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SURVIVAL OF *Erysiphe betae* CLEISTOTHECIA ON CHARD (WEED BEET) AND IT'S SEED TRANSMISSION TO SUGAR BEET IN EGPYT.

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ABSTRACT

Powdey mildew disease of sugar beet incited by *Erysiphe betae* (Vanha) Weltzien is an important disease of sugar beet (*Beta vulgaris* var *vulgaris*). Very little information is available on weather or how the pathogen survives. Experiments were conducted to study the role disease transmission by chard (*Beta vulgaris* var *cicla*) that found as a common weed beet in sugar beet fields in Egypt. Powdery mildew infection and cleistothecia of the pathogen were abundantly occurred on both chard and sugar beet plants. . Microscopic observation during the sugar beet growing season indicated that by May more than 90% of the cleistocthia had generated under field conditions. In the several sample taken from sugar beet and chard plants from two different locations, the dimensions of conidia, cleistothecia and asci confirm that the isolates are typically belong to *E. betae*. Cleistothecia were formed on seed clusters of chard plants and 65% of cleistothecia contained intact ascospores. When chard seeds infected with cleistothecia planted in greenhouse with sugar beet under aseptic conditions, 30% of the plants examined developed powdery mildew symptoms. The percentage of mature cleistothcia on sugar beet leaves were 44- 45% and 47-52% in chard plants. The pathogenicity of ascospores from stored cleistothecia with different inoculation methods either by detached leaf method or by whole plant spray were succefull and lead to 45-50 % disease incidence in detached leaves under laboratory conditions while disease incidence was 55-75% in whole plant spray method in March and April 2007 trials

Keywords: *Erysiphe betae*, Cleistothecia, sugar beet,

INTRODUCTION

Sugarbeet powdery mildew was first recorded in Egypt by El-Kazaz *et al*., 1997. The disease became widespread in sugarbeet field especially in fileIds cultivated in late sowing dates (Abdalla, 2004). Like most powdery mildew fungi, the sugarbeet powdery mildew pathogen *Erysiphe betae* (Vanha) Weltzien has relatively narrow host range. Taxonomically, the genus Beta is divided into four sections: Beta (formerly Vulgares), Corollinae, Procumbentes (formerly Patellares), and Nanae, represented by a single species endemic to Greece. The Section Beta includes the cultivated beets (*Beta vulgaris* subspecies *vulgaris*), which are divided into four Culti-groups: Leaf beet group, Garden beet group, Fodder beet group, and Sugar beet group (Lange et al. 1999). Chard (*Beta vulgaris* L. subsp. *cicla* (L.) W. D. J. Koch) (Cicla Group) is one of the common weed in sugar beet fields in Egypt and other sugarbeet producing countries in the world. The Arabic name of the weed is SALK and the English names are (Foliage beet, Leaf beet, Spinach beet). In Egypt; it grows in all fileds during autmun and winter and produce seed clusters in summer. Occurrence of such weed beet complicate the

powdery mildew control due to no herbicide exist against it due to its congeniality with cultivated varieties. Egypt import seeds of sugar beet annually because the breeding system of sugar beet is complex. The crop is biennial; flowerings requires vernalization, at 10°C or lower for 80–120 days coincidental with or followed by long-day photoperiod which lengthens the generation time to almost 1 year (Owen *et al.* 1940). Pernnation or survival from generation to the next, of the sugarbeet powdery mildew pathogen has been thought to be accomplolished by ascospores in and/or mycelium or haustoria in crowns of escaped plants and wild Beta spp. Also, the fungus can be survived in auxillary bud tissue of beet seed cluster (Whitney and Duffus, 1991).

MATERIALS AND METHODS

Characteristics of *E. betae* isolates

Based on previous observations in sugar beet fields at Dakahlia and Gharbia governorates that cleistothecia of sugarbeet develop late in the growing season. A hand lens (10X) was used to observe cleistothecia on the plant materials in the field before sample collection. Microscopic studies on E. betae isolates from sugarbeet and chard plants were made by a disecting microsope (35-60X) was used to assess the cleitothecia maturity, frequency and abundance. Also, compound microscope (200-400X) was used to identify both conidial and cleistothecial charateristics of asci and ascospores development. Ten plants of chard and sugar beet with highly abundant cleistothecia were taken from two field locations. Examinations of cleistothecia were made on leaves and seeds of chard and on leaves only of sugar beet plants. Ten seed replicates from each chard seed cluster were examined. Samples of 30 - 50 cleistothecia were removed from each infected leaf or seed clusters samples and gently crushed on glass slides. To determine the viabilty of cleistothecia; the asci were categorized as asci containing mature spores; immature spores with grannular cytoplasm or degenerate spores containing dark cytoplasm with numerous lipid droplet as in methods described by Mmbaga, 2000. The percentage of cleistothecia containing ascospres of each category was recorded. In this study; pathogenicity proof of the *E. betae* isolates was done by simulataneous inoculation using conidial suspension and ascosopres on its host plants, either sugarbeet or wild beet. Also, reciprocal inoculation experiments were conducted between sugarbeet and wild beet isolates.

Density of cleistothecia

Twenty five samples of young and old leaves of both sugar beet and wild beet were assessed. The mean number of cleistothecia per gram of heavily infected leaf or chard seed cluster was demonstrated.

Seed Transmission of *E. betae*

By the end of sugar beet growing season; highly infected seed clusters of chard plants with abundant cleistothecia were collected from sugar beet fields in Belqas County at Dakahlia governorate, Egypt. The seed clusters of chard were kept in paper bags and preserved in two sites under

room temperature with 60-70% relative humidity. The seed clusters were examined every 2 months until the next growing season of suga beet. The seed samples were soaked for 30 min. The wetted cleistothecia were removed gently fro the surface of the seeds with needle, scalpel and brush, immersed in a drop of distilled water, and mounted in lactophenol on a glass slide. The cleistothecia were opened to release their content by a mild presure on the cover slide placed over them. About 40 to 50 cleistothecia were examined and percent cleistothecia with ascopspores were recorded. The pathogenicity of ascospores from stored cleistothecia were determined on next March and April 2007 during the next sugar beet growing season by two different techniques. The first technique was by attaching chard seeds with cleistothcia to Petri dishe lid suspended over 20 disease-free sugar beet leaves inside plastic bags containing 5% sucrose solution instead of Petri dishes as described in detached leaf assay by Warkentin et al.,1995. In the second technique, the seeds with cleistothecia were crushed in water in mortar and pestle and the ascopore suspension was sprayed on healthy sugar beet plants grown in protected and isolated greenhouse (far from other beet plants). The experiments were repeated twice during March and April using 20 plants where temperature and relative humidity were suitable to normal infection. The inoculated plants were water moisted and covered overnight with plastic bag after inoculation. The control plants were sprayed with sterilized water. Inoculated pants were observed during 2 weeks for disease symptom development and percentages of disease incidence were recorded.

Growing on experiment

Infested chard seed taken from heavily infected chard plants were used in a growing on test in the next season (October, 2007) under isolated greenhouse conditions to determine the role of chard seed in transmission of the pathogen. Seeds were grown in pot experiments and each pot contained one seed of each chard and beet (susceptible cv; Betapoly). The seeds of chard were either naturaly infested with cleistothecia or healthy without cleistothecia and all the beet seeds were healthy and treated with seed dressing fungicide (thiram). The following treatments were conducted in the growing on experiment at the greenhouse; 1) infested chard seed + healthy beet seed; 2) healthy chard seed + healthy beet seed; 3) healthy beet seed only and 4) Infested chard seeds only. The seeds were sown in 50 cm diam pots within a complete randomized design with 10 replicates. Pots with beet plants only were served as control and placed isolated from other pot treatments. All plants in pots received recommended agricultural practices of irrigation and fertilizers and left to grow in pots until next May, 2007. Plants were inspected for disease development; percentage of disease incidence (*No. of infected leaves / Total No. of leaves X 100*) was recorded.

RESULTS

Characteristics of *E. betae* isolates

The comparison results of microscopic examination of *E. betae* isolates isolated from both sugar beet and chard plants from two locations

indicated that mean width and length of conidia were 19-46 µm and 14-46 µm; respectively. Conidia were mostly ellipsoid to cylindric and typically resemple the species betae of the genus *Erysiphe*. Cleistothecia formed on both sugar beet and chard plants were globose shiny yellowish to light brown(immature) that turned later to dark brown (mature) scattered or in groups on the leave surfaces and on heavily infected chard seed clusters that covered with the mycelium growth. The cleistothecia mean diameters in sugar beet and chard plants were 118-120 µm and 100-118 µm while asci number inside cleistothecium were ranged from 5-7 and 4-6 cleistothecia; respectively. Cleistothecia density per g of leaves on both sugar beet and chard plants were 30-34 and 39-46 cleistothecia on leaves, respectively. The density of cleistothecia on 1g of chard seed cluster was 18-25 cleistothecia and less than density on leaves. The percentage of mature cleistothcia on sugar beet leaves was 44- 45% and 47-52% in chard plants (Table 1).

The pathogenicity of ascospores from stored cleistothecia

Microscopic examination of cleistothecia samples taken from seed clusters stored under field and under room conditions revealed that percentage of cleistothecia with viable ascospores after 6 months of storage (until next october, 2006) in samples stored under field conditions were higher than cleistothecia stored under room conditions. After October 2006, the percentage of cleistothecia with viable asci and ascospores decreased rapidly under field condition and slowly under room condition. By April 2006, only 25% of the cleistothecia contained ascospores in saamples stored at field and 30% in samples stored under room temperature (Fig. 1).

Table 1: Characteristics of *E. betae* isolates from two locations on sugar beet and chard plants

Characteristics	Location 1 (Belqas county, Dakahlia Governorate)			Location2 (Gemmiza county, Gharbia Governorate)		
	Isolates					
	Sugar beet	Chard		Sugar beet	Chard	
	Leaves	Leaves	seeds	Leaves	Leaves	seeds
Conidia length (µm)	45 ±1.93 [a]	42 ± 1.18	40 ± 0.96	46± 1.17	44± 1.25	43± 0.75
Conidia width (µm)	19 ± 0.63	17 ± 0.53	16 ± 0.57	20 ± 0.72	18 ± 0.28	16 ± 0.66
Cleistothecia diam.(µm)	120 ± 1.44	110 ± 1.11	100 ± 0.92	118 ± 0.72	115 ± 0.94	105 ± 0.69
Asci per Cleistothecia	6 ± 0.57	5 ± 0.75	4 ± 0.60	7 ± 0.53	6 ± 0.52	5 ± 0.57
Asci length (µm)	65 ± 0.74	63 ± 0.89	59 ± 0.75	70 ± 0.74	65 ± 0.69	61 ± 0.82
Asci wedth (µm)	47 ± 0.87	44 ± 0.66	41 ± 0.66	48 ± 0.92	45 ± 0.80	42 ± 0.66
Cleistothecia density [b]	34	46	25	30	39	18
Cleistothecia (mature) %	45	52	49	44	50	47

a = Mean ± standard deviation (measurements of 75 – 100 conidia)

b = Mean of cleistothecia number per g of leaf or seed.

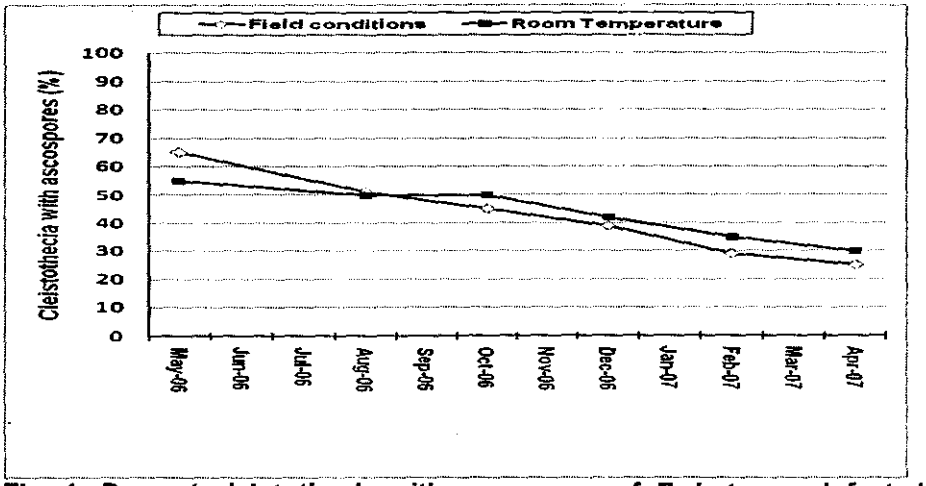

Fig. 1: Percent cleistothecia with ascospores of *E. betae* on infested chard plants with seed cluster under field conditions at Dakahlia Gov. Egypt and at room temperature stored during the summer and autmn 2006 - 2007.

Seed Transmission of *E. betae*

The pathogenicity of ascospores from stored cleistothecia with different inoculation methods either by detached leaf method or by whole plant spray were succefull and lead to 45-50 % disease incidence in detached leaves under laboratory conditions while disease incidence % was 55-75 % in whole plant spray method in March and April trials, respectively. In the control plants, percentage of disease incidence was 15-25% due to natural infection (Fig.2).

Table 2: Percentages disease incidence (DI %) of powdery mildew developed on chard and beet plants during growing on experiments under isolated greenhouse conditions.

Treatments	Disease Incidence (%)					
	March		April		May	
	Chard	Beet	Chard	Beet	Chard	Beet
+ Infested chard seed* + healthy beet seed	25 f	16.7 g	60 b	25 f	60 b	30 e
-Healthy chard seed + healthy beet seed	0 h	0 h	0 h	0 h	0 h	0 h
-Healthy beet seed only	0 h	0 h	0 h	0 h	0 h	0 h
-Infested chard seeds only	40 d**	0 h	50 c	0 h	75 a	0 h

* Infested chard seeds = contaminated with cleistothecia; healthy seeds = without cleistothecia

** Mean values followed by the same letter are not significantly difference according to Duncan's multiple range test (*P=0.05%*). - The seeds were sown in 50 cm diam pots within a complete randomized design with 10 replicates.

- Percentage of disease incidence calculated as = (No. of infected leaves / Total No. of leaves x 100)

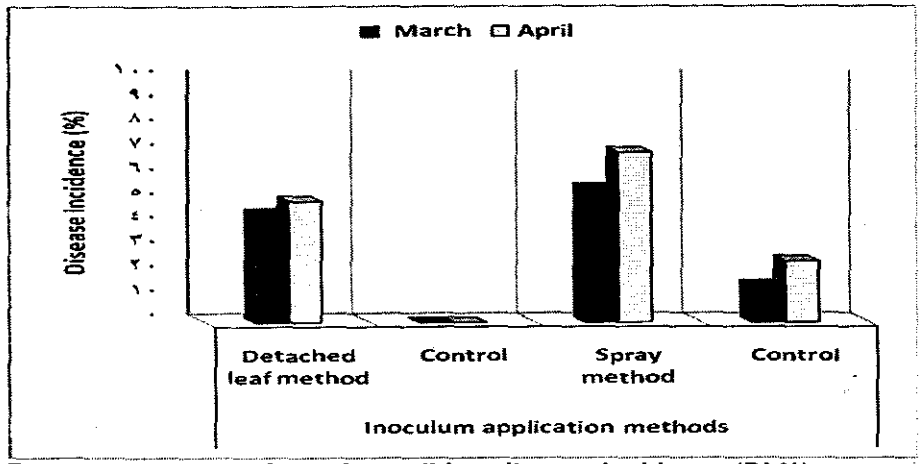

Fig. 2: Percentage of powdery mildew disease incidence (DI %) on sugar beet plants using two different inoculation methods in laboratory and greenhouse conditions repeated twice in March and April, 2007.

DISCUSSION

In the several sample taken from sugar beet and chard plants from two different locations, the dimensions of conidia, cleistothecia and asci revealed that the isolates are typically belong to *E. betae*. This is in agreement with simillar records by other workers (Weltzien, 1963, Kapoor, 1967, Saenz and Taylor, 1999; Francis, 2002 and Fernández-Aparicio, 2009) . In the present investigation, artificial inoculation with ascospores taken from cleistothecia on chard seeds lead to succeful infection to sugar beet and chard plants. There are several reports of succeful infection of different hosts by other species of Erysiphe (Hirata, 1986 and Dixon 1978). Observation in Dakahlia and Gharbia governorates in Egypt showed that powdery mildew first appears early in mid March to April (Abdalla, 2004). If cleistothecia were responsible for oversummering of powdery mildew, then an earlier development of symptoms would be expected. The annual nature of the chard weed excluded pathogen survival as mycelium on host leaves or stems, but perennation in seed clusters are possible alternative. Data from the seed transmission study showed that *E. betae* is transmitted through infested chard seed with ceistothecia; these results were supported by microscopic examination of cleistothecia contained viable ascospore through out extended period of storage of cleistothecia after sugarbeet harvest til sowing it in the next season. Ruppel and Tomasovic (1977) in USA reported that *E. betae* overwintering mycelia, haustoria, or conidia of the sugar beet powdery mildew fungus did not survive long enough in plant debris or on seed to serve as primary inoculum for subsequent beet crops. If the fungus could overwinter in vegetative stage in cold climates, it would expect the disease to occur much earlier in the growing season. In our case, the fungus can survive on chard plants or seed during summer period (3-4 months) in Egypt until the next growing season of beat that started in september and symptoms

appeared early in Febrauary or March as observed in several fields in southern and northern regions of Egypt.

In northern Egypt it was shown that cleistothecia are the prevailing form of overwintering (Abdalla, 2004), two years of field syrveys, indicates that both mycelium and cleistothecia can be sources of primary inoculum and can play an important role in the onset of E. beate epidemics. Degenration of cleistothecia on leaves and dehiscence of asci and ascospores when released outside is important factors control the survival of cleistothecia and success of infection process that depend on many factors related to weather (temperature and moisture) and host susceptibility and can be considered as main reasons for the failure of more than 75% of ascospores to survive until the next season under field conditions (Fig.1). In the present study, disease transmission was demonstrated by the ability of mature cleistothecia with less than 25% viable ascospore to infect sugar beet leaves. Unless, large numbers of cleistothecia were used, the small proportion of viable ascospores with high inoculum efficiency 25-60% would render most inoculation ineffective. Also, maturation of cleistothecia during sugar beet growing season (April to May) and release of ascospore are coincided with availability of the host and suitable temperature that consider main factor determine the rate of cleistothecia and ascospore maturation. Observations on cleistothecia formation on either sugarbeet or chard revealed that temperature lower than 20 °C seems to delay maturation and reduce number of dark globose cleistothecia with viable ascospore. Further investigaions are needed to study the effect of temperature (higher and lower degrees) on the viability and dehiscence of asci and ascospores under different field conditions.

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قدرة الفطر أريسفى بيتا على البقاء فى صورة أجسام ثمرية على حشيشة السلق وانتقاله عن طريق بذور السلق إلى بنجر السكر فى مصر.

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يعتبر الفطر *Erysiphe betae* (Vanha) Weltzien المسبب لمرض البياض الدقيقى فى بنجر السكر (*Beta vulgaris* var *vulgaris*). ولاتوجد إلا معلومات قليلة عن كيفية قدرة الفطر على البقاء بين المواسم الزراعية. أجريت التجارب لدراسة دور حشيشة السلق (*Beta vulgaris* var *cicla*) فى انتشار الفطر والسلق من أكثر من أكثر الحشائش الشائعة فى حقول محصول بنجر السكر فى مصر وتكون بذورها فى نهاية الموسم. ويصاب كلا النوعين بمرض البياض الدقيقى والذى لوحظ ايضا تكون الأجسام الثمرية عليها بكثافة. أظهرت نتائج الفحص الميكروسكوبى لمقاييس وابعاد الجراثيم الكونيدية والأجسام الثمرية وما بداخلها من اعداد وكذلك مقاييس الأكياس والجراثيم الأسكية وجود تطابق لمتوسطات المقاييس الممثلة لتعريف نوع الفطر *E. betae* وذلك فى عينات مأخوذه من موقعين مختلفين. واظهرت نتائج الفحص لنبات السلق المصابة بالبياض الدقيقى قرب النضج تكون الأجسام الثمرية على ثمار او بذور السلق. اجريت تجارب لدراسة قدرة وحيوية الأجسام الثمرية على البقاء حتى الموسم التالى من خلال تخزينها تحت ظروف الحقل وجرارة الغرفة وكذلك انتقال الفطر من بذور السلق إلى نباتات السلق وبنجر السكر فى الموسم التالى.. وجد ان ٣٠% من النباتات قد اصيبت بالفطر وظهور اعراض المرض. وكانت نسبة حيوية الأجسام الثمرية المتكونة على اوراق البنجر ٤٤-٤٥% وعلى أوراق السلق ٤٧-٥٢%. أظهرت النتائج نجاح حدوث العدوى الصناعية بطريقتين للعدوى هما طريقة عدوى الورقة المنفصلة وطريقة العدوى برش معلق الجراثيم على النباتات السليمة فى الصوب المعزولة وتراوحت نسبة حدوث المرض ٤٠-٥٠% فى الطريقة الأولى و ٥٥-٧٥% فى الطريقة الثانية خلال شهرى مارس وابريل من الموسم التالى.