# POTATO RESISTANCE TO CYST NEMATODES - PECULIARITIES FOR ROMANIA

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**Abstract.** Potato cyst nematodes (PCN) are pests of phytosanitary quarantine with high economic implication on potato production and commercialization. Both nematode species *Globodera pallida* and *G. rostochiensis* have the origin in Peru and evolved with the potato dissemination all over the world during the last two centuries. *G. rostochiensis* was recorded in Romania for the first time in 1984 and starting with 2007 all the country is under phytosanitary quarantine for both species. The analysis of the official potato management programme reveals that there are no measures in place to address abiotic (dry wind, run off water) and biotic factors (i.e. sheep and goat transhumance and boars movement in arable land) as well as climate change impact. The interview of 122 city halls in Sibiu and Braşov counties revealed the constant presence of boars into arable land as well as of sheep and goat movement or transhumance that is positively correlated with the presence of both nematode species in soil. The article renders the need to integrate scientific achievements regarding potato resistance, environmental factors impact and new agricultural practices into the current potato management programme.

**Keywords:** genetic resistance, potato cyst nematodes, environmental factors, transhumance.

**Rezumat. Rezistența cartofului la nematozii cu chiști - particularități pentru România.** Nematozii cu chiști ai cartofului (PCN) sunt dăunători de carantină fitosanitară, cu importante implicații economice asupra producției și comercializării cartofului. Ambele specii de nematode *Globodera pallida* și *G. rostochiensis* își au originea în Peru și au evoluat odată cu răspândirea cartofului la nivel global în ultimele două secole. *G. rostochiensis* a fost înregistrată în România pentru prima dată în 1984 și începând cu anul 2007 toată țara se află în carantină fitosanitară pentru ambele specii. Analiza programului oficial de management al cartofului relevă faptul că nu există măsuri în vigoare pentru a aborda factorii abiotici (vânt uscat, apa de infiltrație) și biotici (transhumanța cu ovine și caprine și migrația porcului mistreț în terenurile arabile), precum și impactul schimbărilor climatice. Interviul a 122 primării din județele Sibiu și Brașov a relevat prezența constantă a porcilor mistreți în terenuri arabile precum și transhumanța ovinelor și caprinelor care este corelată pozitiv cu prezența în sol a ambelor specii de nematode. Acest articol discută necesitatea integrării în actualul program de management al cartofului realizările științifice în ceea ce privește rezistența la cartof, impactul factorilor de mediu și al noilor practici agricole.

**Cuvinte cheie:** rezistență genetică, nematozii cu chiști ai cartofului, factori de mediu, transhumanță.

## INTRODUCTION

Potato is today one of the major crop species for the entire world according to the UN Food and Agriculture Organization (FAO) being ranked on the fifth place in 2015. The inheritance of resistance to pests in potato continues to be a major challenge in practice and research from the beginning of the last century (SALAMAN & LESLEY, 1923). After the Second World War, it was published one of the most impressive works on potato regarding geographical origin, genetic relatives, the dramatic infestation that took place in 1840 in Ireland due to *Phytophtora infestans*, the importance in breeding of the potato germplasm collection of the International Potato Centre of Lima in Peru, including historical, economic and social relevance for Europeans (REDCLIFFE, 1949). The first comprehensive compendium regarding potato diseases was published in 1981 and at least six nematodes have been described such as: potato cyst nematodes (PNC), root-knot nematodes, false root-knot nematodes, lesion nematodes, potato rot nematodes and stubby-root nematodes (HOOKER, 1981). According to this compendium *Globodera pallida* and *G. rostochiensis* were recorded up to 1980 in South America and in all Europe. Today it is a fact that *G. rostochiensis* is recorded all over Europe and that *G. pallida* is not present in only five European countries (i.e. Denmark, Estonia, Latvia, Lithuania and Slovakia) (ROSSI, 2012).

Once accepted the idea of centre of origin of crop species proposed in 1917 by VAVILOV, after 1920, there started scientific expeditions in South America that ended with an impressive germplasm collection comprising at least 200 wild and 8 tuber-producing cultivated species belonging to the genus *Solanum*. In this period of time potato became the subject for high standard scientific research all over the world especially due to its nutrition potential as it became one of the seven pillar crops in the world (HAWKES, 1990; GEBHARDT & VALKONEN, 2001).

Potato was the subject of Potato Genome Sequencing Consortium (PGSC) aiming to elucidate the complete genome sequence of potato that ended by 2011. As potato is a tetraploid species (2n=4x=48) the above mentioned authors consider that it is far from an ideal species for genetic analysis. Thus, at least 25 genetic models should be considered in inheritance studies only for four alleles, per locus, supporting the studies on inheritance and linkage of single genes for potato resistance made by COCKERHAM in 1970. Tremendous efforts have been made for the analysis of potato genome in direct relationship with the genetic basis of resistance to PCN. It is accepted today that PCN comprises 8 pathotypes (Ro1 to Ro5 of *G. rostochiensis* and Pa1 to Pa3 of *G. pallida*) making really difficult the research of testing and further breeding programme for improving resistance to PCN (ASANO et al., 2012).

In 1999, it was proved the presence of forty-five SNPs at six loci spanning 2 cM in the interval between markers *GP21* and *GP179* that were associated with resistance to *G. pallida* (ACHENBACH et al., 2009). QTL markers for resistance to PCN among other pests were identified on all 12 chromosomes of potato genome (GEBHARDT & VALKONEN, 2001). The authors revealed the complexity of potato resistance and proved that the most prominent resistance allele was associated with the linkage disequilibrium group C and positioned close to R genes on the V chromosome. After years of research, in 2008, it was proved for the first time that the origin of the Western European populations of *G. pallida* is in the Peruvian population showing a clear south to north phytogeographical pattern originating from Peru and reconfirming the results published by STONE in 1979 (PLANTARD et al., 2008). Thus, studying the phylogenetic tree of the mitochondrial haplotypes of *G. pallida* by using molecular markers it was proved that western European pathotypes (UK1-4; FR 1, 3, 5, 7 and 9; NL 1 and 2; SW and SP) have correspondence in the Arapa population in Peru.

The genetic variability of the Western European strains is very high and comparable with that of two clades of Peruvian origin. Genetic studies proved that the *Grp1* locus detected on chromosome V seems to confer resistance both against *G. rostochiensis* and *G. pallida* (FINKERS-TOMCZAK et al., 2009). *Ro1* is the only one pathotype found in Japan and researchers are working mainly on the resistance gene *H1* which confers almost full resistance against *G. rostochiensi* (ASANO et al., 2012). The same pathotype was recorded in Russia and resistant cultivars (i.e. cv. 'Hydra', 'Gelda' and 'Kardia') developed in Germany and placed on the market on 1977 have been used as genetic resources for new potato cultivar breeding (SIMAKOV et al., 2008). In Spain, it is relevant the presence of the pathotypes *Pa2/3* (RITTER et al 2008). In 2013, it was published the most complete result regarding the molecular basis of resistance to *G. pallida* and *G. rostochiensis*, different loci belonging to different chromosomes and contributing to the expression of this characteristic (GEBHARDT, 2013).

The rapid dissemination of new potato cultivars all over the world for the last century opened new challenges due to agricultural practices, the novelty of new agro-ecosystems and the interaction with other management practices or species (i.e. domesticated or feral native species) as well as new climatic conditions. There are at least two facets of the issue: the continuous scientific knowledge generation and on the other hand the transfer to the economic domain of this knowledge and to be integrated into issues related to agricultural practices, trade and commercialization. Thus, if science is looking for provable evidences in connection to the genetic basis of potato resistance to PCN and vectors for disseminating it related to strict conditions, agricultural practices and commercialization may further contribute to perturbing the consistency of scientific results due to added factors. One major problem recognized by Romania after 2007 is the continuous infestation of arable land with PCN (DG SANCO, 2010), including the potato seed area (GROZA et al., 2011). Moreover, from a country exporting seed potato after 2007, Romania is under phytosanitary quarantine, complying with a strict monitoring plan of phytosanitary measures (DG SANCO, 2010). The scope of this article is to discuss connections between the current potato monitoring programme and environmental factors contributing to the huge infestation of Romania arable land with PCN.

## MATERIAL AND METHODS

This paper is developed based on an integrative approach regarding the SWOT analysis (strengths, weaknesses, opportunities, and threats) of the national Potato Programme. In this regard there have been used different data bases: the European Cultivated Data Base, the National Catalogue for Plant Varieties and screening literature for PCN resistance. All city halls from Brașov County (i.e. 58 city halls) and Sibiu County (i.e. 64 city halls) have been interviewed for the presence of PCN and boars in the arable land in the period 2009-2014.

## RESULTS AND DISCUSSIONS

**Potato cultivation in Romania.** Potato as a crops species is mentioned to be cultivated in the gardens of Transylvania of the 18$^{th}$ century but not for commercial use, being considered as not important at that time for fiscal system of that region in Europe (GYÉMÁNT et al., 2009). In less than 200 years this gardening crop becomes more and more important in our country due to its remarkable adaptation to climatic conditions. After 1989, Romania passed a political change that is more and more addressed by scientists analysing the socio and economic factors evolution in connection with different subject including land use and crop production (FRASER & STRINGER, 2009). According to these authors the political climate installed after 1989 had major negative impact especially on land use change and crop production making from potato one of the most important crop of the country. Still, according to FAO database, the largest potato harvesting area was of 351,400.00 ha recorded in 1989 and followed by a dramatic decrease in 1992 (i.e. 218,695.00 ha). After 1992 the total harvesting area is about 250,000.00 ha up to 2011 and another decrease is registered in 2014 (i.e. 202,657.00 ha). In terms of productivity, Romania recorded the highest productivity in 1985 (6,631,200.00 t), followed by that of 1989 (i.e. 4,420,000.00 t). Starting with 1989 the potato productivity decreased continuously until 2014 even if it became one of the most important crops for the countryside (i.e. 3,519,329.00 t). It can be considered that after 2007, the year of accessing the European Union, Romania continued to lose potato in terms of harvesting area and productivity.

**National Collections.** Gene Bank of Suceava is in charge to preserve the germplasm collection of the country and currently there are 801 accessions. A single one accession is described as a landrace and collected from high altitude in 1986. In 2009, a complete study regarding potato germplasm collection, including landraces was published in Târgu Secuiesc (BACIU, 2009).

**Potato Breeding Programme.** The National potato breeding programme is implemented in four major research institutions namely the National Institute of Research and Development for Potato and Sugar Beet Brasov (NIRDPSBB), Research and Production Stations Târgu Secuiesc, Research and Production Stations Miercurea Ciuc and Agricultural

Research Station Suceava (STRAJERU & BODEA, 2000). Romania designated a national institute for running research on potato in 1977 based on resources taken from the older research stations dating from 1949. Today, the NIRDPSBB also supports the national potato phytosanitary system. *G. rostochiensis* was for the first time signalled by researchers of the NIRDPSBB in 1984 in Lăzarea, Harghita County, a place for virus free potato seed production (ROJANCOVSCHI & DEHELEANU, 1986; DONESCU & ENOHJ, 1987). In 1991, it was stated that 40-45% of potato production may be destroyed by the presence of these PCN in the soil when the concentration may be over 20 eggs/100 g of soil with a persistence of up to 28 years (BOȚOMAN, 1991). In 2005, the NIRDPSBB stated that it is compulsory that infested lands with *G. rostochiensis* and *G. pallida* to be excluded from the potato seed production (CHIRU, 2005).

Results regarding resistance testing for couple of potato cultivars (Astral, Victoria, Magic and Claudiu) to *G. rostochiensis* pathotypes *Ro1* have been published (BODEA, 2004). According to the Official Catalogue only three varieties have been placed on the market in Romania (i.e. cultivars 'Astral' in 2001, 'Magic' in 2001 and 'Claudiu' in 2003). After four years, there were officially promoted for commercial purpose new potatoes varieties such as Luiza and Ioana (MIKE et al., 2008) not yet accepted into the European Cultivated Potato Database. Still, cultivars such as 'Hydra', 'Gelda' and 'Kardia', with proved resistance against *G. rostochiensis* developed in Germany and placed on the market on 1977, have never been tested in Romania for placing on the market.

**Official Catalogue Analysis.** By investigating the Official Catalogue for plant varieties in Romania for registering years and varieties origin new gaps may be revealed for the current phytosanitary management system. Thus, based on this analysis it can be considered that Romania officially started importing seed potato cultivars for commercialization and cultivation in 1961, after becoming a Party to the International Convention for the Protection of New Varieties of Plants of the International Union for the Protection of New Varieties of Plants (UPOV Convention). Romania ratified the Convention in 2001 but the plant variety protection system is in place in Romania since 1955. The three cultivars: 'Bintje', 'Ostara' and Desiree are considered today as the more susceptible potatoes to the PTC attack (Table 1). 'Bintje' was erased from the Official Catalogue before 1986 but 'Ostara' and 'Desiree' continued to be promoted up to 2004, when they were erased from the Official Catalogue. It can be considered that there was a serious gap between science results and the policy for promoting new potato cultivars and responsible for the spreading of PCN. Cultivars such as 'Roxy', 'Hilta', 'Nicola', 'Gloria', 'Anosta', 'Concorde', 'Koretta' and 'Sante' entered the market place after 1988 (Table 1) and have been officially promoted for being cultivated as resistant to PCN after 1993 (DRAICA & CHIRU, 1994). In case of the cultivar 'Roxy' that was placed on the Romanian market in 1988, it was officially promoted after 5 years in the complex fight against PCN due to its proved resistance to *G rostochiensis* pathotypes *Ro1* and *Ro5*. The same delay is registered for all other cultivars.

Table 1. Potato cultivars imported before 1989 based on the survey of the Official Catalogue for Plant Varieties.

| No. | Cultivar | Country of origin | Year for placing on the Romanian market | Year for placing on the market according to potato database* | Resistance testing to *G. pallida* | Resistance testing to *G. rostochiensis* |
|---|---|---|---|---|---|---|
| 1. | Bintje | Netherlands | 1961 | 1910 | Low: Pa1 and Pa2 | Low: Ro1 and Ro3 |
| 2. | Ostara | Netherlands | 1971 | 1961 | Very low to low: Pa2 | Very low/low: Ro1 |
| 3. | Desiree | Netherlands | 1965 | 1962 | Low: Pa1, Pa2, Pa3 | Low: Ro1, Ro3 |
| 4. | Procura | Netherlands | 1976 | 1971 | -* | High: Ro1 |
| 5. | Gloria | Germany | 1988 | 1972 | - | High: Ro1 |
| 6. | Manuela | Germany | 1976 | 1972 | - | Low: Ro1 |
| 7. | Nicola | Germany | 1985 | 1973 | Low: Pa1 and Pa2 | High/very high: Ro1<br>Very high: Ro4 |
| 8. | Adretta | Germany | 1978 | 1975 | Low: Pa1 and Pa2 | Low: Ro1;<br>Very low to low: Ro5 |
| 9. | Anosta | Netherlands | 1988 | 1975 | - | High: Ro1 |
| 10. | Astarte | Netherlands | 1989 | 1976 | Low: Pa2 and Pa3 | Moderate to high: Ro1<br>Moderate: Ro2<br>Low/moderate: Ro3 |
| 11. | Frezja | Poland | 1984 | 1979 | - | Low: Ro1 |
| 12. | Darwina | Netherlands | 1989 | 1981 | High: Pa2 | High: Ro1, Ro2, Ro3, Ro4, Ro5 |
| 13. | Roxy | Germany | 1988 | 1981 | - | High: Ro1, Ro5 |
| 14. | Koretta | Germany | 1989 | 1983 | - | High: Ro1 |
| 15. | Hilta | Germany | 1988 | 1983 | - | High: Ro1, Ro2, Ro3, Ro5 |
| 16. | Sante | Netherlands | 1989 | 1983 | Low to moderate: Pa1, Pa2, Pa3 | High: Ro1, Ro2, Ro3, Ro4 |
| 17. | Concorde | Netherlands | 1989 | 1986 | Low: Pa1 and Pa2 | High: Ro1<br>Very high: Ro4 |

*- no data available

**The Potato Monitoring Programme** (PMP) is adopted by the National Phytosanitary Agency working within the Ministry of Agriculture and it is implemented with the support of County Phytosanitary Units, County Phytosanitary Inspectorates and four Phytosanitary Laboratories in close cooperation with customs. The national PMP between 2007 and 2015 proposed strict quarantine measures for PCN. Starting with 1991 the NIRDPSBB communicated clear specifications regarding PCN sampling period such as September 15 for start and July ending 15 (COJOCARU, 1991).

The subject of these measures includes: potatoes as commodity, seed potato, soil, producers and equipment (NAN, 2005). Thus, even in 2005, the NIRDPSBB stated that drastic measures should be put in place; however, the official monitoring system did not include potential activities that may further contribute to PCN spreading. Finally, the PMP for 2015-2016 proposed the following major measures: to be used only tested seed potatoes for commercial purpose, to keep a healthy environment on the arable land, to limit circulation between parcels, to maintain a constant surveillance on the plots and waste management. However, no indications regarding associated costs to such a programme were in place and no definition for limiting the circulation between potatoes parcels either. Furthermore, analysing the chapter II of the management programme, regarding the monitoring of native production and environment, there miss measures regarding abiotic and biotic factors as vectors in spreading PCN. In this regard it will be discussed environmental factors (i.e. dry wind, water runoff, wild herbivores) and agricultural practices (i.e. sheep and goats transhumance) that are not mentioned into the current PMP.

**Environmental factors.** White, in 1953, documented the spreading of PCN by hot dry winds and run off water that was supported by further studies (TURNER & EVANS, 1998; REPŠIENĖ & MINEIKIENE, 2006). Romania comprises a broad range of agricultural landscapes due to relief on one hand and to land management on the other hand (HARTEL et al., 2016). Still, Romania faces now the climate change effects all around the Carpathian Mountains (SPINONI et al., 2015). Thus, the diversity of agricultural landscapes provided conditions for implementing different agricultural practices that contributed further to the spreading of this pest.

It is accepted today that PCN can be spread by biotic factors such as contaminated livestock (HODDA & COOK, 2009). The process become too complex for Romania where livestock represented by sheep and goats or boars passing the fields are not taken into consideration into the national monitoring programme. Similar cases have been already described and the probabilities of PCN establishment in new areas beyond the current distribution were both studied in Australia in 2009 (HODDA & COOK, 2009).

Considering that the transhumance of sheep and goats facilitates the spread of PCN in the field it can be seen that in 1985 it was recorded the highest number of these species (i.e. 19,391,000.00) that continuously decreased up to 2002 (i.e. 7,776,300.00). This increase may be associated to the first official recording of PCN in 1984. Starting with 2002 the number of sheep and goats continuously increased up to 2014 (i.e. 10,448,645.00) as well as the covering area with this livestock, which also may be associated with the spread of the PCN all over the country up to potato seed production areas. It is relevant to underline that in Romania, even diminished, the transhumance is still in practice (STANCIU et al., 2012) and no phytosanitary management measures are in place to reduce the spreading of the PCN.

Moreover, in Romania agro-ecosystems are closely connected to wild biodiversity and mainly with large herbivores species that consume potatoes such as boars (i.e. *Sus scrofa*). Only in 2015 there have been recorded about 28,153.00 boars according to the data base of the Ministry of Environment and in Sibiu or Brașov County the arable land is constantly the subject of boars migrations. Between 2007 and 2014, Romania was under phytosanitary quarantine for exporting pork meat (i.e. in Romania the swine population comprises 6,815,000 individuals in 2007) due to swine fever and boars were accepted to be the most important pest reservoir (INDRIE & MÂNZAT, 2009) with major economic loss in the region (BELTRÁN-ALCRUDO et al., 2009). According to the results of the interview applied to all 122 city halls (i.e. in Sibiu and Brașov counties), boars are a constant presence in the arable land that can be associated with the presence of PCNs. Thus, it should be taken into account restriction measures for the cultivation of seed potatoes in the bordering areas with boars in order to obtain free PCN zones.

**Agricultural practices** in Europe had a major historical contribution to the spreading of PCNs, as well as to the increase of their genetic variability (GEBHARDT, 2013). Potato fields in Europe are ploughed to higher depth compared to Peru and further homogenising potato cyst densities in soil, supporting a hypothesis earlier published (MUGNIÉRY & ZAOUCHI, 1976). Although, there are couples of new agricultural practices promoting the cultivation of potato with pests' repellent plant species (SAND et al., 2013). It can be added that the current PMP should also include the need to promote new agricultural practices use.

## CONCLUSIONS

Potato monitoring programme should put in place measures according to the latest scientific achievements. It should also follow the national and international breeding programmes in order to substantiate the promotion for placing on the market of the most resistant potato cultivars. On the other hand it should take into account all environmental peculiarities of the country including measures for adaptation and mitigation to climate change considering that the PCN may resists up to 28 years. Livestock transhumance and boars migration should be the subject of this potato monitoring programme in direct connection with the type of potato culture in re-establishing free PCN zones. New agricultural practices should be further developed in supporting the full implementation of quarantine phytosanitary measures.

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