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An International Organization to Improve Knowledge on *Potato Virus Y*

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ABSTRACT

The *Potato Virus Y* (PVY) is one of the most economically important potato pathogens. PVY isolates are described according to their biological, serological and molecular characteristics. Such data have been used to classify PVY isolates in groups (including the PVY^{N} and PVY^{O} main groups) and variants (PVY^{N}-W (also described as $PVY^{N:O}$) and PVY^{NTN}). However, the high variability of the PVY genome, together with the restricted knowledge on molecular determinant(s) linked to PVY biological properties and the characteristics of commonly used detection tools have impaired the description of several PVY isolates. Indeed, numerous studies have reported isolates with a set of properties that do not fit in the already described classification. It has been shown that most of these unconventional isolates result from original genomic recombination events between PVY^{N}- and PVY^{O}-like sequences. These viral isolates have to be more efficiently detected and characterized to 1) improve the description of the diversity of this potato pathogen, 2) better understand the PVY evolutionary processes and 3) identify selection pressures applied to the PVY genome during its evolution history. To reach these aims, 22 scientists and team leaders from European, African and American laboratories have created the "PVY^{wide} Organization" (www.inra.fr/pvyorganization). Through this organization, members coordinate research on variability and evolution of PVY and analyse jointly the characteristics of thousands field-collected isolates. In addition to the studies of the plant-virus-vector interactions (e.g. pathogenicity, aphid transmission, host range, etc.), viral genomes of PVY isolates will be analysed using either an innovative technology for viral genotyping (multiplex icosaSNaPshot) or a regular sequencing procedure. All together, the resulting data could potentially offer new understandings on PVY.

Keywords: biological and genomic diversities, potato network, viral evolution, worldwide collection

POTATO VIRUS Y AND THE POTATO SEED INDUSTRY

Potato is the fourth most important food crop in the world after rice, wheat and corn, with a yield of 315 million tons in 2006 (www.potato2008.org/). The recent introduction (during the last 20-30 years) of potato into the Asian and African markets has modified the world balance of tuber consumption. According to the economical importance of potato, China (the world leader in potato production) has initiated programs to increase its potato production potential. Europe (ranked first in the world for potato production) and North America (ranked first in the world for yield production) resist against the current increase of foreign markets. The potato industry in the world is currently being affected by several pests including potato viruses. These viruses reduce commercial yields and the quality of seed. *Potato Virus Y* (PVY) (Potyviridae: *Potyvirus*) is rapidly becoming the most economically important potato virus and causes severe yield losses (10-80%) to potato growers in all potato growing regions in the world (Valkonen 2007). Seed certification agencies in many countries indicate that PVY infection is also jeopardizing the seed production industry. For these reasons, the production of high quality tubers is a real economic and agronomical challenge of worldwide importance. However, despite the improvement of diagnostic tools (from visual cues to molecular tools), the official procedures resulting from the United Nations Organization recommendations (or from more stringent regulations) are based on a outdated scheme that restricts the management of situations to act only after the spread of the pathogen at regional, country or continental scales. In current context of a highly competitive international market worth several billion Euros, the limited knowledge of PVY diversity and consequently the lack of reliable PVY diagnostic tools are unacceptable for the major potato producing countries. The description of the evolutionary potential of this highly damaging potato pathogen will improve the management of risks associated with PVY and will also allow to anticipate the next emerging problems associated with the genetic variability of this virus. This will increase the quality of tubers sold by organizations that implement the results generated by this research. The value of these improvements in potato tuber quality would potentially generate millions of Euros in the potato tuber markets.

POTATO VIRUS Y: A HIGHLY VARIABLE PATHOGEN

PVY possesses a single-stranded positive sense RNA genome of approximately 9700 bases (Kerlan 2006). The PVY genome presents a large open reading frame (ORF), a viral genome-linked protein (VPg) covalently attached to the 5′-RNA by a tyrosine, a poly adenine tail at the 3′-end of the genome and untranslated regions flanking this ORF. A recent study has reported the presence of a second short ORF (PIPO; Chung *et al.* 2008) embedded within the previously described large ORF (**Fig. 1**). The polyprotein (3061 amino acids) encoded by the large ORF is cleaved by three viral proteases (NIa, HC-Pro and P1; Oh and Carrington 1989; Verchot *et al.* 1992; Carrington *et al.* 1993) in 9 products required to achieve the viral cycle. PVY infects a wide

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Research Note

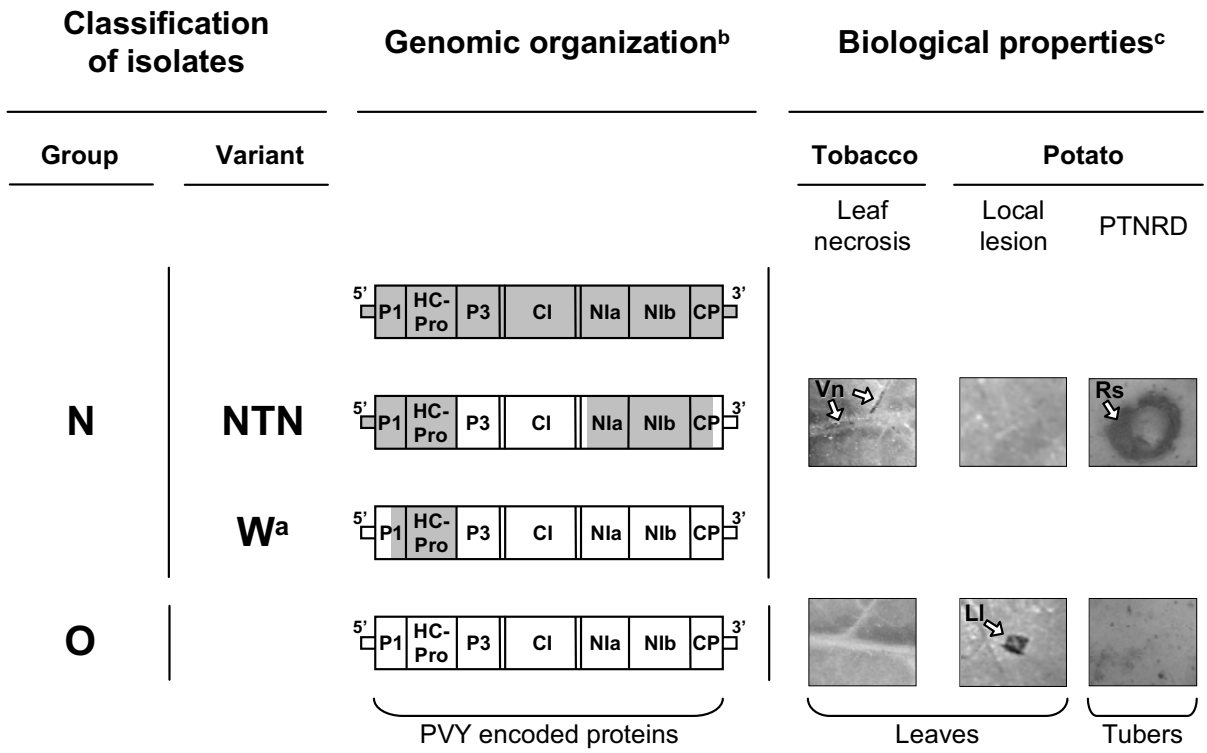

Fig. 1 Molecular and biological variability of PVY isolates belonging to the main described PVY groups and variants. a: PVY^{N}-W variant are closely related to the North American $PVY^{N:O}$ variant. b: Boxes correspond to PVY genes and to non translated sequences. Grey and white areas indicate the PVY^{N}- and PVY^{O}-like sequences, respectively. c: Biological properties are based on symptoms observed on *Nicotiana tabacum* cv. 'Xanthi' and on leaves and tubers of selected potato cultivars (*Nytbr*; e.g. cv. 'Désirée'). PTNRD: Potato Tuber Necrotic Ringspot Disease. Vn: vein necrosis, Rs: necrotic ringspot, Ll: local lesion.

range of plant species (De Bokx and Hunttinga 1981), in particular species from the family Solanaceae (including potato, tobacco, pepper, tomato and solanaceous weeds). It is transmitted in a non persistent manner by more than 70 species of aphid, including species that feed both exclusively and non-exclusively on the PVY host plants (Sigvald 1984). The PVY is highly variable (Singh *et al.* 2007) at biological and molecular levels (**Fig. 1**). To date, molecular data obtained from PVY detection and characterization studies showed that PVY evolved by mutation and recombination events (for a review see Glais *et al.* 2001). However, little is described about details of the genetic variability of this plant pathogen. The data available in databases, scientific literature, and the wider scientific community are mainly constituted by partial characterisations of few tens PVY isolates using different biological and/or molecular tools. Yet, the absence of standardized procedures for description and characterization of isolates makes difficult the use of available data to evaluate the diversity and the evolution of PVY populations at a world scale.

At the beginning of the 20th century, two types of "ancestral" PVY isolates were identified and categorized as either PVY^{O} (ordinary) or PVY^{N} (necrotic) group members, according to the infection symptoms they induced on tobacco (*Nicotiana tabacum*) and potato (*Solanum tuberosum*) plants. Isolates of the PVY^{O} group induce mottling and mosaic symptoms on tobacco and mild to severe mosaic and leaf drop on potato, while isolates belonging to the PVY^{N} group induce veinal necrosis on tobacco and cause very mild mottling with occasional necrotic leaves on potato. Following the initial identification of isolates (PVY was first identified in 1931 from potato plants from South America (Smith 1931), epidemiological data (1930-2000) from Europe and North America revealed the establishment of the necrotic isolates in natural populations of PVY (at the expense of non necrotic PVY^{O} isolates; Piche *et al.* 2004; Lindner and Billenkamp 2005; Glais L., pers. comm.). At the beginning of the 80's, variants of PVY were described in Poland (PVY^{N}-W; Chrzanowska 1991) and Hungary (PVY^{NTN}; Beczner *et al.* 1984). PVY^{N}-W isolates, closely related to the $PVY^{N:O}$ described few years ago in North American countries (Singh *et al.* 2003), are different from ancestral isolates by exhibiting PVY^{O} serology but inducing group PVY^{N} necrosis symptoms on tobacco plants during infection. PVY^{NTN} isolates have all the biological characteristics of the PVY^{N} group and are distinguished from standard PVY^{N} isolates by their ability to induce necrosis on potato tubers (Potato Tuber Necrosis Ringspot Disease, PTNRD). Since their first description, the PVY variants (PVY^{N}-W and/or PVY^{NTN}) have spread and now represent (at least in France, Germany and The Netherlands) the majority of PVY isolates within natural populations. The increased virulence and aggressiveness of these variants may be the cause of recent important losses in potato cultivation.

Recent published studies have revealed two point mutations, occurring in the gene coding for the multi-functional HC-Pro protein, linked to ability of PVY to induce necrosis on tobacco leaves (Tribodet *et al.* 2005). However, molecular determinants involved in necrosis ability of PVY that have not been characterized are present within the viral sequence (Schubert *et al.* 2007; Lorenzen *et al.* 2008; Tribodet M., pers. comm.). Investigations performed by numerous international research groups into the genomic organisations of PVY variants revealed from one to three recombination events between PVY^{N}- and PVY^{O}-like sequences (Glais *et al.* 2002). Data recently collected in Europe (France, Glais L., pers. comm.; Germany, Schubert *et al.* 2007; Netherlands, Van der Vlugt R., pers. comm.) and North America (United States; Lorenzen *et al.* 2006; Gray S., pers. comm.) revealed PVY isolates with original serological and pathogenicity phenotypes, resulting from novel genomic recombination events. Viruses possessing these novel genomic organisations have been identified in different host plants (including potato plants) and in different countries and continents. In addition, bioinformatics analyses of complete PVY sequences available in public databases revealed that several PVY^{N} or PVY^{O} isolates seem to result from not yet reported recombination events.

Table 1 The "PVYwide organization" network.

Member	Institute	Country
Alvarez Juan Manuel	University of Idaho	USA
Balmelli Carole	Station de recherche Agroscope Changins-Wädenswil ACW	Switzerland
Barker Ian	International Potato Center	Peru
Bellstedt Dirk	University of Stellenbosch	South Africa
Blanchard Alexandra[a]	National Institute of Agronomical Research	France
Boonham Neil	Central Science Laboratory	England
Crosslin James	United States Department of Agriculture	USA
Dedic Petr	Potato Research Institute Havlíčkův Brod	Czech Republic
Fox Adrian	Scottish Agricultural Science Agency	Scotland
Gray Stewart	Cornell University	USA
Jacquot Emmanuel[a]	National Institute of Agronomical Research	France
Karasev Alexander	University of Idaho	USA
Lindner Kerstin	Plant Virology, Microbiology and Biology Institute	Germany
Pompe-Novak Maruša	National Institute of Biology	Slovenia
Rolot Jean-Louis	Walloon Agronomical Research Center	Belgium
Spetz Carl	Norwegian Institute for Agricultural and Environmental Research	Norway
Tomassoli Laura	Plant Pathology Research Center	Italy
Valkonen Jari	University of Helsinki	Finland
Van der Vlugt René	Plant Research International	The Netherlands
Varveri Christina	Benaki Phytopathological Institute	Greece
Whitworth Jonathan	United States Department of Agriculture	USA
Zimnoch-Guzowska Ewa	Plant Breeding and Acclimatization Institute at Radzikow	Poland

[a] current chairmen of the organization

The description of such recombinant genomes, for isolates belonging to the PVY^{O} or PVY^{N} groups, exemplifies the limitations of our current understanding of the PVY genetic diversity.

To date, the description of new genomic organization is limited. Indeed, new genomic organizations that do not induce biological differences in environmental and/or experimental conditions would remain unknown with the use of currently available tools. Thus, PVY isolates with recombinant genomic organization which have been reported in scientific literature represent only a fraction of the genetic diversity of this plant pathogen. In addition, described and not yet described recombinant genomes could potentially belong to the next important potato pathogens. It is therefore important to study the biological and molecular variability as well as the evolutionary potential of PVY. Analyses of data produced through an international cooperation could i) lead to an improved understanding of the events that have led to the current situation of this pathosystem (PVY/Solanaceae) and ii) facilitate the estimation of both the probability of emergence of novel PVY entities and their agricultural and/or environmental impacts.

A WIDE ORGANIZATION TO IMPROVE PVY RESEARCH

In response to the extent of the problems associated with the spread of the necrotic PVY isolates in potato fields throughout the world, which were reported during the last European Association for Potato Research meeting (Edinburgh, Scotland, June 2007) and the American Phytopathological Society congress (San Diego, Ca, USA, July 2007), team leaders of numerous international research institutes (from Belgium, Czech Republic, France, Finland, Germany, Greece, Italy, Norway, Peru, Poland, Scotland, Slovenia, South Africa, Switzerland, The Netherlands and The United States) have expressed their interest in participating in cooperative research projects related to PVY evolution, emergence, and spread. Consequently, an international network of collaborators, "The PVY^{wide} Organization" (http://www.inra.fr/pvyorganization) was constituted in September 2007 (**Table 1**). The main objective of this network is to coordinate researches on variability and evolution of biological and molecular properties of PVY performed in numerous laboratories in the world. Twenty-two scientists and team leaders located in Europe, Africa and America are analysing jointly the biological and molecular characteristics of thousands field-collected PVY isolates from wide and intensive surveys. Each laboratory involved in this program contributes to create a wide library of PVY isolates. All together, these viral isolates form one of the most complete library of a plant virus species (>5000 PVY isolates collected to date) ever constituted. Each isolate of this library is characterized by some members of the PVY^{wide} Organization using appropriate standardized tools and/or procedures to describe its biological (using indicator host plants), serological (using appropriate monoclonal/polyclonal antibodies) and/or molecular (using standard RT-PCR procedures and/or real-time RT-PCR assays) properties. The diversity and the complementarity of the skills of the PVY^{wide} members (i.e. entomology, molecular biology, classical "green" virology and bioinformatics), as well as the different equipments and facilities available in their institutes, offer an excellent possibility to study and analyze different aspects (such as epidemiology, pathogenicity and plant-virus-vector interactions) of the complex PVY pathosystem. In complement to the characterization of PVY biological properties, the viral genome of PVY isolates will be genotyped (using an innovative multiplex SNaPShot procedure) and sequenced (partial or full length sequence). Produced data will be analysed using computer-based procedures. All together, the resulting data would offer the opportunity to better understand processes supporting the high diversity of PVY and would potentially make possible the identification of biotic and abiotic pressures applied to the PVY during its evolutionary history.

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