trace amounts, in healthy leaves is a general feature of hypersensitive responses of plants to pathogens.

Careful comparisons among different plant species could help in recognizing the basic features of the biological mechanism involved.

Considerable modification of protein synthesis seems to be a general response of cells to stress; nevertheless, it seems likely that some specificity exists in response to different stresses. Comparisons using uniform experimental methods are needed to establish this point.

References

Abu-Jawdah, Y., 1981. Changes in the patterns of soluble proteins of bean leaves infected with *Colletotrichum lindemuthianum* (Sacc. & Magn.). Parasitica 37: 35-38.

Abu-Jawdah, Y., 1982. Changes in the soluble protein patterns of bean leaves upon fungal or viral infections or after chemical injury. Phytopath. Z. 103: 272-279.

Altschuler, M. & Mascarenhas, J.P., 1982. Heat shock proteins and effects of heat shock in plants. Plant Mol. Biol. 1: 103-115.

Andebrhan, T., Coutts, R.H.A., Wagih, E.E. & Wood, R.K.S., 1980. Induced resistance and changes in the soluble protein fraction of cucumber leaves locally infected with *Colletotrichum lagenarium* or tobacco necrosis virus. Phytopath. Z. 98: 47-52.

Antoniw, J.F. & Pierpoint, W.S., 1978. The purification and properties of the 'b' proteins from virus-infected tobacco plants. J. gen. Virol. 39: 343-350.

Antoniw, J.F., Ritter, C.E., Pierpoint, W.S. & Loon, L.C. van, 1980. Comparison of three pathogenesis-related proteins from plants of two cultivars of tobacco infected with TMV. J. gen. Virol. 47: 79-87.

Camacho Henriquez, A. & Sänger, H.L., 1982. Gelelectrophoretic analysis of phenol-extractable leaf proteins from different viroid/host combinations. Arch. Virol. 74: 167-180.

Conejero, V., 1981. Stimulation of a viroid-like syndrome and inhibition of viroid symptom expression by Ag^+ ion. Abstr. Fifth Int. Congr. Virol., Strasbourg, nr. P 21/03. p. 228.

Conejero, V., 1982. Do viroids elicit host preexisting mechanisms of response? Abstr. Fourth Int. Conf. Comp. Virol Banff.

Conejero, V., Picazo, I. & Segado, P., 1979. Citrus exocortis viroid (CEV): protein alterations in different hosts following viroid infection. Virology 97: 454-456.

Conejero, V. & Semancik, J.S., 1977. Exocortis viroid: alteration in the proteins of *Gynura aurantiaca* accompanying viroid infection. Virology 77: 221-232.

Cooper, P. & Ho, T.D., 1983. Heat shock proteins in maize. Pl. Physiol. 71: 215-222.

Coutts, R.H.A., 1978. Alterations in the soluble protein patterns of tobacco and cowpea leaves following inoculation with tobacco necrosis virus. Pl. Sci. Lett. 12: 189-197.

Coutts, R.H.A. & Wagih, E.E., 1981. Alterations in RNA and protein metabolism in uninoculated half-leaves of cowpea adjacent to tobacco necrosis virus infected halves. Pl. Sci. Lett. 21: 51-59.

Dawson, W.O. & Grantham, G.L., 1981. Inhibition of stable RNA synthesis and production of a novel RNA in heat-stressed plants. Biochem. Biophys. Res. Comm. 100: 23-30.

Devash, Y., Biggs, S. & Sela, I., 1982. Multiplication of tobacco mosaic virus in tobacco leaf disks is inhibited by (2′-5′) oligoadenylate. Science 216: 1415-1416.

Fleck, J., Durr, A., Fritsch, C., Vernet, T. & Hirth, L., 1982. Osmotic-shock 'stress proteins' in protoplasts of *Nicotiana sylvestris*. Pl. Sci. Lett. 26: 159-165.

Fraser, R.S.S., 1981. Evidence for the occurrence of the 'pathogenesis-related' proteins in leaves of healthy tobacco plants during flowering. Physiol. Pl. Path. 19: 69-76.

Gat-Edelbaum, O., Altman, A. & Sela, I., 1983. Polyinosinic-polycytidylic acid in association with cyclic nucleotides activates the antiviral factor (AVF) in plant tissues. J. gen. Virol. 64: 211-214.

Gera, A. & Loebenstein, G., 1983. Further studies of an inhibitor of viral replication from tobacco mosaic virus-infected protoplasts of a local lesion-responding tobacco cultivar. Phytopathology 73: 111-115.

Gessler, C. & Kuc, J., 1982. Appearance of a host protein in cucumber plants infected with viruses, bacteria and fungi. J. Exp. Bot. 33: 58-66.

Gianinazzi, S., 1982. Antiviral agents and inducers of viral resistance: analogies with interferon. In: R.K.S. Wood (Ed.), Active defense mechanisms in plants. Pp. 275-297. Plenum Press, New York and London.

Gianinazzi, S., 1983. Genetic and molecular aspects of resistance induced by infections or chemicals. In: E.W. Nester & T. Kosuge (Eds), Plant-microbe interactions. Molecular and genetic perspectives, Vol 1. Macmillan publishing Co., Inc., New York (in press).

Gianinazzi, S., Martin, C. & Vallée, J.C., 1970. Hypersensibilité aux virus, température et protéines solubles chez le *Nicotiana* Xanthi nc. Apparition de nouvelles macromolécules lors de la répression de la synthèse virale. C.R. Acad. Sci. Paris 270D: 2383-2386.

Gianinazzi, S., Pratt, H.M., Shewry, P.R. & Miflin B.J., 1977. Partial purification and preliminary characterization of soluble leaf proteins specific to virus infected tobacco plants.

J. Gen. Virol. 34: 345-351.
Key, J.L., Lin, C.Y. & Chen, Y.M., 1981. Heat shock proteins of higher plants. Proc. Natl Acad. Sci. USA 78: 3526-3530.
Loebenstein, G. & Gera, A., 1981. Inhibitor of viral replication released from tobacco mosaic virus-infected protoplasts of a local lesion-responding tobacco cultivar. Virology 114: 132-139.
Loon, L.C. van, 1976. Specific soluble leaf proteins in virus-infected tobacco plants are not normal constituents. J. gen. Virol. 30: 375-379.
Loon, L.C. van, 1982. Regulation of changes in proteins and enzymes associated with active defence against virus infection. In: R.K.S. Wood (Ed.), Active defense mechanisms in plants. Pp. 247-273. Plenum Press, New York and London.
Loon, L.C. van & Antoniw, J.F., 1982. Comparison of the effects of salicylic acid and ethephon with virus-induced hypersensitivity and acquired resistance in tobacco. Neth. J. Pl. Path. 88: 237-256.
Loon, L.C. van & Kammen, A. van, 1970. Polyacrylamide disc electrophoresis of the soluble leaf proteins from *Nicotiana tabacum* var. 'Samsun' and 'Samsun NN'. II. Changes in protein constitution after infection with tobacco mosaic virus. Virology 40: 199-211.
Loon, L.C. van & Ritter, C.E., 1978. Occurrence, specificity and properties of new tobacco leaf proteins induced by virus infection. Abstr. Fourth Int. Congr. Virol., The Hague, nr. W 17/3, p. 271.
Orchansky, P., Rubinstein, M. & Sela, I., 1982. Human interferons protect plants from virus infection. Proc. Natl Acad. Sci. USA 79: 2278-2280.
Pennazio, S., 1981. Changes in the soluble protein constitution of *Gomphrena globosa* leaves showing spontaneous local lesions. Riv. Patol. Veg. 17: 127-135.
Pennazio, S. & Redolfi, P., 1980. Studies on the partly localized reaction of *Gomphrena globosa* to potato virus X. Phytopath. Medit. 19: 97-102.
Pierpoint, W.S., Robinson, N.P. & Leason, M.B., 1981. The pathogenesis-related proteins of tobacco: their induction by viruses in intact plants and their induction by chemicals in detached leaves. Physiol. Pl. Path. 19: 85-97.
Redolfi, P., 1983. Protein changes and hypersensitive reaction in virus-infected bean leaves. Riv. Patol. Veg. 19: 7-14.
Redolfi, P. & Cantisani, A., 1981. Protein changes and hypersensitive reaction in virus infected bean leaves. Abstr. Fifth. Int. Congr. Virol., Strasbourg, nr. P 26/06, p. 264.
Redolfi, P., Vecchiati, M. & Gianinazzi, S. 1982. Changes in the soluble leaf protein constitution of *Gomphrena globosa* during the hypersensitive reaction to different viruses. Phytopath. Z. 103: 48-54.
Rohloff, H. & Lerch, B., 1977. Soluble leaf proteins in virus infected plants and acquired resistance. I. Investigations on *Nicotiana tabacum* cvs. 'Xanthi nc' and 'Samsun'. Phytopath. Z. 89: 306-316.
Sela, I., 1981. Plant-virus interactions related to resistance and localization of virus infections. Adv. Virus Res. 26: 201-237.
Tas, P.W.L. & Peters D., 1977. The occurrence of a soluble protein (E_1) in cucumber cotyledons infected with plant viruses. Neth. J. Pl. Path. 83: 5-12.
Wagih, E.E. & Coutts, R.H.A., 1981. Similarities in the soluble protein profiles of leaf tissue following either a hypersensitive reaction to virus infection or plasmolysis. Pl. Sci. Lett. 21: 61-69.
Wagih, E.E. & Coutts, R.H.A., 1982a. Peroxidase, polyphenoloxidase and ribonuclease in tobacco necrosis virus-infected or mannitol osmotically-stressed cowpea and cucumber tissue II. Qualitative alterations. Phytopath. Z. 104:124-137.
Wagih, E.E. & Coutts, R.H.A., 1982b. Comparison of virus-elicited and other stresses on the soluble protein fraction of cucumber cotyledons. Phytopath. Z. 104: 364-374.

Wagih, E.E., Raftopoulos, A.E., Archer, S.A. & Coutts R.H.A., 1983.Characterization of an apparently novel soluble protein fraction found in pathogen and osmotically stressed cucumber cotyledons. Phytopath. Z. 107: 233-243.

Webster, P.L., 1980. 'Stress' protein synthesis in pea root meristem cells? Pl. Sci. Lett. 20: 141-145.

Wit, P.J.G.M. & Bakker, J. de, 1980. Differential changes in soluble tomato leaf proteins after inoculation with virulent and avirulent races of *Cladosporium fulvum* (syn. *Fulvia fulva).* Physiol. Pl. Path. 17: 121-130.

Ziemiecki, A. & Wood, K.R., 1975. Changes in the soluble protein constitution of cucumber cotyledons following infection with two strains of cucumber mosaic virus. Physiol. Pl. Path. 7: 79-89.