International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Evaluation of the Effect of Previous Cultural of Sugar Beet on the Viral Inoculum Load of "*Beet Necrotic Yellow Vein Virus*" in Morocco

F. Nouayti [1, 2], A. Tahiri [1], I. Madani [2], A. Blenzar [2]

[1]Laboratory of phytopathology, Department of Protection the Plants & Environment, Ecole National of Agriculture, BP S 40 Meknès, Morocco

[2]Laboratory of Environment & Health, Department of Biology, Faculty of Sciences, BP 11201, Zitoune, Meknes, Morocco

Abstract: ***This study aimed to evaluate the effect of previous crop of sugar beet on the viral inoculums load 'Beet Necrotic Yellow Vein Virus' (BNYVV). In Morocco wheat, corn, sesame and alfalfa are used in rotation with sugar beet the role of this plants to transmit BNYVV and Polymyxa betae was determined by growing plants in naturally infested soils from rhizomania outbreaks in Morocco. After 28 days of culture, the analysis of their roots by DAS- ELISA and under the microscope showed the absence of virus and its vector fungus in the roots of these plants. Only P.betae isolates from sugar beet were found to be able to transmit BNYVV back to sugar beet. Therefore it seems likely that the plants used in rotation in Morocco play not a role in the spread of rhizomania disease compared to that of sugar beet.***

Keywords: Morocco, Rotation, rhizomania, Polymyxa betae and Sugar beet.

1. Introduction

Rhizomania is caused by *Beet Necrotic Yellow Vein Virus* (BNYVV) [1]. Rhizomania causes serious disease of sugar beet. It was first reported in Italy in the 1950 [2], but now it is present in sugar beet areas all over the world [3]. BNYVV is transmitted in soil by zoospores of plasmodiophorid, *Polymyxa betae* [4], and is member of the genus *Benyvirus* [5].

The first sign of rhizomania disease in a sugar beet crop appears as light green or yellow irregularly shaped patches in the field. Individual plants show the characteristic proliferation of fibrous roots around the tap root, "the root madness symptoms" of rhizomania. In severely infected plants, the tap root and lateral roots become necrotic and die then and the vascular tissue develops a pale brown coloration [6]. BNYVV leads to serious decreases in root yield and quality of sugar. Virus reduces sugar content in the roots by 3-4% and yields of sugar beet more than 50-60% [7].

In Morocco, Rhizomania was first detecting in 2004 at Tadla Sugar Refinery area [8]. Later the presence of the disease was report in Doukkala [9]. Sugar beet is a crop rotation. It is grown on the same parcel once every 3-5 years. Important rotation crop, it operates as a break crop in the main cereal rotations, prevents the development of disease and reduces the need for fertilizer and pesticides succeeding crops. The objective of this study is to evaluate the effect of previous crop of sugar beet on the viral inoculum load BNYVV.

2. Materials and Methods

2.1. Plant Growing

Five plant species were grown in soil infected with rhizomania, sugar beet (susceptible variety), wheat, corn, alfalfa and sesame. The soil used in this study was collected from a plot Souk Sebt Beni Mellal [10] - [11]. These plots are recognized infested by rhizomania. Sample collection was performed according to the standards recommended by the official method of virus detection rhizomania in sugar beet [12].

The soil was thoroughly mixed to allow its homogenization. Then activated charcoal was incorpo rated at 0.673 g / 150 ml soil. For each plant, 12 repetitions were prepared. Then, 12 pots of 150 ml were filled to 7/8th with soil - coal mixture and the seeds of the test plants were sown on the surface and covered by the soil. The negative control was composed 6 pots of 6 autoclaved soil (120°*C* for 2 h with 2 autoclaving at 24 -hour intervals) and mixed with charcoal (at a rate of 0.673 g / pot). The pots were incubated 28 days in a greenhouse shelter under temperatures of 25°C ± 5°C and humidity between 50%. The pots were watered daily with well water. After 28 days of culture, each pot was decanted individually to identify the rootlets debris.

A gentle wash with running water on a mesh sieve 1 to 2 mm was made in order to avoid losses of samples of rootlets. The sample of lateral roots was divided to two parts, of which one was subjected to transmission back to sugar beet and the other one used for detection of BNYVV and *P.betae.*

2.2. Detection of BNYVV

Detection of BNYVV in roots was by Double Antibody Sandwich - Enzyme Linked Immuno Sorbent Assay (DAS-ELISA) according to the protocol provided by the manufacture (SEDIAG, France). Each sample was tested in two replicates, and wells each of positive and negative controls were included. Reading was done after incubations of 1h and 2h with substrate pNPP (p-nitrophenyl phosphate) at 37°C. Mean experimental reading at least three times the mean of the negative controls was measured using ELISA ANTAI Reader.

2.3. Detection of *P.betae*

The roots were cut into pieces of 0.5 to 1 cm, and then transferred into a potassium hydroxide solution 10%. The whole was autoclaved at 120 ° C for 10 min. The fragments are then were washed with sterile distilled water. The remaining KOH was neutralized by a lactic acid bath for 3-4 minutes. This treatment has the purpose of emptying the cells of their cytoplasmic contents and allows good color roots [13].

Root fragments clouds were immersed in a methylene blue solution and placed in an oven for 15 minutes at 90 °C. They were subsequently mounted in glycerin between slide and coverslip, at a rate of 15 fragments per slide. Microscopic observation involved 30 random fragments per plant species.

2.4. Transmission Back to Sugar Beet

The roots were air dried for 3 weeks to provide inoculum for transmission rhizomania back into sugar beet. This was done by grinding the air dried root to a fine powder and sprinkling it around the roots of a 5 day old sugar beet seedling in autoclaved soil. These were then grown for 8 weeks under the conditions previously described. Plants were then harvested, tested for BNYVV by DAS-ELISA and RT-PCR and examined under microscope for *P.betae* [14].

2.5. RT-PCR

Total RNA was extracted from 100 mg of root tissue essentially according to (Henry *& al) & (*Hughes and Galau) [15] – [17]. Transcription and amplification of viral RNA was carried out using the kit (SuperScriptTM III Partinium One Step Quantitative RT -PCR System with Rox, Invitrogen, USA) as described. RT-PCR was carried out using specific primers (5F1/5R1), which amplify a 520bp fragment of the read through region of the coat protein gene located on RNA-2 of BNYVV [15] - [16] Temperatures used for the cycling reaction were as follows : 30 mn at 37°C; 2 mn at 94°C; followed by 30 cycles of 1 mn at 94°C, 1mn at 55 °C, 1 mn at 72°C; finally, 3 mn at 72°C. PCR products were analyzed by electrophoresis on 2% Agarose using the 50pbDNA Step Ladder (Promega, USA) for size estimation.

3. Results and Discussion

With the exception of sugar beet the plant species tested were found to be free from any characteristic structures of *P.betae* (plasmodia, sporangia or cystosori) when examined under the light microscope and were not infected by BNYVV when tested by DAS-ELISA. Table 1 shows that's only sugar beet became infected with either *Polymyxa* or BNYVV. *Beta vulgaris* cultivars were always infected by *polymyxa* and BNYVV, indicating that the soils contained a high level of viruliferous *P.betae* cystosori [14].

Table 1: Plant species with roots infected by *P. betae* or BNYVV

Plants	Presence *of P. betae*	Infection by BNYVV
Sugar beet	+	+
Wheat	-	-
Corn	-	-
Alfalfa	-	-
Sesame	-	-

- + infection
- - no infection

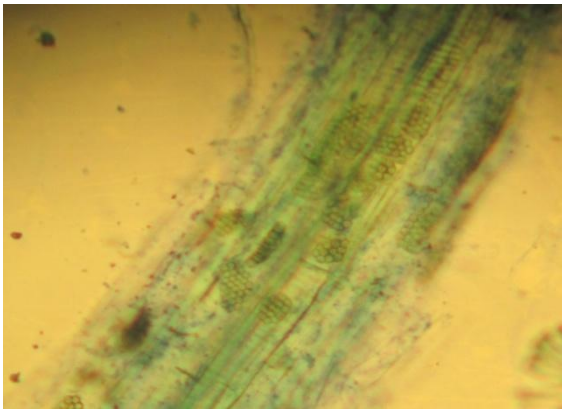

Figure 1: Microscopic observation (X40). A sugar beet root colonized by *P. betae* cystosores

Attempts were made to transmit BNYVV from viruliferous *P.betae* cystosori from the roots back to sugar beet. BNYVV and Polymyxa betae were successfully transmitted to sugar beet only from sugar beet root (Table 2) [14] – [18].

Table 2: Transmission back to sugar beet

Plants	DAS-ELISA	RT- PCR	*P.betae*
Sugar beet/ sugar	+	+	+
Beet/Wheat	-	-	-
Beet /Corn	-	-	-
Beet /Alfalfa	-	-	-
Beet/ Sesame	-	-	-

- + infection
- - no infection

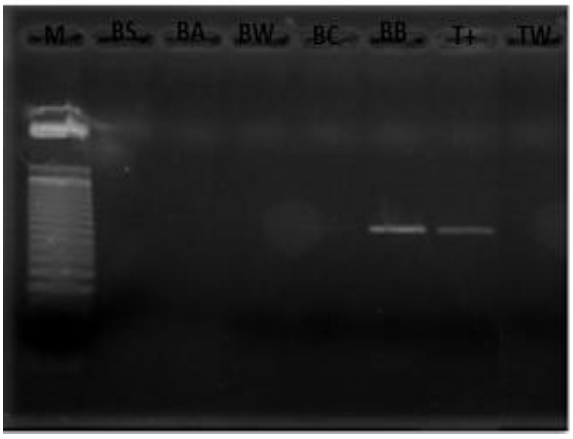

Figure 2: Amplification profile of beet roots inoculated with the tested plants.

M: molecular weight marker ; BS beet inoculated sesame roots ; BA : beets inoculated alfalfa roots ; BW: beet inoculated wheat roots ; BC: inoculated beet roots of corm; BB: beets inoculated beet roots ; T+: positive control ; & TH : water control.

The PCR reaction produced yielded the expected band only with beet inoculated beet roots. Furthermore, no amplification was obtained with the other samples tested (Figure 2).

The absence of the virus and the *P.betae* in four species (wheat, alfalfa, corn and sesame) studied allows us to conclude that the transmission of the BNYVV and the vector P. *betae* only ensured by roots of sugar beet. [22]

As expected, sugar beet was heavily infected by *P.betae* and BNYVV from naturally infested soil. The other plants tested are not host plants for the virus and its vector.

The previous cultures of sugar beet used in Morocco don't have role in the buildup of rhizomania inoculum in soil and its spread to sugar beet crops.

The severity and spread of the rhizomania disease is caused primarily by agricultural practices. According to the ITB (Technical Institute for Beet France) (2010) [19] risk practices are irrigation and rotation. Several studies have found that in the cultivation of sugar beet, irrigation frequency is highly correlated with the intensity of some diseases, including rhizomania [20].

In the US, Picinni et al., Showed the presence of a strong correlation between the intensity of the disease and frequency and the irrigation dose [21].

According to the ITB crop rotation of at least 4 years is recommended in case of ground shown by rhizomania. In the rotation, crop selection is important. Indeed, crops that can retain the virus and ensure its multiplication in the ground should be avoided especially spinach [22] and cultures of the genus Beta.

Fortunately cropping precedents used in rotation with sugar beet in Morocco can't retain the virus and don't allow multiplication in the soil but the cultivation practices; namely rotation every 2 years instead of 4 years, more spring are hot and humid; help to create favorable conditions for the development and growth of fungus *P.betae* and subsequently maintaining BNYVV in the soil.

However weed hosts could, in theory, act as reservoir for rhizomania disease in the absence of sugar beet crop, but the impact of this is debatable because inoculum can persist in the soil for at least 15-20 years in the absence of a host [23].

References

[1] T.Tamada, T.Baba, "Beet necrotic yellow vein virus from rhizomania affected sugar beet in Japan", Ann. *Phytopath. Soc. Japan* 39, 325–332, 1973.
[2] A. Canova, "On the pathology of sugar beet". *Inf Fitopatology, 9*, 390-396, 1959.
[3] S. Chiba, H. Kondo, M. Miyanishi, I.B. Andika, C. Han, & T. Tamada, "The evolutionary history of *Beet necrotic yellow vein virus* deduced from genetic variation, Geographical origin and spread, and the breaking of host resistance". *Molecular Plant- Microbe Interaction, 24*(2), 207-218, 2011.
[4] B. Keskin, "*Polymyxa betae* n.sp., ein Parasit in den Wurzeln von *Beta vulgaris* Tournefort, besonders der Jugendent wicklung der Zuckerrübe". *Archivi für Mikrobiologie, 49*, 348-374,1964.
[5] T. Tamada, "Production and pathogenicity of isolates of *Beet necrotic yellow vein virus* with different numbers of RNA components". *Journal of General Virology, 70*, 3399-3409, 1989.
[6] A.A. Brunt, & K.E. Richards, "Biology and molecular biology of furoviruses". *Advances in Virus Research, 36*, 1-32, 1989.
[7] C. Henry, "Rhizomania-its effect on sugar beet yield in the UK". *British Sugar Beet Review, 64*, 224-26, 1996.
[8] Anonyme (2005) Premier Signalement du Beet necrotic yellow vein benyvirus au Maroc. EPPO Bulletin Service d'Information, No.7
[9] K. Raji, Diagnostic de la rhizomanie de la betterave sucrière et évaluation de la charge virale de quelques échantillons de sol provenant de la région de Doukkala. Mémoire de fin d'étude, *Ecole Nationale d'Agriculture de Meknès*, Maroc. PPE, 2011.
[10] K. Akallouf, Cartographie de la rhizomanie dans la région du Tadla et essai d'évaluation du comportement et d'adaptation des variétés tolérantes à la rhizomanie. Mémoire de fin d'étude, *Ecole Nationale d'Agriculture de Meknès, Maroc*. PPE, 2008.
[11] S. Chofqi, "Cartographie et Pathotypage de la rhizomanie de la betterave sucrière dans la région du Tadla et évaluation du comportement de quelques variétés tolérantes à la rhizomanie". Mémoire de fin d'étude, *Ecole Nationale d'Agriculture de Meknès, Maroc*. PPE, 2009.
[12] J. Mathurin, "Méthode officielle de détection du virus de la rhizomanie de la betterave sucrière par test biologique suivi du test ELISA". VS/04/06 version b, 2007.
[13] A.Nidkahman, 2002. "Descriotion d'endomycorhizes associés aux Citrus dans deux sites d'étude au Maroc Effets de la mycorhization sur les zoospores de *Phytophtora citrophthora* (Smith & Smith) Leon". Mémoire de fin d'étude, *Ecole Nationale d'Agriculture de Meknès, Maroc*. PPE, 2002.
[14] A.S. Hugo, C.M. ., Henry, C.M. & V. Harju, "The role of alternative host of in transmission of beet necrotic yellow vein virus in England". *Plant virology* 45,62-666, 1996.
[15] C.M. Henry, I. Barker, J. Morris & S.A. Hugo, "Detection of beet necrotic yellow vein virus using reverse transcription and polymerase chain reaction". J. Virol. Methods 54, 15-28, 1995.
[16] Anonyme (2011) Diagnostic protocols for regulated pests. Beet necrotic yellow vein benyvirus. EPPO Bulletin 34, 229-237, 2004.
[17] D.W. Hughes, G. Galau, "Preparation of RNA from cotton leaves and pollen". *Plant Mol. Biol. Rep*. 6 253-257, 1988.
[18] K.J. Barr & M.J.C. Asher, "The host range of polymyxa betae in Britain". *Pant pathology* 41, 64-68, and 1992.
[19] Anonyme (2010) Nématode à kystes et rhizomanie : quelle gestion durable ? La technique betteravière. ITB, N° 935 du 21 Septembre 2010.
[20] R.M. Harveson, C.M. Rush, "The Influence of Irrigation Frequency and Cultivar Blends on the Severity of Multiple Root Diseases in Sugar Beets". Plant Dis. 86:901-908, 2002.
[21] G. Piccinni, C.M. Rush, "Determination of Optimum Irrigation Regime and Water Use Efficiency of Sugar

Beet Grown in Pathogen-Infested Soil". Plant Dis. 84:1067-1072, 2000.

[22] K. Richardson, B. Mou S. Benzen & H.L. Liu, " Effects of *Beet necrotic yellow vein virus* in Spinach Cultivars". Plant Dis. 96:618-622, 2012.

[23] H.Abe T. Tamada, "Association of beet necrotic yellow vein virus with isolates of *polymyxa betae* Keskin". *Annals of the phytopathological society of japan*. 52, 235-47, 1986.