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Assessment of Resistance in some Sugar Beet Varieties to Powdery Mildew using Crossed Immunoelectrophoresis Technique

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The present study was performed at Gemmeiza Agricultural Research Station in 2015 season. Antisera were raised in white New-Zealand rabbits against antigens of three sugar beet varieties, Puma (resistant), Kawemira (moderately susceptible) and Herkl (susceptible) as well as conidia of the fungus *Erysiphe betae*. Using crossed immunoelectrophoresis technique (CIE), 7, 7 and 8 precipitin peaks were detected when antigens of Puma , Kawemira and Herkl varieties reacted with its specific antibodies (homologous reactions). Also, eight precipitin peaks were detected when antigens of the fungus *Erysiphe betae* reacted with its specific antibodies. However, in heterologous reactions, 3, 5, 6 common precipitin peaks (common antigens) were detected when antigens of Puma, Kawemira and Herkl reacted with antibodies of *Erysiphe betae*, respectively. The phenomenon of common antigens sharing plant hosts and its parasites has been interpreted in an attempt to explain the basis of susceptibility or resistance to diseases. The more antigenic similarity means the most susceptibility in host - pathogen interaction. According to these phenomena, the Puma variety could be classified as the most resistant one which gave the lowest number of common antigens. While, the Herkl variety could be classified as the most susceptible followed by Kawemira variety. These results are coincide with those obtained under greenhouse and field conditions and gave evidence that CIE technique could help to have a quick knowledge about the response of sugar beet varieties to powdery mildew disease. However, this study is the first report to assessment of resistance in sugar beet powdery mildew using crossed immunoelectrophoresis technique.

Keywords: Antigens, antisera, *Erysiphe betae*, heterologous reaction, immunoelectrophoresis technique and sugar beet.

Sugar beet (*Beta vulgaris* L.) is considered one of the most important sugar crops all over the world. In Egypt, it is ranked the second crop after sugar cane for sugar production (Eweis *et al*., 2006). Powdery mildew of sugar beet caused by *Erysiphe betae* (Syn. *Erysiphe polygoni*) is a serious foliar disease in Egypt. Disease symptoms like a radiating dusty white coating over leaf surfaces and also undersurface could be observed during summer time. Disease spread occurs mostly by conidial infections, which dispersed by wind overwintering takes place. Moderate resistance is obtainable in commercial varieties appearing in “slow-mildewing” phenotypes. The losses of 20-30% in gross sucrose can occur when sugar beet powdery mildew is not controlled (Frate *et al*., 1979 and Kontradowitz and Verreet, 2010). Byford (1996) and Karaoglanidis and Karadimos (2006) found that severe

attacks of powdery mildew occurred in Southern Europe and north American caused reduction in root yield exceed 22% and root sucrose exceed 13%.

Partial resistance of slow-mildewing type has been identified in sugar beet germplasm (Whitney *et al*., 1983). High levels of resistance were identified in wild beet (*B. vulgaris* subsp. *maritima*) accessions, *i.e.* WB97 and WB242 which could be used in breeding program as a source of resistance (Lewellen and Schrandt, 2001).They reported that, resistance to powdery mildew was inherited as a single dominant major gene in both sources.

Protein-protein interactions are a critical element of biological systems and the analysis of interaction partners can provide valuable hints about unknown functions of a protein (Braun *et al*., 2013). This hypothesis has been developed by comparing antigens of different varieties of plants with those of virulent strains of pathogens. Compatibility between plants and their potential invaders depends on number of common antigens. Antigens sharing between different cells have been of special interest because of its coincidence in compatible host-parasite relationships (El-Kazzaz *et al*., 1996; El-Shamy, 2006 and Awad and El-Ghonemy, 2015).

The objective of this work was to detect resistance / susceptibility in some sugar beet varieties to powdery mildew disease using crossed-immunoelectrophoresis technique (CIE).

Materials and Methods

Three varieties of sugar beet, chosen on the bases of their responses to powdery mildew disease, *i.e.* Puma (resistant), Kawemira (moderately susceptible) and Herkl (susceptible), according to El-Sayed *et al*. (2014), were used in this study (Table 1).

Table 1. Response of three sugar beet varieties to powdery mildew under greenhouse and field conditions

Variety	Disease severity (%)			
	Greenhouse	Field		
	2012/13	2012/13	2013/14	Mean
Puma	8.71	4.19	5.04	4.62
Kawemira	32.13	19.63	21.44	20.54
Herkl	69.26	39.81	48.15	43.98

Preparation of antigens:

The conidia of pathogenic fungus *Erysiphe betae* were collected from the infected leaves of the highly susceptible Herkl variety using collector machine, or the healthy leaves (200 gm) of the three sugar beet varieties. The fungal biomass was ground up in a mortar with 20 g glass beads under liquid nitrogen and diluted with HCl-Tris buffer (0.05M), pH 7.2 and kept at 5ºC in a refrigerator. The extractions were centrifuged at 10,000 rpm for 20 min. The supernatant was collected and protein content was adjusted into 20 mg/ml before injection in male rabbits according to Lowry *et al*. (1951) using spectrophotometer at 750 nm.

Immunization and production of antisera:

Tested antigens, either of *Erysiphe betae* fungus or sugar beet varieties, were mixed with incomplete freund adjuvant (1:1) and then administered intramuscularly and subcutaneously in white New-Zealand rabbits. Each rabbit received a course of injections (two/week), the volume of the antigen was 0.5 ml for the first five injections and 1.00 ml for the remained injections (Harboe and Ingild, 1973). The blood was bled by cutting the lateral veins, 7, 9 and 11 days after the last injection. The blood was received in sterilized tubes. The blood was left to clot at room temperature overnight. The clot was loosened from the wall of the tube using a fine glass rod and then the tubes were kept at 5ºC in a refrigerator overnight to allow the clot to retract and express the straw-colored serum. The antisera were obtained by centrifuged at 10,000 rpm for 20 min to remove any precipitates then kept at 4ºC until use. Sodium azide was added at 0.02% to prevent any contaminations. The immunoglobulin was concentrated by adding ammonium sulphate (37%, w/v).

Crossed Immunoelectrophoresis (CIE):

CIE technique was carried out according to Axelsen *et al*. (1973), where 1% agarose gel (Litex, Glostrup, Denmark) was mixed with barbital buffer (pH 8.6; ionic strength 0.02). The first dimension of electrophoresis of the fungus or the varieties was run at 12ºC, applying 10 V/ cm for 60 min. The second dimension was run at 12ºC, 3 V/cm for 18 h. through a gel containing 10 µl of abs/cm2. The used plates were 10x10 cm for the first dimension and 6X10 cm for the second dimensions. The non-precipitated proteins were removed by washing the gel in 0.1 ml NaCl (20 min.) then distilled water (15 min.). After drying, the plates were stained for 15 min. in a solution of 0.5% (w : v) Coomassie brilliant blue R-250 (Sigma) in ethanol: glacial acetic acid: water (45:10:45). Excess dye was removed by repeated washing in distaining solution (ethanol : glacial acetic acid : water, 45:10:45).

Results

Homologous reactions of the sugar beet varieties and Erysiphe betae:

Data in Table (2) and Fig. 1 (A , B , C and D) show that seven, seven and eight precipitin peaks were detected when antigens and antisera of Puma (A), Kawemira (B) and Herkl (C) sugar beet varieties, respectively, reacted in homologous reactions. However, eight precipitin peaks were detected when antigens of *E. betae* reacted with its antisera (D).

Table 2. Number of precipitin peaks detected in homologous reactions of the sugar beet varieties or *E. betae* isolate

Antigen / antibodies	Puma	Kawemira	Herkl	*E. betae*
Puma	7	-	-	-
Kawemira	-	7	-	-
Herkl	-	-	8	-
E. betae	-	-	-	8

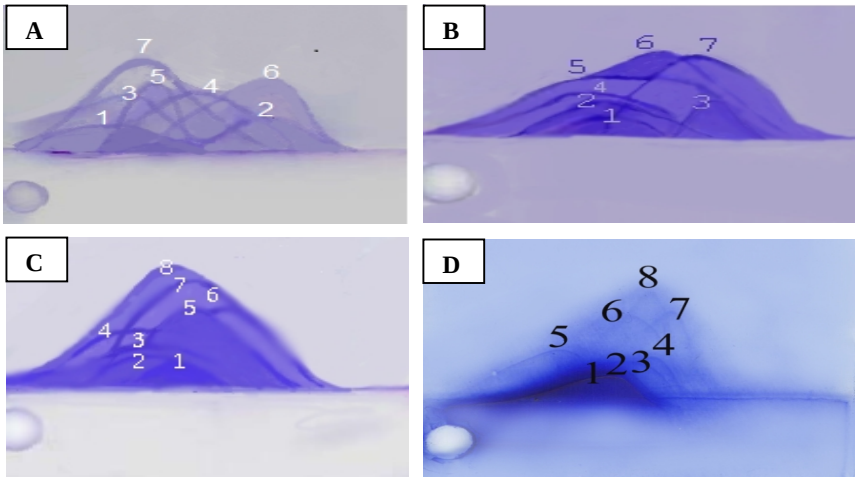

Fig. 1. Homologous reactions between antigens and antisera of Puma (A), Kawemira (B), Herkl(C) and *E. betae* isolate (D) using Crossed Immunoelectrophoresis (CIE) technique.

Heterologous reactions between the sugar beet varieties and E. betae:

Different numbers of common precipitin peaks (antigens) were detected when antigens of the sugar beet varieties were reacted with antisera of *E. betae* isolate. Data in Table (3) and Fig. 2 (E , F and G) reveal that the resistant sugar beet variety Puma(E) gave the lowest number of common precipitin peaks (3 precipitin peaks), while the moderately susceptible Kawemira variety and the susceptible Herkl variety gave 5 and 6 common peaks, respectively (F and G). These data run in a parallel line with those obtained under field conditions.

Table 3. Number of precipitin peaks detected in heterologous reactions of antigens of the sugar beet varieties and antibodies of *E. betae* isolate

Antigen / antibodies	Puma	Kawemira	Herkl
E. betae	3	5	6

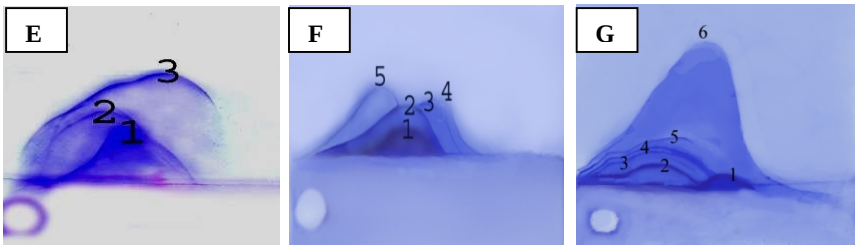

Fig. 2. Heterologous reactions between antigens of Puma (E), Kawemira (F), Herkl (G) sugar beet varieties and antibodies of *E. betae* using Crossed Immunoelectrophoresis (CIE) technique.

Discussion

The greater similarity between pathogens and its hosts or any two organisms means that they completely recognized each other, according to the definition of gene for gene concept (Flor, 1971). In plant host-parasite systems, the resistance and susceptibility to infection and disease development may be depended on the antigenic relationship between the host plants and their pathogens named as common antigen (Devay and Thornton, 1995 and El-Shamy, 2006). It could be noticed that the number of the detected common antigens were associated with the degree of resistance and/or susceptibility, since more antigens were common between the fungus and the susceptible varieties Herkl and Kawemira and vice versa with the partially resistant variety Puma. Similar results were obtained with other crops and pathogens using the same technique (El-Kazzaz *et al*., 1996; El-Shamy *et al*., 2008; Ghoniem, 2013; Awad *et al*., 2014 and Awad and El-Ghonemy 2015). Also, similar results were obtained with other hosts-pathogens relationship. In this concern, Dasguta *et al*. (2005) stated that pathogenicity of *C. eragrostis* to different varieties of tea was found to be related to the number of common antigens present between host and pathogen. Also, Chakraborty and Sharma (2007) found that pathogenicity of *Exobasidium vexans* to different tea varieties is related to the level of antigenic similarity between host and pathogen. According to the available literature, this is the first study in this respect.

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تقدير المقاومة فى بعض أصناف بنجر السكر لمرض البياض الدقيقى بإستخدام تكنيك الكروسد أميونوالكتروفريسيز

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نفذت هذه التجربة فى محطة البحوث الزراعية بالجميزة موسم ٢٠١٥ . تم تجهيز الأنتيجينات والسيرم (الأجسام المضادة) للثلاثة أصناف وهى بوما (مقاوم)، كاوميرا (متوسط القابلية للإصابة) و هرقل (قابل للإصابة) وكذلك كونيديا الفطر إريسيف بيتا وذلك بالحقن في أرانب نيوزيلندي بمعدل أرنب لكل منها. أوضحت النتائج بإستخدام تكنيك التفريد الكهربي المناعي في اتجاهين لمعرفة التركيب الأنتيجيني لكل من الأصناف الثلاثة والفطر قدرت ب٧، ٧، ٨ أنتيجينات للأصناف الثلاثة على التوالى وكذلك ٨ أنتيجين لعزلة الفطر وذلك عند تفاعل الانتيجينات مع الأجسام المضادة الخاصة بها على التوالي (homologous reactions)، بينما تم الحصول على٦,٥,٣ أنتيجينات مشتركة عند تفاعل أنتيجينات الأصناف بوما ،كاوميرا و هرقل مع الأجسام المضادة للمسبب المرضى (heterologous reaction). إستخدم تعريف الأنتيجينات المشتركة بين النباتات ومسبباتها المرضية لتوضيح صفة المقاومة أو القابلية للإصابة بالمرض. كلما زاد التشابه الأنتيجينى (عدد الأنتيجينات المشتركة) بين الصنف و المسبب المرضي فإن ذلك يعني زيادة شدة الاصابة بالمرض .طبقا لذلك فانة يمكن تصنيف الصنف بوما على أنه مقاوم للمرض حيث أعطى أقل عدد من الأنتيجينات المشتركة مع الفطر المسبب للمرض ،بينما يمكن تصنيف الصنف هرقل على أنه شديد القابلية للإصابة يليه الصنف كاوميرا. و لقد وجد أن هذه النتائج تتوافق مع النتائج المتحصل عليها من تجارب الصوبه والحقل وطبقا لهذه النتائج يمكن القول بان التكنيك المستخدم يساعد في الحصول على معلومة مسبقة وسريعة لتفاعل الأصناف مع المرض، وتعد هذه الدراسة أول تطبيق لهذا التكنيك لتقدير المقاومة لمرض البياض الدقيقي في بنجر السكر.