

Common Antigens in Relation to Potato Resistance to Late Blight Disease

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Abstract: The role of common antigen in relation to potato resistance was studied using the indirect ELISA and dot blot immunoassay (DIA) to detect the cross-reactivity between antigens of potato cultivars (resistant and susceptible) and *P. infestans* antiserum. Results indicating that, the higher reaction and more specific antigens were detected in the susceptible cultivars than in the resistant ones.

Key words: Late blight, Potato, common antigen, resistance.

INTRODUCTION

The presence of specific common antigen (s) between a pathogen and host has been discussed by several authors (Abd-El-Rehim and Hashem 1970; Abd-El-Rehim and El-Meleigi 1973; and Abd-El-Rehim *et al.*, 1974). The work of Fedotova, (1935) can be considered as a rapid advance in the applicability of serological methods to differentiate between resistant and susceptible plant varieties to certain pathogens. She tested serologically the susceptibility of different cotton cultivars to *Verticillium dahliae* and found that, the more the precipitates, the more the susceptibility. She added that, the serological reactions intensity correlated with the cultivar resistance in the field. DeVay, *et al.*, (1972) suggested that, common antigens affect the plant host range and specificity of bacterial and fungal pathogens. Afterwards, Abd-El-Rehim and Hashem (1970) found that the antigen of Fusarium-susceptible cotton cultivar gave a large precipitin zone when compared with that formed by the Fusarium-resistant cotton cultivar. Also, Abd-El-Rehim *et al.*, (1971) showed that seed globulins used in sero-diagnosis reflected the relative susceptibility of watermelon varieties to damping-off caused by *F. semitectum*. Abd-El-Rehim and El-Meleigi (1973) and Abd-El-Rehim *et al.*, (1974) found that, the serological methods could be applicable to determine the tolerance of tomato seedlings to *Pythium irregulare*. Abd-El-Rehim *et al.*, (1988a and 1988b) reported that the antisera of *F. oxysporum* f. sp. *vasinfectum* reacted with seed globulins of cotton cultivars, more precipitin lines were formed when the Fusarium antiserum reacted with the globulins of the susceptible cultivars than with those of resistant cultivars. Tohamy, (1985), found that, using the double diffusion technique, when the seed globulins of the susceptible cotton cultivars reacted with antiserum of the *F. oxysporum* f.sp. *vasinfectum* and more precipitation lines were formed than the resistant cultivars. No obvious reaction was detected in case of *F. moniliforme* antiserum. El-Kaffash, (1990) found that, the susceptible cultivars of *Faba bean* had more reactive antigen with *Botrytis fabae* antiserum than the resistant cultivars and the nonhost plant (wheat). These results indicated the presence of common antigens between the susceptible host and the tested pathogen. In the present work, using the indirect ELISA and dot blot immunoassay (DIA), the role of common antigens in relation to potato resistance to late blight pathogen were studied.

MATERIALS AND METHODS

Potato Cultivars:

Four potato cultivars (*S. tuberosum*) varied in their resistibility to late blight disease were used in this study (El_Komy, 2007); Hanna, Cara (resistant cvs.), Lady-Rosetta and Diamant (susceptible cvs.). Potato cultivars were obtained from International Potato Center (CIP) Kafr El-zayat, Egypt.

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Preparation of Antigen from Potato Cultivars:

Total soluble proteins extracted from healthy leaves of resistant and susceptible potato cultivars as described by Niderman *et al.*, (1995). Approximately, 5g of potato leaves tissues were immersed in liquid nitrogen, ground into a powder using a mortar and pestle, and then suspended in 5ml protein extraction buffer (0.1M Phosphate buffer pH7). The solution was centrifuged at 10,000×g for 20min at 4°C, and the supernatant was collected. The protein content was estimated according to Bradford, (1976) and adjusted to 50Cg ml-1 in Phosphate buffer and stored at -20oC as antigen.

Preparation of Antigen from *P. infestans*:

The antigen was prepared from total protein extracted from mycelium of *P. infestans* isolate P14 (highly virulent isolate). The mycelial mat was filtered through muslin, washed twice with phosphate buffer (pH 7), and lyophilized. Lyophilized mycelium (1g) was ground with liquid nitrogen. Phosphate buffer saline (PBS; 10 ml) containing 0.1% cystein, 0.1% ascorbic acid, and 17% sucrose was added to the ground mycelium (El-Nashaar *et al.*, 1986) and the suspension was centrifuged at 10000×g for 30 min at 4oC. The supernatant was concentrated over polyethylene glycol 6000 and then dialyzed against PBS (0.1 M, pH7) for 24h. The pellet was re-suspended in 1ml of PBS. The protein content was determined (Bradford, 1976) and adjusted to 1mg ml-1 in PSB and stored at - 20oC as antigen.

Preparation of Antiserum Against *P. infestans*:

Antiserum against *P. infestans* (isolate P14) was prepared using New Zealand white rabbit by four weekly intramuscular injections with purified preparation of the fungus at concentration of 1mg mL⁻¹. For the first injection the purified fungus was emulsified with an equal volume of Freund's complete adjuvant (Difco lab, U.S.A.) and for subsequent injections the purified fungus was emulsified with an equal volume of Freund's incomplete adjuvant. The injections were administered at one week interval. Two weeks after the last injection, rabbits were bled and the blood was collected from the marginal ear vein then left for, two hours at room temperature for clot formation then kept in refrigerator overnight. The antiserum was clarified by centrifugation at 3000×g for 30 min at 4oC and stored at - 20 oC until use (Srivastava and Arora, 1997).

Determination the Cross-reactivity Between Tested Potato Antigens and *P. infestans* Antiserum:

Indirect ELISA:

The indirect enzyme-linked immunosorbent assay (ELISA) was carried out as described by Fegla *et al.* (1997). Disposable polystyrene flat bottom micro- ELISA plates were used. The tested samples were immersed in liquid nitrogen, ground into a powder using a mortar and pestle, and then suspended in 5mL phosphate buffer pH7 and centrifuged at 10000xg for 10 min. The protein content was determined and adjusted to 0.5 mg mL⁻¹ in PSB. (Bradford, 1976). Wells were coated with antigens by adding 100 µL of each sample to the bottom of the well and incubated for 3 hrs at 37°C or overnight at 4°C. The plates were rinsed thrice by flooding wells with phosphate buffer saline + tween 20 (PBST), 5 minutes each. Aliquots (100 µL) from the diluted antiserum (1:500 diluted in PBST) were added to each well, after which the plates were incubated at 37°C for 2 hrs or at 4°C overnight, then washed as mentioned earlier. Goat anti-rabbit gamma globulin conjugated to alkaline phosphatase (whole molecule, enzymatic activity 457 units /mL⁻¹) obtained from Sigma Chem. Co St Louis, Mo (Production # A8025) was diluted 1:1000 in serum buffer and 100Cl was added to each well, followed by one hour incubation at 37 °C, then the plates washed as mentioned earlier. Enzyme substrate (100 µL), 0.5 mg mL⁻¹ paranitrophenyl phosphate (Sigma# 104) in 10% diethanolamine buffer, pH 9.8 were added to each well and incubated at room temperature (25°C) for about 30 min. The enzyme activity was stopped by adding 50 µL of 3M NaOH. The ELISA values measured by Universal automated microplate reader El X 800 and were expressed as absorbency at 405 nm. Absorbency values, of at least double of that of the healthy control, were considered positive. In each set of test, wells lacking antigen (coating buffer only) were included as blanks. The cross-reactivity between tested potato antigens and *P. infestans* antiserum were detected. Cross-reactivity with phosphate buffer was served as control.

Dot Blot Immunoassay (DIA):

DIA was carried out as described by Fegla *et al.* (2001). A grid consisting of 1cm squares was drawing on nitrocellulose membrane sheet (NCM, 0.45nm Bio-Rad Laboratories, Richmond, CA) with a pencil. The sheet was then cut to a size that would accommodate the number of samples in an individual test. The tested

samples (leaves and tuber-discs) were immersed in liquid nitrogen, ground into a powder using a mortar and pestle, and then suspended in 5mL phosphate buffer pH7 and centrifuged at 10000×g for 10min. The protein content was determined and adjusted to 0.5 mg ml⁻¹ in PSB. (Bradford, 1976). The nitrocellulose membrane was dipped in phosphate buffer pH7 and placed on filter paper for 5min to dry. Each sample (2μL) was spotted on the nitrocellulose membrane in the center of each grid square and dried for 5min. The membrane was then placed in a Petri-dish containing 10ml blocking solution (2% bovine serum albumin and 2% Triton x -100 solution in phosphate buffer pH7), gently agitated for one hour (40 oscillations min⁻¹). The membrane was removed from the blocking solution, dipped in distilled water and washed twice by agitation for 10 min in phosphate buffer + tween 20, then the membrane was transferred to another Petri dish containing 10ml of fungal-antiserum (1:500 diluted in PBST), and gently agitated for 2hrs. The membrane was removed from the first antibody solution, dipped in distilled water, and washed twice by agitation for 10min in phosphate buffer+tween20. The membrane transferred to 1:1000 dilution of goat anti-rabbit *IgG* conjugate to alkaline phosphatase in PBST and gently agitated for one hour. Finally, the membrane was removed from the second antibody dilution, dipped in distilled water, and washed twice by agitation for 10 min each in phosphate buffer + tween 20. The 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT) substrate solution was prepared for the final washing in which membrane was incubated for color development. After color development, the reaction was stopped by washing the membrane in 0.01 M Tris- HCL containing 0.05 M EDTA, pH 7.5. The cross-reactivity between tested potato antigens and *P. infestans* antiserum were detected. Cross-reactivity with phosphate buffer was served as control.

RESULTS AND DISCUSSION

In the present work, using the indirect ELISA and dot blot immunoassay (DIA), the role of common antigens in relation to potato resistance to late blight pathogen were studied. The possible involvement of common antigens as a basic compatibility factor was evident in a study involvement of cotton and the soil borne fungal pathogens *F. oxysporum* f.sp. *vasinfectum*, *F. solani* and *V. dahliae* which infect root and hypocotyls tissue of cotton (Charudattan and DeVay 1972). Investigation concerning common antigens between host plants and pathogens and possible involvement of an immunologic response in disease resistance or susceptibility might be most clearly recognized in host-pathogen interactions where sharply defined host specificity is displayed by the pathogen. Doke *et al.*, (1975) reported that, binding of proteinaceous substances from two cultivars of potato to zoospores component from two races of *P. infestans* showed differences which were possibly related to host-pathogen specificity. Palmerly and Callow (1978) reported that, common antigen were not detected between *P. infestans* and leaves of nonhost plants. Also, Alba and DeVay (1985) found that, cross-reactive antigens exhibited a more intense reaction with *P. infestans* antiserum for susceptible potato cultivars than for resistant ones, which demonstrating antigenic disparity. In the present work, total protein extracted from healthy leaves of 4 potato cultivars; resistant (Hanna and Cara) and susceptible, (Lady-Rosetta and Diamant), and were served as antigens. The cross-reactivity between tested antigens and *P. infestans* antiserum were detected. Data in Table (1) revealed that, indirect ELISA absorbance values (E405nm) were higher in case of susceptible potato cultivars, Lady-Rosetta (0.065) and Diamant (0.048), compared with the resistant cultivars Hanna (0.012) and Cara (0.035). Also, the same results were detected using dot blot immunoassay (DIA) according to the density of purple color (Fig. 1). These results indicating that, higher reaction and more specific antigens were detected in the susceptible cultivars than in the resistant ones. Such results are in agreement with those reported by Abd-El-Rehim and Hashem (1970); Abd-El-Rehim and El-Meleigi (1973); Abd-El-Rehim *et al.*, (1974); Palmerly and Callow (1978); Alba and DeVay (1985); Tohamy, (1985) and El-Kaffash, (1990), who indicated the presence of more specific antigens between the susceptible host and the pathogen than the resistant host. However, there are some reports which contradict these findings which support the common antigen. Barna *et al.*, (1978) reported that the protein sharing between hosts and pathogens was poor if any. Likewise, Alba *et al.*, (1973) did not detect any common antigens between uredospores antiserum of *Hemileia vastatrix* and their corresponding resistant and susceptible *Coffea Arabica*.

Table 1: Serological relationship between potato cultivars and *P. infestans* antiserum as indirect ELISA absorbance values (E405nm) in 50 µg of total protein extracted from healthy potato leaves

Potato cultivars	Absorbance values (E405nm)	Infection reaction
Hanna	0.012	Resistance
Cara	0.035	Resistance
Lady-Rosetta	0.065	Susceptible
Diamant	0.048	Susceptible
Phosphate buffer (control)	0.004	-

- The experiment was repeated twice and ELISA absorbance values at 405nm are average of two replicates each.
- One hundred CI from the diluted *P. infestans* antiserum (1:500 diluted in PBST) were used for each ELISA reaction.
- Absorbance values of at least double of that of the Phosphate buffer (control) were considered positive.

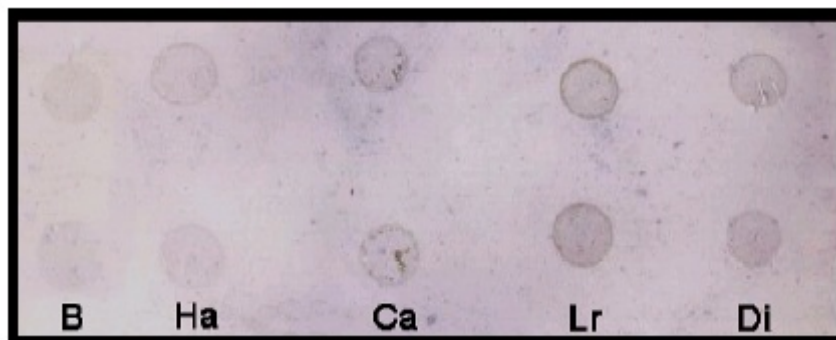


Fig. 1: Serological relationship between *P. infestans* antiserum and potato cultivars varied in their resistance to *P. infestans* using dot blot immunoassay (DIA).

- B = Phosphate buffer (Control).
- Ha = Potato cv. Hanna (resistant).
- Ca = Potato cv. Cara (resistant).
- Lr = Potato cv. Lady-Rosetta (susceptible).
- Di = Potato cv. Diamant (susceptible).

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