

Using Protein(s) Marker to Differentiate Between Resistant and Susceptible Cantaloupe Plants to Sudden Wilt Disease

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Cantaloupe plants became increasingly suffer from sudden wilt disease caused by *Monosporascus cannonballus*. This disease start to appear on plants at fruit set stage. The present study was aimed to find out biochemical marker could be differentiate between resistant and susceptible plants to this disease. The attention was focused on protein marker. Soluble protein was extracted from healthy and diseased plant roots as well as from 16th leaf of diseased and healthy ones. Moreover, protein of cantaloupe seeds was also extracted. Protein extraction was subjected to SDS-electrophoresis. Band with M.W 72.08 K.Da was found only in healthy roots, and a band with M.W 25.31 K.Da was found in healthy plant leaves and absent from diseased ones. From seeds band with M.W 25.66 K.Da was found in 50% of cantaloupe seeds (Galia hybrid). This protein marker would be useful for selection of transplants for cultivation.

Keywords: Cantaloupe, *Monosporascus cannonballus*, protein marker and sudden wilt.

Searching for marker related to plant disease resistance became very attractive models for searching to resistant plants among plant population. These markers could be classified as: biological, morphological, biochemical, cytological and molecular (Ribaut and Hoisington 1998; and Rosyara, *et al.*, 2007 and Benali, *et al.*, 2011). In the recent years, many investigators directed their interest for searching for protein marker correlate with plant disease resistance (Ribaut *et al.*, 2001; Dekkers, 2004; Rosyara, 2006 and Benali, *et al.*, 2011). This protein marker could be traced by very simple procedure, *i.e.* ELISA.

Sudden wilt disease (Martyn, 2002) also named as *Monosporascus* root rot and vine decline of melons (MRR/VD) (Martyn, 2007), has become increasingly more prominent in the last years. Diseased vines exhibited severe stunting, yellowing and at a late stage complete collapse of the leaf canopy occurs. The roots showed discoloration, discrete lesions on all root systems and loss of secondary and tertiary feeder roots. Numerous perithecia were observed on the secondary and tertiary roots only (Karlatti *et al.*, 1997). Fruit load is always associated with vine decline symptom expression (Wolff, 2000). According to Bruton *et al.* (1995) cucurbit crops that develop vine decline, generally, exhibit no foliar symptoms until about 10 to 20 days before harvest.

Survey of such phenomenon in widely cultivated Governorates by cantaloupe revealed that disease incidence varied between nil to 100% (Helmy, 2009).

The present investigation aimed to find out biochemical marker could be differentiate between plants predispose or not to the disease in order to detect it in cantaloupe plants population. This marker could be useful for selection of seedlings for plantation.

Materials and Methods

This study was carried out at plastic house and molecular plant pathology Lab. Faculty of Agriculture, Ain Shams University during 2008-2009.

Preparation of plant materials:

Under plastic chamber conditions, Galia seeds (Melon Galia hybrid F1 PMR imported from France) were planted in trays (84 cell) contained pasteurized peat moss-vermiculite mixture 2:1 to prepare transplants (12 days old). Transplants were transplanted in pots 30 cm diameter containing clay soil obtained from subsoil surface of Faculty of Agriculture, Ain Shams University at Shubra El-Kheima, Qalybeia Governorate, Egypt. Eighty pots were cultivated, one plant was left for each pot, and pots were fertilized and irrigated as usual. When plants reached the flowering stage and fruit set, symptoms of sudden wilt began to appear on some plants (75 days from transplanting). Healthy and diseased plants were used to determine some molecular proteins.

Extraction of soluble protein:

Plant leaves or roots of infected and healthy plants were removed then one gram of plant sample was treated with liquid nitrogen then ground with 2 ml Lan's buffer (2x), using mortar and pestle. Samples were transferred in Eppendorf tubes, and kept at 0°C over night, centrifuged for 20 min at 12.000 rpm at 4°C. Supernatants containing water soluble protein fraction were subjected for further analysis by SDS electrophoresis (Okuno *et al.*, 1991). Also, total protein of sixteen seeds of Galia hybrid was randomly extracted.

Sodium dodecyl sulphate gel electrophoresis (SDS-PAGE) was performed on protein fraction of root, foliage and seeds according to the method of Laemmli (1970) as modified by Studier (1973).

Visualization of protein bands was carried out using Coomassie Brilliant Blue R-250 (Borejdo and Flynn, 1984).

Results

SDS - Polyacrylamide electrophoresis of total proteins:

a. Roots:

Total proteins were extracted from roots of healthy and diseased cantaloupe plants at the first stage of disease incidence (75 days after transplanting). Such protein was subjected to SDS-PAGE electrophoresis. Seven protein bands were visualized using Coomassie Brilliant blue reagent indicating molecular weights as 161.97 (P1), 101.48 (P2), 89.47 (P3), 73.31 (P4), 72.08 (P5), 54.14 (P6) and 49.07 (P7) kilo Dalton (K.Da) (Table 1 and Fig. 1).

Table 1. Electrophoretic pattern for protein bands of roots of healthy and diseased cantaloupe plants cultivated in naturally infested soil with *Monosporascus cannonballus*

Number of bands * and K.DA(K.Da)	Lane number of roots																	
	Diseased									Healthy								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
P1	161.97	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-
P2	101.48	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P3	89.47	-	-	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+
P4	73.31	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P5	72.08	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
P6	54.14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P7	49.07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

* P= protein band and K.Da= Molecular weight.

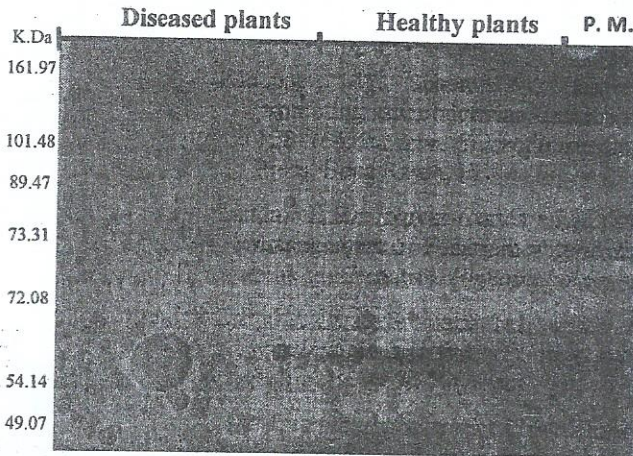


Fig. 1. Electrophoretic profile of protein bands (P) of roots of healthy and diseased cantaloupe plants cultivated in non-autoclaved soil (P.M.= Protein marker).

The most interesting band was the band with molecular weight 72.08 K.Da. This band is completely absent in diseased cantaloupe plant roots. However, it was present in all healthy ones. The same observation was recorded for P3 (73.31 K.Da), except that detected at one lane of diseased sample (lane No 3), but disappeared from the other 8 lanes of diseased samples.

On the other hand, the band No 1 (161.97, K.Da) was absent in all samples of diseased roots as well as the healthy ones, except on two lanes of the healthy roots (lanes 10 and 13), where it was detected. However, the rest bands of protein, i.e. P2 (101.48, K.Da), P4 (73.31, K.Da), P6 (54.14, K.Da) and P7 (49.07, K.Da) were clearly detected at all lanes in diseased cantaloupe roots as well as in healthy ones.

b. Leaves:

Protein content of 16th leaf of both healthy and diseased cantaloupe plants were separated during the appearance of first symptoms of sudden wilt (75 days after transplanting). Soluble protein was extracted and subjected to SDS-PAGE electrophoresis. Nine bands were visualized with different molecular weights, i.e. 141.25, 77.74, 66.89, 57.14, 43.50, 29.85, 25.31, 21.15 and 14.25 K.Da (Table 2 and Fig. 2).

Table 2. Electrophoretic pattern of protein bands of leaves of healthy and diseased cantaloupe plants grown in non-autoclaved soil

Number of* bands and K.DA (K.Da)	Lane number of leaves																				
	Diseased									Healthy											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
P1 141.25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P2 77.74	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P3 66.89	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-
P4 57.14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P5 43.50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
P6 29.85	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
P7 25.31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+
P8 21.15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P9 14.25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

* P= protein band and K.Da= Molecular weight.

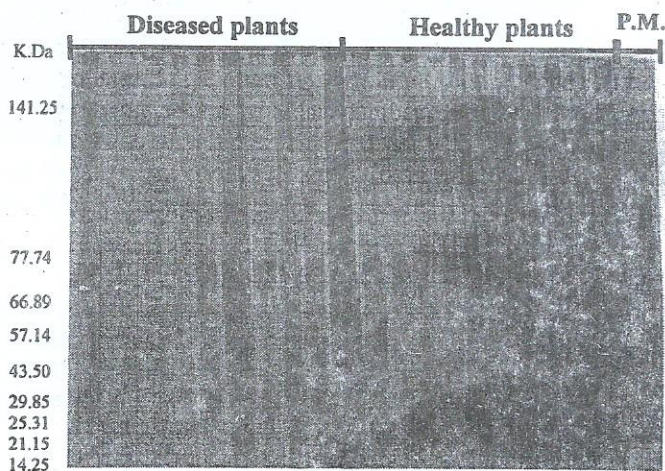


Fig. 2. Electrophoretic profile of protein bands of leaves of healthy and diseased cantaloupe plants cultivated in non-autoclaved soil.

The bands No. 1, 4, 8 and 9 were detected at all lanes of diseased as well as healthy leaves without any difference. However, band No 7 (P7, 25.31K.Da.) was completely disappeared at all lanes of diseased samples, in spite of it was separated on all lanes of healthy samples. Similar results were recorded for P2 (77.74, K.Da) as it disappeared at 7 lanes only (No. 1-7), but still present at lanes No. 8, 9 and 10 of diseased samples as in the healthy. On the other hand, another different record was detected with three bands, *i.e.* P3 (66.89 K.Da), P5 (43.50K.Da) and P6 (29.86 K.Da) as they were completely disappeared in all lanes of diseased samples, but were detected on some, but not all, lanes of healthy (lanes 15-18; lanes 17 and 18; lanes 16-21) for the three bands, respectively.

c. Seeds:

Sixteen Galia seeds were randomly taken for soluble protein extraction and subjected for SDS-PAGE electrophoresis. Eight bands were visualized with molecular weights, of 115.60, 96.21, 67.38, 29.60, 27.39, 25.66, 23.43 and 21.55 K.Da for bands No 1-8, respectively (Table 3 and Fig. 3).

Table 3. Electrophoretic pattern of protein bands of cantaloupe seeds (Galia hybrid)

Number of bands * and K.DA (K.Da)		Lane number of cantaloupe seeds (Galia hybrid)															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
P1	115.60	-	-	+	+	+	+	+	+	+	+	-	-	-	+	+	+
P2	96.21	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P3	67.38	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P4	29.60	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-
P5	27.39	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P6	25.66	-	-	+	+	+	+	+	+	-	-	+	-	-	-	+	-
P7	23.43	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+
P8	21.55	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	+

* P= protein band and K.Da= Molecular weight.

Bands with molecular weight 96.21, 67.38 and 27.39 K.Da were found in all seed samples. Other bands were found differentially in all seeds. The band with M.W. 115.60 K.Da was absent in seed samples at lanes No. 1, 2, 11, 12 and 13, whereas, the band with M.W. 29.60 K.Da was absent in seed samples at lanes No. 1, 2, 13, 14, 15 and 16. However, the band with M.W. 23.43 K.Da was absent in seed samples at lanes No. 10, 11, 12 and 13. The band with M.W. 21.55 K.Da was absent in most seed samples. The band with M.W. 25.66 K.Da was appeared in 50 % of seed sample. This band was the band that found in roots of healthy cantaloupe plants and absent of roots in diseased ones.

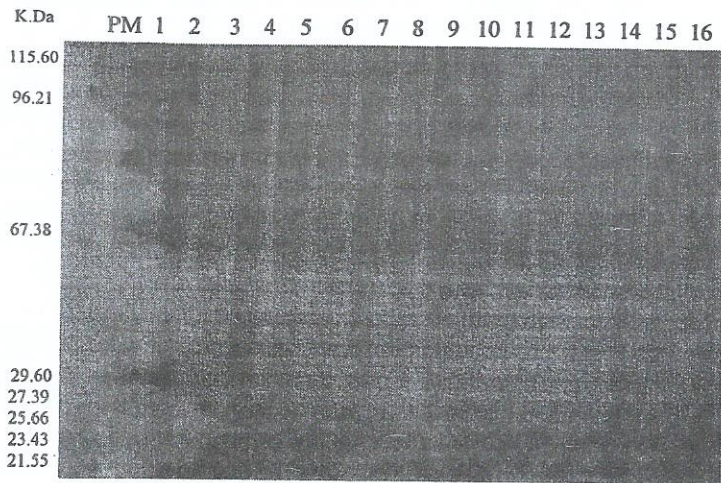


Fig. 3. Electrophoretic profile of protein bands of cantaloupe seeds (*Galia* hybrid).

Discussion

Cantaloupe plants are suffer from sudden wilt disease caused by *Monosporascus cannonballus*, which start to appear at fruit set stage causing very great economic losses. All practical means of disease management were failed to reduce such losses (Hames and Rickwood, 1990; Gentzbittel *et al.*, 1998; Martin *et al.*, 2003; Belkhadir *et al.*, 2004; Dubcovsky, 2004; Goodman, 2004 and Collard and Mackill, 2007).

The present investigation was aimed to search for molecular marker could differentiate between susceptible and resistant plants to the disease. The efforts were focused to find out this marker among soluble protein fraction.

Soluble protein was extracted from healthy plant roots and leaves (75 days after transplanting), as well as from diseased ones and subjected to SDS-PAGE. Moreover, soluble protein was extracted from cantaloupe seeds prepared for cultivation and subjected to the same procedure.

SDS-PAGE electrophoresis of roots of healthy or diseased plants revealed the presence of seven protein bands, their K.DA ranged from 49.07 to 161.97 K.DA. From these protein bands, bands of K.DA 72.08 and 89.47 K.DA. are consider very interesting and existed bands, they were appeared in healthy plant roots, and completely absent in roots of diseased plants.

Samples of leaves represented healthy and diseased plants were collected when the first sign of sudden wilt appeared (75days after transplanting). Proteins were extracted then subjected to SDS-PAGE.

SDS-PAGE separated leaf proteins to 9 bands with K.DA ranged from 14.25 to 141.25 K.Da. The most interesting band was the band with K.DA 25.31 K.Da., as it appeared in healthy plant leaves, and completely absent from leaves of diseased plants. This result was previously found in roots with two bands.

This finding could be used to differentiate between susceptible and resistant plants to sudden wilt pathogen(s). Using these protein bands, could be help in detection of gene(s) of resistance for further breeding and could be used to separate plants that could resist infection using ELISA technique (Crosby, 2001 and Crosby *et al.*, 2002)

In order to prove the occurrence of genetic segregation in cantaloupe seeds prepared for commercial plantation, some imported seeds of cantaloupe were taken and their protein was extracted and subjected to SDS-PAGE.

Data obtained indicated the presence of great variation among seeds. Protein band with K.DA 25.66 K.Da. may be the same band of leaves (K.DA 25.31 K.Da.), which could be used as a marker of resistance.

In this direction, Crosby (2000) studied the inheritance of melon root volume in relation to its infection by *M. cannonballus*, and he found existence of quantitative inheritance and he postulated of occurrence of segregation for resistance. Although the number of genes that control tolerance is unknown, results indicate gene action is additive in nature (Cohen *et al.*, 1996) and tolerance inheritance is likely complex (Crosby, 2000). However, high heritability of several root traits suggests the possibility of making efficient selections for improved tolerance. Although the tolerance mechanisms are possibly both morphological and biochemical, the mechanisms are not extremely important as long as improvement is possible in breeding programs (Crosby, 2000). Correlation analysis indicated that by selecting for shorter vine length and more substantial root systems disease tolerance could be improved (Martyn and Miller, 1996). Since, certain non-genetic factors such as fruit maturity and environmental stresses may contribute to disease progression (Pivonia, *et al.*, 1999; Pivonia *et al.*, 2002 and Wolff, 1995). These factors need to be studied further and taken into account in breeding programs when selecting for tolerant plants (Cohen *et al.*, 2000).

An important point of view from the whole data in the present study, that different genetical backgrounds were detected in cantaloupe seeds. So, it must be taken in to consideration, in order to prevent losses due to sudden wilt phenomenon, the selection of genomes carrying resistant genes during seed production.

References

- Belkhadir, Y.; Subramaniam, R. and Dangl, J. 2004. Plant disease resistance protein signaling: NBS-LRR proteins and their partners. *Curr. Opin. Plant Biol.*, 7: 391-399.

- Benali, S.; Mohamed, B.; Jamel Eddine, H. and Neema, C. 2011. Advances of molecular markers application in plant pathology research. *Europ. J. Sci. Res.*, **50**: 110-123.
- Borejdo J. and Flynn, C. 1984. Electrophoresis in the presence of Coomassie brilliant blue R-250 stains polyacrylamide gels during protein fractionation, *Analytical Biochem.*, **140**: 84-86.
- Bruton, B.; Davis, R. and Gordon, T. 1995. Occurrence of *Acremonium* sp. and *Monosporascus cannonballus* in the major cantaloupe and watermelon growing areas of California. *Plant Dis.*, **79**: 754-758.
- Cohen, R.; Pivonia, S.; Burger, Y.; Edelstein, M.; Gamliel, A. and Katan, J. 2000. Toward integrated management of *Monosporascus* wilt of melons in Israel. *Plant Dis.*, **84**: 496-505.
- Cohen, R.; Elkind, Y.; Burger, Y.; Offenbach, R. and Nerson, H. 1996. Variation in the response of melon genotypes to sudden wilt. *Euphytica*, **87**: 91-95.
- Collard, B.C.Y. and Mackill, D.J. 2007. Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. *Philos. Trans. R. Soc. Lond. Biol. Sci.*, **17**: 1-16.
- Crosby, K. 2000. Impact of *Monosporascus cannonballus* on root growth of diverse melon varieties and their F1 progeny in the field. *Subtropical Plant Sci.*, **52**: 8-11.
- Crosby, K.M.; Miller, M.E. and Wolff, D.W. 2002. Screening plant introductions of *Cucumis melo* L. for resistance to *Monosporascus cannonballus*. Pages: 188-191. In: *Cucurbitaceae*. Maynard, D.N. (ed.). ASHS Press, Naples, FL, USA.
- Crosby, K.M. 2001. Screening *Cucumis melo* L. agrestis germplasm for resistance to *Monosporascus cannonballus*. *Rio Grande Hort. Soc.*, **53**: 24-26.
- Dekkers, J.C.M. 2004. Commercial application of marker- and gene-assisted selection in livestock: Strategies and lessons. *J. Anim. Sci.*, **82**: 313-328.
- Dubcovsky, J. 2004. Marker-assisted selection in public breeding programs: the wheat experience. *Crop Sci.*, **44**: 1895-1898.
- Gentzbittel, L.; Mouzeyar, S.; Badaoui, S. and Mestries, E. 1998. Cloning of molecular markers for disease resistance in sunflower (*Helianthus annuus* L.). *Theor. Appl. Genet.*, **96**: 519-25.
- Goodman, M.M. 2004. Plant breeding requirements for applied molecular biology. *Crop Sci.*, **44**: 1913-1914.
- Hames, B. and Rickwood, D. 1990. *Gel Electrophoresis of Protein: A Practical Approach*. TRL Publishing Co., London, UK.
- Helmy, Karima G. 2009. Molecular Studies on Sudden Wilt Disease of Cantaloupe in Egypt. Ph.D. Thesis. Plant Pathol. Dept., Fac. Agric., Ain Shams Univ., Cairo, Egypt. 147pp.

- Karlatti, R.; Abdeen, F. and Al-Fehaid, M. 1997. First report of *Monosporascus cannonballus* on melons in Saudi Arabia. (Abstract) *Plant Dis.*, **81**: 1215.
- Laemmli, U. 1970. Cleavage of structural head of bacteriophage. *Nature*, **227**: 680-685.
- Martin, G.B.; Bogdanove, A.J. and Sessa, G. 2003. Understanding the functions of plant disease resistance proteins. *Ann. Rev. Plant Biol.*, **54**: 23-61
- Martyn, R. 2002. *Monosporascus* root rot and vine decline of melons. *The Plant Health Instructor*, <http://nsdl.org/resource/2200/20101027202339119T> (available on line 2011).
- Martyn, R. 2007. Late-season vine declines of melons: Pathological, cultural, or both. *Acta Hort. Sci.*, **731**: 345-356.
- Martyn, R. and Miller, M. 1996. *Monosporascus* root rot/vine decline: An emerging disease of melons worldwide. *Plant Dis.*, **80**: 716-725.
- Okuno, T.; Nakayama, M.; Okajima, N. and Furusawa, I. 1991. Systemic resistance to downy mildew and appearance of acid soluble proteins in cucumber leaves treated with biotic and abiotic inducers. *Ann. Phytopathol. Soc. Japan*, **57**: 203-211.
- 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 Mole. Plant Pathol.*, **60**: 39-49.
- Pivonia, S.; Cohen, R.; Kigel, J.; Katan, J. and Levite, R. 1999. Effect of soil temperature on the development of sudden wilt disease of melons. (Abstract) Presentations at the 20th Cong. Israeli Phytopathol. Soc.
- Ribaut, J.M., Betran, J.; Dreher, K.; Pixley, K. and Hoisington, D. 2001. Marker assisted selection in maize: Strategies, examples and costs. Abstract, Plant and Animal Genome IX Conference W40 06.
- Ribaut, J.M. and Hoisington, D.A. 1998, Marker assisted selection: new tools and strategies. *Trends Plant Sci.*, **3**: 236-239.
- Rosyara, U.R.; K.L. Maxson-Stein; K.D. Glover; J.M. Stein; J.L. Gonzalez-Hernandez. 2007. Family-based mapping of FHB resistance QTLs in hexaploid wheat. Proc. National Fusarium head blight. Kansas City, MO, USA.
- Rosyara, U.R. 2006. Requirement of robust molecular marker technology for plant breeding applications. *J. Plant Breed. Gr.*, **1**: 67-72.
- Studier, F.W. 1973. Analysis of bacteriophage T 7 early RNAs and proteins of slab gels. *J. Mole. Biol.*, **79**: 237-248.
- Wolff, D. 1995. Fruit load affects *Monosporascus* root rot/vine decline symptoms expression. Pages: 87-88. In: *Melon Production System in South Texas*. Miller, M.E. (ed.). Ann. Res. Rep. Texas Agric. Exp. Station, Weslaco, TX, USA.

Wolff, D. 2000. Genotype, fruit load, and temperature affect *Monosporascus* root rot/vine decline symptom expression in melon (*Cucumis melo* L.). (Abstract) Proc. 6th Eucarpia Meeting on Cucurbit Genetics and Breeding Cooperative. Pp. 280-284.

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استخدام علامات بروتينية للتفريق بين نباتات الكتنلوب المقاومة والقابلة للأصابة بمرض الذبول المفاجيء

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تعاني نباتات الكتنلوب بشكل متزايد من مرض الذبول المفاجيء المتسبب عن الفطر *Monosporascus* كائناتولوس. هذا المرض يبدأ في الظهور على النباتات في مرحلة الإثمار. وتهدف هذه الدراسة لمعرفة العلامات الجزيئية التي يمكن التفريق بها بين النباتات المقاومة والقابلة للأصابة بهذا المرض. وقد تركزت الاهتمامات على العلامات البروتينية. تم استخلاص البروتينات من جذور و أوراق نباتات سليمة و أخرى مصابة بمرض الذبول المفاجيء في الكتنلوب. وتم استخلاص البروتين من بذور كتنلوب بعد التفريد الكهربائي للبروتين ، تم الحصول على حزمة ذات وزن جزيئي ٧٢,٠٨ ك دالتون في جذور النباتات السليمة ، الحزمة ذات الوزن الجزيئي ٢٥,٢٦ ك دالتون وجدت في أوراق النباتات السليمة ولم تتواجد في أوراق النباتات المصابة بالمرض. وجدت الحزمة البروتينية ذات الوزن الجزيئي ٢٥,٦٦ ك دالتون في ٥٠% من بذور الكتنلوب (هجين جاليا). يعد هذا البروتين علامة جيدة تفيد في انتخاب شتلات الكتنلوب لزراعتها.