ABSTRACT

Abed Abd Algaleel Ata: Variability of *Uromyces betae*, the Cause of Sugar Beet Rust and Its Control. Unpublished Ph.D. Thesis, Department of Plant Pathology, Faculty of Agriculture, Ain Shams University, 2009.

Beet rust disease incited by *Uromyces betae* Tul. ex Kick is considered among the most destructive diseases attacking sugar beet causing quantitave and qualitative losses of yield world wide and Egypt.

Rust spores on beet infected trash or carried on/ in contaminating imported seeds could not be considered as a primary source of inoculum, so it is probable that, the first spores of *U. betae* reach sugar beet in winter are windborne spores produced on beet in European countries in late autumn.

Twenty three isolates of *U. betae* were collected from different locations in four governorates of Egypt and used in the present study to evaluate the genetic diversity among the fungus isolates using SDS-Protein Electrophoresis and Random Amplified polymorphic DNA (RAPD) analysis.

Total protein electrophoresis analysis exhibited 85.714% polymorphism among the twenty three tested isolates. Fourteen different protein bands were detected; two of them were recorded as monomorphic bands, in addition three bands were recorded as isolate specific bands.

Dendrogram analysis based on total protein polymorphism separated the twenty three tested isolates into two main groups at approximately 26 % dissimilarity. There was no correlation between clustering in the protein dendrogram and geographic origin of the tested isolates.

Results of this study suggested that protein profiles data can differentiate *U. betae* isolates. The RAPD analysis with primer OP2 in a preliminary study gave twenty polymorphic bands. It showed 100 % polymorphism among the twenty three tested isolates; in addition five bands were recorded as isolatespecific bands. These isolate-specific markers could distinguish four isolates out of the twenty three. Dendrogram analysis based on DNA polymorphism with primer OP2 separated the twenty three tested isolates into three main groups at approximately 26 % dissimilarity.

The RAPD analysis with the six primers in a complementary study gave 56 different DNA fragment bands with wide molecular weights. Thirteen of them were expressed as isolate-specific bands and could distinguish seven isolates out of the fourteen tested isolates. The complied data for the six primers recorded 98.33 % polymorphism among the fourteen tested isolates.

Dendrogram analysis based on DNA polymorphism of the six primers separate isolate No. 2 from the tested isolates at dissimilarity 25 % and divided the reaming isolates into two main groups at approximately dissimilarity 16 %.

RAPD groups were not associated with geographic origin of the tested isolates.

Among five chemical inducers evaluated for their capabilities to induce resistance against beet rust in greenhouse and field trials during 2006/ 07 growing season in Damietta and Kafr EI-Sheikh governorates, Hydrogen peroxide at 1.0 % and 0.5 %, salicylic acid and di-basic potassium phosphate at 8 mM were the most effective treatments. Moreover these inducers recoded the highest sucrose, quality percentage and maximum reduction of non-sucrose chemical components. Hydrogen peroxide, at 1.0 % showed the highest level of oxidative enzymes (PO, PPO, PAL and TAL) activity. The higher free phenols and lignin contents were recorded by hydrogen peroxide 1.0 % and salicylic acid at 8 mM.

Among five microelements and their mixture evaluated for their efficacy to induce resistance against beet rust, the most

effective microelements were the mixture at 400 and 200 ppm and Boron at 400 ppm. The highest sucrose, quality percentage and the maximum reduction of non-sucrose chemical compounds were obtained by B at 400 ppm. Microelement's mixture at 400 ppm induced the highest level of oxidative enzymes (PO, PPO, PAL and TAL) activity followed by Band Fe at 400 ppm. Maximum amounts of free phenols and lignin were observed in leaves sprayed with the mixture followed by B, Mn and Zn at 400 ppm.

Among three growth regulators, ethephon at 0.80 % followed by indole butric acid at 50 ppm were the most effective treatments. These inducers also increased sucrose, quality percentage and decreased non-sucrose chemical components. Ethephon at 0.80 % showed the highest level of oxidative enzymes (PO and PPO). Higher free phenols and lignin contents were recorded by ethephon at 0.80 %.

Among twelve isolates of different bio-agents isolated from the phylloplane of healthy leaves of sugar beet plants or from different hosts and three bio-fungicides, *Bacillus pumilus* and *Pseudomonas fluorescens* isolated from the phylloplane of healthy leaves of sugar beet plant grown in heavily infected fields were the most effective bio-agents. These isolates significantly increased root weight/ plant, recorded the highest percentage of sucrose and juice quality and reduced nonsucrose chemical components.

Key words :

Sugar beet (*Beta vulgaris*), rust, *Uromyces betae*, source of primer infection, SDS-Protein Electrophoresis, PCR, RAPD, induced resistance, H₂O₂, SA, KH₂PO₄, K₂HPO₄, Bion (BTH), Mn, Zn, B, Fe, Cu, GA₃, IBA, ethephon, biological control, *Bacillus subtilis*, *B. pumilus*, *B. mycoides* and *Pseudomonas fluorescens*.

CONTENTS

CONTENTS	n
	Page
LIST OF FIGURAS	V TV
	1
	1
	3
1. Rust of sugar beet	3
1.1. Disease symptoms	3
1.2. Causal organism	3
1.3. Disease history	4
1.3.1. In the world	4
1.3.2. In Egypt	4
1.4. Host range	5
1.5. Economic importance of the disease	6
2. Source of primary inoculum	6
2.1. Source of primary inoculum of rust fungi	6
2.2. Source of primary inoculum of sugar beet rust	8
3. Differentiation among <i>U. betae</i> isolates	9
3.1. Differentiation among <i>U. betae</i> isolates based on virulence	9
3.2. Differentiation among <i>U. betae</i> isolates based on biochemical	
and molecular markers	10
3.2.1. Differentiation among U. betae isolates based on protein	
patterns obtained by SDS. PAGE	10
3.2.2. Differentiation among <i>U. betae</i> isolates based on DNA	
(RAPD) analysis	13
4. Induction of resistance to diseases in plants	16
4.1. Induced resistance in sugar beet plants	18
4.2. Induced resistance by chemical compounds	20
4.3. Induced resistance by microelements	24
4.4 Induced resistance by growth regulators	25
4.5 Effect of the different inducers of resistance on yield	20
components	27
4.6 Biochemical changes associated with induced	21
resistance	28
4.6.1 Ovidetive enzymes	20
4.6.2 Dhenolic compounds	20 20
4.6.2. Fileholic compounds	29
4.0.3. Light content	29
	31
MATERIALS AND METHODS	51
1. Source of primary inoculum of beet rust in Egypt	37
1.1. Survival of uredio and telito-spores in soil	37

1.2. Survival of rust spores on-and in beet infected trash	37
1.3. Spores carried on/ in contaminating imported seeds	38
1.4. Air born oversees spores	38
2. Differentiation among <i>U betae</i> isolates	39
2.1. Collection of rusted leaf samples	39
2.2. Urediniospores collection and single uredinium isolation	39
2.3. Greenhouse tests	40
2.4 Electrophoretic detection of protein by sodium dodecyle	
sulphate polyacrylamide gel electrophoresis (SDS-PAGE)	40
2.5 Polymerase chain reaction (PCR) technique	44
2.5.1 DNA isolation and RAPD technique	<u>4</u> 4
2.5.7. Britt isolation and ferring teening teening teening (RAPD)	45
2.5.2. Aundoin amplified polymorphism D101 teeningue (10 if D) 2.5.3. Amplification product analysis	46
2.5.4 Gel analysis	46
3 Inducing resistance against rust disease	46
3.1 Greenhouse tests	46
3.2 Field tests	47
3.3 Rust disease assessment	47
3.4 Effect of treatment with different inducers on yield components	48
3.5 Biochemical changes associated with induced resistance	40 40
3.5.1 Determination of oxidative enzymes activity	49
3.5.2 Extraction and determination of free phenolic compounds	51
3.5.3. Extraction and determination of lignin	51
4 Biological control of beet rust	52
4.1 Source of microorganisms	52
4.1. Solution of microorganisms	52
4.3. Identification of microorganisms	53
4.4 Preparation of biocontrol agents inoculua	54
4.5 Evaluation of antagonists under greenhouse conditions	55
4.6 Evaluation of antagonists under field conditions	55
4.7 Effect of the antagonists on yield components	56
RESULTS	57
1 Rust of sugar beet	57
1.1 Disease Symptoms	57
1.2 Causal organism	57
2 Source of primary inoculum of <i>U betae</i> in Fount	60
2.1 Survival of uredinio and telito-spores in soil	60
2.2. Survival of rust spores on-and in beet infected trash	60
2.3. Spores carried on/ in contaminating imported seeds	60
2.5. Spores carried on in containing imported seeds	60
3 Differentiation among Uromyces hetae isolates	61
5. Enterendation among cromyces betae isolates	01

3.1 SDS-Protein Electrophoresis	61
3.1.1. Evaluation the efficiency of the used biochemical technique	~
(Protein electrophoresis) in U. betae isolates discrimination	65
3.1.2. Survey of biochemical isolate-specific markers for the tested	
isolates	65
3.1.3. Phylogenetic relationships	65
3.2. Random Amplified polymorphic DNA (RAPD) analysis	<i>(</i> 0
(preliminary study)	0ð
5.2.1. Evaluation the efficiency of OF2 arbitrary primer in U.	68
3.2.2. Survey of isolate-specific markers for the tested isolates	71
3.2.3. Phylogenetic relationships	71
3.3 Random Amplified polymorphic DNA (RAPD) analysis (a	/1
complementary study)	74
3.3.1. Evaluation of the efficiency of RAPD-PCR analysis in U.	/ 4
betae isolates discrimination	87
3.3.2. Survey of RAPD-PCR isolate-specific markers for the	
tested isolates	90
3.4. Phylogenetic Relationships	92
4. Inducing resistance against beet rust disease	96
4.1. Inducing resistance by chemical compounds	96
4.1.1. Greenhouse experiment	90 00
4.1.2. Field experiments	90
incidence and severity	98
4.1.2.B. Effect of treatment with chemical inducers on yield	70
components	98
4.1.3. Biochemical changes associated with chemical compounds	103
4.1.3.1. Effect of chemical inducers on activity of oxidative	
enzymes	103
4.1.3.2. Effect of chemical inducers on free phenols content	106
4.1.3.3. Effect of chemical inducers on lignin content	107
4.2. Inducing resistance by microelements	107
4.2.1. Greenhouse experiment	107
4.2.2.A. Effect of some microelements on both DI and DS	108
4.2.2.B. Effect of some microelements on yield components	111
4.2.3. Biochemical changes associated with some microelements.	114
4.2.3.1 Effect of some microelements on activity of oxidative	
enzymes	114

4.2.3.2. Effect of some microelements on free phenols and lignin
4.2. Inducing register on hy growth regulators
4.3. Inducing resistance by growth regulators
4.3.1. Greenhouse experiment
4.3.2. Field experiments
4.3.2. A. Effect of growth regulators on both DI and DS
4.3.2. B. Effect of growth regulators on yield components
4.3.3. Biochemical changes associated with growth regulators
4.3.3.1. Effect of growth regulators on activity of oxidative
enzymes
4.3.3.2. Effect of growth regulators on free phenols content
4.3.3.3. Effect of growth regulators on lignin content
5. Biological control of beet rust
5.1 Effect of spraying sugar beet plants with the tested
microorganisms isolated from the phylloplane of healthy leaves
of sugar beet plants on disease incidence (DI) and severity (DS)
5.1.1. Green house experiment
5.1.2. Field experiments
5.1.2.1. Effect of spraying sugar beet plants with the tested
microorganisms on disease incidence and severity
under field natural infection
5.1.2.2. Effect of spraving sugar beet plants with the tested
microorganisms on vield components
5.2 Effect of spraving sugar beet plants with five known bacterial
and fungal isolates and three biofungicides on disease
incidence severity and yield components
5.2.1 Greenhouse experiment
5.2.7. Greenhouse experiment
5.2.2.1 Effect of spraying sugar beet plants with five known
bacterial and fungal isolates and three bio functional solates
DI and DS under field natural infaction
5.2.2.2. Effect of spraying sugar best plants with five known
5.2.2.2 Effect of spraying sugar deet plants with five known
viold components under field activity linfortier
yield components under field natural infection
KEFERENCES
ARABIC SUMMARY