Case Study 2: Virus Resistant Potato

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PART I: THE NON-TRANSGENIC PLANT

GENERAL DESCRIPTION OF THE PLANT.

Potato (\textit{Solanum tuberosum} L.) has its center of origin in the Andean region of South America and it has been in use as food for centuries. Two subspecies are cultivated: \textit{S. tuberosum} subsp. \textit{andigena} and \textit{S. tuberosum} subsp. \textit{tuberosum}. The first subspecies comprises the local traditional varieties used in the Andes, from Argentina to Ecuador. The second subspecies comprises the cultivars intensively used worldwide.

In Brazil, potato is grown in the Center-south region of the country. There is only one important growing area in the Northeast of Brazil, at the altiplan of Chapada Diamantina. No potato is grown in the Amazon Region. The crop cycle of cultivars grown in Brazil varies from 80 to 110 days. Potato has two main growing seasons in the country. Nevertheless, the planting period depends on each region, which results in having potato fields growing around the year. The main phytosanitary problems are diseases caused by fungi (\textit{Alternaria solani} and \textit{Phytophthora infestans}), bacteria (\textit{Ralstonia solanacearum} and the complex \textit{Dickeia-Pectobacterium}) virus (\textit{Potato virus Y} - PVY and \textit{Potato leafroll virus} - PLRV), nematodes (\textit{Meloidogyne} spp.); and insects (aphids of several species, cucumber beetles- \textit{Diabrotica speciosa} - and potato moth - \textit{Phthorimaea operculella}). In general, there is an intensive use of pesticides and very few commercial materials with pest resistance are available.

REPRODUCTIVE BIOLOGY OF THE SPECIES.

The potato plant is a functional hermaphrodite, although several cultivars have cross pollination mechanisms, such as the absence of pollen or self-incompatibility. Once pollinated, a berrylike fruit develops and may contain up to thousands of viable seeds that generally have a natural dormancy.

Although botanical seeds germinate and are able to generate an adult plant, the crop is commercially propagated by tubers. Botanical seeds generate an impressive desuniformity, based in the genetic variability backed by the potato tetraploid genome, which is not compatible with the commercial use of the plant.

Various cultivars do not flower in Brazilian conditions and, even when blossom, they are not able to set fruits. This happens mainly to the short days we experience in the country, especially in latitudes lower than 22°. Cultivar Achat, which was used in the present case study, do not
flower under Brazilian conditions (Jabuonski and Furumoto, 1987). Moreover, there are no reports of blooming in cultivar Achat under any environmental conditions.

**Center of Origin and center(s) of Genetic Diversity.**

Potato originated in South America, particularly in the highlands around the Titicaca Lake in Peru and Bolivia. The widely cultivated subspecies also received an extensive genetic contribution from one subpopulation whose center of origin is the Chiloé Island (South Chile). The variability of the species lies along the Andes in both traditional local cultivated varieties and wild sexually compatible relatives.

In Brazil, some wild relatives occur as weeds, mainly the species *Solanum viarum* (joá) and *Solanum nigrum* (nightshade). None of them are sexually compatible with the cultivated potato. These species do not belong to the Petota section that comprises the tuber bearing *Solanum* species and that are sexually compatible to the potato.

**Means of Dispersal and Establishment (e.g., Pollen, Seed Dispersal, Vegetative Dispersal etc.).**

Pollen dispersal is exclusively via insects, mainly bumble bee species (*Bombus* sp). The insect lands on the flower and due to its weight, the flower bends. To prevent from falling from the flower and to collect the nectar, the bee moves its wings continuously in high speed. As result, the flower vibrates and the pollen dehisces over the insect body and the stigma, leading to the fertilization of self compatible genotypes. Having the body covered by pollen, the insect either cross pollinates another flower upon visiting it or gives the means to another self-pollination. Larger bees, such as the africanized European bees can, although less efficiently, pollinate the potato flower. Other small insects are not able to act as pollinators in potato, due to the dehiscence system observed in the species. Other larger insects but the bumble bee usually do not visit the potato flower, except some butterflies that, due to their low body weight, are not able to induce the pollen release.

There is no information on the long term viability of the pollen when over the insect body under natural conditions. In controlled experiments, potato pollen is viable up to 72 h at 4°C. When kept under low air humidity and freezing temperatures, the potato pollen can be kept viable for several years, although the fruit bearing efficiency is reduced when compared with fresh pollen.

Natural crossings were not reported in distances larger than 20 m (Tynan et al., 1990; Dale et al., 1992; McPartlan e Dale, 1994; Skogsmyr, 1994, Conner e Dale, 1996).

**Intra-Specific, Inter-Specific and/or Inter-Generic Hybridization:**

In all Brazilian ecosystems, several *Solanum* species occur, such as Dutch eggplant (*Solanum aculeatissimum*), nightshade (*S. nigrum*), cockroach-berry (*S. ciliatum*), tobacco tree (*S. erianthum*), lobeira (*S. lycocarpum*), jurubeba nightshade (*S. paniculatum*), soda-apple (*S. viarum*) and sticky nightshade (*S. sisymbriifolium*). These species do not belong to the Petota section (tuber bearing species). There are also two *Solanum* species widely grown as vegetables
in Brazil: *S. melongena* (eggplant) and *S. gilo* (gilo). Nevertheless, just like the weeds, they are sexually incompatible with potato.

Two species of potato wild relatives, namely *Solanum commersonii* and *S. chacoense*, occur in Brazil, in the states of Minas Gerais, Paraná, Santa Catarina and Rio Grande do Sul. These *Solanum* species belong to the section Petota, thus they bear tubers and are potential donors and receptors of pollen. *Solanum chacoense* has three subspecies that occur in Brazil: *S. chacoense* subsp. *chacoense*, *S. chacoense* subsp. *muelleri* and *S. chacoense* subsp. *calvescens*, the two latter not find elsewhere. *S. chacoense* subsp. *calvescens* is a natural triploid, whereas all others are diploids.

There is only one report of crosses between potato and nightshade (Eijlander and Stiekema, 1994). However, these were obtained in artificial conditions, using emasculation, embryo rescue and resulted in fully sterile hybrids.

Regarding the wild relatives that belong to Section Petota, natural crosses are theoretically possible. Nevertheless, at the field, without human assistance, their frequency is practically null owing to differences in the ploidy level (the cultivated potato is tetraploid). In addition, if a cross takes place, the zygote is aborted due to the lack of endosperm balance between pollen and ovule of different ploidy levels. Even though, these species are regularly used in breeding programs (Jacobsen e Jansky, 1989; Sanford *et al.*, 1994, 1995; Masuelli e Camadro, 1997).

**PART II: THE RECEIVING ENVIRONMENT**

*CULTIVATION OF THE CASE STUDY PLANT SPECIES IN BRASIL.*

The GM potato of this case study was release into the environment only under experimental conditions, in small field experiments of maximum 625 m² area. The releases were used to evaluate the field resistance to PVY (Dusi et al., 2009) and other environmental and food safety issues (data not published). In total, eight experiments were conducted from 1999 to 2007.

*PRESENCE OF ANY SEXUALLY COMPATIBLE RELATIVES IN THE RECEIVING ENVIRONMENT.*

No sexually compatible relatives occur in the experimental areas.

*ECOLOGICAL INTERACTIONS IN THE RECEIVING ENVIRONMENT (E.G., IDENTIFY SIGNIFICANT PEST COMPLEXES).*

In the experiments, which used both the GM and its non-transformed parental, the main potato pests in Brazil were reported, such as viruses (PVY and PLRV), early and late blight, bacterial wilt, aphids, cucumber beetles and other insects. No significant differences regarding the reaction to these biotic stresses were observed between the GM and its parental.
PART III: THE TRANSGENIC PLANT

THE PURPOSE OF THE TRANSFORMATION (E.G., INSECT RESISTANCE, DROUGHT TOLERANCE, OUTPUT TRAIT, ETC.)

Potato cultivar Achat was transformed with the PVY coat protein gene to provide PVY resistance (Romano et al., 2001).

THE ANTICIPATED CULTIVATION REGION (E.G., SMALL SCALE IDENTITY PRESERVED CROP OR WIDELY CULTIVATED CROP ETC.)

The GM was experimentally released in two specific areas under controlled conditions in Brasília (DF) and Canoinhas (SC). The experimental area varied from 125 to 625 m² maximum. If approved, the GM was intended to be grown in all national production areas.

A SUMMARY OF THE INTRODUCED GENETIC ELEMENTS AND THE SOURCE OF THESE (E.G., DONOR ORGANISM ETC.)

The plant was transformed via Agrobacterium tumefasciens. The construction contained the PVY coat protein (CP-PVY) and the nptII genes. The CP-PVY was under the control of the constitutive 35S promoter from the Cauliflower mosaic virus (CaMV) and of the transcription terminator of the nopalina sintase gene (nos). The nptII was under the control of both the promoter and the terminator.

INHERITANCE AND STABILITY OF EACH INTRODUCED TRAIT (E.G., SEGREGATION ANALYSIS, STABILITY OF THE INSERT ETC.).

The inheritance of the introduced trait was never studied, due to two main aspects: (1) the vegetative nature of commercial propagation in potato makes the knowledge of the inheritance not relevant and (2) the absence of flowering in cultivar Achat makes it impossible to investigate it.

The insert was stable after several in vitro multiplications (Romano et al., 2001).

DIFFERENCES IN GENETIC AND PHENOTYPIC VARIABILITY FROM NON-TRANSGENIC CROP.

No differences, either genetic or phenotypic, were reported between the GM and its parental, except for the presence of the CP-PVY transgene, the marker gene and their associated promoter and termination codons.

DIFFERENCES IN MODES AND/OR RATE OF REPRODUCTION FROM NON-TRANSGENIC CROP (E.G., ANY AVAILABLE OUT-CROSSING DATA ETC.).

No differences between the GM and its parental were reported.

EXPRESSION LEVELS OF NOVEL PROTEINS IN DIFFERENT TISSUES OVER TIME.

No protein expression was ever reported. The mechanism of resistance to PVY in the GM plant is, apparently, RNA mediated (co-suppression).
DIFFERENCES IN AGRONOMIC CHARACTERISTICS FROM NON-TRANSGENIC CROP.

No differences between the GM and its parental were reported, except for the target trait, i.e. PVY resistance, which was reported under greenhouse and field conditions (Dusi et al., 2001, 2009).

DIFFERENCES IN DISEASE AND/OR PEST SUSCEPTIBILITY FROM NON-TRANSGENIC CROP.

Unpublished data of controlled greenhouse and field experiments for PLRV, bacterial wilt, late blight, early blight, the root-knot nematode, cucumber beetles and other field occurring insects show that, as far as we could assess, the genetic transformation did not have any effect on the field occurrence, and on the resistance or susceptibility to these pests, when the GM potatoes are compared to the parental cultivar.

POTENTIAL IMPACT ON NON-TARGET ORGANISMS IN THE RECEIVING ENVIRONMENT:

Unpublished data of controlled greenhouse experiments for PLRV, bacterial wilt, late blight, early blight, the root-knot nematode and cucumber beetles show that, as far as we could assess, the genetic transformation had no potential impact. For cucumber beetles, a preference for non GM plants was recorded in free choice cage tests, where the insect was placed in cages containing the GM and the conventional parental. However, when no choice was offered (cages with GM or conventional parental), the insect completed its life cycle in plants of both treatments.

There are unpublished data of controlled field experiments for cucumber beetles. Apparently, there was preference of larvae for tubers of the conventional parental. Regarding adults, no significant difference was observed. This data was confirmed in greenhouse experiments. The natural insect population during the crop cycle was recorded in the same studies. Again, no significant difference between treatments was observed.

AVAILABLE EXPOSURE DATA (E.G., POLLEN MOVEMENT, PROTEIN DISSIPATION, ETC.).

GM plants do not flower and the transgene is not translated into protein. No other information is available.

REFERENCES FOR ANY RISK ASSESSMENTS UNDERTAKEN IN OTHER JURISDICTIONS.

No references available.
REFERENCES:


Eijlander, R. e Stiekema, W. J. (1994). Biological containment of potato (Solanum tuberosum): outcrossing to the related wild species black nightshade (Solanum nigrum) and bittersweet (Solanum dulcamara). Sex Plant Reproduction 7:29-40.


