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Chromatographic and Electrophorotic Separation of Lectin Preparation from Resistant and Susceptible Plants of Tomato to *Pseudomonas solanacearum*[1]

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Abstract : Lectin preparations from wilt resistant and susceptible tomato plants to *Pseudomonas solanacearum* they have similar movement in Sephadex G-200 columns and SDS-polyacrylamide gels. Column fractions with highest protein content have the highest agglutination titers. Protein content, agglutination titers of column fractions, and the staining intensity of gels after electrophoresis are related to the susceptibility or resistance of the plants used for lectin extraction, inoculation of these plants, and the virulence of the *Pseudomonas solanacearum* isolate used for inoculation.

Key words : *Pseudomonas solanacearum,* Lectin, Chromatographic separation, Electrophoretic separation.

INTRODUCTION

Previous research[4,5,6] has shown that inoculation of tomato plants with incompatible isolates of *P. solanacearum* initiates a hypersensitive type of response. Numbers of inoculated bacteria initially increase then decrease and amounts of lectins increase with time after inoculation in incompatible tomato lines. A relationship between the resistance of tomato plants to *P. solanacearum* and the presence of lectins in stem extracts has been noted[3,5,6].

Sequeira et. al.[8,9,10,11] postulated that lectins in plants possess the ability to attach to bacteria and thus limit their multiplication. Our research results suggest that increases in lectin concentration can be elicited by inoculation with avirulent isolates of *P. solanacearum* in resistant and susceptible plants or by inoculation with virulent isolate in resistant plants. Furthermore, the types of lectins elicited may vary dependent on the isolate of bacteria used for inoculation or susceptibility of the host plants. Lectin types can be separated

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on the basis of whether they agglutinate virulent or avirulent cells of *P. solancearum* and the effect of various carbohydrates on agglutination inhibition. The objective of this study was to partially purify lectins from tomato plants inoculated with different isolates of *P. solanacearum* and to determine similarities or differences among those lectin preparations.

MATERIALS AND METHODS

Growth of tomato plants—Tomato seeds of the resistant (VC-8) and susceptible (Campbell-28, C-28) tomato lines were sown in a 1 : 1 (v/v) mixture of soil and sand. Seedlings were grown for twenty days in the greenhouse and were transplanted singly into six inch plastic pots containing a 3 : 1 (v/v) mixture of soil and sand. Plants were grown in the green house at 30±3°C for thirty days after transplanting.

Inoculation of tomato plants—Uniform plants with ten expanded leaves were selected and individually inoculated with isolate #64 and #64-β by injecting twenty-five ul of bacterial suspensions (6.1×10^9 cells/ml) into each of 4th, 6th, 8th leaf axil below the growing point of each plant. Bacterial suspensions was obtained by suspending bacteria grown on TZC medium[1]at 28°C for 48 hours into sterile distilled water. Plants inoculated with sterile distilled water served as controls. Inoculated plants wre then re turned to the 30 ±3°C greenhouse.

Lectin preparation and agglutination assay—Lectins were extracted from ten plants for each of six treatments. Treatments included inoculation of the VC-8 line and C-28 cultivar with isolates #64-β or sterile distilled water. Lectins were extracted by the modified method of Sequeira[6]as described in Part II 120 hrs. after inoculation. Final volumes of lectin extracts were adjusted at a ratio of 1 ml 0.1 M acetate buffer (pH 3.6) per gram dry weight of extracted tissue.

Lectin preparations (1ml) for each treatment were applied to a column (1.5cm×10cm) of Sephadex G-200 containing 0.1M acetate buffer (pH3.6) . Columns were eiuted with buffer at a rate at a rate of 7 ml/hr. in a 4°C cold room. Fractions (0.5ml) were collected for 3 hrs. Total protein content of each fraction was determined by the Lowry method[3]. The agglutination activity of each fraction was measured by an agglutination bioassay using the avirulent isolate #64-β as descrbed in Part II.

Polyacrylaimide-gel disc electrophoresis—Sodium dedecyl sulfate (SDS) electrophoresis was performed by a modification of the method used by Laemmli[2]. Fifty ul g tomato lectin extracted directly from the tomato plants were placed in the sample slots of a gel slab (14×9×0.1 cm) which contained 12.5% acrylamide, 3.75 mM HCl (pH8.9), 0.058 mM TEMED and 0.1% SDS. Electrophoresis was conducted at room temperature at 25 mA throughout the running gel until the tracking dye reached the bottom of the gel. Gels were stained in Comassive blue (0.1% in 45% methanol and 9% acetic acid.

RESULTS

Sephadex G-200 Column separation of lectins—The protein content of fractions from each of six lectin preparations generally displayed two main peaks representing protein content when run through a Sephadex G-200 column. The first peak eluted in fractions 1-8, contained low amounts of protein and did not exhibit agglutination activity for the avirulent isolate (#64-β) of *P. solanacearum.* The second peak was eluted in fractions 8 to 34, generally contained higher amounts of protein, and exhibited agglutination activity (Fig. 1-6) .

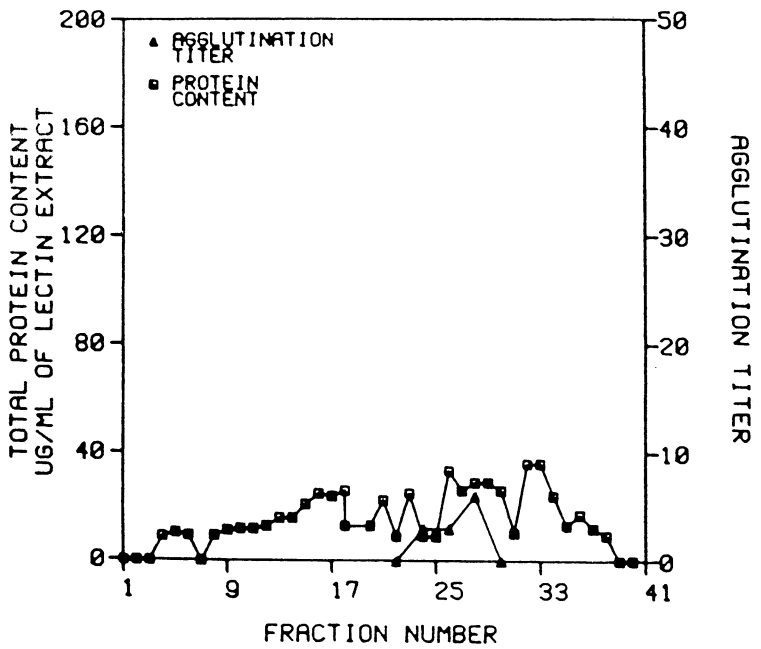

Fig. 1. Protein content and agglutination titer of lectin fractions from uninoculated resistant tomato plants (VC-8) eluted from a Sephadex G-200 column with 0.1 M acetate buffer (pH3.6). Agglutina tion titer is the reciprocal of the highest dilution of lectins that caused agglutination of the avirulent isolate (#64-β) of *Pseudonomas solanacearum.*

Lectins which agglutinated the avirulent isolate were detected in fractions 12 to 34 of preparations from resistant plants inoculated with avirulent or virulent isolates or uninoculated. Highest agglutination titers of these preparations appeared in fractions 24, 20 and 28, respectively (Figures 1,3,5) . Lectins were detected in fractions 12 to 25, with maximum agglutination titer in the fraction 16 of each lectin preparation from susceptible plants inoculated with avirulent or virulent isolates or uninoculated (Figures 2,4,6) . Lectin preparations from uninoculated plants (resistant VC-8 and susceptible C-28 lines) or susceptible plants (C-28) inoculated with the virulent isolate contained low amounts of protein. Fractions of these preparations with detectable protein were characterized by low agglutination titers (Figs. 1,2,4) .

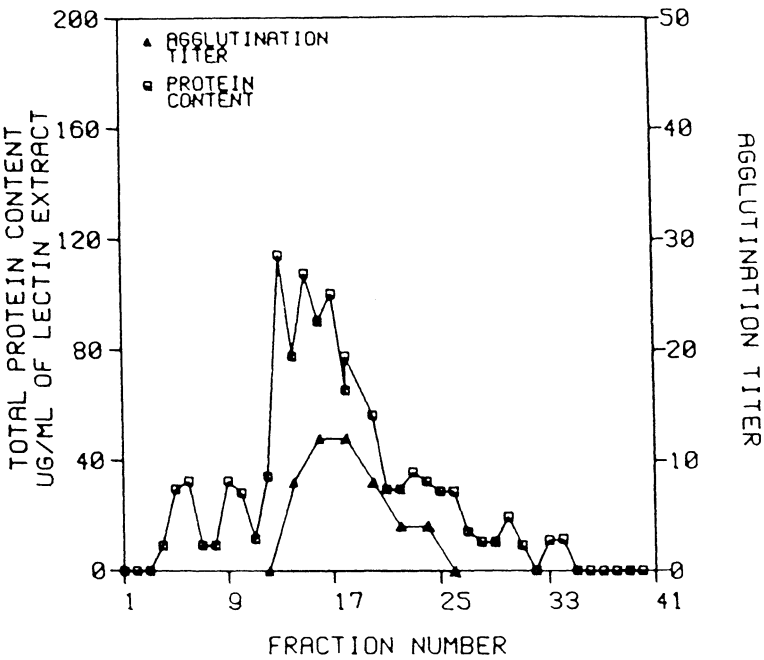

Fig. 4. Protein content and agglutination titer of lectin fractions from inoculated susceptible tomato plants (C-28) , inoculated with the virulent iso late #64 of *Psuedomonas solanacearum,* eluted from a G-200 column with 0.1 M acetate buffer (pH3.6) . Agglutination titer is the reciprocal of the high est dilution of lectins that caused agglutination of the avirulent isolate (#64-B) of this bacterium.

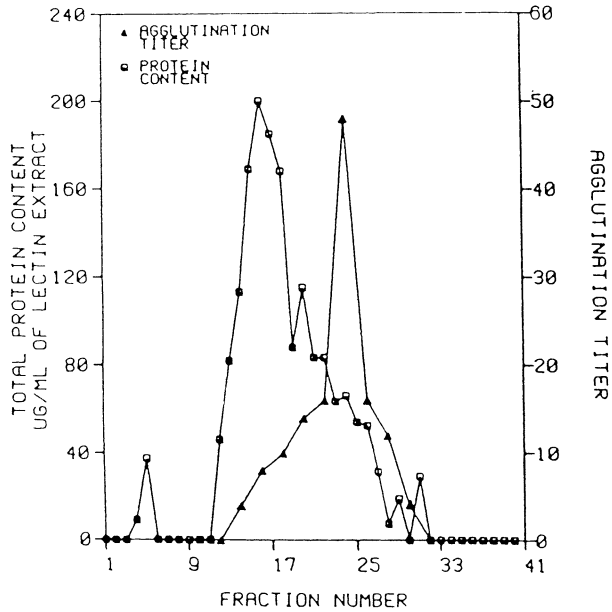

Fig. 5. Protein content and agglutination titer of lectin fractions from resistant plants (VC-8) inoculated with the avirulent isolate #64-β of *Psuedomonas solanacearum*, eluted from a G-200 column with 0.1 M acetate buffer (pH3.6) . Agglutination titer is the reciprocal of the highest dilution of lectins that caused agglutination of the avirulent isolate(#64-β) of this bacterium.

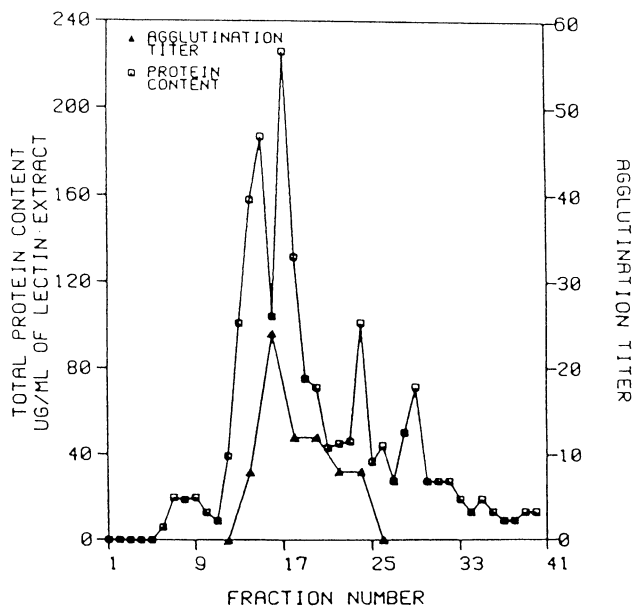

Fig. 6. Protein content and agglutination titer of lectin fractions from susceptible plants (C-28), with the avirulent isolate (#64-β) of *Psuedomonas solanacearum*, eluted from a G-200 column with 0.1 M acetate buffer (pH3.6) . Agglutination titer is the reciprocal of the highest dilution of lectins that caused agglutination of the avirulent isolate (#64-β) of this bacterium.

Gel electrophoresis of lectin preparations — Lectin preparations isolated from six different host-pathogen com binations (i.e. the resistant VC-8 and susceptible C-28lines inoculated in the avirulent, virulent isolates of *P. solanacearum* or uninoculated) were characterized by similar band patterns upon electrophoresis of polyacrylamide gels in the presence of 0.1% sodium dodecyl sulfate. Differences between lectin preparations wre observed, however, in the intensity of bands 2 ABD 3. Differences were not detected in the number and mobility of bands for these six lectin preparations (Fig. 7) .

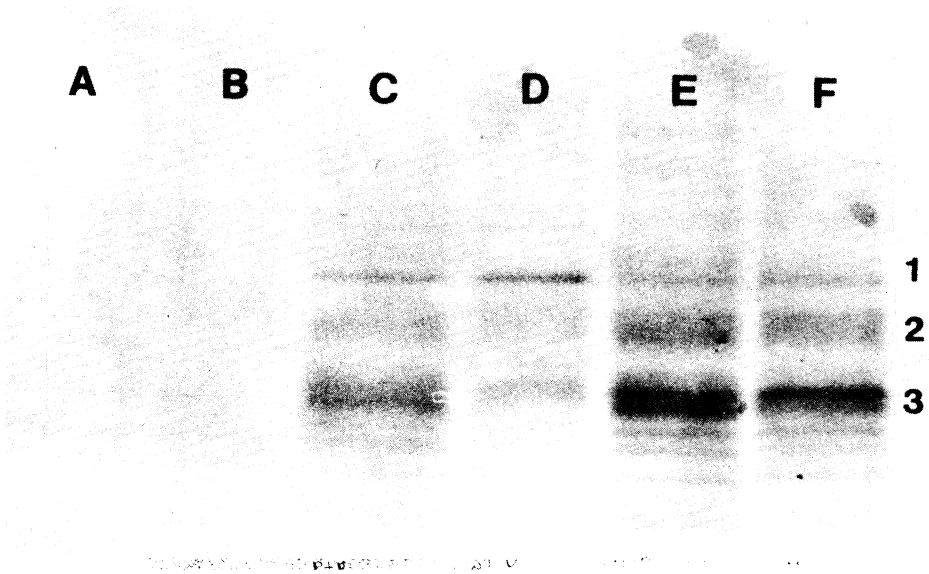

Fig. 7. SDS-PAGE patterns of lectin preparation from A) Uninoculated resistant (VC-8) plants ; B) Uninoculated susceptible plants ; C) Resistant plants inoculated withe the virulent isolate of *Psuedomonas solanacearum* ; *D*) Susceptible plants inoculated with the virulent isolate ; E) Resistant plants inoculated with the avirulent isolate ; F) Susceptible plants inoculated with the avirulent isolate.

DISCUSSION

Previous results[3,4,5,6] suggest that at least two types of lectin are elicited in tomato plants depending on the host susceptibility and the isolate of *P. solanacearum* used for inoculation. Lectin types can be separated on the basis of agglutination with virulent (#64) or avirulent (#64-β) cells of *P. solanacearum*[3,5,6] In addition, agglutination of bacterial cells by lectins is inhibited by simple carbohydrates[4]. Results of sephadex G-200 column analysis and SDS -PAGE patterns suggested relatively few differences in terms of fractions with elevated protein content and agglutination titer and electrophoretic bands, respectively, between lectin preparations from susceptible and resistant plants uninoculated or inoculated with avirulent and virulent isolates of *P. solanacearum.* However, lectin preparations from uninoculated resistant and susceptible plants or susceptible plants inoculated with the

virulent isolates of *P. solanacearum* were characterized by lower total proteins, lower agglutination titers (Figures1,2,4) and electrophoretic bands (especially bands 2 and 3) that were less intensely stained (Figure7) than other lectin preparations. These results supported previous conclusions[3,4,5] that higher amounts if lectins are elicited in tomato plants by incom patible isolates of *P. solanacearum.* Differences in the staining intensity of electrophoretic bands supported the hypothesis that more than one type of lectin is present in inoculated tomato plants depending on the susceptibility of the host and virulence of the inoculum.

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利用色層分析與電泳法探討番茄聚血素與抗青枯病菌之關係[1]

林 俊 義[2]

摘 要

利用色層分析管（Sephades G-200 Cdumns）及十二烷硫酸鈉聚丙烯醯胺膠（SDS-polyacrylamide gels）分離番茄聚血素（lectins）.結果顯示，不論聚血素是來自抗番茄青枯病菌之抗病品系或感病品系，其在色層分析管收集或聚丙烯醯胺膠上之移動速率均無差異。惟在色層分析管方面，收集蛋白質含量愈高之分離管（Column fractions）其凝聚作用值（agglutination titer）愈高；而色層分析管所收集的蛋白質含量或濃度值以及電泳分離後之膠質染色強度亦發現，聚血素的含量與萃取自抗感性番茄植株，或番茄被接種不同致病力之青枯病菌菌株有密切關係。

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