Molecular characterization of Beet necrotic yellow vein virus (BNYVV) infecting sugar beet in Tadla

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Abstract

Sugar beet is the most important sugar crop in Morocco. The main production areas of sugar beet are Doukkala, Tadla, Gharb, and Moulouya. Rhizomania, caused by Beet necrotic yellow vein virus (BNYVV, genus *Benyvirus*), was first described in Italy and has spread in most sugar beet-growing areas of the world within a few decades. In the Tadla region, Rhizomania is one of the major phytosanitary problems for the sugar-beet industry. In 2010, five isolates from different locations in the Tadla region were collected for molecular characterization studies by sequencing the coat protein gene (CPg). The obtained results showed that all the sequenced isolates were in the B type of BNYVV and revealed the highest nucleotide similarity with the Belgian isolate B2 (AY696077), with a sequence homology of 100%. To the best of our knowledge, this is the first molecular characterization of BNYVV isolates in Morocco.

Keywords: Rhizomania, sugar beet, molecular characterization, coat protein gene, Morocco.

Caractérisation moléculaire du virus des nervures jaunes nécrotiques de la betterave (BNYVV) infectant la betterave à sucre au Tadla

Résumé

La betterave sucrière est la principale source de sucre au Maroc. Les principales zones de production de la betterave à sucre sont Doukkala, Tadla, Gharb, et Moulouya. La rhizomanie, causée par le virus des nervures jaunes nécrotiques de la betterave (BNYVV, genre *Benyvirus*), a été décrite pour la première fois en Italie et s'est propagée dans la plupart des régions productrices de la betterave sucrière. Dans la région de Tadla, la rhizomanie est l'un des principaux problèmes phytosanitaires majeurs pour la culture de la betterave sucrière. En 2010, cinq isolats de différentes localités de la région de Tadla ont été collectés pour des études de caractérisation moléculaire par le séquençage du gène de protéine de la capside (CP). Les résultats obtenus ont montré que les isolats marocains étaient dans le groupe B de BNYVV; ces isolats ont montré une grande similarité nucléotidique avec l'isolat Belge B2 (AY696077), avec une homologie de séquence de 100%. Au mieux de nos connaissances, il s'agit de la première caractérisation moléculaire des isolats de BNYVV au Maroc.

Mots-clés: Rhizomanie, betterave sucrière, caractérisation moléculaire, gène de la protéine de la capside, Maroc.

INTRODUCTION

Production of sugar beet is the most important sugar crop in Morocco. With an area of cultivation of 58.400 ha, it produces 3.6 million tons of roots (Anonymous, 1015). The main sugar beet production areas in Morocco are Doukkala, Tadla, Gharb, and Moulouya. Sugar beet production is suffering from many problems, especially from diseases and pests. Very scarce information is available on viral diseases of sugar beet, especially on *Beet necrotic yellow vein virus* (BNYVV, genus *Benyvirus*), the causal agent of Rhizomania, which is one of the major phytosanitary problems for the sugar-beet industry worldwide. Heavy infection with rhizomania can cause a yield loss of up to 70% (Johansson, 1985) and a decrease in sugar content from 16-18% to less than 7% (Bongiovanni and Lanzoni, 1964).

BNYVV is transmitted by soil-borne plasmodiophorid (protozoan) vector, *Polymyxa betae* Keskin (Abe and Tamada, 1986; Tamada, 2002). The virus is cystosorus-borne and survives many years in soils even without susceptible plants (Tamada, 2002). The disease appears as foci (patches) in low laying areas of sugar beet fields. The infected sugar beet plant shows mild to severe chlorosis particularly in vascular bundle, followed by venial necrosis (necrotic yellow vein). The virus also produces characteristic symptoms on roots by exhibiting proliferation of lateral rootlets (rhizomania) and stunting (Tamada, 2002).

BNYVV has a multipartite single-stranded RNA genome with all natural isolates containing four RNA species, although some isolates have an additional RNA-5 (Tamada et *al*., 1989). Three major serologically indistinguishable BNYVV groups named type A, B and P have been identified (Kruse et *al*., 1994; Koenig et *al*., 1995; Miyanishi et *al*., 1999; Koenig and Lennefors, 2000). BNYVV A type isolates are distributed worldwide, whereas the B type isolate is prevalent in limited areas of Europe, mainly in France and Germany, but occurs also in China and Japan (Miyanishi et *al*., 1999; Sohi and Maleki, 2004; Schirmer et *al*., 2005; Li et *al*., 2008). BNYVV A and B types typically contain only RNAs 1 to 4. BNYVV P type isolates that contain RNA-5 are detected only in France, Kazakhstan and in the UK (Koenig et *al*., 1997; Koenig and Lennefors, 2000; Harju et *al*., 2002; Ward et *al*., 2007). P type

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isolates appear to be more virulent than both the A and B types (Heijbroek et *al*., 1999) and may evade activation of plant defense responses.

BNYVV was first reported from Italy (Canova, 1959) and Tamada and Baba (1973) identified the causal agent of this disease as a virus and later they called it BNYVV from the leaf symptoms it induces. It has since been reported from many sugar beet producing countries all over the world (Chiba et *al.,* 2011). In Morocco, Rhizomania was first detected in 2004 in Tadla region, and then the presence of the virus was confirmed in Doukkala region (EPPO, 2005).

Although BNYVV has occurred in Morocco since 2004, no attempt has been carried out to characterize the local virus type. Therefore, the present study aims to characterize local BNYVV isolates.

MATERIAL AND METHODS

Five BNYVV sources were collected from different locations in the Tadla region (Afourer, Beni Amir, Dar Ouled Zidouh, Ouled M'Bark and Souk Sebt) and were used to perform RNA extractions using RNaesy Plant Mini Kit (Qiagen, Germany) according to the manufacturer's instructions; then, tested by RT-PCR using specific primers to amplify a 567-bp fragment of the coat protein (CP) gene of BNYVV (Schirmer et *al*., 2005). The PCR program used was 94°C for 3 min, 35 cycles of 94°C for 1 min, 61°C for 1 min and 74°C for 1 min, followed by final extension at 72°C for 10 min. PCR products were electrophoresed in 1.2% agarose gel in 1X TAE buffer and stained with ethidium bromide (25 ng/ml) and visualized on UV trans-illuminator. Fragment sizes were determined by comparison with a 1 Kb DNA standard marker (GeneRulerTM DNA Ladder, 0.5 ug/ul).

PCR products corresponding to the CP (567 bp) gene, from five local BNYVV isolates form Tadla region (TDL1, TDL2, TDL4 and TDL5), were purified using a DNA extraction kit (Fermentas, Lithuania) and sequenced. Sequencing was carried out in two directions and each sample was sequenced twice. The obtained sequences were aligned using the BioEdit software version 7.0.9 (Hall, 1999) and the MEGA software version 5.05 (Tamura *et al.,* 2011). The CP gene sequence of one Moroccan isolate from Tadla region (RHZ-TDL-Mor) was deposited in the GenBank database under the accession number KY771166. RHZ-TDL-Mor was then confronted with other strain references available in the NCBI database. Calculation of pair wise nucleotide distances between sequences and clustering were performed using the phylogenetic and molecular evolutionary genetic analysis software MEGA version 5.05 for the estimation of the nucleotide homology (Tamura *et al.,* 2011).

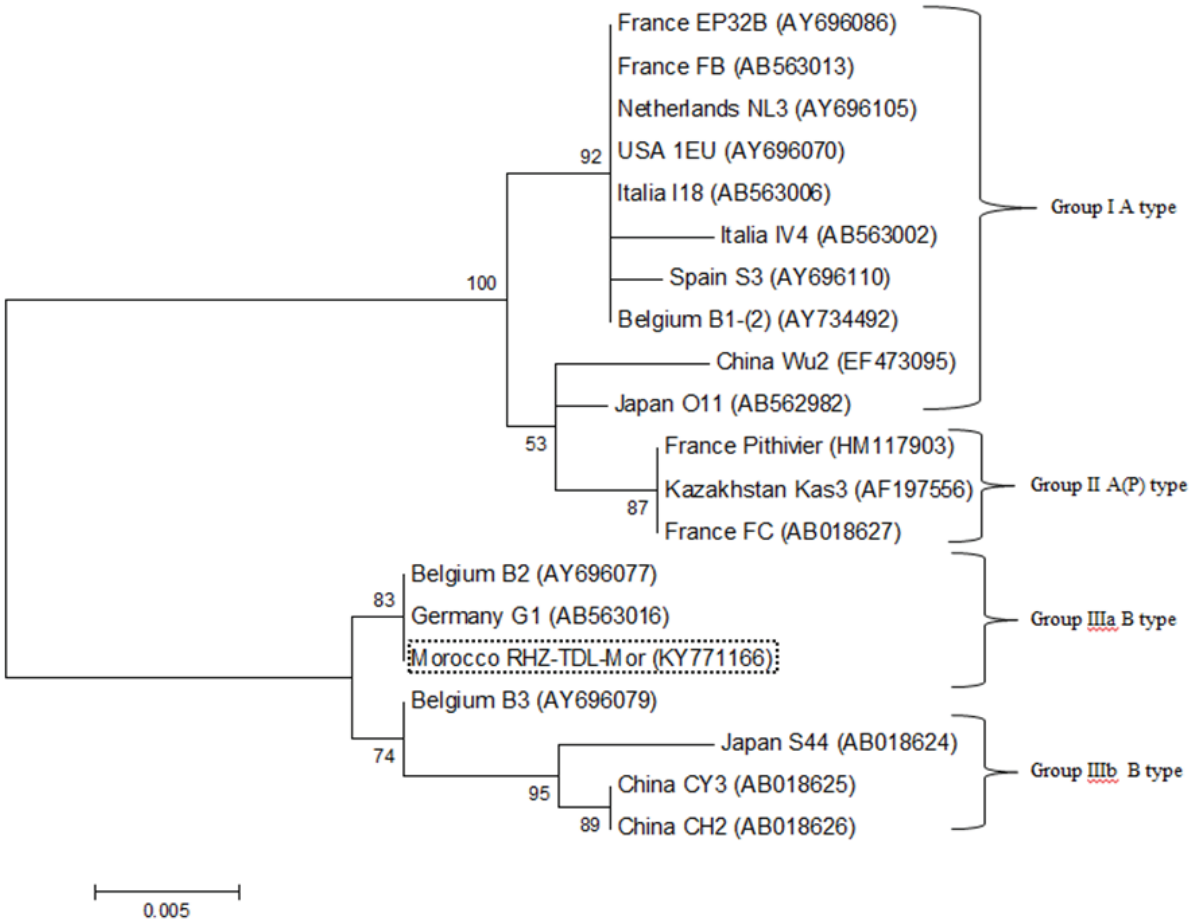

Figure 1: Phylogenetic tree of Beet necrotic yellow vein virus encoded CP sequences using the neighbour-joining method. The names indicate the country of origin and name of the isolate and are followed by accession number (in parentheses). Scale bar indicates the phylogenetic similarity index (bar size refers to 0.005 amino acid changes per site). Numbers on branches represent the bootstrap values out of 1000 replicates. Only bootstrap values over 50 are shown.

RESULTS AND DISCUSSION

Multiple alignments and the sequence identity matrix of the CP gene showed a high sequence similarity among the five sequenced Moroccan BNYVV isolates in the Tadla region (100 % nucleotide identity). Thus, a low genetic diversity exists within the Moroccan BNYVV population; suggesting that the virus was not introduced into Morocco from multiple sources. The obtained results showed that the Moroccan isolate RHZ-TDL-Mor (KY771166) was in the B type of BNYVV and showed the highest nucleotide similarity with the Belgian isolate B2 isolate (AY696077), with a sequence homology of 100% (Figure 1).

RHZ-TDL-Mor clustered with Belgian (AY696077) and Deutsch (AB56306) isolates indicating a possible common entrance or introduction from these regions. We also found that coat protein gene of local RHZ-TDL-Mor isolate was closely related to other sequences of different BNYVV isolates available in the database with a sequence homology ranging from 96.2% to 98.9%; supporting the idea that coat protein is a relatively stable sequence in BNYVV genome.

Comparisons based on CP-encoding nucleotide sequences indicated that percentage identity is highly conserved for all the isolates reported worldwide, suggesting that either the virus has a very stable genome or this might be the incidence of a recent introduction of the virus in different sugar beet growing areas. The result is quite in agreement with the findings of Bouzoubaa *et al.,* (1987) where high level of genome conservation was reported for BNYVV reported isolates.

Rhizomania is one of the most economically important diseases affecting sugar beet, and is widely distributed in most sugar beet growing areas of the world. The studies on BNYVV worldwide revealed that there are three pathotypes; A-type, B-type, and P-type (Kruse *et al.,* 1994; Koenig *et al.,* 1995; Schirmer *et al.,* 2005). The A-type is distributed throughout most sugar beet growing regions of the world and is, so far, the only form present in the Tadla region.

Fields remain infested with BNYVV indefinitely by *Polymyxa betae* cystosori that remain dormant for up to 25 years. It has been reported that the environmental conditions and cultural practices do not alter the viability of the virus inside the resting spores (Tamada, 2002; Rush, 2003). Therefore, rotation to non-host crops or lengthening rotations is ineffective at reducing disease incidence, and the only viable means of control has been natural host-plant resistance. Indeed, the virus detection and timely diagnosis is of utmost importance.

CONCLUSION

BNYVV is one of the most economically important viruses infecting sugar beet in Morocco. Molecular characterization studies showed low genetic diversity among BNYVV isolates in the Tadla region. Also, these local isolates were all of the BNYVV B type and showed the highest nucleotide similarity with the Belgian BE isolate. Results of the present study are important in terms of future efforts aiming at restraining the spread of BNYVV to new areas. As with other soil-borne viruses, efforts to eradicate BNYVV from newly contaminated fields could be very difficult if not impossible without abandoning cultivation of susceptible crops for years. In areas where BNYVV occurs, introduction of rhizomania-resistant sugar beet cultivars is the most important option whose sustainability depends on the genetic variability of the local BNYVV populations. This study provides information for planning future strategies to control rhizomania in Morocco.

Aspects which require further investigations include determining the density of *Polymyxa betae* carry BNYVV, and the stimulation of cystosori in the soil to release zoospores in absence of the host or the needed trap plants to attract zoospores before planting sugar beet in infected soils. Additionally, more investigations are needed to determine the predominant BNYVV type in the other beet sugar cultivation areas. Answers to these questions may help to develop control strategies against BNYVV and other viruses transmitted by plasmodiophorid vectors.

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