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Review

Achievements and prospects in breeding for rhizomania resistance in sugar beet

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ABSTRACT

Economic viability of a sugar beet crop largely depends on its successful protection against rhizomania, a most devastating disease that causes severe losses in root yield, sucrose content and quality. Rhizomania disease is caused by *Beet necrotic yellow vein virus* (BNYVV), a virus present in most sugar beet growing regions being vectored by the widely spread soil borne protoctist *Polymyxa betae* Keskin. The only practical means to control the disease is the use of genetically resistant varieties and, to date, such resistance is mainly based on a dominant gene (*Rz1*) that when present confers a sufficiently high level of protection against BNYVV. However, the emergence of virus strains capable of compromising the resistance employed in commercial varieties as well as a possible spread of more pathogenic isolates threatens crop's protection efficiency in the future. All these point to the necessity for exploiting new and more effective genetic sources of rhizomania resistance, both by classical and molecular breeding approaches, a practice that is being pursued by the relevant breeding firms. This article critically reviews the various issues related to the disease and its management and particularly to the ones pertaining to pathogen genetic diversity, types of genetic resistance currently employed, as well as to novel biotechnological approaches aiming at the development of better resisting cultivars.

Contents

1. The rhizomania disease

Historically, *Beet necrotic yellow vein virus*, the etiological agent of rhizomania disease (Tamada and Baba, 1973), is considered as one of the most important threats in worldwide sugar beet cultivation (Tamada, 1999; Lennefors et al., 2005). In the absence of efficient control measures, the disease causes severe economic losses due to a dramatic reduction in root yield, sugar content and purity (Tamada, 1999). The virus is the type species of the genus *Benyvirus* (Torrance and Mayo, 1997; Tamada, 1999) and is transmitted by the widely spread soilborne protoctist *Polymyxa betae* Keskin (Fujisawa and Sugimoto, 1976) which, due to its thick-walled resting spores, can survive in soil for years (Abe and Tamada, 1986). Rhizomania disease root symptoms mainly include a massive proliferation of secondary and tertiary roots that eventually become necrotic and give the root a bearded appearance, a profound constriction of the main taproot, a general plant stunting and a brown discoloration of the vascular stele (Richard-Molard,

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1985; Putz et al., 1990). Foliar symptoms are mostly manifested by a bright fluorescent yellowing which can be easily confused with nutrient deficiencies. The yellow vein appearance, that provides the name for the disease causal agent, is only rarely found and mostly confined to fields infected by a specific virus pathotype (Tamada, 1975). Diseased plants usually occur in patches, but can also be found scattered throughout the field. Disease responses at the physiology level include a reduced transpiration and CO_2 uptake, a reduced content of nitrogen, chlorophyll and carotenoid and an elevated amino nitrogen, sodium and potassium in the root sap (Steddom et al., 2003). For a detailed review on morphophysiological consequences of the disease the reader is referred to Rush (2003).

Disease diagnosis is usually performed by immunological tests such as DAS-ELISA, whereas pathotype differentiation was in the past mainly based on molecular techniques such as single-strand conformation polymorphism (SSCP) and restriction fragment length polymorphism (RFLP) analysis (Kruse et al., 1994; Koenig et al., 1995; Suarez et al., 1999). Since some years differentiation is mainly performed by partial or complete (re)sequencing (Koenig and Lennefors, 2000; Meunier et al., 2003; Schirmer et al., 2005).

2. Genetic features and diversity of BNYVV

BNYVV has a multipartite genome consisting of four genomic messenger-like RNAs, with some isolates also possessing a fifth RNA species, RNA 5. All genes required for basic house-keeping functions including replication, encapsidation and cellular translocation reside on RNAs 1 and 2, whereas the small RNA species RNA 3, 4 and the isolate-specific RNA 5 encode for genes involved in vector transmission and pathogenicity (Fig. 1) (Tamada, 1999) (for details see Tamada, 2002; McGrann et al., 2009). RNA 3-encoded p25 is a major determinant of disease expression in the sugar beet host but also acts as an avirulence factor in resistant sugar beet lines. The outcome of BNYVV-host-specific resistance interactions is mainly controlled by single amino acid changes in p25 (Acosta-Leal et al., 2008, 2010b; Chiba et al., 2008, 2011; Koenig et al., 2009; Pferdmenges et al., 2009).

BNYVV has been classified in three major pathotypes, referred to as A, B, and P (Koenig et al., 1995; Koenig and Lennefors, 2000). Type A is widespread in most European countries, the USA, China and Japan (Schirmer et al., 2005). Type B has a limited spread and is primarily found in Germany and France (Kruse et al., 1994), while it has been also incidentally reported in Sweden, China, Japan and Iran (Miyanishi et al., 1999; Lennefors et al., 2000; Sohi and Maleki, 2004; Koenig et al., 2008). BNYVV type P contains an additional genomic RNA (RNA 5) and is closely related to the A-type (Miyanishi et al., 1999; Schirmer et al., 2005). P-type was originally discovered in Pithiviers, France (Koenig et al., 1997) and was later also encountered in Kazakhstan (Koenig and Lennefors, 2000) and recently, in the UK (Ward et al., 2007) and Iran (Mehrvar et al., 2009). Other RNA 5-containing isolates have been reported in Japan, China (Tamada et al., 1989; Kiguchi et al., 1996; Miyanishi et al., 1999), the UK (Harju et al., 2002; Ward et al., 2007) and in Germany, where an Asian RNA 5-containing BNYVV isolate has been recently found to occur (Koenig et al., 2008). BNYVV isolates containing a fifth RNA species are generally considered as more aggressive than those containing RNAs 1-4 (Tamada et al., 1989, 1996; Heijbroek et al., 1999), presumably due to *in planta* transcription by the RNA 5-encoded p26 (Link et al., 2005).

Recent studies on the evolutionary history of BNYVV, based on the magnitude and complexity of sequence variation of four genes (RNA 2-*CP*, RNA 3-*p25*, RNA 4-*p31* and RNA 5-*p26* genes), revealed the existence of various reassortant isolates in China and Japan, as a result of mixed infections of different source isolates. It was thus suggested that the virus most probably originated in East Asia long before the beginning of sugar beet cultivation and that wild beet or related species might not have been the natural hosts of both BNYVV and *P. betae* (Chiba et al., 2011). The spread of BNYVV into the crop dates in recent years. Since the initial reports for the disease (Canova, 1959), the virus has colonized most sugar beet growing areas worldwide, yet generally showing a considerable genetic stability among virus populations separated in space and time (Koenig and Lennefors, 2000). BNYVV populations also present a relatively low incidence of reassortants or natural recombinants (Schirmer et al., 2005). Given its multipartite genome and the frequent occurrence of mixed infections with different BNYVV strains (Koenig et al., 1995), it has been assumed that natural selection poses constraints in an expected BNYVV diversification and conditions virus evolution by acting as a filter controlling the mutations that eventually become fixed (Acosta-Leal et al., 2008).

Despite the relatively low general genetic diversity among BNYVV isolates, different degrees of selection pressure seem to operate, depending on the gene and geographic location. In this framework, the RNA 3-p25 was found to be subjected to the strongest selection pressure and, more importantly, it has been demonstrated that certain amino acid changes in p25 are associated with the emergence of BNYVV strains capable of compromising the commercially exploited partial resistance sources in the last decade (Schirmer et al., 2005; Acosta-Leal et al., 2008, 2010a; Chiba et al., 2008, 2011; Koenig et al., 2009). More specifically, it has been suggested that amino acids 67–70 of p25 are linked with symptom development in resistant cultivars (Schirmer et al., 2005), although other amino acid residues in p25 may also influence isolate virulence (Acosta-Leal and Rush, 2007; Liu and Lewellen, 2007; Chiba et al., 2008). However, conclusive evidence has been to date obtained only for positions 67 and 68. In this framework, it has been shown that the amino acid change A → V at position 67 is associated with increased virulence in cultivars endowed with the *Rz1* and/or *Rz2* resistance genes (see later section) (Acosta-Leal et al., 2008, 2010a,b; Koenig et al., 2009; Pferdmenges et al., 2009; Pferdmenges and Varrelmann, 2009). In addition, it has been reported that amino acid residue 68 plays a major role in pathogenicity (Acosta-Leal et al., 2008; Chiba et al., 2008) and furthermore, changes from F or Y to C, H, L, Q lead to an increased virulence and thus, to the manifestation of the *Rz1*-resistance breaking (RB) trait (Chiba et al., 2011). Although, based on these studies, the $V_{67}C_{68}$ motif of p25 is generally considered as responsible for RB, the occurrence of such isolates was not always found associated with more pronounced disease symptoms, higher virus accumulation in the roots and reduced performance of the varieties tested as a result of *Rz1*-RB (Liu and Lewellen, 2007; Pavli et al., in press).

The possible association between genetic diversity of the virus and resistance breaking ability has been investigated in a study of host effect, employing *Rz1*, *Rz2* and susceptible plants, on genetic diversification of BNYVV. Although no direct such association was evidenced, the study revealed a significant increase of virus diversity in proportion to the strength of host resistance and it was argued that the genetic structure of BNYVV populations is correlated with virulence and the magnitude of defence barriers to be defeated for disease occurrence (Acosta-Leal et al., 2010a).

3. Conventional and molecular breeding for rhizomania resistance

Rhizomania incidence and severity can be only very moderately reduced by preventive cultural practices such as rotation, avoidance of excessive soil moisture and early plantings. Consequently, the only substantial means to ensure a viable crop production in