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ATPase ASSOCIATION WITH THE SUDDEN WILT DISEASE OF CANTALOUPE.

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ABSTRACT

Sudden wilt disease of Cantaloupe (*Cucumis melo* var. *cantaloupensis*) is considered one of the main problems face planting of this crop in Egypt and all over the world. This disease led to great conflict between researchers concerning disease etiology, which ranged between specialists from agricultural practices till phytopathogenic fungi (i.e., *Monosporascus cannonballus, Fusarium oxysporium and Pythium* sp.).

In the present study, it was postulated that the causal of such phenomenon may lead to imbalance of water absorption by plant roots. Therefore, ATPase activity and sugar fractions were determined in roots of diseased and healthy plants.

Obtained data indicated that ATPase activity was greatly depressed in roots of diseased plants as compared to healthy ones. Data also indicated that the total and reducing sugars were very low in diseased plant roots compared with healthy ones.

Irrigation of uninfected plants at flowering stage by water contained specific ATPase inhibitor (Omeprazole) led to appear disease syndromes on 62.5 % of treated plants.

So the obtained results led to postulate that plant suffer from reduction of ATPase activity and reducing sugars may be predisposing factors that plants easily infected by different phytopathogenic fungi.

Key Words: Cantaloupe – ATPase – Sugars - ATPase inhibitor - Sudden wilt.

INTRODUCTION

Cantaloupe (*Cucumis melo* var. *cantaloupensis*) is one of the most important vegetable crops either for exportation or for local consumption in Egypt. This crop suffers from many diseases; the most dangerous one is sudden wilt. It causes devastating losses of plant productivity and may led to plant death.

The disease appeared when cantaloupe hybrids became widely spread in agriculture (Martyn, 2002), therefore the etiology of such phenomenon varied according to the specialists. From one hand, horticulturists see that such phenomenon may attribute to agricultural practices (Bruton *et al*., 2000); however, plant pathologists attribute this phenomenon to phytopathogenic fungi (Pivonia *et al*., 1997; Aegerter *et al*., 2000 and Sales *et al*., 2003)

However, the nomenclature of this phenomenon was varied; it is sudden wilt or *Monosporascus* Root Rot and Vine Decline of Melons (MRR/VD). Also referred to as sudden death, melon collapse, *Monosporascus* wilt, and black pepper root rot.

Symptoms of that phenomenon appear when plants reached flowering stage, that, epinecity, wilt and collapse of shoot system followed by complete death of plants are occurred.

In this study, it was postulated that water absorption by root system is a critical point of disease development. Since water absorption is an physiologically active process (Pivonia *et al.,* 2002), therefore, the activity of ATPase in roots of diseased and healthy plants were determined, in addition to total, reducing and non reducing sugars as well.

MATERIALS AND METHODS

1. Plant materials

A pot experiments were conducted under green house conditions. Cantaloupe seeds (Galia hybrid) were planted in pots (30 cm in diameter) containing natural clay soil obtained from the land of Faculty of Agriculture, Ain Shams University, Shoubra El Kheima, Kaloubiya Governorate.

Eighty pots were planted, and then one plant was left in each pot. Plants were fertilized and irrigated as usual. When plants reached the flowering stage (72days after seeding), early symptoms of sudden wilt began to appear on some plants.

Plants were divided into two categories, healthy and diseased. Twenty plants of each category were taken-off carefully; roots were individually separated, washed several times by tap water then left to dry. Roots were cut into small pieces, then two grams (fresh weight) were taken represented the roots system. One gram was used to extract protein containing ATPase and the other was used for extracting and determination of sugars.

2. Determination of ATPase activity

ATPase activity was determined according to the method adopted by Bisswanger, (2004) as follows:

One gram (fresh weight) of root was grinded in 3 ml Tris-Hcl buffer (0.05 M, pH 7.4) containing 1 ml sucrose (0.25 M). The homogenates were taken in Eppendorf tube for centrifugation at 1500 rpm for 10 min at 4 ^{0}C. Reaction mixture consisted of : half ml of crude extract, 1 ml Tris-Hcl, 0.5 ml ATP solution (by dissolving 80 mg ATP in 30 ml distilled water), 0.5 ml KCL (400 mM) and the volume was completed to 4 ml by distilled water in a test tube. The mixture was incubated at 30 ^{0}C for 30 min., then one ml of TCA was added to the mixture to stop the reaction.

After incubation, 2 ml of the mixture were mixed with one ml of ferrous sulfate-ammonium molybdate (5g ammonium molybdate and 200ml H_2SO_4 5N) then left for 2 hours. Color density was determined at 820 nm using spectrophotometer Unico-2100 model. The amount of liberated phosphate (function of ATPase) was calculated using standard curve of potassium phosphate (KH_2PO_4).

3. Determination of soluble sugars

The concentrations of total soluble, reducing and non reducing sugars, were determined according to **Schales and Schales (1945)**

Preparation of crude ethanolic extract:

Sugars were extracted according to **Ackerson (1981)** as the following procedure:

One gram of fresh samples was extracted using 80% ethanol at 70^{o}C by refluxing for 1 hr for three times. The supernatant was evaporated at 55^{o}C, then the dried film was dissolved in 5 ml of 10% isopropanol and the total volume was made up to 15 ml.

3.1. Determination of reducing sugars

Reducing sugars were determined colorimetrically by using alkaline potassium ferricyanide solution as the following procedure:

Sample extract (0.3 ml) was pipette out into a series of test tubes, then the volume was completed up to 1 ml with isopropyl solution, 1.5 ml of alkaline potassium ferricyanide solution was added, mixed well and boiled in water bath for 20 min, cooled and then the volume was raised up to 10 ml with distilled water. Optical density was recorded against isopropyl solution at 420 nm. The amount of reducing sugars was calculated according to the standard curve of glucose.

3.2. Determination of total soluble sugars

One and half ml of HCl (2N) was added to 3ml of the sugar extract and heated in a water bath at 60^{o}C for 30 min. The solution was cooled then neutralized and the total volume was made up to 15 ml with distilled water. The total soluble sugars were determined colorimetrically by using alkaline potassium ferricyanide solution. The amount of total soluble sugars was calculated according to the standard curve of glucose.

Non-reducing sugars = total soluble sugars - reducing sugars.

4. Effect of ATPase inhibitor on disease incidence of sudden wilt

Sixteen healthy plants of cantaloupe (Galia hybrid) cultivated under plastic house conditions in sterilized soil (pots 30 cm diameter) were chosen to study the effect of inhibition of ATPase activity on sudden wilt incidence. The specific inhibitor of ATPase, Omeprazole ($C_{17}H_{19}N_3O_3S_1$): benzimidazole, 5-methoxy-2-[[(4-methoxy-3, 5-dimethyl-2-pyridinyl) methyl] sulfinyl]-1h-benzimidazole) was produced by Schwartz Pharma Co., was dissolved in distilled water to get a concentration of 50 ppm. Eight plants were irrigated one time by water containing ATPase inhibitor (50 ml/pot), meanwhile, other 8 plants were irrigated by water free of ATPase inhibitor. Then plants were irrigated when need and left for further observation of sudden wilt incidence as leaf epinecity and vein collapse.

5. Effect of ATPase inhibitor on morphological characters of ATPase inhibitor treated and untreated plants.

Healthy and diseased plants were subjected for measuring the growth characters of booth root and shoot systems.

6. Statistical analysis:

All experiments were set up in a complete randomized design. Data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS Institute, Inc., 1996). Means were separated by Duncan's multiple range test at P< 0.05 level.

RESULTS AND DISCUSSION

1. Activity of ATPase in healthy and diseased cantaloupe plant roots.

Liberation of phosphate from ATP was determined as a function of ATPase activity in roots of diseased or healthy plant roots. Such activity was determined in plant roots when disease symptoms started to appear on plants (72 days from seeding).

Data presented in fig (1) illustrated that the activity of ATP was significantly higher in healthy plants comparing with diseased ones. The activity in healthy roots was three folds that of the diseased plants.

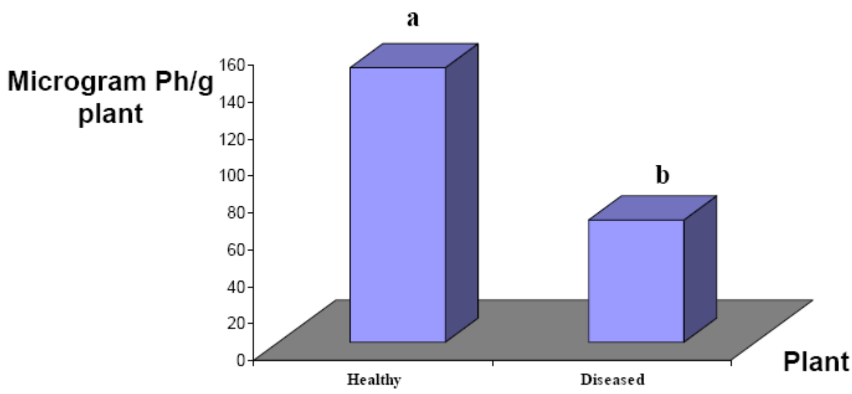

Fig (1): Effect of sudden wilt disease on root ATPase activity.
Significant difference using Duncan's multiple range test at P< 0.05 level.

2. Total, reducing and non-reducing sugars in roots of diseased and healthy cantaloupe plants.

All figures of sugars (i.e. total, reducing and non reducing) were determined in roots of healthy and diseased plants. Data are presented in Table (1).

Total soluble sugars in healthy roots were significantly higher than in diseased ones. They reached 3.82 mg/g fresh weight in healthy roots while it was 1.41 mg/g fresh weight in diseased roots.

On the other hand no significant difference was observed between healthy and diseased plants in non reducing sugars.

Reducing sugars were clearly reduced in root tissues of the diseased plants. They reached 2.65 mg/g fresh weight in healthy roots and 0.37 mg/g fresh weight in diseased roots.

Table (1): soluble sugars analysis of root system of both healthy and diseased plants (mg/g fresh weight).

Plant	Soluble sugars		
	Reduced Sugars	Non Reduced Sugars	Total Soluble Sugars
Healthy	2.65[a]	1.22[a]	3.83[a]
Diseased	0.37[b]	1.05[a]	1.41[b]

Values followed by the same latter are not significantly different at $P = 0.05$ according to Duncan's multiple range tests.

3. Effect of ATPase inhibitor on disease incidence and morphological parameters of cantaloupe plants.

After 35 days from treatment of plants by ATPase inhibitor, disease syndrome started to appear on some of the treated plants only and no any disease syndrome were observed on non-treated plants. Five plants out of 8 treated plants showed disease syndrome (table 2).

Morphological parameters of diseased plant were determined. Treated plants were divided into two categories, first one is plants showed symptoms (5 plants) and second one is plants appeared healthy.

Table (2): Effect of ATPase inhibitor on incidence of sudden wilt (sterilized soil) after 35 days from the treatment.

Treatment	No. of treated plants	No. of plants showed sudden wilt.	% Plants showed sudden wilt.
Treated by ATPase inhibitor	8	5	62.5
Without treatment	8	0	0.0

Growth characters of both root and shoot systems were measured. Data of this study are presented in table (3).

Comparing morphological parameters of diseased plants and healthy ones that treated by ATPase inhibitor indicated that such parameters were significantly lower in diseased plants than of healthy plants.

No significant differences were observed between healthy roots of plants treated by ATPase inhibitor or non treated healthy plants, but significant increase was observed in foliage of healthy treated plants.

Table (3): Effect of ATPase inhibitor on morphological features of cantaloupe plants showed sudden wilt comparing to healthy ones after 35 days from the treatment.

Treatment	Root system			Shoot system		
	L.(cm)	W. (gm)	V. (ml)	L.(cm)	W. (gm)	V. (ml)
Diseased treated	32^{b}	16.282^{a}	16.76^{a}	221^{b}	241.964^{b}	272.8^{b}
Healthy treated	51.5^{a}	19.1366^{a}	19.3^{a}	367.33^{a}	925.986^{a}	1072.7^{a}
Healthy non treated	54.1^{a}	20.758^{a}	22.02^{a}	268.86^{ab}	399.99^{ab}	481.88^{ab}

Values followed by the same latter are not significantly different at P = 0.05 according to Duncan's multiple range tests.

DISCUSSION

Sudden wilt disease of cantaloupe started to breakthrough when hybrid cvs began to widely cultivated (Martyn, 2002). Disease etiology led to great conflict between specialists. Etiology of such disease ranged from agricultural practices (i.e., excess or decrease of available water for plants, soil type (sandy or sandy loam etc.) or fertilizers to phytopathogenic fungi such as *Monosporascus cannonballus* (Reuveni *et al*., 1983 and Hamza *et al.,* 2007), *Acremonium cucurbitacearum* (Garcia - Jimenez *et al*., 1989) , *A. cucurbitacearum , Monosporascus cannonballus , Rhizopycnis vagum , Fusarium solani, Macrophomina phaseolina , Pythium sp. and Verticillium dahliae* (Bruton *et al*., 2000) and three factors have been implicated with the cause of the disease complex; i.e.: agronomic, pathogen and plant factors (Horlock and Akem 2004).

Disease syndrome started to appear on plants at flowering stage and progress on plant till its death. Syndrome started as leaf epinecity till sever wilt without discoloration (i.e., chlorosis) and ended by plant death.

Since mineral and water absorption is active process depend on energy liberated from hydrolysis of ATP by ATPase (Levitt, 1946), therefore, the activity of ATPase was determined in healthy and diseased plant roots when the first symptom of syndrome started to appear.

Obtained data indicated that the activity of ATPase in roots of diseased plants was less than that found in the roots of healthy plants to very great extent. Do such decrease in ATPase activity is a primary reason of disease incidence?

In this case, inhibition of ATPase led to induce such phenomenon on 62.5 % of treated plants. If inhibition of ATPase activity is the causal of such phenomenon, why disease syndrome did not appear on all treated plants? It could be suggested that there were genetic variation between plants whereas 5 plants were responded to the inhibitor out of 8 treated plants.

For answering such question, sugar content (i.e., reducing, non reducing and total sugars) was determined in healthy and diseased plant roots. Data obtained clearly indicated that total and reducing sugars were very high in root of healthy plants than those found in diseased plant roots. These results are in agreement with Jang Hoon (2004) who found that quick fruit removal of plant, led to reduce disease severity than on plants with fruits. This treatment led to increase root growth and carbohydrate accumulation in cantaloupe roots. He postulated that root sugar concentrations affected infection efficiency and disease progress of *Monosporascus* root rot and vine decline.

Reducing sugars undergo different metabolic processes, i.e., glycolysis and tricarboxylic acid cycle, through which ATP is synthesized. Dose the decrease of ATPase refer to the great decrease of reducing sugars in plant root? This point needs further studies.

The role of ATPase activity in disease incidence was studied by inhibit its activity using specific ATPase inhibitor (Omeprazole). In order to exclude the role of phytopathogenic fungi in disease incidence, eight plants cultivated in sterilized soil were irrigated by 50 ppm of Omeprazole, then irrigated by water free of inhibitor and left for further observation; other 8 plants were served as control. Thirty five days later, symptoms of disease started to appear on 5 plants out of 8 treated plants. Non treated plant still healthy.

As the writer as aware, no previous attempts was carried out before to induce disease syndromes artificially.

According to the obtained results it could be concluded that reduction of ATPase activity may be the predisposing agent for the presence of many phytopathogenic fungi associated with root system of diseased plants and owing to the decrease of total and reducing sugars and ATPase activity, that the plant became unable to defend itself against these pathogens.

REFERENCES

Ackerson, R.C. (1981).Osmo regulation in cotton in response to water stress, II- Leaf carbohydrate states in relation to osmotic adjustment. *Plant Physiol. 67:* 489-493.

Aegerter ,B. J., Gordon, T.R., and Davis, R.M. (2000). Occurrence and pathogenicity of fungi associated with melon root rot and vine decline in California. *Plant Disease* 64:224-230.

Bisswanger,H.(2004). Practical Enzymology. WILEY-VCH Verlag Gmbh&Co. KGaA,Weinheim, pp.:255.

Bruton B.D., Garcia-Jimenez J, Armengol J, Popham TW, (2000).Assessment of virulence of *Acremonium cucurbitacearum* and *Monosporascus cannonballus* on *Cucumis melo*. *Plant Disease 84:* 907–13.

Horlock,C. and Akem, C. (2004). Sudden wilt of melons (watermelon, rockmelon and honeydew). Dept. Primary Industries and Fisheries(DPI&F) note, Queensland Government. 21 Jan. 2004. http://www.dpi.qld.gov.au/horticulture/11648.html.

García-Jiménez, J., Veláquez, M. T., Jordá, C., and Alfaro-García, A. (1994). *Acremonium* species as the causal agent of muskmelon collapse in Spain. *Plant Disease* 78:416-419.

Hamza, H., Belkadhi, M.S., Triki, M.A., and Zouba, A. (2007). Morphological and biological studies of *Monosporascus cannonballus,* the cause of root rot and vine decline of melon in Southern Tunisia. *Tunisian Journal of Plant Protection 2*: 71-77.

Jang Hoon, (2004) Effect of fruit removal on carbohydrate concentrations of cantaloupe *(Cucumis melo L.)* roots in naturally infested soil with *Monosporascus cannonballus*. http://handle.tamu.edu/1969.1/259.

Levitt, J. (1946).The Thermodynamics of active (Non-osmotic) water Absorption. *Plant Physiol.89*: 514-525.

Martyn, R.D. (2002). *Monosporascus* root rot and vine decline of melons. The Plant Health Instructor. DOI: 10.1094/PHI-I-2002-0612-01

Pivonia, S., Cohen, R., Kafkafi, U., Ben Ze'ev, I. S., and Katan, J. (1997). Sudden wilt of melons in southern Israel: Fungal agents and relationship with plant development. *Plant Disease 81*:1264-1268.

Pivonia, S., Cohen, R., Katan, J. and Kigel, J. (2002) Effect of fruit load on the water balance of melon plants infected with *Monosporascus cannonballus*. *Physiol. and Molec. Plant Pathol. l60*: 39-49.

Reuveni, R., Krikun, J., and Shani, U. (1983).The role of *Monosporascus eutypoides* in a collapse of melon plants in an arid area of Israel. *Phytopathology 73*:1223-1226.

Sales,R. J., Oliveira, O. F., Senhor, R. F. and Alves, M. Z. (2003) *Monosporascus cannonballus* causal agent of collapse in melon plants in the State of Rio Grande de Norte, Brazil. *Fitopatol.Bras. 28 (5):* 567

SAS Institute, Inc. (1996). SAS/STAT User's Guide, Version 6, 12th ed. Vol. *2*, 846 pp. SAS Institute, Inc. Cary, North Carolina.

Schales, O. and Schales, S.S. (1945). A simple method for determination of glucose in blood. *Arch. Biochem. 8*:285

نشاط انزيم ATPase وعلاقتة بمرض الذبول المفاجئ على الكنتالوب.

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يعد مرض الذبول المفاجئ أحد أهم المشاكل التى تواجه زراعة الكنتالوب فى مصر والعالم. أدى ذلك المرض الى تضارب واضح بين الباحثين فيما يخص المسبب المرضى، حيث أرجعه بعض المتخصصين الى العمليات الزراعية واخرون الى الفطر كمسبب مرضى.

فى هذه الدراسة ، افترض بأن المسبب لهذه الظاهرة قد يعزى لخلل فى امتصاص الماء بواسطة جذر النبات ناشئ عن اصابة جذر النبات بالفطر الممرض. لذا تم تقدير نشاط انزيم ATPase والسكريات فى الجذور السليمة والمصابة.

أوضحت النتائج انخفاض نشاط انزيم ATPase بشدة فىجذور النباتات المصابة بالفطر مقارنة بالجذور السليمة. أظهرت النتائج كذلك ان السكريات الكلية والمختزلة صارت شديدة الأنخفاض فى جذور النباتات المصابة مقارنة بالسليمة.

أدى رى نباتات غير معدية بالفطرفى مرحلة الأزهار بواسطة ماء محتوى على مثبط لأنزيم ATPase الى ظهور أعراض المرض على خمسة نباتات من ثمانية (بنسبة 62.5 %).

النتائج المتحصل عليها أدت الى وضع فرضية بأن انخفاض نشاط ATPase والسكريات المختزلة قد يكون العامل المهئ والمؤدى لسهولة اصابة النباتات بالمسببات المرضية المختلفة مما يؤدى لظهور أعراض مرض الذبول المفاجئ على الكنتالوب.