O.63 - Risk assessment of cyst nematode evolution and durability of potato cyst nematode resistance

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Abstract

To understand the process that leads to breakdown of a plant resistance gene, we need to understand the processes that govern pathogen evolution. By studying two of the main evolutionary forces (migration and mutation) operating on nematode populations, we show that cyst nematodes appear to present a higher risk than previously thought. In particular we showed that cyst nematodes exhibit a high level of gene flow at the intra-regional level and that parasitism genes tend to accumulate non synonymous substitutions and were subjected to strong diversifying selection pressures.Different oligogenic resistances to the potato cyst nematode Globodera pallida were introduced from wild species (Solanum sparsipilum, S. spegazzinii and S. vernei) in cultivated potato. At INRA we have focused most on these 20 last years on the S. vernei resistance and the first consumable variety carrying this resistance will be soon available. Its durability was estimated by (1) studying the efficiency of the resistance against the variability of the parasite in its native area, (2) studying the heritability of aggressiveness of hybrids made by crossing virulent and non virulent nematodes and (3) studying the circumvention of the S. vernei resistance after five years of continuous selection pressure on a field nematode population. The results strengthen the need to manage the use of these resistances to nematodes, to continue with the development of quarantine measures to avoid any new introduction of PCN in Europe and to increase our knowledge of the processes that govern pathogen evolution.

Potato cyst nematodes (PCN) are of major agronomic importance. In maritime temperate climates, PCN species *Globodera pallida* and *G. rostochiensis* are amongst the most damaging potato pests, besides *Phytophthora infestans*. The two *Globodera* species can lead to yield decrease of up to 70%. The PCN are biotrophic sedentary endoparasites with obligatory sexual reproduction. Their lifecycle involves the J1 to J4 juvenile and the adult stages, separated by moults. The first moult occurs in the eggs contained in the female. Potato root exudates stimulate the hatching of the infective second stage juveniles (J2). The J2 penetrate the roots, migrate intracellularly, establish a permanent feeding site forming a syncytium close to the vascular tissues, and become sedentary. The J2 undergo three moults during development to the adult stage. Males leave the roots to fertilise the swollen females. Females produce hundreds of eggs which remain enclosed in a cyst constituted of the hardened phenolysed dead female body. Nematodes remain viable in the cyst for many years. Sex is determined epigenetically, with the frequency of males increasing under crowding or poor nutrition conditions at the J2 stage.

PCN originate from South America. They were introduced in Europe at the end of the XIXth century and, despite they are considered as quarantine pests, they are nowadays dispersed in most of the potato producing countries. Building on earlier work showing a clear south to north phylogeographic pattern in Peruvian populations, we have been able to identify the origin of European populations with high accuracy. They are all derived from a single restricted area in the extreme south of Peru, located between the north shore of Lake Titicaca and Cusco. Only four cytochrome B haplotypes are found in Europe, one of them being also found in some populations of this area of southern Peru. The allelic richness at seven microsatellite loci observed in the European populations, although only one third of that observed in this part of southern Peru, is comparable to the allelic richness observed in the northern region of Peru. This result could be explained by the fact that most of the genetic variability observed at the scale of a field or even of a region is already observed at the scale of a single plant within a field (see also later results on the level of gene flow observed). Thus, even introduction via a single infected potato plant could result in the relatively high genetic variability observed in Europe.

Resistant crops have been developed and represent an efficient and environmentally friendly means to control this pest. However, two major limits to the use of these resistances exist: (1) the resistance is often specific to a species or even a group of populations in this species, (2) nematode populations can adapt to the pressure imposed by the resistance and then breakdown the resistance. To understand the process that leads to breakdown of a plant resistance gene, we need to understand the processes that govern pathogen evolution. McDonald and Linde have proposed an assessment of pathogen risk based on the characteristics of the main evolutionary forces that act on populations. By studying two of the main evolutionary forces (migration and mutation) operating on nematode populations, we show that cyst nematodes appear to present a higher risk than previously thought.

Very few studies of population genetics have been done on plant parasitic nematodes. However, to be able to manage resistance in a durable way it is first necessary to gain knowledge of the neutral genetic variability of the populations. This genetic variability can then be used to quantify gene flow at different spatial scales or to obtain genetic knowledge of the reproduction of these animals, two elements that will form the basis of any future device for durable management of resistant cultivars to nematodes. Study of the G. *pallida* population genetic structure in its native area (Peru) revealed massive gene flow (Fst < 0,06) even between populations several kilometres distant and with a clear pattern of isolation for populations more than 100km distant. This absence of genetic differenciation at this spatial scale is most probably the consequence of a massive dispersion of cysts through natural factors (wind, rain) or human activities (field labour, exchange of contaminated plants/tubers). Results obtained also reveal a high level of consanguinity among the populations. All these data confirmed those obtained previously on the beet cyst nematode *H. schachtii*. Therefore, we can assume that this population's genetic functioning is common to cyst nematodes.

Durability of resistances will be also strongly dependent on the process that will take place during the plant–nematode interaction and in particular will depend on products secreted by the nematodes and that will allow them to penetrate their host, migrate into the plant tissues, initiate a feeding site and avoid the plant defences. Pathogenicity factors secreted by the nematode into plant cells from subventral and dorsal oesophageal gland cells via the stylet are assumed to play key roles during parasitism. Some of these pathogenicity factors may also represent virulence genes as it is postulated for the soybean cyst nematode chorismate mutase and the Rbp1 gene isolated in *G. pallida* for which the protein product was recently shown to interact with the product of the *Gpa2* nematode resistance gene. Study of the sequence variability of RBP1 in a set of more than 13 G. *pallida* populations allowed us to identify polymorphisms in some structural domains of the proteins involved in protein-protein interaction. The nature of the selection occurring on this gene was defined through the estimation of the Ka/Ks ratio along the gene sequences and using maximum likelihood methods we were able to demonstrate that some residues have evolved under diversifying selection. One of these residues correlates with the virulent or avirulent phenotype observed when co-expressing these RBP1 variants with the potato resistance gene *Gpa2*.

Different oligogenic resistances to the potato cyst nematode *Globodera pallida* were introduced from wild species in cultivated potato. At INRA we have focused on three diploid species : *Solanum sparsipilum*, *S. spegazzinii* and *S. vernei*. Following the results obtained on the reassessment of the evolutionary risk concerning PCN, the durability of these resistances was estimated by (1) studying the efficiency of the resistance against the variability of the parasite in its native area, (2) studying the heritability of aggressiveness or virulence of hybrids made by crossing virulent and non virulent nematodes and (3) studying the circumvention of the *S. vernei* resistance after five years of continuous selection pressure on a field nematode population.

The F1 and F2 nematode progenies issued from crossbreeding between Peruvian (P5A) and European (Pa2/3) individuals were non aggressive towards *ex-S. vernei* resistance, but were virulent towards resistance from *S. spegazzinii* and *S. sparsipilum*. The resistances conferred by the 3 QTL of *S. spegazzinii* or the major QTL of *S. sparsipilum* may only be durable if no corresponding virulence gene exists in European populations of the nematode.

The circumvention of *S. vernei* resistance was studied after continuous selection pressure of this resistance source on a natural nematode population (St Malo). Different French tetraploid genotypes were evaluated like 60.96.1 ((AM78.3778 x Fanette) x Melissa) and 96F376.16 ((AM78.3778 x Mondial) x 90F136.3). No evolution was observed after 5 years of cultivation of 96F376.16, but for 60.96.1 selected nematodes develop in females in a significantly larger extent on this resistant

genotype as on the resistant genotype from *S. spegazzinii*. It may be inferred that the resistance from *S. vernei* is durable in some genotypes and not in others even when using the same resistant source.

The results strengthen the need to manage the use of these resistances to nematodes, to keep developing quarantine measures to avoid any new introduction of PCN in Europe and to increase our knowledge of the processes that govern pathogen evolution.

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