• A NEW VIRUS THREAT TO SEED-POTATO CERTIFICATION IN BRAZIL: THE WHITEFLY-TRANSMITTED *TOMATO CHLOROSIS VIRUS* (GENUS: CRINIVIRUS) *

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- ***Supported by FUNDAG/Souza Cruz S.A/MICROGEO/ ABBA/ABVGS***

Central potato pots with plants showing PLRV-lime symptoms are ToCV infected progeny tubers (PCR+/Dr J.Rezende, ESALQ-USP), from a grown on test performed by Natalino Shymoyama, CEO of the Potato Assoc of Brazil (ABBA)

Exposition on a recent **Whitefly Seminar,** organized by the Potato Association of Brazil (ABBA), held in Uberlândia – Minas Gerais (Brazil), 4-5/November/2013

"The potato production in Brazil, before and after the identification of the whitefly-transmitted Tomato chlorosis virus – ToCV: Some factors and facts "

-Original title: ***A bataticultua no Brasil, antes e depois de constatada a mosca branca transmitindo o Tomato chlorosis virus (ToCV): Fatores e fatos***

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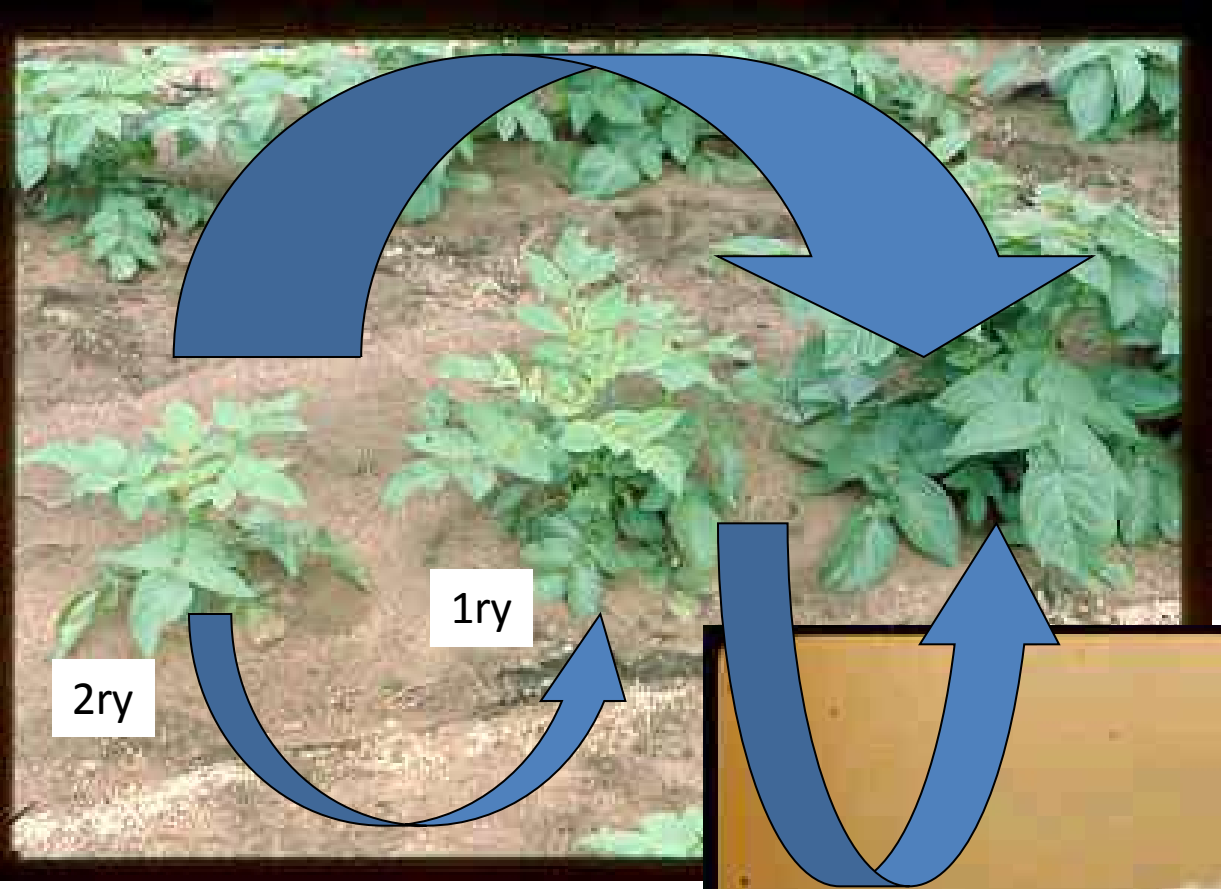
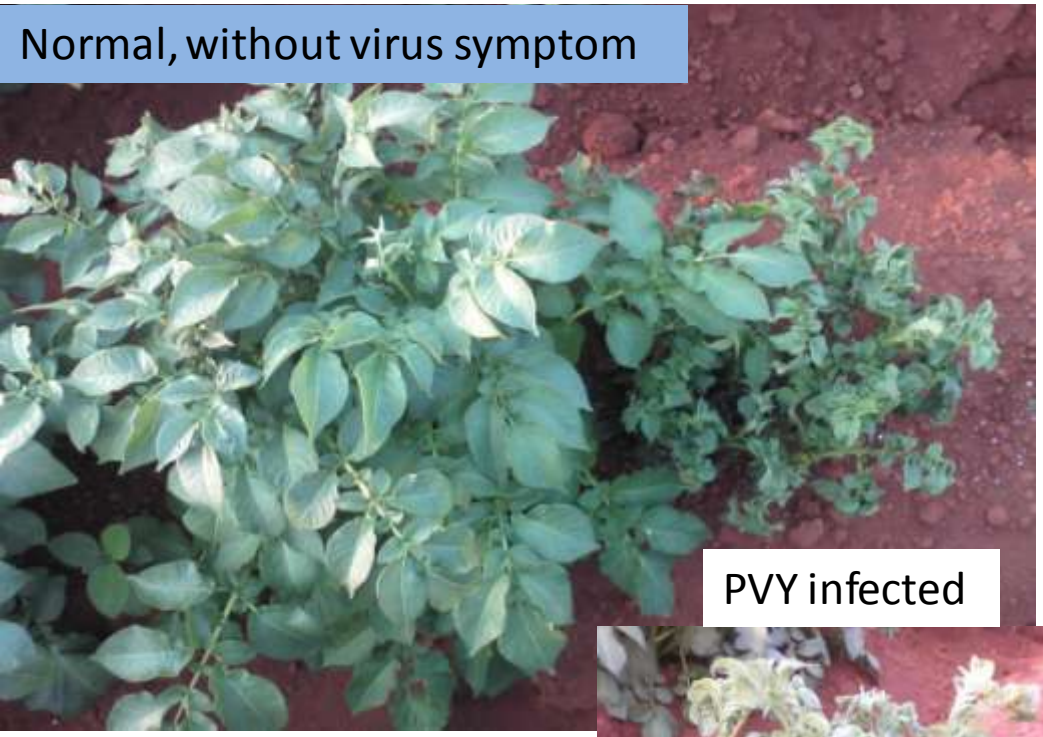


PLRV, cirulative persistantly.
Transmitted by few aphid
Species; mainly *Myzus persicae*.

Aphid Transmission is dependent on chaperonin GroEL proteins Produced by endosymbiotic bacteria Genus Buchnera

Potato virus Y –PVY, Potiviridae , genus Potevirus, not circulative-persistant transmitted bay various species of aphids.

Normal, without virus symptom



PVY infected

PVYntn = circular necrotic superficial Rongs, or curves on the tuber epiderm.
Sensitive cvs: Monalisa, Caesar, Mondial

PVY, stream "N" = PVYn

PVY, stream "O" = PVYo

Bemisia tabaci on leaves of potato plant: High (huge) infestation associated mostly with PLRV-like symptoms; in **field fields where aphids were on "total" absence (unseen all season long).**

ESPECIAL

US$ 5 billion loss

“ The pest of the Centuries”

Praga dos séculos

Favorecida por fatores como condições climáticas, ampla oferta de hospedeiros, variedades suscetíveis, cultivos escalonados, extensivos e mistos cultivos, à mosca branca (*Bemisia* spp.) tem provocado perdas superiores a R$ 10 bilhões em lavouras de tomate do Brasil, com danos diretos e indiretos à cultura. Para conter o avanço do inseto é indispensável lançar mão de um conjunto de estratégias, adotadas de forma integrada e raci

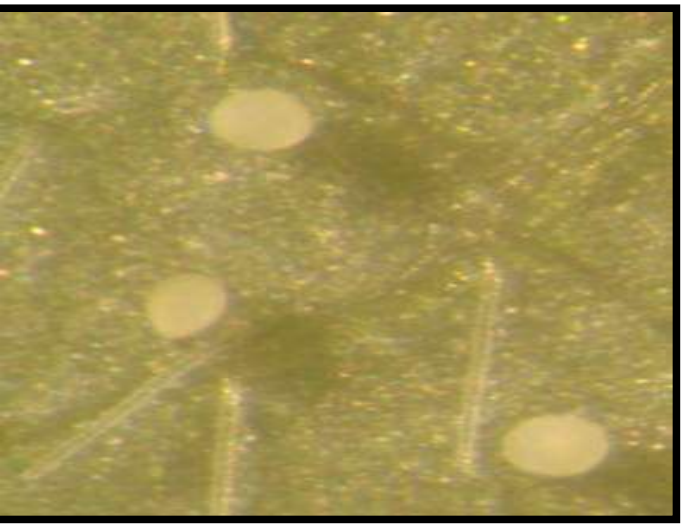
Revista Cultivar 8(55):23-28, 2009

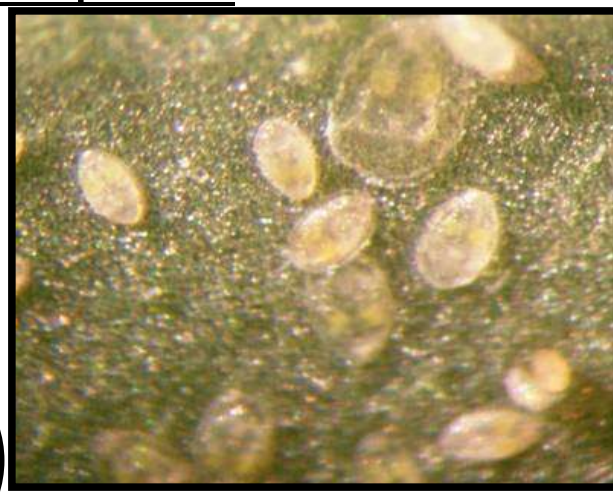
Cecilia Czepak,
Jácomo Divino Borges e
Jardel Barbosa dos Santos,
Universidade Federal de Goiás
Hellen Geórgia Santana,
AHL Distribuidora

Atualmente, a mosca branca (*Bemisia* spp.) está presente em todos os estados brasileiros, provocando perdas superiores a R$ 10 bilhões, tanto pelos prejuízos diretos na sucção de seiva e injeção de toxinas, como indiretos, como vetor de viroses, transformando-a em um dos insetos de maior impacto na entomologia agrícola, tendo sido denominada

po S; Grupo 4 - Índia, biótipo H; Grupo 5 - Sudão, biótipo L, Egito, biótipo não identificado, Espanha, biotipo Q, Nigeria, biotipo J; Grupo 6 - Turquia, biotipo M, Hainan, biotipo nao identificado, Coréia, biótipo não identificado e Grupo 7 - Austrália, biótipo AN. Esses biótipos são populações com potenciais de maximizar suas ati-

Whitefly (*Bemisia tabaci*) Bio cycle highly adapted and colonizing on potato plants





21 - 45 days;
300 eggs =
27 million
in 90 days

General Aspects:

Large genotype diversity: *Bemisia tabaci (biotype B, Q,etc), Trialeirodes vaporariarum, T. spp*

Large host range**: > 600 plant species**

Efficient virus vector: **> 70 virus species**

Geminivirus identificado em batatal no Brasil
ToYVSV, 1996 (Souza-Dias et al.) and 1997 (Farias, et al.)
ToSRV (2008 (Souza –Dias et al.) = **Não tem sido um problema para a bataticultura como tem sido para outras solanaceas (ex.:Tomateiro)**

From begining 2000, basically without any aphid presence throughout the season, near 40% PLRV-like symptomatic potato plants, in cerfied tuber/seed lot, started to observed in association with high infestation of whiteflies (*Bemisia tabaci*). Suspects of whitefly-transmitted PLRV were raised.

POTATO LEAFROLL VIRUS - PLRV: WOULD IT BE RE-EMERGING IN POTATO FIELDS AFTER A DECADE OF NEAR ABSENCE ?

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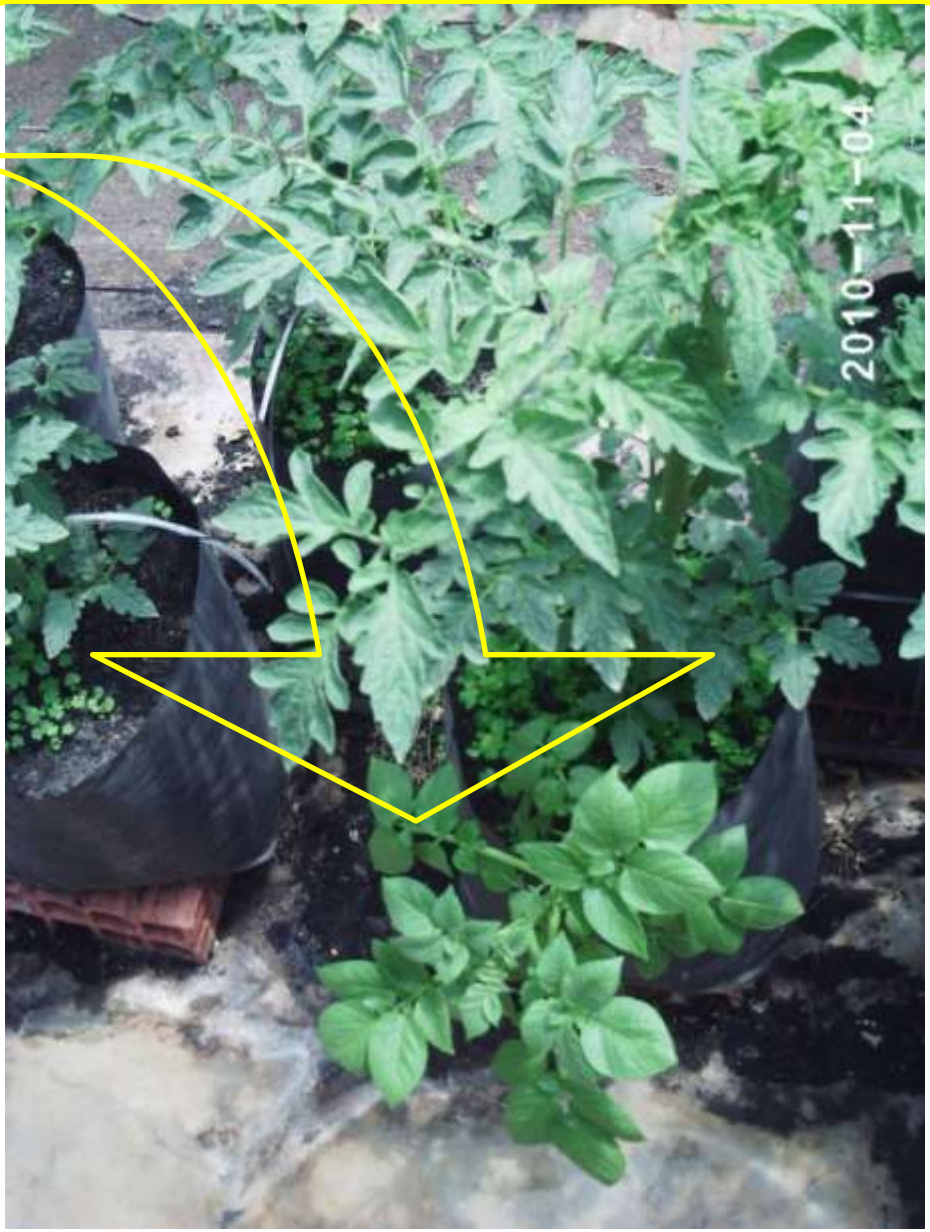
[1e3] CNPq Projs. 314018/2009-3 e 382608/2010-0; Apoio: FundAg.

Preliminary experiments to evaluate potato cvs response to tomato whitefly transmitted viruses

Preliminary experiments on evaluation of potato cvs response to tomato whitefly transmitted viruses



Greenhouse 2 – Evaluating whitefly transmitted potato viruses to seven potato cvs (10 plants random distribuited per cv), inside commercial tomato greenhouses (with history of high whitefly and Begomoviruses and Crinivirus)

D. stramonium = Interveinal yellowing on large, older leaves, similar to Potato leafroll virus – PLRV, Luteovirus , known to be aphid transmitted , circulative, persistent). Crinivirus (ToCV), not suspected during this trials

D. metel = Most symptomless , a few with shrinks (waving) on top leaves.

Tomate cv Ikran = mid and bottom leaves showing yellow (interveinal and blotches). Older leaves crispy and rolled upward, Similar to PLRV symptom in tomato plants

Symptom similarities in *Datura stramonium* indicator (test) plants, wich has been exposed in potato fields to monitor PLRV and ToCV (the presence of whitefly and absence of aphids would indicate ToCV

Interveinal yellow and rolled upward of older leaves, crispy tisssues

Tomato plants (cv Ikran) showing interveinal yellow on bottom leaves, due to ToCV infection. At right, a normal (symptomless) tomato leaf

These symptoms are just like those described to PLRV in tomato plants, confirmed by bio (Costa, 1964), imuno (Souza Dias & Costa, Summa Phytopatologica 21(1):50, 1994), and molecular assays (Souza-Dias et al, 2003)

Tomato chlorosis virus (ToCV)
Symptoms similar to Potato Leafroll virus (PLRV)

Evaluation of 7 (seven) potato cv s Inside a ommercial tomato greenhouse (Oct 2010-Jan 2011)

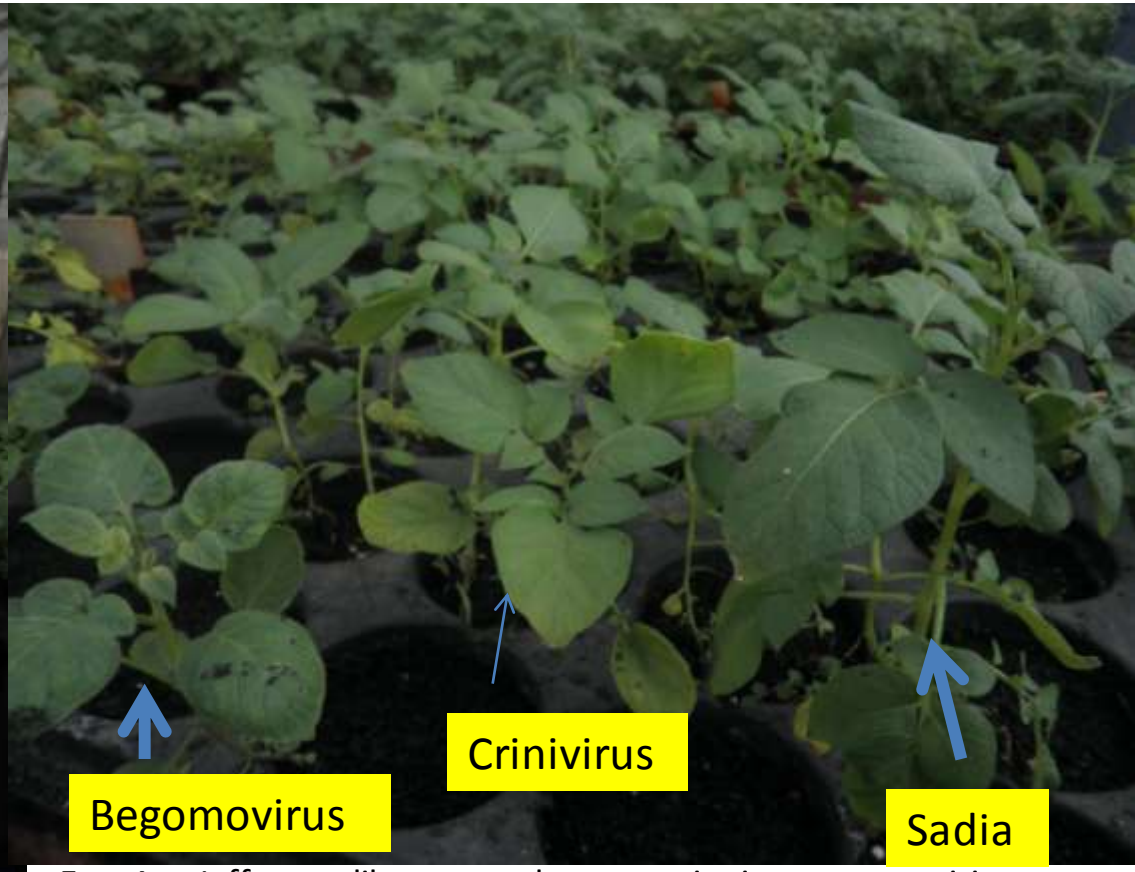
with history of high level of whitefly transmitted viruses

Sprout evaluation (tuber progenies from potato cvs on the Tomato greenhouses) . PLRV-like symptoms were confirmed ToCV postivie by PCR (performed in ESALQ-USP, at Dr Rezende's Lab)

Potato plants Cv Mondial growing from sprout , detached from a tuber produced by a plant that had shown yellow deforming mosaic symptoms, at the Tomato experiments. Confirmed Begomovirus

Sprout growing plant from a PLRV-like mother plant at the tomato grenhouse experiments) . For most of the seven potato cvs, PLRV-like symptoms (as above) were randomly chosen for PCR testes and confirmed ToCV+ and PLRV-

Sprout evaluation of tuber progenies from seven potato cvs inside tomato greenhouses, for whitefly transmitted viruses (May-June 2011. Meanwhile, Dr. Rezende's student Debora Freitas identified Crinivirus in potato plants showing PLRV-like symptoms for the first time in Brazil (Freitas, et al. 2012). Some PLRV-like plants in this picture were PCR tested and confirmed ToCV+ (by Dr Rezende/ESALQ-USP)



Eng. Agr. Jefferson, like many other extensionists came to visit examining plant progenies from the tomato x whitefly x virus experiments . Symptoms of deforming mosaic begomovirus (ToSRV and ToYVSV) and leafroll with interveinal yellowing (PLRV-like): May-June 2011.

plant disease

Editor-in-Chief: R. Michael Davis
Published by The American Phytopathological Society

April 2012, Volume 96, Number 4
Page 593

http://dx.doi.org/10.1094/PDIS-12-11-1068-PDN

Disease Notes

First Report of *Tomato chlorosis virus* in Potato in Brazil

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Potato plants (*Solanum tuberosum* cv. Ágata) exhibiting symptoms of leaf roll and interveinal chlorosis, especially on older leaves, were found in a commercial crop in the County of Cristalina, State of Goiás, Brazil in June 2011. The crop was severely infested by whitefly *Bemisia tabaci* biotype B. Four potato tubers from symptomatic plants were indexed for the presence of the following viruses: *Tomato chlorosis virus* (ToCV), *Potato leaf roll virus* (PLRV), *Tomato severe rugose virus* (ToSRV), and *Potato virus* Y (PVY). Total RNA was extracted separately from each tuber and used for reverse transcription (RT)-PCR using the HS-11/HS-12 primer pair, which amplifies a fragment of 587 bp from the highly conserved region of the heat shock protein (HSP-70) homolog gene reported for ToCV. The RT-PCR product was subsequently tested by nested-PCR for detection of ToCV with specific primers ToC-5/ToC-6 (2). Amplicons of 463 bp, amplified from total RNA separately extracted from three tubers, were purified and directly sequenced. Comparisons among the three consensus sequences of 448 bp (GenBank Accession Nos. JQ288896, JQ288897, and JQ288898) revealed respectively, 98, 100, and 100% identity with the

Tuber progenies (tuber and sprouts) under evaluation for whitefly transmitted virus from seven potato cvs: Preliminary experiments inside tomato greenhouses at Sumaré county.

Deforming yellow mosaic, in potato progenies of cvs. **Itararé,** and **Mondial** (putative ToSRV),

Tuber progenies from the seven potato cvs on grown on test (inside greehouse – May-June 2011).

Potato plants grown on from seed-tubers perpetuating **ToCV** and **Begomovirus**, were both molecularly confirmed.

ToCV + (showing PLRV–like symptoms), **were negative for PLRV** (ELISA kits from SASA, AGDIA,

Crinivirus= ToCV

Healthy ToCV- : discrepancies among tuber progenies

Crinivirus= ToCV

Geminivirus=ToSRV

By June 2011, when tuber x sprouts progenies from the seven potato cvs produced inside the tomato greenhouses, were grown on tested , PLRV was ELISA testes (due to typical symptom expression), but results were negative for PLRV.

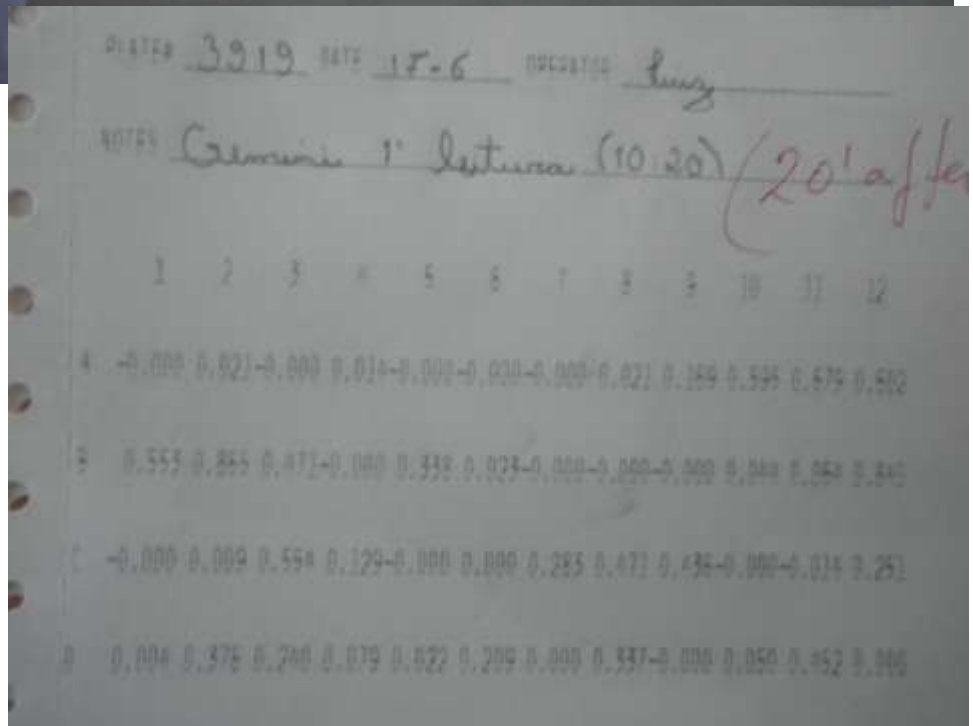
Meanwhile, with the first discover of ToCV in potato crops associated with huge whitefly infestation, draw attention to ToCV to be tested in these potato progenies.

PCR and sequencing analysis, were performed by Debora Freitas, a PhD student at Dr Jorge Rezende (ESALQ-USP) lab). Only ToCV, but not PLRV was confirmed molecularly.

CRINIVIRUS ToCV

All potato cvs, except '**Cupido'**, showed similarly moderate susceptibility to ToCV. For Begomovirus, results indicated good resistance among the seven potato cvs (inoculum pressure was considerably high inside greenhouses)

Like performed with potato plants in the tomato greenhouses, PLRV-like symptoms in potato plants were checked for PLRV, only. Most negative results, until mid June/2011, when ToCV was first identified in potato plants (Cristalina region, Goiás State) showing PLRV-like symptoms, in field with no aphid records, but huge whitefly infestation. The pictures shows ELISA on sentinels plants (*Datura stramonium and Datura metel*) exposed within potato fields for virus bio-monitoring, nearby tomato plantations.



Some evidences on *Tomato chlorosis virus* – ToCV, genus Crinivirus (in Latim, Crini = "hair")

- Wisler, G.C., et al., Plant Disease 82:270, 1998.
- **"...Potato plants showed symptoms symilar to those caused by Potato leafroll virus (PLRV) when experimentally infected with ToCV from tomato."**

- **Tomato plants** infected with ToCV shows an irregular chlorotic mottle that appears first on lower leaves and gradually advances towards the growing point. Interveinal yellow areas on leaves also develop red and brown necrotic flecks

Greenhouse 1

Cultivars	Number of observed virus-like symptoms(3)(R = leaf roll-interveinal yellowish-PLRV-like; D= deforming yellow mosaic; RD = both / number of plants), grown from a virus-free single sprout(4)	Number of plants grown from three tuber-progeny size and respective apical sprout (5), of each test plant / number of plants showing each virus-like symptoms (R; D and RD) (6), at 30-40 days after planting (Jun, 2011)																	
		Tuber sizes/plant									Sprout/respective tuber size								
		Small			Mid			Large			Small			Mid			Large		
		R	D	RD	R	D	RD	R	D	RD	R	D	RD	R	D	RD	R	D	RD
Monalisa	3/ 10	2	1	1	3	0	0	3	2	0	-	-	-	3	0	0	1	2	0(7)
Ibitu-Açu	2/8	1	0	2	1	0	2	2	1	1	0	1	1	1	0	2	1	1	1
Itararé	7/10	3	1	5	3	1	5	4	3	3	-	-	-	2	2	3	2	3	4
Agata	3/10	3	0	0	4	2	0	4	1	1	3	0	0	4	1	1	4	2	0
Mondial	9/10	6	2	2	6	2	2	8	0	2	6	3	0	5	3	1	6	0	2
Asterix	2/10	4	0	1	4	0	1	4	0	1	-	-	-	4	0	0	4	0	0
Cupido	0/10	0	2	0	0	1	1	0	1	1	0	2	0	0	1	1	0	2	0

Greenhouse 2

Cultivars	Number of observed virus-like symptoms(3)(R = leaf roll-interveinal yellowish-PLRV-like; D= deforming yellow mosaic; RD = both / number of plants), grown from a virus-free single sprout(4)	Number of plants grown from three tuber-progeny size and respective apical sprout (5), of each test plant / number of plants showing each virus-like symptoms (R; D and RD) (6), at 30-40 days after planting (Jun, 2011)																
		Tuber sizes/plant									Sprout/respective tuber size							
		Small			Mid			Large			Small			Mid			Large		
		R	D	RD	R	D	RD	R	D	RD	R	D	RD	R	D	RD	R	D	RD
Monalisa	1/ 10	1	0	0	1	0	0	4	0	2	-	-	-	1	0	0	2	2	0(7)
Ibitu-Açu	2/9	1	0	1	1	0	2	1	1	2	1	0	0	1	0	2	1	1	1
Itararé	8/10	2	3	3	2	3	5	2	3	5	0	1	5	4	0	6	1	3	6
Agata	2/10	3	0	0	3	0	0	3	0	1	3	0	0	3	0	0	3	1	0
Mondial	4/10	6	4	0	5	4	1	5	4	1	-	-	-	5	3	1	5	2	0
Asterix	1/10	3	0	0	3	0	1	3	0	0	-	-	-	1	0	0	2	0	0
Cupido	0/10	0	0	0	1	1	0	2	1	0	0	0	0	0	1	1	2	1	0

Conclusion

- The seven potato cultivars in the two trials showed a moderate good resistance to the predominate Tomato Begomovirus (putatively ToSRV and ToSRV).
- Cv Cupido showed the lowest Begomovirus incidence among the other six cvs, as observed on grown on tests.
- PLRV-like symptomatic plants (PCR+ for ToCV) showed a tendency of small tubers to be less infected (symptomless plants, tested PCR and graft into test plant, were free of ToCV).
- Uneven distribution of ToCV within and among daughter-tubers sustain previous observation (**Fortes & Navas –Castillo, 2012**)

ELISA kits for whitefly transmitted viruses: Has become a priority demand for seed-potato certification in Brazil

ELISA kits were obtained soon after ToCV was first identified in potato crops in major potato producing areas of the State od Goipas (Brazil). Kits supplied by Dr Stephan Winter (DSMZ, Germany), with support from: FUNDAG/ IHARABRAS and Souza Cruz S.A.

Since 2003, potato fields planted with basic class seed-potato tuber lots, at the Southeast and West Center regions of Brazil, have been rising frequency of records for:

(1) Plants showing PLRV-like symptoms (**primary** = top yellow, erect and rolled up leaflets; and **secondary** = stunted and bottom leaves crisp and rolled, some interveinal yellowing, may also show purple color on leaf edges); and

(2) **APHIDS** controlled to "total absent" ,throughout the season, but highly infested with whitefly (***Bemisia tabaci***).

PLRV symptoms:
Secondary, seed-tuber perpetuation
Primary, curent season

Feb. 2011 – South of the State of Minas Gerais (Southeast region): Absence of aphids but hight whitefly infestation (considered uncontroled). Potato cvs Agata and Asterix showing > 80% PLRV-like symptoms of 1ry and 2rd types.

Soybean plantation favor whitefly population exponential increase and constant movement into seed-potato field, nearby.

>45% PLRV (1ry)-like symptoms on plants cv Asterix.
No rizoctoniose damaged stems.

Results from real time PCR, performed at SASA (Scotland), confirming only ToCV in potato samples cv Agata, showing PLRV-like symptoms in a Brazilian major potato region (Perdizes, MG) in Brazil (Aug 2012)

Table 1 – Bio-assays: **1**=Mecanic; **2**=Graft, for viruses in potato ´plants showing PLRV-like symptoms, from differente cvs in the states of MG and SP (Brazil) . Confirmation of potato viruses by molecular (PCR) analysis were carried at ESALQ-USP (Brazil) and SASA .(Scotland, UK).

Crop Regions	Cultivars	Symptoms on foliage			Test plant/bio-assays 1 and 2: records 45 days after inoculation(*)					
					Datura stramonium		D. metel		N. tabacum cv Burley-21	
		PLRV-like	Mosaic	Def.Ylw Mos	1	2	1	2	1	2
Reg. Perdizes-MG	Agata-1	YES for all	No for all	No for all	SS	FE	SS	SS	SS	AI
	Agata-2				SS	AI	SS	FE	SS	AI
	Agata-3				SS	AI	SS	SS	SS	FE
	Agata-4				SS	FE	SS	FE	SS	AI
	Agata-5				SS	AI	SS	SS	SS	SS
	Agata-6				SS	AI	SS	SS	SS	SS
	Agata-7				SS	FE	SS	SS	SS	FE
	Agata-8				SS	AI	SS	SS	SS	AI
	Agata-9				SS	AI	SS	SS	SS	SS
	Agata-10				SS	AI	SS	SS	SS	SS
	Agata-11				SS	AI	SS	SS	SS	AI
ToCV ck+ / potato	Agata ck+				SS	AI	SS	SS	SS	AI
ToCV ck - / potato	Agata ck-	no			SS	SS	SS	SS	SS	SS
Reg. Perdizes-MG	Cupido-1	YES for all	No for all	No for all	SS	AI	SS	FE	SS	AI
	Cupido-2				SS	FE	SS	SS	SS	SS
	Cupido-3				SS	AI	SS	FE	SS	SS
	Cupido-4				SS	SS	SS	FE	SS	AI
	Cupido-5				SS	AI	SS	SS	SS	FE
	Cupido-6				SS	FE	SS	FE	SS	FE
	Cupido-7				SS	FE	SS	SS	SS	SS
	Cupido-8				SS	SS	SS	SS	SS	AI
ToCV ck+ / potato	Cupido ck				SS	AI	SS	SS	SS	FE
ToCV ck - / potato	cupido ck-	no			SS	SS	SS	SS	SS	SS
Reg. Itapetininga-SP	Atlantic-1	YES for all	No for all	No for all	SS	SS	SS	SS	SS	AI
	Atlantic-2				SS	AI	SS	FE	SS	AI
	Atlantic-3				SS	AI	SS	SS	SS	FE
	Atlantic-4				SS	FE	SS	SS	SS	AI
	Atlantic-5				SS	AI	SS	SS	SS	SS
	Atlantic-6				SS	AI	SS	SS	SS	SS
	Atlantic-7				SS	FE	SS	FE	SS	FE
	Atlantic-8				SS	FE	SS	FE	SS	AI
PLRV ck+ / potato	Atlant ck+				SS	AI	SS	FE	SS	AI
PLRV ck+ / potato	Atlant ck+				SS	AI	SS	SS	SS	SS

(*) inoculated at 15-20 cm high: symptoms legend : FE = graft failure; AI = Interveinal Yellow(ToCV +PCR); SS = no

Table 2 – Immuno assay (ELISA) on virus identification in potato plants showing PLRV-like symptoms, from differente cvs in the states of MG and SP (Brazil) . Confirmation of potato viruses via molecular (PCR) analysis were carried at ESALQ-USP (Brazil) and SASA .(Scotland, UK).

Crop Regions	Cultivars	Symptoms on foliage			ELISA results (2 x > ck- =+ positive). Kit from DSMZ (Germany): ToCV, TYLCV/gemini-grp), TSWV; from SASA (UK): PLRV, PVY, PVX, PVS						
		PLRV-like	Mosaic	Def Ylw Mos	PLRV	ToCV	PVY	PVX	PVS	TYLCV-gemini-group	TSWV
Reg. Perdizes-MG	Agata-1	YES for all(*)	No for all	No for all	(-)	(+)	(-)	(-)	(-)	(-)	(-)
	Agata-2				(-)	(+)	(-)	(-)	(-)	(-)	(-)
	Agata-3				(-)	(+)	(-)	(-)	(-)	(-)	(-)
	Agata-4				(-)	(+)	(-)	(-)	(-)	(-)	(-)
	Agata-5				(-)	(+)	(-)	(-)	(-)	(-)	(-)
	Agata-6				(-)	(+)	(-)	(-)	(-)	(-)	(-)
	Agata-7				(-)	(+)	(-)	(-)	(-)	(-)	(-)
	Agata-8				(+) ?	(+)	(-)	(-)	(-)	(-)	(-)
	Agata-9				(-)	(+)	(-)	(-)	(-)	(-)	(-)
	Agata-10				(+) ?	(+)	(-)	(-)	(-)	(-)	(-)
	Agata-11				(-)	(+)	(-)	(-)	(-)	(-)	(-)
ToCV ck+ / potato	Agata ck+				(-)	(+)	(-)	(-)	(-)	(-)	(-)
ToCV ck - / potato	Agata ck-	no			(-)	(-)	(-)	(-)	(-)	(-)	(-)
Reg. Perdizes-MG	Cupido-1	YES for all	No for all	No for all	(-)	(+)	(-)	(-)	(-)	(-)	(-)
	Cupido-2				(-)	(+)	(-)	(-)	(-)	(-)	(-)
	Cupido-3				(-)	(+)	(-)	(-)	(-)	(-)	(-)
	Cupido-4				(-)	(+)	(-)	(-)	(-)	(-)	(-)
	Cupido-5				(-)	(+)	(-)	(-)	(-)	(-)	(-)
	Cupido-6				(-)	(+)	(-)	(-)	(-)	(-)	(-)
	Cupido-7				(-)	(+)	(-)	(-)	(-)	(-)	(-)
	Cupido-8				(-)	(+)	(-)	(-)	(-)	(-)	(-)
ToCV ck+ / potato	ck+/kit				(-)	(+)	(-)	(-)	(-)	(-)	(-)
ToCV ck - / potato	cupido ck-	no			(-)	(-)	(-)	(-)	(-)	(-)	(-)
Reg. Itapetininga-SP	Atlantic-1	YES for all	No for all	No for all	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	Atlantic-2				(-)	(+)	(-)	(-)	(-)	(-)	(-)
	Atlantic-3				(-)	(-)	(-)	(-)	(-)	(-)	(-)
	Atlantic-4				(-)	(+)	(-)	(-)	(-)	(-)	(-)
	Atlantic-5				(-)	(+)	(-)	(-)	(-)	(-)	(-)
	Atlantic-6				(-)	(+)	(-)	(-)	(-)	(-)	(-)
	Atlantic-7				(-)	(+)	(-)	(-)	(-)	(-)	(-)
	Atlantic-8				(-)	(+)	(-)	(-)	(-)	(-)	(-)
PLRV ck+ / potato	Atlant ck+				(+)	(-)	(-)	(-)	(-)	(-)	(-)
ToCV ck +/D.stram	D.stramon				NÃO TEST	(+)	(-)	(-)	(-)	(-)	(-)

(*) All eleven samples of cv Agata, showed Real Time PCR positive for ToCV, only; and, negative for TICV (another Crinivirus). PCR negative also for PLRV, and Begomovirus . **(?)** PLRV not confirmed by PCR, but did confirm ToCV/ELISA; thus, confirming a satisfactory efficacy of the ELISA kit

"What to do, so?"

- **Breeding** resistant/tolerant commercial potato varieties; Applying **IPM** whitefly **strategies**, as virus vector.
- **But in addition:**
- **It should now become mandatory:**
- **1-** To include **ToCV tests (ELISA)** on seed-potato certification;
- **2-**To apply **the Sprout/Seed-Potato Technology** (Souza –Dias & Costa, 1985. Summa Phytop. 11:52; PAA & EAPR congs) for a safety, no virus degeneration multiplication rate of virus-free basic (imported or tissue culture) seed-potato stocks – **Therefore, no more delay on having the SPROUT /SEED-POTATO as an official seed-potato propagating material .**

For considering whitefly not a virus vector to potato plants, most seed-potato producer and certification agents considered as **"low or normal" countings of 20-30 whiteflies per leaf. But "very intolerable" countings of a few aphid , as they are well recognized as virus-vector (PLRV, PVY)**

In Central and Southeastern Brazil, an increasing number of potato fields have shown typical primary or secondary *Potato leafroll virus* (PLRV)-like symptoms on 20-100% of plants, with zero aphid counts, but with heavy whitefly (*Bemisia tabaci*) presence.

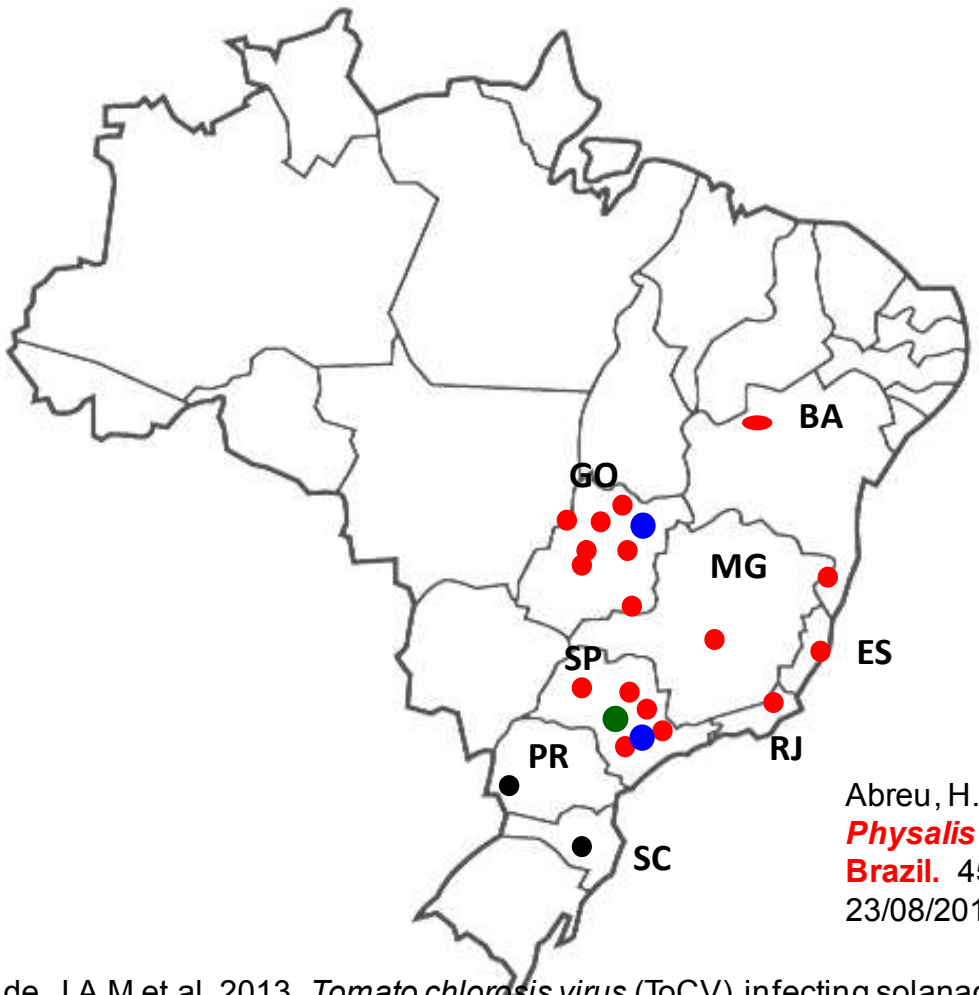
. Main Potato Producing Regions of Brazil (Source Agrianual 2011)

2009	Cultivated area (Hectars = ha)	Production metric tons	Productivity tons/ha
Northeast (NE)	8,102	293,730	36.25
Southeast (SE)	69,019	1,816,002	26.31
South (S)	57,829	1,076,643	18.61
West Center (WC)	6,027	232,250	38.53
Whole Country	140,987	3,418,625	24.25

BRAZIL: Sites of ToCV identifications

(photo courtesy of Dr. Jorge A. M. Rezende / ESALQ-USP)

Barbosa et al., 2011
Barbosa, Rezende, Bergamin Filho não publicado
Freitas et al., 2012



- Tomato
- Sweet pepper
- Potato
- Evidences

Abreu, H. et al., ***Tomato chlorosis virus* infecting the weed *Physalis angulata* within tomato fields in São Paulo State, Brazil.** 45°Cong Bras. Fitopatologia. Amazonas , MA, 18-23/08/2012

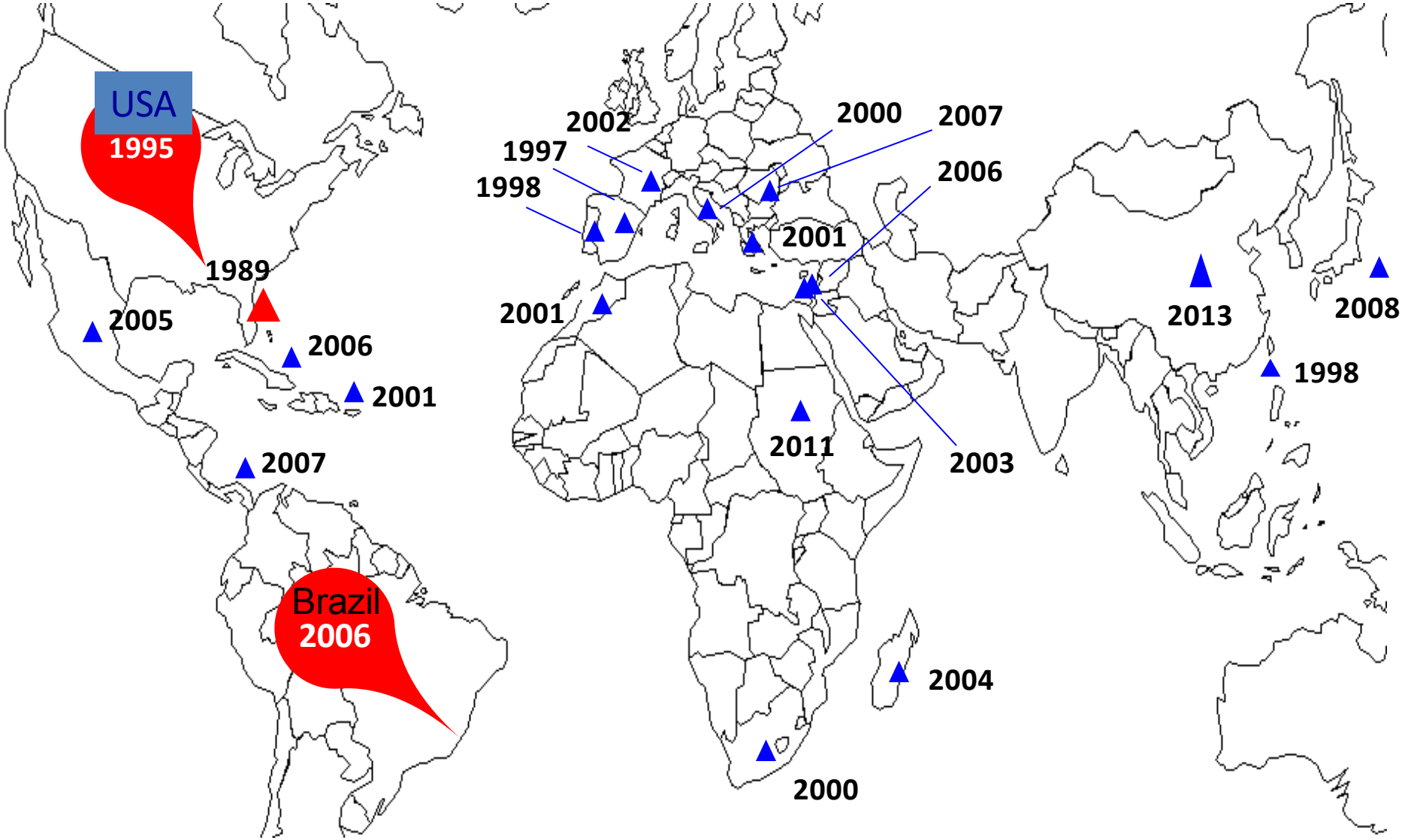
Rezende, J.A.M et al. 2013. *Tomato chlorosis virus* (ToCV) infecting solanaceous crops in Brazil: occurrence, epidemiology and management . 46° Cong Bras Fitopatologia, Ouro Preto, MG, CD rom, 20-25/10/2013. "…**ToCV is transmitted most efficiently by *Bemisia tabaci* biotypes A, B; *Trialeurodes vaporariorum,*** *T. abutilonea* (not present in Brazil) .

Lourenção, A.L./APTA-IAC, personal communication : ***T. vaporariorum is also present in field crops in Brazil.***

ToCV – A worldwide spread by year of first record

(photo courtesy of Jorge A. M. Rezende. ESALQ-USP)

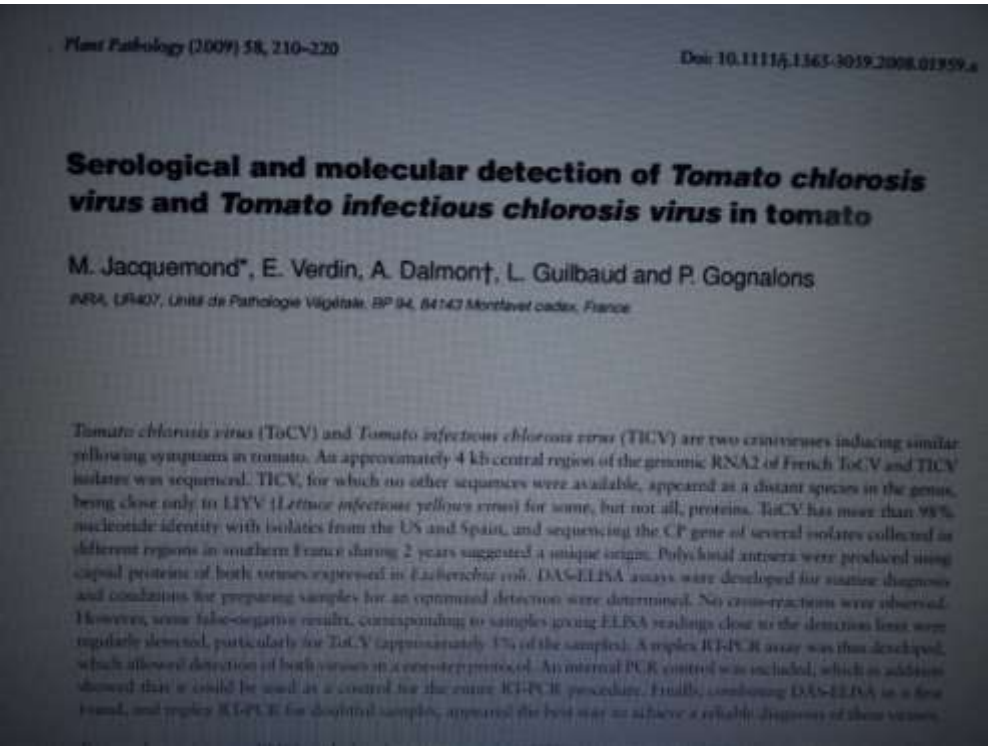
- BRAZIL is seed-potato importer for basic (virus-free) seed-potato stock renewal – annually
-Imported or national virus-free stocks must be Multiplied (G-0;>G-1>G2 up to G3 or more?)



New Disease Report- Plant Disease-APS

Navas-Casillo et al. 2011 Annual Review Phytopathology

ToCV recommended detection: Combining DAS-ELISA in a first test and then triplex RT-PCR assay can achieve a realiable diagnosis for tomato Crinivirus (ToCV x TICV) (Jacquemond, M. et al., 2009. Plant Pathology 58:210-220)



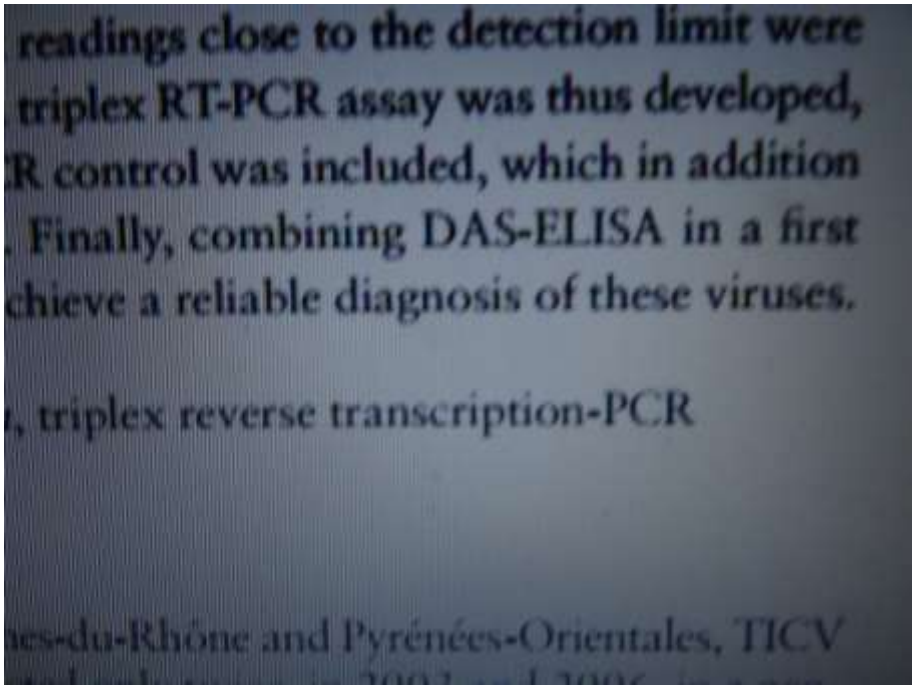
Plant Pathology (2009) 58, 210–220 Doi: 10.1111/j.1365-3059.2008.01959.x

Serological and molecular detection of *Tomato chlorosis virus* and *Tomato infectious chlorosis virus* in tomato

M. Jacquemond*, E. Verdin, A. Dalmon†, L. Guilbaud and P. Gognalons

INRA, UR407, Unité de Pathologie Végétale, BP 94, 84143 Montfavet cedex, France

Tomato chlorosis virus (ToCV) and *Tomato infectious chlorosis virus* (TICV) are two criniviruses inducing similar yellowing symptoms in tomato. An approximately 4 kb central region of the genomic RNA2 of French ToCV and TICV isolates was sequenced. TICV, for which no other sequences were available, appeared as a distant species in the genus, being close only to LIYV (*Lettuce infectious yellows virus*) for some, but not all, proteins. ToCV has more than 98% nucleotide identity with isolates from the US and Spain, and sequencing the CP gene of several isolates collected in different regions in southern France during 2 years suggested a unique origin. Polyclonal antisera were produced using capsid proteins of both viruses expressed in *Escherichia coli*. DAS-ELISA assays were developed for routine diagnosis and conditions for preparing samples for an optimized detection were determined. No cross-reactions were observed. However, some false-negative results, corresponding to samples giving ELISA readings close to the detection limit were regularly detected, particularly for ToCV (approximately 5% of the samples). A triplex RT-PCR assay was thus developed, which allowed detection of both viruses in a one-step protocol. An internal PCR control was included, which in addition showed that it could be used as a control for the entire RT-PCR procedure. Finally, combining DAS-ELISA as a first round, and triplex RT-PCR for doubtful samples, appeared the best way to achieve a reliable diagnosis of these viruses.

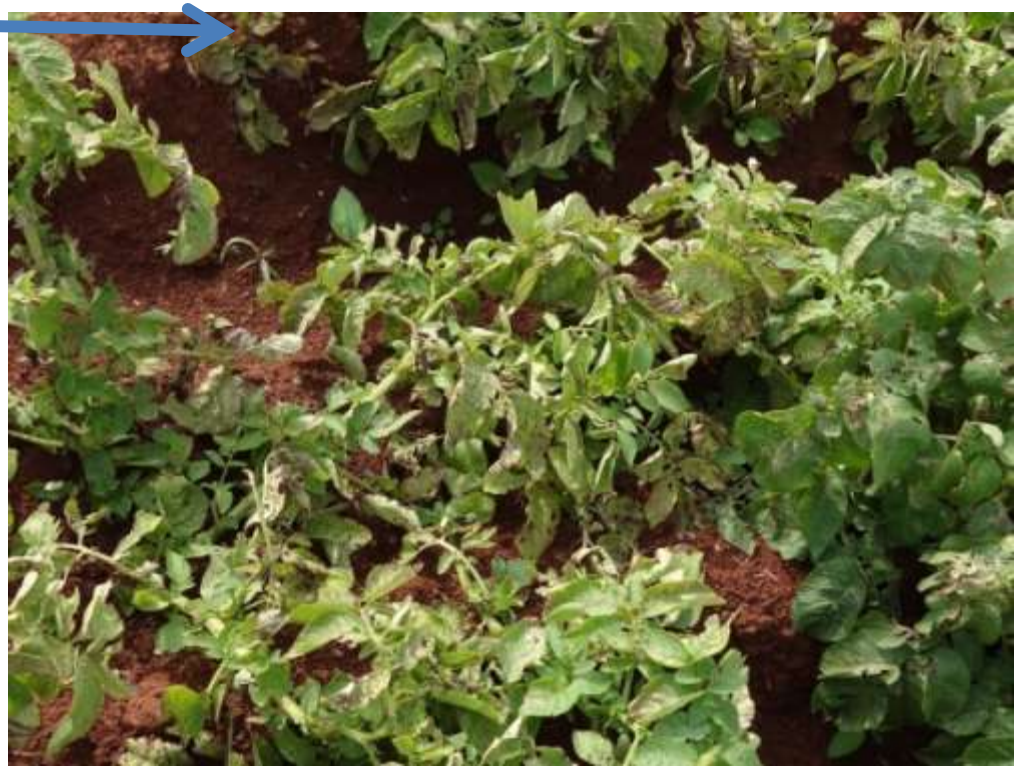


readings close to the detection limit were
triplex RT-PCR assay was thus developed,
R control was included, which in addition
Finally, combining DAS-ELISA in a first
chieve a reliable diagnosis of these viruses.

, triplex reverse transcription-PCR

es-du-Rhône and Pyrénées-Orientales, TICV

Field visit and sampling for PLRV-like potato plants has become normal (routine) in most warmer regions of Brazil (Southeast, Central and Northeast –Chapada). These scenario of no aphids but huge whitefly population has been increasing, repeating in most major potato producing areas and cultivars. ToCV detection has been increasing and constant in various phyto-diagnose labs in Brazil.



Datura stramonium e *D. metel*: Biomonitoramento de viroses transmitidas por insetos vetores nas plantações de batata e fumo. *

(*Datura stramonium* e *Datura metel*: Bio-monitoring insect transmitted viruses in potato and tobacco plantations.)

J A Caram de Souza-Dias(1); Haiko E. Sawasaki(2); Angenilson Delfrate (3); Armando T. Tomomitsu, (4); Silvia R. Galleti(5).

(1e4)APTA-Instituto Agronômico (IAC) / CPD-Fitossanidade, cep:13020-902, Campinas, SP, / (2)CG-Molecular; (3)Souza Cruz S/A, Rio Negro, PR; (5)APTA-I. Biológico (IB)/CMEletronica, SP.
E-mail: jcaram@iac.sp.gov.br.

*Apoio FUNDAG/Grupo Ishida; ABVGS; Souza Cruz AS;ABBA

XLII CONGRESSO BRASILEIRO DE FITOPATOLOGIA. Rio de Janeiro, 3 a 7 de agosto de 2009.

RESUMO

As culturas da batata (*Solanum tuberosum* L.,) e fumo (*Nicotiana tabaccum*.) são afetadas por virus transmitidos por insetos vetores, destacando-se: Potyvirus (PVY, TVMV); Polerovirus (PLRV, TVDV), Begomovirus (ToYVSV, ToSRV;), Ilarvirus (TSV) and Tospovirus (TSWV, ToCSV). Fatores bio ou abióticos dificultam inspeção visual. Desde 2005, 20 a 100 mudinhas (2-3 folhas) sadias das espécies *D. stramonium* e *D. metel*, têm sido expostas em plantações de batata e fumo. Em todos os ensaios, *D.metel* observa-se clorose e enrugamento severo a Potyvirus, oposto da *D. stramonium* que é imune a potys mas muito sintomática para: PLRV (amarelo internerval); TSV (folhas necróticas e denteadas); Tospovirus (riscas, anéis necróticos e morte do topo); Geminivirus (nervuras com riscos amarelados em folhas deformadas), que se assemelham a Tospovirus. Testes imuno e moleculares têm confirmado a "bio" ausência/presença desses virus nas duas culturas. Incidência de moscas Lyromiza e Bemisia com ausência de afideos sob alta incidências de PVY e PLRV suscita nova relação virus/vetor. São reflexos práticos desses resultados: maior eficiência na diagnose e na erradicação.

Introdução

As plantas indicadoras Datura stramonium e D. metel, são utilizadas na pesquisa biológica das fitoviroses mais comuns das culturas da batata (*Solanum tuberosum* L.) e do fumo (*Nicotiana tabaccum*), destacando-se: **Potyvirus** (*Potato virus Y* - PVY, *Tobacco vein mottle virus* - TVMV); **Polerovirus** (*Potato leafroll virus* - PLRV, *Tobacco vein distortion virus* - TVDV). **Begomovirus** (*Tomato yellow vein streak virus* - ToYVSV, *Tomato severe rugose virus* - ToSRV;), **Ilarvirus** (*Tobacco virus S* - TSV) and **Tospovirus** (Tomato spotted wilt virus - TSWV, *Tomato chlorotic spot virus* - ToCSV). (Jeffries, C. 1998. Potato – FAO/IPGRI No. 19: 177p; Salazar, L.S. 1996. Potato Viruses and Their Control. CIP-Peru: 213p; Walkey, DGA, 1985. Applied Plant Virology. Chapmann & Hall, London; 338p.; Costa, A.S. 1975-92. Apostilas PG-ESALQ/Fitovirologia).

Nesse trabalho, procurou-se avaliar o potencial valor auxiliar nas inspeções de campo focada em questões de viroses dessas espécies de solanáceas, incluindo o tomate (Lycopersicon esculentum))

MATERIAL E MÉTODOS

Nos últimos 4 anos, mudinhas sadias das espécies *D. stramonium* e *D. metel*, produzidas em estufa anti-afideos, foram transplantadas (estádio de 2-3 folhas), em campos ou talodos de cultivo comercial. O transplante das mudinhas foi feito logo após aplicações de herbicidas pré/pós emergência . As plantas indicadoras expostas receberam tratamentos iguais aos da espécie cultivada, durante todo o ciclo. As inspeções de sintomas foram feitas pelo menos duas vezes durante o ciclo. Amostras de folhas com e sem sintomas suspeitos de viroses foram coletadas tanto das espécies de *Datura*, como das plantas cultivadas, e submetidas para análises imunoenzimáticas (DAS ou TPA ELISA), moleculares (PCR com ou sem sequenciamento) e em microscopia eletrônica.

RESULTADOS e DiscUSSÃO

Polerovirus, PLRV e Potyvirus, PVY: *D. stramonium* comportou-se imune ao PVY; suscetivel ao PLRV (amarelo internerval). *D. metel*, a resposta foi oposta à da *D. stramonium* e distinta para as duas viroses. Conjuntamente, tiveram testes confirmando a visual ausência ou presença e disseminação de PVY e PLRV em campo. Ilarvirus, TSV: *D. stramonium* expressou sintomas de folhas necróticas e denteadas mais definidos e mais suscetível que a *D. metel*, esclarecendo sintomas em folhas de fumo; Tospovirus: ambas daturas manifestaram sintomas de pontuações e riscas brancas internervais, anéis necróticos, seguido ou não de morte do topo; Geminivirus, ToSRV e ToYVSV, apenas *D. stramonium* mostrou sintomas de clorose internerval e riscos amarelados em folhas deformadas: semelhantes aos de Tospovirus. Testes imuno e moleculares confirmaram a "bio" ausência/presença desses virus nas plantações onde os sintomas nas duas espécies de Datura foram ou não observados.

Conclusão

Plantas de *D. metel* e *D. stramonium*, auxiliam respostas a questões comuns em campo, tais como origem da virose: semente/muda ou primária; o foco inicial e demanda de proteção localizada; (2) Eficiência no controle de fitoviroses , reduzindo movimentação dentro da cultura; (3) Direcionamento objetivo de material (amostras do campo) para laboratorio de análises, tornando a relação custo/beneficio melhor ao produtor. Fatores favoráveis adicionais pode vir da possivel redução na (1) disseminação do PVY via "limpeza" de estiletes de afideos vetores em *D. stramonium*; e (2) infestação de mosca branca (*Bemisia tabacci*) em barreiras de *D. metel* (Souza-Dias et al., 2008 Summa Phytop. 34(suplm):186,).

D stramonium em batatal: Mostrando Geminiviru e PLRV

D. metel monitorando (Potyvirus) dentro d telado/produção de minitubérculos de batata-semente

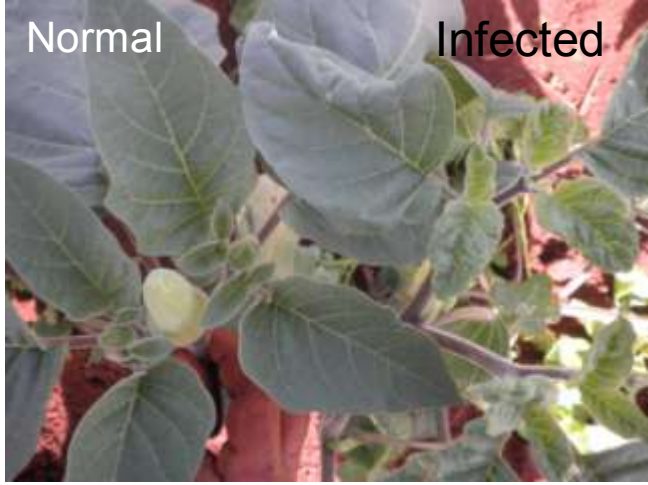
D. metel monitora PVY e Reduz vetores de viroses em fumo

Datura stramonium com sintomas típicos de Tospovirus. Notar planta de fumo (seta)

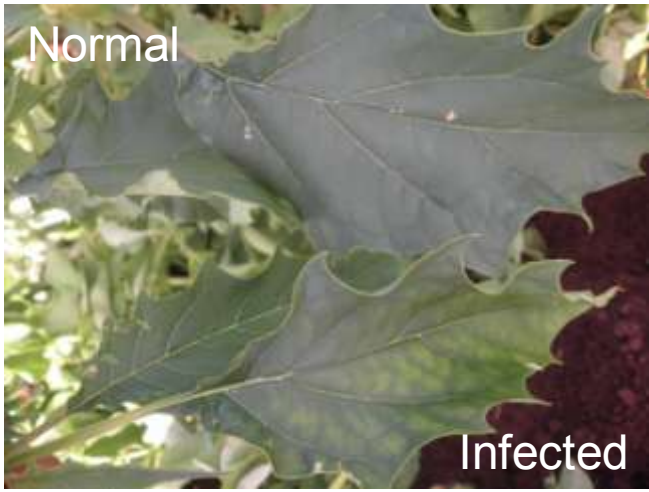
SECRETARIA DE AGRICULTURA E ABASTECIMENTO

GOVERNO DE SÃO PAULO

D metel
PVY, only(90%)



D stramonium
PLRV or ToCV
Similar interv yellow



D stramonium
Begomovirus
(ToYVSV or ToSRV
similar

Bio-monitoring insect-transmitted potato virus via exposition of *Datura metel* & *D stramonium*
(flower removal are recommended and done at weekly inspections)

The Sprout/Seed-potato Technology

(Souza-Dias et al, 2000-2012 have been presenting at PAA and EAPR Meetings)

It is ready to become a new (Innovative) safe movement of basic seed-potato stocks

(www.carambatatasemvirus.blogspot.com.br)

The sprout/seed-potato technology: similar performance on yield of minituber as compared with tissue culture plantles (in vitro)

The Sprout/Seed-Potato system for large scale, virus-free, (mini)tuber/seed-potato production.
The picture below shows production of a single sprout, detached from virus free (basic class) tuber-seed-potato (imported stock, cv Agata):
Planted on 15/May/2013; Harvested on 01-05/Aug/2013

Field production of tuber/seed-potato which had sprouts removed x not removed, cv Agata

Sprout **not** removed

24 tubers

Sprout removed

26 tubers

THE SPROUT/SEED-POTATO (S/SP) TECHNOLOGY: NOT A CHILLING BUT A CHALLENGING SEED-POTATO PRODUCING SYSTEM.

José Alberto Caram de Souza-Dias (1); Valdir J. Ramos (2); Kerstin Lindner (3).

(1) APTA-IAC/CPDFitossanidade,13020-902 - Campinas, SP (Brazil); (2) APTA-UPD-Itararé,SP; (3)Fed. Research Centre Cultiv. Plants – Julius Kühn-Institute, Braunschweig, Germany.

e-mail: jcaram@iac.sp.gov.br .

XXXII CONGRESSO Paulista de Fitopatologia, Ituverava, SP, 2-4 de Fevereiro de 2010.

*Apoio: FUNDAG/13-002/93; CNPq/MAPA 578746/2008-5

RESUMO

Before field planting, high-grade tuber/seed-potato stocks (*Solanum tuberosum*) generates hundred tons of sprouts. Due to the S/SP system (Patent PI 0604078-0; C1 0604078-0) which has been introduced worldwide (Souza-Dias, et al. 2009. 93thPAA Mtg, Canada, abst 54), these sprout/by-products is detoured from dump to aphid-proof greenhouses. Just like tissue culture plantlets, sprouts are planted to produce hundred of thousands virus-free minituber/seed-potato (Souza-Dias & Costa, 1998. A Granja. 597(54):12-18). The S/SP has become a new source of income for small farmers, revealing a new commodity. The challenge of increasing seed-potato supply without environmental impact, has drawn attention to the S/SB by seed-potato experts from exporter and importer countries. The recent Brazil-China coop. research project is pioneering on evaluating the S/SP by shipping sprouts from APTA-IAC bred varieties to China (Souza-Dias et al., 2009. Virus Rev & Res; 14(1):256-257). Due to its low: production cost; freight and risk of soil-borne pathogens movement, the S/SP strikes the seed-potato market as an alternative to fight hunger with sanitary safe movement of seed-potato.

Technology transfer: proceedings and achievements

The innovative "Sprout/Seed-potato" system (APTA/IAC/CPDFitossanidae. Patent pending INPI PI 0604078-0, alter C1 0604078-0), consist of moving (transporting) only sprouts as "seed-potato" (*Solanum tuberosum*) for large scale production of virus-free, "pre-basic" minituber/ seed-potato stocks, inside an aphid proof-screenhouse (Souza-Dias & Costa, 1998. A Granja. 597(54):12-18; Souza-Dias et al, 2001, A.J.P.Research. v.78; Souza-Dias ET AL., 2005. Batata-Show, 5(11):11).

Over the past ten years, sprouts has been a new source of income for small as well as highly technically structured potato producers whose basic minituber/seed-potato stokes, produced from tissue culture are de-sprouted after >8 months storage in moderate room temperature. A potential new commodity for the seed-potato marketing has also been considered by regular tuber/seed-potato producer/exporter countries, such as the USA/Alaska (Souza-Dias, et al. 2007 http://www.saprvirology2007.org.uk/25%20Souza-Dias.pdf.; Kuhl, J.C. et al., Abstracts 93rd Annual PAA Meeting, N.B. Canada, 9-13/August, 2009, p. 80 (abst 107); Tewa, R. 2008, http://magissues.farmprogress.com/WFS/WS02Feb08/wfs018.pdf);and Canada/ New Brunswick (Souza-Dias, et al., 2009. 93rd. Annual PAA meeting. 9-13/Aug/2009, p. 52-53, abstract 54).

In this presentation we are pointing out the potential impact of the sprout/seed-potato technology that has been transferred to China (Brazil-China Cooperative research program for transferring and evaluating the Sprout/Seed-potato system (Gomes, C. 2009. Diário Oficial ESP, IV-119(4), 08/01/2009). The same cooperation has now been planned with African countries (Moçambique and Benin). Besides being a low cost propagating material, with low overseas freight cost (weighing 10 fold less than tuber/seed-potato), sprouts offer less risk of moving soil-borne pathogens as compared to the tuber/seed-potato system. These advantages sustain the sprout/seed-potato technology as another economic/social Brazilian phytopathological contribution to fight hungry in developing nations and promote a more sanitary safe agro-business as movement of basic stocks of seed-potato between exporter and importer countries are concerned.

MOÇAMBIQUE

-----Original Message -----

From: Carolino Martinho
To: Jose Alberto Caram de Souza Dias
Cc: Jacinto Matalacusser ; Calisto Bias ; Ricardo M. Coelho
Sent: Tuesday, January 19, 2010 10:48 AM
Subject: Re: tecnologia "broto / batata-semente»

Prezado Dr. Caram Souza-Dias,

Muito obrigado pelo seu email e pelas informações dadas. Estamos a contactar o Ministerio de Agricultura de Moçambique para a permissão de importação em conformidade com as orientações dadas. O Director Geral do =IAM, Dr. Calisto Bias, aprovou a intensão de cooperação. Assim esyamos a espera do draft do projecto de cooperação a ser elaborado por voces para comentarmos e dar sugestões da nossa parte.

Melhores cumprimentos,

Carolino MartinhoTo

CHINA

USA / ALASKA

BENIN

CANADA

CNPq

SECRETARIA DE AGRICULTURA E ABASTECIMENTO

GOVERNO DE SÃO PAULO

Thank you!

Questions?

Special thanks to my research support from:

APTA-IAC;

CNPq;

FUNDAG/

Souza Cruz S.A.;

MICROGEO;

ABBA; ABVGS)