Occurrence of rhizomania of sugarbeet in Egypt associated with beet necrotic yellow vein benyvirus infection

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ABSTRACT

The presence of beet necrotic yellow vein virus (BNYVV) associated with rhizomania of sugarbeet (Beta vulgaris L.) was confirmed for the first time in Egypt in some cultivations of sugarbeet in El-Fayoum and Giza governorates. The causative virus was soil-transmitted through the viruliferous Polymyxa betae Keskin fungus. Confirming results were brought about through mechanical inoculation, host range studies, visual and light microscopy examination of infected sugarbeet roots, infectivity studies using the viruliferous fungus, as well as serological testing using authentic monoclonal and polyclonal antisera.

The virus was transmitted mechanically to sugarbeet, Spinacia oleracea L., Chenopodium amaranticolor Coste & Reyn, and C. quinoa Willd.. Infected roots showed malformation necrosis, and proliferation of large numbers of lateral roots. Light microscopy examination of such roots indicated the presence of fungal cystosori associated with root cell death and root discoloration.

Serologic examination using monoclonal and/or polyclonal antisera for BNYVV and beet soil borne mosaic virus (BSBMV) confirmed reactions of these antisera with infected roots of sugarbeet collected from fields as well as sugarbeet seedlings exposed to soil contaminated with the viruliferous fungus.

Key words: *Benyvirus, Furovirus pladmodiophoromycetes, Polymyxa betae Keskin, rhizomania, dot and tissue blotting immunoassays*

INTRODUCTION

Beet necrotic yellow vein virus (BNYVV), is a benyvirus (Tamada and Mayo, 1997; van Regenmortel *et al.*, 2000) previously located among furoviruses (Richards and Tamada 1992; Rush and Heidel, 1995; Tamada and Baba, 1973; Wisler *et al.*, 1999a). Currently, the Benyvirus group includes BNYVV and sugarbeet soil borne mosaic virus (BSBMV) (van Regenmortal *et al*, 2000) and possibly rice stripe necrosis virus (Morales *et al.*, 1999). BNYVV is transmitted by the fungus *Polymyxa betae* Keskin, a member of the *Plasmodiophoromycetes* (Abe and Tamada, 1986), and causes rhizomania, crazy root, root madness, of sugarbeets (*Beta vulgaris* L.). The fungus can survive in infested soil for many years in thick-walled fungal resting structures called cystosori (Abe and Tamada, 1986). Rhizomania is one of the most serious diseases of sugarbeet (Tamada, 1975; Torrance *et al.*, 1988; Rush and Heidel, 1995).

Rhizomania can greatly reduce sugar yield by reducing either the tonnage or sugar content, or both, upon harvesting (Heidel and Rush, 1994; Rush and Heidel, 1995).

Symptom expression varies greatly, with some infected plants occasionally appearing healthy. Root symptoms observed after early infection include a massive proliferation of feeder roots that appear brown due to infestation by cystosori and root cell death. Infected taproots show necrosis in the vascular system. The storage roots in susceptible varieties are rotted and constricted, thus resembling the shape of a wineglass. Very late infections may result in no obvious symptoms. Foliar symptoms sometimes include the proliferation of small leaves at the crown. Due to inefficiency of infected roots for water and nutrient uptake, leaves are narrow, with long petioles, flaccid, and express symptoms of water stress or nitrogen deficiency. Usually infected plants exhibit temporary wilting in hot weather. Rarely veinal yellowing and necrosis of leaf tissues are seen unless infection becomes systemic and the virus can be recovered from leaves. Diseased plants usually occur in patches and not scattered. Disease severity is usually great in depressed or compacted poorly drained portions of the field that tend to remain wet (Franc *et al.*, 1993; Heidel and Rush, 1994; Rush and Heidel, 1995; Tamada, 1975; Tamada and Baba, 1973).

BNYVV is probably distributed worldwide where there are major sugarbeet industries. It was first described in Italy (Canova, 1959). It occurs now in several European regions, the Mediterranean region (Syria, Turkey, Lebanon, and Iran (Al-Chaabi *et al.*, 1999), the North American region, and the Eastern Asian region (Brunts *et al.*, 1986; Franc *et al.*, 1993; Kuszala *et al.*, 1986; Rush and Heidel, 1995; Tamada and Baba, 1973). Recently rhizomania has also been reported in Sweden (Tynelius, 1998).

BSBMV, previously designated as Texas 7, is also transmitted by *P. betae* Keskin (Heidel and Rush, 1994, 1997; Liu and Duffus, 1988; Rush and Heidel, 1995; Wisler *et al.*, 1994). BSBMV- infected-leaves may show mottling or slight distortions; however, roots are often asymptomatic. Some infected plants may exhibit root symptoms more typically associated with rhizomania. Such roots have been positive in ELISA for BSBMV but negative for BNYVV (Rush and Heidel, 1995). BSBMV has not been identified outside the United States of America (Heidel and Rush, 1997). Often BSBMV and BNYVV are present in the same field and even in the same plant (Heidel and Rush, 1997). According to some investigators, the two viruses can be differentiated serologically (Heidel and Rush 1997; Liu and Duffus, 1988; Mahmood and Rush, 1999; Rush and Heidel, 1995; Wisler *et al.*, 1999a). Other results; however, showed serologic cross reactivity between the two viruses upon using different sources of antisera (Wisler *et al.*, 1993, 1994).

Screening for virus diseases of sugarbeet started in Egypt a few years ago. The presence of beet curly top virus, beet yellows virus, beet western yellows virus, and the spinach strain of cucumber mosaic virus were detected (Abdel-Salam and Amin , 1990; Abdel-Salam *et al.*, 1991, 1992). Several rhizomania -like symptoms were observed in Kafr El Sheikh governorate and attributed to physiological disorders, the cultivation in heavy soil, or the presence of unknown viruses (Abdel-Salam 1987). However, with the appearance of rhizomania-like symptoms again in El-Fayoum governorate, a promising area for sugarbeet production, it became obvious that these symptoms are due to disease agent (s). Upon comparing these symptoms with those caused by beet necrotic

yellow vein benyvirus on sugarbeet, a great similarity was observed. However, due to complexity of rhizomania symptom expression, positive identification of rhizomania of sugarbeet is heavily dependent on serologic diagnosis (Franc *et al.*, 1993; Heidel and Ruch, 1994; Torrance *et al.*, 1988; Wisler *et al.*, 1999 a). The present paper describes the serologic identification and other disease aspects of this virus for the first time in Egypt. With the sugarbeet tonnage reaching 25 % of the total sugar production in Egypt (Afifi, 1999), such positive identification of BNYVV in Egypt should ring a bell for those interested in expanding the cultivation of sugarbeet in Egypt.

MATERIALS AND METHODS

Virus sources

Sugarbeet plants with suspected rhizomania-like symptoms were collected from commercial fields in El-Fayoum governorate. Additional sugarbeet plants were obtained from the Experimental Farm, Fac. of Agric., Cairo Univ., Giza. Infected plants were transferred to sterilized pots and soil and maintained in the greenhouse. Upon recovery of plants, they were used for serologic testing and virus transmission tests.

Mechanical transmission

Healthy plants of sugarbeet cv. Athospoly and other differential hosts including *Chenopodium quinoa* Willd., *C. amaranticolor* Coste & Reyn, and *Spinacia oleracea* L, were used for transmission of BNYVV from infected beet plants obtained from El-Fayoum and Giza governorates. Tested plants were grown in sterilized pots and soil. Fifteen plants from each species were used for inoculation. Five healthy plants, for each plant species, were inoculated with buffer to serve as a control. Plants were dusted with 600-mesh carborundum and inoculated with infected sap obtained by grinding infected tissues of leaf petioles or lateral roots with 0.1 M potassium phosphate buffer, pH 7.4 containing 0.02 M Na_2SO_3 with a ratio of 1:2, w/v. Plants were rinsed with tap water and monitored for symptom development.

Detection of BNYVV associated with rhizomania

Two techniques were used to verify the incidence of rhizomania

A) Soil survey for BNYVV

Nine soil samples were collected from three-sugarbeet fields (three samples/field) in the Fayoum governorate. Samples were obtained from soil surrounding sugarbeet roots exhibiting rhizomania syndrome. Samples were placed in 9-cm^2 square pots using three replicates per sample and planted with seed of sugarbeet cv. Pleno. The plants were grown for 75 days in the greenhouse to allow adequate-time colonization by *P. betae* Keskin and infection by BNYVV if viruliferous *P. betae* Keskin was present in the soil (Beemster and Heij, 1987; Gerik, 1992; Heidel and Rush, 1994). Roots were harvested, and assayed serologically (see below) for BNYVV. Negative control included roots grown in sterilized soil and pots. Samples were also taken from these roots for light microscopy detection of *P. betae* Keskin.

B) Serologic techniques

The techniques of dot blotting immuno binding assay (DBIA) and tissue blotting immuno binding assay (TBIA) were followed as previously described (Abdel-Salam *et al.*, 1997; Abdel-Salam, 1999 a, b). For TBIA, leaf petioles of plants exhibiting suspected symptoms were cut with a razor blade. The exposed edges were then blotted onto 200 µm-nitrocellulose membrane (NCM). For DBIA, samples of rhizomania-infested sugarbeet

hairy roots, leaf petioles, and healthy controls were ground (1:2, w/v) in an extraction buffer (EB) composed of 0.1 M Na_2HPO_4-NaH_2PO_4, pH 8.3, containing 0.02 M of Na_2SO_3 and ethylene diamine tetracetate (EDTA), and 1.5 % Triton X-100 [TX-100]) using mortar and pestle. The resulting sap was centrifuged at 1000 g. Samples were blotted onto NCM and developed. For root samples driven from beet seedlings sown in infested soil under greenhouse conditions, each small single root was packed in an Epindorf tube in the presence of 0.5 ml of EB, sonicated (10 min) in an ice bath and ground using ELISA- sample extractor. Tubes were frozen overnight, thawed, sonicated, vortexed again, centrifuged (1000g/10 min), then sample were blotted on NCM and developed.. Positive controls included antigens for BNYVV and BSBMV (see Table 1). A Negative control included sap from healthy sugarbeet roots grown in sterilized pots and soils in the greenhouse. In both DBIA and TBIA, antisera were diluted using TBS-Tween solution, TBST (10 mM Tris-HCl buffer, pH 8.0, 150 mM NaCl, 0.05% Tween 20).

Sources of authentic virus antigens and antisera

Virus antigens and antisera used in this study are depicted in Table 1:

Table (1): Sources of authentic different antigens and antisera used in serologic detection of rhizomania-associated viruses of sugarbeet in Egypt.

Anigens	Antisera	Sources of Antigens and Antisera
BNYVV* *(California isolate)*	***Polyclonal antibodies (PAB)***	C. Rush, Texax A&M. Univ., Bushland, TX, USA.
	BNYVV (Whole virus) #5422*	
	BNYVV (denatured coat protein) #5423*	
BSBMV* *(EA isolate, Colorado)*	BSBMV (Whole virus) #5425*	
	BSBMV (denatured coat protein) # 5427*	
	Monoclonal antibodies (MAB)	L. Torrance, Invergowire, Dundee, Scotland, UK
	BNYVV SRC* # 84, 86, & 87	

* = Preserved in glycerol,

RESULTS

Disease symptoms:

Foliar symptoms:

Infected sugarbeet plants exhibited disease syndrome typical to rhizomania of sugarbeet (Fig. 1-I A, B). Temporary wilting is obvious at noontime where leaves appear flabby when temperatures are high. These infected plants usually regain their turgidity in the evening and early morning (Fig.1-II A, B). Infected leaves show narrow blade and long petioles (Fig.1-II B, C). In some instances, foliar proliferations of small leaves are observed in the root crown areas (Fig.2-E-2, 3). Sometimes infected plants exhibit no chlorosis or yellowing of foliage, which confuse them with healthy plants. Vein yellowing associated with necrotic areas of tissues), after which the virus was named, is extremely rare and seldom seen under field conditions (Fig.1-II C). Vein yellowing and necrosis occur when infection becomes systemic and the virus can be recovered from the leaves. (Fig. 1-II D).

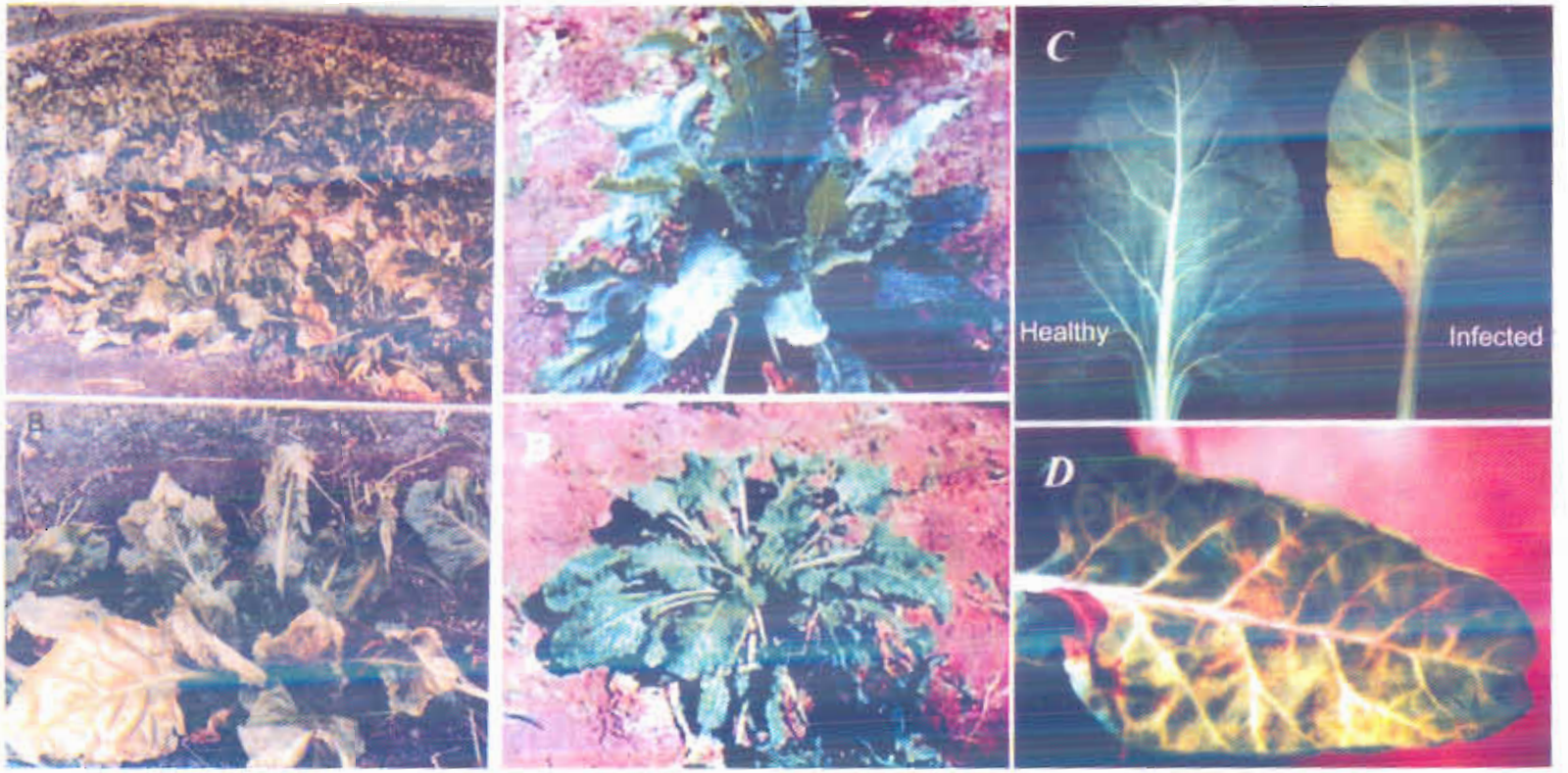


Fig. (1): Foliar symptoms of rhizomania of sugarbeet plants. I-A, a general view of an infected-sugarbeet field showing typical rhizomania syndrome including yellowing and plant recumbence; I-B, a close up photography to one of the infected plants. II-A, an infected plants showing leaf vigor in the early morning; II-B, an infected plant showing temporary wilting at noontime. II-C, vein yellowing and necrosis of sugar beet leaves in the field. Notice the long petiole of the infected leaf comparing to the healthy control. II-D, vein yellowing and veinal necrosis of a mechanically inoculated plant with BNYVV in the greenhouse.

Root symptoms

Disease symptoms of infected roots vary greatly depending on the time of infection. The invading fungus causes the killing of secondary roots and even the young tap roots. This leads to the proliferation of more lateral feeding roots (Fig. 2-A). Usually early infection may lead to severely stunted roots (Fig. 2-E-1,2). In highly susceptible sugarbeet varieties, continuous killing of the proliferating roots by the invading fungus may induce the symptom known as the bearded roots (Fig. 2-A). Usually small tumors exist at the base of proliferating roots as a result of active cell division. This can be used as a positive identification for rhizomania upon making longitudinal section in infected tap roots. The formed lateral roots are necrotic and blackened (Fig. 2-C, D) due to invasion by the fungus cystosori (Fig. 3). Sometimes necrosis may extend to parts of the main tap root (Fig. 2-B). The proliferation of young leaves can abnormally be seen on the tap roots (Fig. 2-E-2,3). Roots infected at late stages of growth take the shape of a wineglass (Fig. 2 - B). Much milder symptoms result in the formation of fewer feeding roots and the main tap root remains intact (Fig. 2-E-4). Transverse sections of the tap roots usually show necrosis and rotting of the central steel.

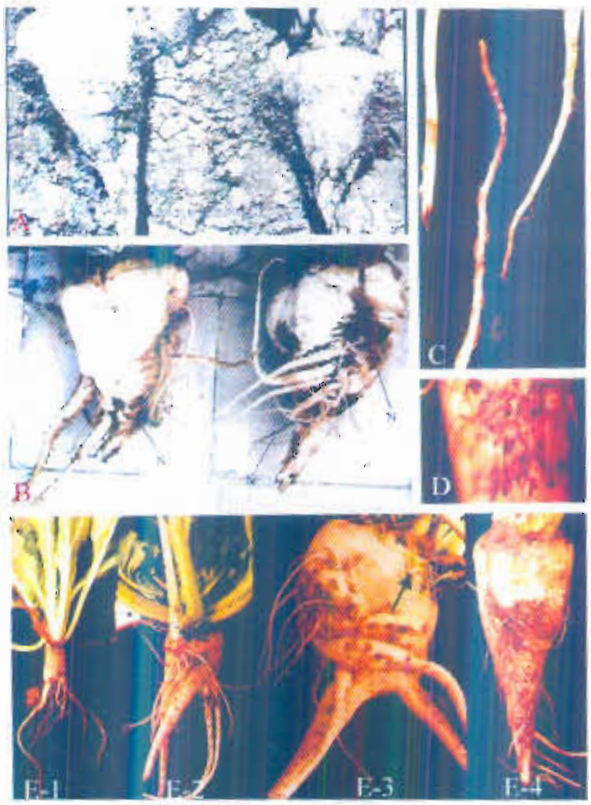


Fig. (2): Rhizomania syndrome of sugarbeet roots. A, bearded roots; B, infected roots showing proliferation of lateral roots and necrosis (N) on both lateral and tap roots. The root on the right takes the shape of a wineglass; C and D, necrosis and blackened lateral roots; E-1, severely stunted roots; E-2 and 3, proliferation of lateral roots and small leaves (see arrows) on the tap roots; E-4 an infected root with a healthy looking showing fewer lateral root proliferation.

Mechanical transmission and differential hosts

Mechanically inoculated beet plants with infected sources (i.e petioles or necrotic roots) from either the Fayoum or Giza fields showed vein yellowing, and veinal necrosis (Fig.1). All inoculated beet plants, grown in the greenhouse, exhibited excessive lateral root proliferation similar to symptoms of rhizomania of sugarbeets seen in the field. Bright diffused chlorotic local lesions were detected on both *Chenopodium quinoa* Willd. and *C. amaranticolor* Coste & Reyn. Chlorotic mottle, flecks and systemic infection were detected on inoculated *S. oleracea* L (results are not shown here).

Detection and Diagnosis of disease elements in infected sugarbeets

1) Detection of Polymyxa Betae Keskin *in infected sugarbeets*

Microscopic examination for infected-sugarbeet rootlets or roots from seedlings exposed to contaminated soil revealed the presence of cystosori of *P. betae* Keskin (Fig.3-A). Cystosori can be seen lined up on the external (Fig.3-B), internal layers (Fig. 3-C), and in the steel (Fig.3-D). Sporangial plasmodia can also be observed arising from infected rootlets (Fig.3-B, C). In developed stages of infection, the steel appears degraded and darkened due to cell death and the brown color of the cystosori (Fig. 3-D).

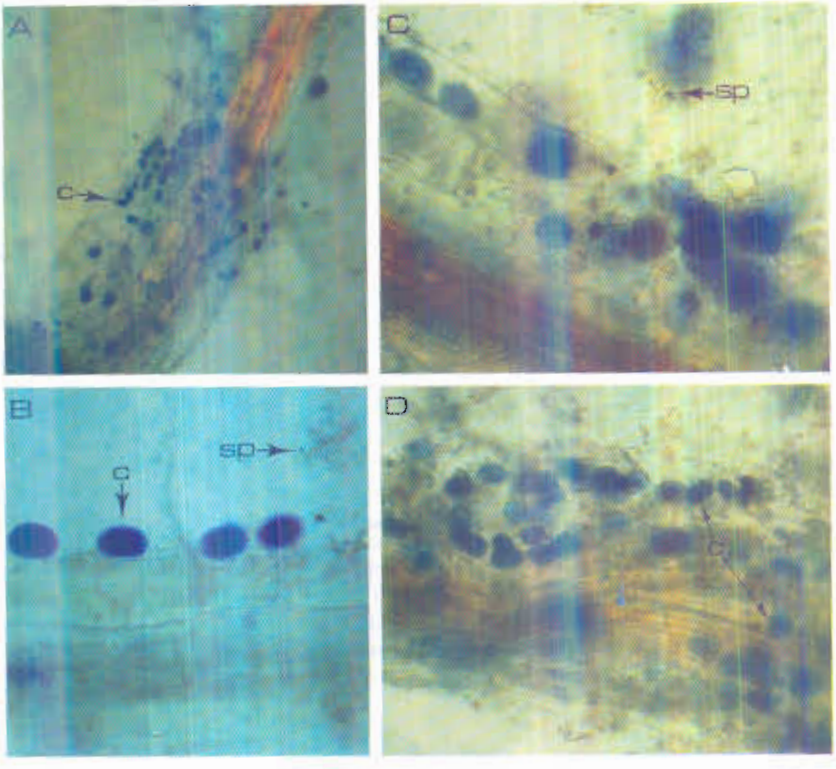


Fig. (3): Microscopic testing showing disease manifestation of sugarbeet lateral roots upon infection with viruliferous-P. betae Keskin. Cystosori. A, a lateral roots infested with cystosori (C) (Mag. X 475). B, lined up cystosori on a root surface showing the presence of sporangial plasmodium (SP) structures (Mag. X 1900); C, Cystosori inside root cells (Mag. X 1900); D, Cystosori inside a root with distorted and darkened steel (Mag. X 1187). Preparations were stained with lactophenol cotton blue. Print magnification factor = 3.8.

2) Detection of BNYVV

Serologic detection of BNYVV was carried out in a) leaf petioles of sugarbeet plants exhibiting rhizomania syndrome either collected from the field or after BNYVV-mechanical inoculation, b) naturally infected sugarbeet roots collected from the field, and c) in roots of sugarbeet seedlings artificially exposed to contaminated soil containing *P. betae* Keskin.

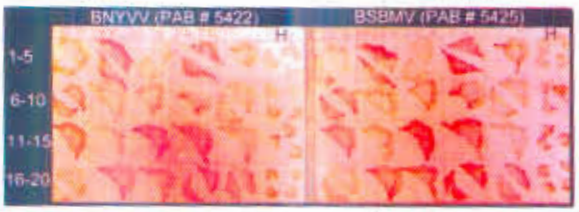
TBIA results indicated that both authentic PABs of BNYVV and BSBMV and MABs of BNYVV reacted positively with blots from leaf petioles of naturally infected sugarbeet plants (Table 2 and Fig. 4) and BNYVV-mechanically inoculated sugarbeet seedlings (not shown).

Table (2): Accumulative results on immunologic detection (TBIA & DBIA) of BNYVV in leaf petioles of sugarbeet plants from El-Fayoum and Giza governorates.

SampleNo.	TBIA							DBIA
	BNYVV (PAB)		BSBMV (PAB)		BNYVV (MAB)	BNYVV (MAB)	BNYVV (MAB)	BNYVV (MAB)
	Whole # 5422	CP # 5423	Whole # 5425	CP # 5427	SRC84	SRC 86	SRC 87	SRC 84
1	-	-	-	-				
2	+++	+	+++	+	+	+	+	+++
3	+	+	+	+	+	+	+	+
4	+++	++	+++	++				
5	+	++	+	++	+	+	+	+
6	++	++	++	+	+	+	+	+
7	+	+	+	++	++	+	++	+
8	+	++	+	+				
9	+	+	++	+				
10	+	+	+	++	+	+	+	++
11	+	+	++	+				
12	+	+	+	+				
13	++	++	+++	-				
14	+	++	+	+				
15	+	++	+	+	-*	+*	+	+
16	+	++	+	++	+	-*	+	+
17	++	++	++	++				
18	+	++	+	++				
19	++	++	+++	++	+	+	+	+
20	++	+	+	+				
21	+	+	+	+	+	+	+	++
22	-	-	-	-	-	-	-	-

Samples # 1 - 19 wee taken from commercial fields, El-Fayoum governorate
Samples # 20 - 21 were taken from commercial fields, El-Giza governorate. Sample # 22 is healthy sugarbeet leaves. PAB antisera for whole virus (Whole) were used at 10^{-3} dilution; while PAB antisera for denatured coat protein (CP) were used at 10^{-2} dilution. MAB antisera were used at 10^{-} dilution. Each sample was tested three times. * = Erotic results not repeatable.

Fig. (4): TBIA for sugarbeet leaf petioles (Blots # 1-19) suspected for rhizomania disease collected from El-Fayoum governorate and exposed to BNYVV (left) and BSBMV (right) antisera. For each five tested samples, in each row, a control of healthy (H) sugarbeet leaves grown in the greenhouse was included.



In El-Giza governorate, 12 out of 17 root samples of sugarbeet plants suspected for rhizomania syndrome, were infected upon applying DBIA assays using MABs and PABs for BNYVV and BSBMV (Table 3 and Fig. 6). Similarly, seven out of nine root samples of sugarbeets collected from El-Fayoum governorate were found infected upon testing them with PABs of BNYVV and BSBMV (Table 3 and Fig.5).

Table (3): Accumulative results on immunologic detection (DBIA) of BNYVV in roots of sugarbeet plants from El-Giza and El-Fayoum governorates .

El-Giza gobvernorate					El-Fayoum governorate		
Sample No.	BNYVV (MAB) SRC 84	BNYVV (MAB) SRC 86	BNYVV (MAB) SRC 87	BSBMV (PAB) Whole #5425	Sample No.	BNYVV (PAB) whole #5422	BSBMV (PAB) whole # 4525
1	+++	+++	+++	+++	1	+	+
2	+++	+++	++	+++	2	+	+
3	+	+	++	++	3	+	+
4	+	+	+	+++	4	-	-
5	+	+	++	++	5	+	+
6	+	+	+	++	6	+	+
7	+	+	+	++	7	+	+
8	++	+	++	++	8	-	-
9	+	+	+		9	+	+
10	+	+	+	++	10[a]	-	-
11	-	-	-	-			
12	-	-	-	-			
13	+	+	+	+			
14	+	+	+	+			
15	-	-	-	-			
16	-	-	-	-			
17	-	-	-	-			
18	-	-	-	+++			
19	+++	+++	++	-			
20[a]	-	-	-	-			

BNYVV-(MAB) antisera were used at 10^{-1} dilution. PABs of BNYVV and BSBMV antisera was used at 10^{-3} dilution. Samples # 18 and 19 are BSBMV (*EA isolate, Colorado*) and BNYVV (*California isolate*), respectively. Samples # 10^a, 20^a = healthy sugarbeet roots.

Fig. (5): DBIA showing the reactions of nine- sugarbeet roots, from El-Fayoum governorate suspected for rhizomania, with PAB antisera of BVYVV (left) and BSBMV (right). Sample # 10 is a healthy control of sugarbeet roots.

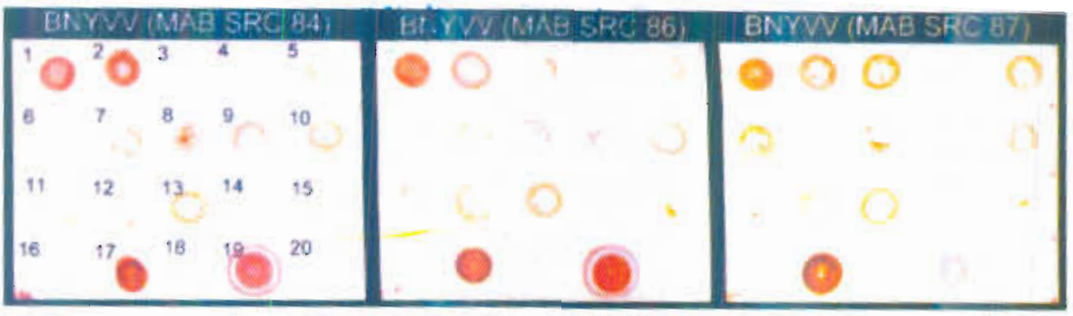


Fig. (6): TBIA showing the reaction of BNYVV (MAB SRC 84, 86, 87) antisera with rhizomania-suspected sugarbeet root samples (blots # 1-17) from El-Giza governorate. Blots 18, 19 are BSBMV (EA isolate) and BNYVV (CA isolate), respectively. Blots # 20 are healthy (control) sugarbeet roots.

Soil assays for the presence of BNYVV vectored by the soilborne fungus *P. betae* Keskin was conducted through exposing seedlings of sugarbeet to contaminated soil. Figure (7) shows the positive reactions of PABs of BNYVVY and BSBMV antisera (Fig. 7-B) as well as the three MABs for BNYVV to those soil-exposed seedlings (Fig. 7-B).

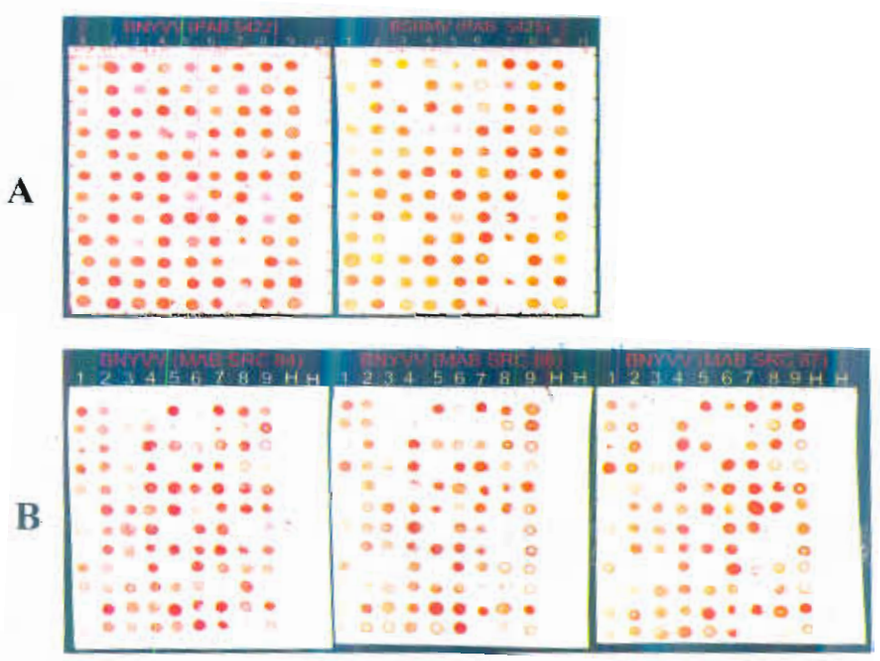


Fig. (7): DBIA test demonstrates the transmission and infection of sugarbeet seedlings upon growing in pots containing contaminated soil with BNYVV-E. Columns 1-9 in (A) and (B) represent soils from nine different fields in El-Fayoum governorate. Each column contains 12 tested root extracts. H = healthy root extracts grown in sterilized soil and pots. Antisera used are BNYVV (5422) on the left-hand and BSBMV (5425) on the right-hand, (A); while the reactions of BNYVV (MAB SRC 84, 86, 87) antisera with the same sample are shown in (B).

Serologic relatedness between the Egyptian isolate of BNYVV (BNYVV-E) and the American isolates of BNYVV and BSBMV

Results in Fig. (8) showed positive serologic relatedness between BNYVV-E and the American tested isolates of BNYVV and BSBMV upon using their induced antisera for the whole virus (Fig. 8-A, C) or for the coat protein (Fig. 8-B,D). The reactions resulted from using the antisera for the coat protein (Fig. 8-B, D) were, however, much weaker but more specific than those antisera raised for the whole virus (Fig. 8-A, C).

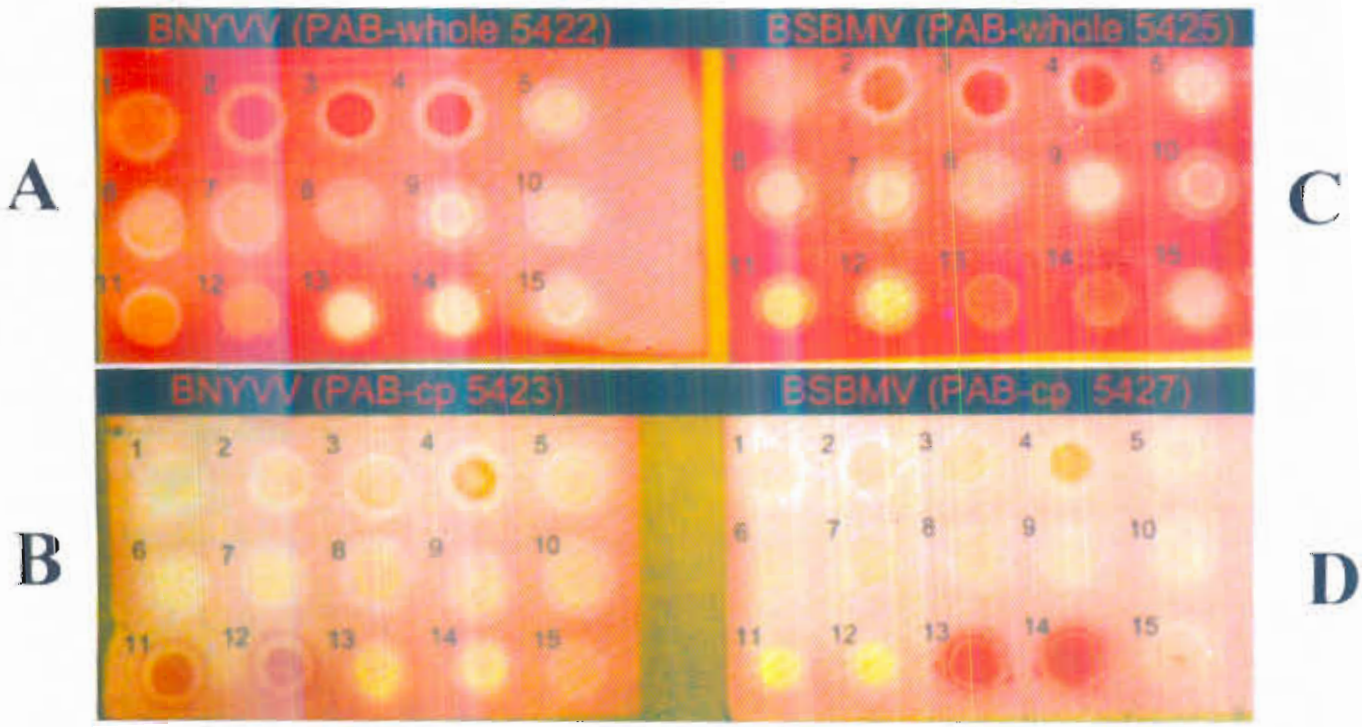


Fig. (8): DBIA showing the serologic relationships between BNYVV-E and the American BNYVV and BSBMV using antisera raised for either BNYVV for the whole virus (A) or the coat protein (B) and BSBMV for the whole virus (C) or the coat protein (D). Blots # 1-10, rhizomania-suspected roots; blots # 11-12, BBNYVV-CA isolate; Blots # 13-14, BSBMV- EA isolate, Colorado; blots # 15, healthy sugarbeet roots.

Similar TBIA results (Fig. 4) and DBIA results (Figs. 5, 7) indicated that BNYVV-E cross-reacted with both BNYVV-*CA* and BSBMV-*EA*.

However, no cross-serologic reactions between BNYVV-*CA* or BSBMV-*EA* were found upon using PABs (see samples # 11,12 and 13, 14 in Fig. 8) or upon using MABs (see samples # 18, 19 in Fig. 6).

DISCUSSION

The confirmation of the presence of rhizomania of sugarbeet in Egypt was based on: 1) disease syndrome observed on both foliar and root parts of infected plants, 2) transmission and differential host studies, 3) disease signs or disease elements through light

microscopy examination for *P. betae* Keskin in roots, and 4) through serologic detection of BNYVV-E in infected tissues.

Infected sugarbeet plants from El-Fayoum or Giza fields exhibited a wide range of foliar disease symptoms including temporary leaf wilting in hot weather, narrow leaf blade and long petioles, and veinal yellowing and necrosis. Infected roots developed excessive lateral roots, root necrosis, and proliferation of small leaves on the crown areas. Such described symptoms are typical to those described for rhizomania of sugarbeet (Franc *et al.*, 1993; Heidel and Rush, 1994;, Rush and Heidel, 1995; Tamada, 1975; Tamada and Baba, 1973).

Transmission studies indicated that BNYVV-E was transmitted mechanically to sugar beet and spinach forming systemic infection and inducing chlorotic local lesions *on Chenopodium amaranticolor* Coste & Reyn *and C. quinoa* Willd. Such results agree with transmission and host range studies reported for BNYVV (Rush and Heidel, 1995; Brunts *et al,* 1996). Soil survey experiments indicated that BNYVV-E is also transmitted through the *P.betae*-contaminated soil (Fig. 7). Such obtained results comply with the nature of BNYVV as a soil-transmitted virus vectored in the soil by *P. betae* Keskin. (Guinchedi and Langenberg, 1982; Beemster and Heij, 1987, Heidel and Rush, 1994).

Examination of infected-lateral roots of sugarbeet plants showed the presence of cystosori (Abe and Tamada, 1986) of *P. betae* Keskin outside and inside root tissues. Root necrosis and tissue degradation were associated with the presence of cystosori as described by Rush and Heidel (1995).

Many factors can interfere in the diagnosis of rhizomania of sugarbeet. Some of these factors might be related to the complexity and the wide range of disease syndrome, the presence of rhizomania-infected but symptomless sugarbeet plants, nitrogen deficiency of sugarbeet, or the presence of several soilborne fungi and adverse soil conditions (e.g. hardpan soil). Therefore, excessive lateral root proliferation and foliar syndrome should not be taken as the sole criteria for rhizomania diagnosis. Currently, serologic tests (e.g. dot blotting, DAS-ELISA, TAS-ELISA, western blotting); are being used by many investigators as the ultimate criteria for the correct diagnosis of rhizomania of sugarbeet (Heidel and Rush, 1994, 1997; Rush and Heidel, 1995; Resca and Biaggi, 1990; Wisler *et al.*, 1999a, b). In the present study, the incidence of BNYVV-E was confirmed through testing naturally infected sugrabeet plants using MABs and PABs for BNYVV as well as PABs for BSBMV). Further soil survey for the presence of BNYVV (Guinchedi and Langenberg, 1982; Beemster and Heij, 1987, Heidel and Rush, 1994) was conducted using sugarbeet seedlings as baits for BNYVV and later on were detected serologically to confirm the soilborne nature of BNYVV-E. This latter technique is invaluable for indexing soil for the presence of BNYVV and should be used before plantation of sugarbeet.

The BNYVV-E isolate reacted with PAB antisera for BNYVV and BSBMV (Figs. 4,5,7,8) indicating its serologic relatedness to the Benyviruses. Since both viruses can be differentiated serologically from each other (see the present results in Figs. 6, 8 and Heidel and Rush 1997; Liu and Duffus, 1988; Rush and Heidel, 1995; Wisler *et al.*, 1999a), the obtained results, in the present study, may suggest the following: 1) BNYVV-E has common epitopes (eps) for both viruses. Such eps are mostly of the cryptope types (external eps), (see Fig. 8-A, C) since poor reaction of BNYVV-E was obtained upon its reaction with antisera induced for coat proteins, containing neotopes (internal eps) of both

BNYVV and BSBMV, (see Fig. 8-B, D). 2) BSBMV may be present as a mixed infection with BNYVV-E. Heidel and Rush (1997) have shown the possibility of the presence of both viruses in the same plant. Rush and Heidel (1995) stated; however, that BSBMV had not been recorded outside the USA. Therefore, the presence of BSBMV in Egypt has to be confirmed first to verify the validity of this suggestion. Future studies on BNYVV-E should include western blot analysis using both antisera for BNYVV and BSBMV. This would tell whether or not mixed infection with both viruses is present.

The present study has supported the presence of rhizomania of sugarbeet for the first time in Egypt. Large-scale survey for all the sugarbeet-cultivated areas with cultivated varieties should be executed to evaluate the spread out of this disease and damage assessment. Further, soil survey for rhizomania should be carried out before sugarbeet cultivation. Such epidemiological and precautionary measures represent a must if we want the sugarbeet industry to continue flourishing in Egypt.

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الملخص العربي

تواجد ظاهرة الجذر المجنون (Rhizomania) في بنجر السكر في مصر والمصاحبة للإصابة بفيروس اصفرار وموت عروق البنجر

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تـم تـأكيد تواجد فيروس اصفرار وموت عروق البنجر beet necrotic yellow vein virus (BNYVV) والمصاحب لظاهـرة الجذر المجنون (Rhizomania) لأول مرة في مصر في بعض زراعات بنجر السكر في محافظتي الجيزة والفيوم. ووجد أن الفيروس المسبب ينتقل خلال التربة الملوثة بالفطر *Polymyxa betae* Keskin الحامل للفيروس. وقد استخدم لتأكيد ذلك اختبارات الـنقل الميكـانيكي لـلفيروس، العوائـل المشخصة، الفحص المجهري لجذور البنجر، العدوى بالفطر الحامل للفيروس، وكذا الاختبارات السيرولوجية باستخدام أمصال أحادية وعديدة الكلون.

وقد بينت اختبارات النقل الميكانيكي إمكانية نقل الفيروس لنباتات بنجر السكر والسبانخ والزربيح.

وقـد اظهر فحص جذور البنجر المصابة حدوث تشوهات في الجذر الأصلي وتكون العديد من الجذور العرضية، مع موت أنسجة الجذور.

وبيـن الفحـص المجهري لجذور البنجر تواجد الجراثيم الساكنة cystosori الخاصة بالفطر الناقل للفيروس ، وصاحب ذلك موت الخلايا .

وقد بينت اختبارات السيرولوجي باستعمال الأمصال أحادية وعديدة الكلون الخاصة ب BNYVV , وكذا الأمصال عديدة الكلون الخاصة بفيروس موزيك البنجر المنقول بالتربة beet soil bone mosaic virus (BSBMV) تواجد الفيروس في جذور البنجر المأخوذة من الحقل وكذا مع جذور البنجر المعرضة لتربة تحتوى على الفطر الحامل للفيروس.