National Institute of Research and Development for Potato and Sugar Beet Brasov, ROMANIA

Tackled problems / Directions

- **DETECTION** of viruses in seed potato, breeding material and other samples
- **OBTAINING** IgG and conjugates used for diagnose potato X, S, M, Y viruses
- **MAINTAINING** the collection of potato virus isolates and test plants

A. Evaluating **THE EFFECTS** of several **MODIFICATIONS of ELISA** on the detection of potato viruses

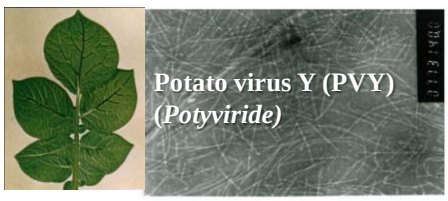
B. Identification of several treatments with **ESSENTIAL OILS** that could have beneficial effects on different quality indicators of potato and tobacco plants inoculated with PVY

THE RESEARCH WORK TARGETS

A. IMPROVE the sensibility and the reliability of ELISA TECHNIQUE by applying several test MODIFICATIONS, specially for PVY and PLRV detection

- Sample prelevation
- Changement of the conjugate incubation modality
- Using grinding buffer with different chemical composition/ Replacement of extraction buffer with McIlvain's phosphate-citric acid buffer (0.18M; pH 7)

WHY PVY AND PLRV FOR OUR RESEARCH ACTIVITY?



CAN CAUSE
stand loss, reduced yields,
undersized tubers
reduced quality

HAS BECOME
an increasingly serious constraint to seed potato production in the world

EFFORTS to CONTROL
= essential for potato seed production

Modifications of ELISA technique used for potato VIRUSES detection
I. Samples prelevation
II. Samples incubation
III. New Buffer Additives

A. Modifications of ELISA technique used for potato VIRUSES indexation

I. SAMPLES PRELEVATION

FROM LEAVES

- Treatment TUBERS with giberellic acid
- Sprouting tuber 20-22°C min. 2 weeks
- Planting in the Greenhouse
- Growth of plants 6 weeks
- Extraction sap from leaf in extraction buffer
- AUTOMATIC distribution in microplates treated with antibody

DIRECTLY FROM TUBERS

- Sprouting tuber 20-22°C min. 3 weeks
- Extraction sap from tuber automaticaly dilution in buffer
- AUTOMATIC/MANUAL distribution in microplates treated with antibody

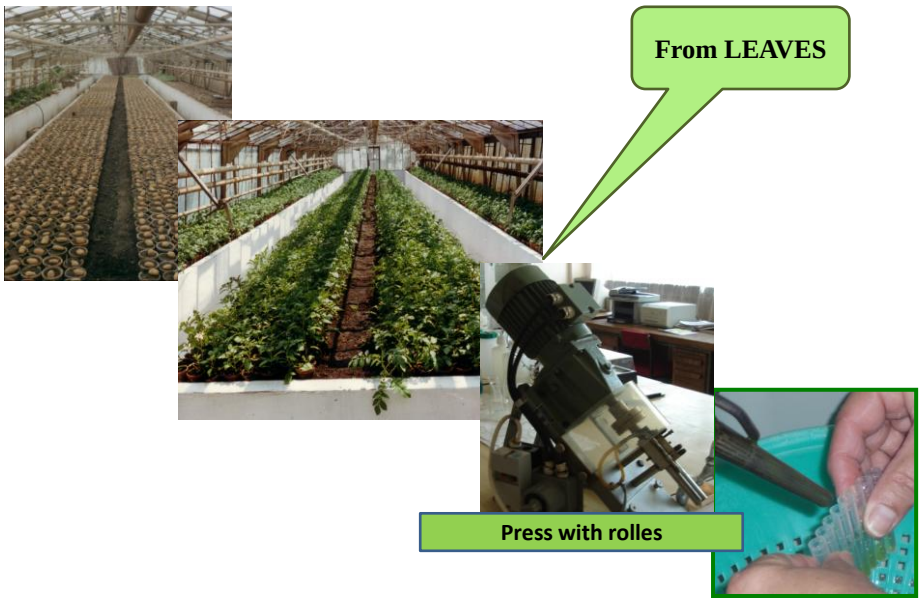
FROM SPROUTS

- Sprouting tuber at 22-24°C until the dimensions of sprouts are 0,3-1cm (2-3 weeks)
- Take sprouts from apical end
- Manual crushed of sprouts and homogenisation with extraction buffer
- MANUAL distribution in microplates treated with antibody

DAS ELISA

Continuation of test with the next stages
(addition conjugate, addition substrate, reading OD at 405nm)

METHODS OF THE SAP'S EXTRACTION



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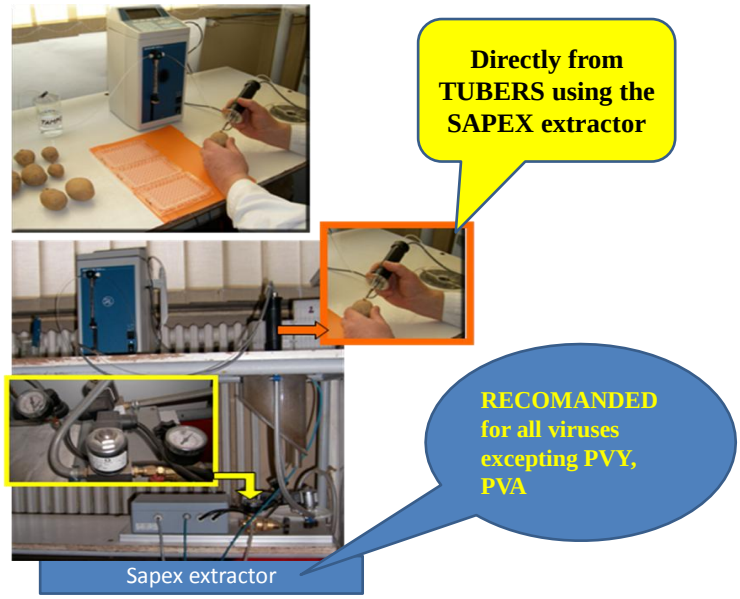
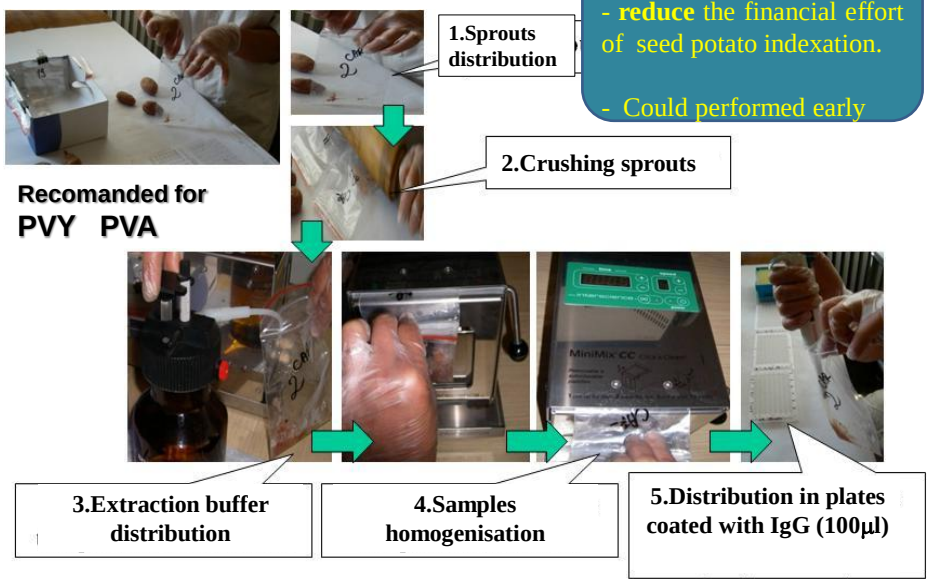


SAP'S EXTRACTION FROM SPROUTS

- **faster**, more **economical**
- **reduce** the financial effort of seed potato indexation.
- Could performed early



Modifications of ELISA technique used for potato VIRUSES detection
II. SAMPLES INCUBATION

Effect of samples incubation on detection of PLRV

Applying **COCKTAIL** ELISA

→ test's **safety** and **sensitivity** increased.

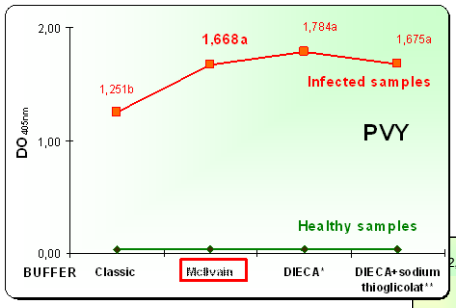
→ **detectability** of potato virus particles was improved.

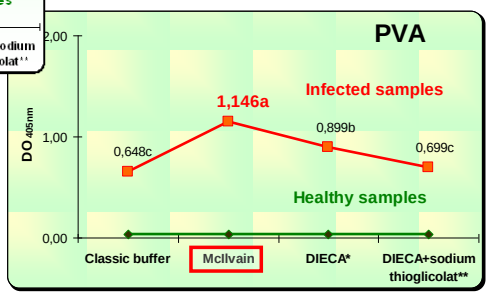
	Incubation time with substrate solution					
	30 minutes		60 minutes		120 minutes	
	V1*	V2**	V1*	V2**	V1*	V2**
Cut off	*0.065*	***0.084***	*0.072*	***0.113***	*0.097*	***0.171***
OD_{405nm}***	0.055 ± 0.014	**0.278 ± 0.119**	0.089 ± 0.030	**0.536 ± 0.230**	0.162 ± 0.063	**1.072 ± 0.450**
OD V1 / OD V2		5.035		6.035		6.6

** V1 – DAS ELISA** V2 – COCKTAIL ELISA (co-incubation samples + IgG-AP)

Modifications of ELISA technique used for potato VIRUSES indexation
III. New grinding buffer with different composition

Effects of McIlvain buffer and several grinding buffer's additives





A. Evaluation THE EFFECTS of ELISA MODIFICATIONS on the viruses detection

Modifications of ELISA technique:
-sap prelevation from tubers / sprouts
-Cocktail ELISA (for PLRV)
-McIlvain's buffer (grinding buffer)

=

POSSIBILITIES for saving TIME + COSTS of several potato viruses diagnostic tests.

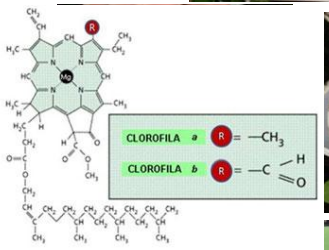
This research work had the financial support of a PROJECT (from the programe "Nucleu"): **PN09-20-01-05** (2009-2012)
"Adaptation and implementation of a fast, modern and economical method for potato seed indexation, according to the demands of ecological agriculture"

Target: Efficiency the indexation of the potato material from basic breeding samples, by adaptation and implementation a more economical methodology

Results: Profitable and competitive method for potato seed indexation (detection of the most important viruses)

THE RESEARCH WORK TARGET

B. ESTIMATE the effects of *several essential* oils (*Lamiaceae* fam.), hydrogen peroxide and ascorbic acid treatments ON:



- photosynthetic pigments
- several quality indicators

- in potato and tobacco plants inoculated with potato virus **Y (PVY)**

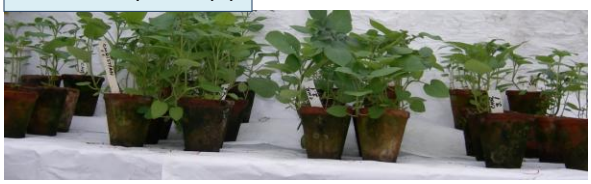
TREATMENTS on potato

Healthy plants (H)

Variants

- H1 +V1 CONTROLS
- H2+ V2 *R.o.* oil (1/100, injected+rinsed)
- H3 +V3 *R.o.* oil (1/100, injected+rinsed) +H_2O_2 (1mM, sprayed)
- H4+ V4 *R.o.* oil (1/100, injected+rinsed) +AA (3mM, sprayed)

Inoculated plants (V)

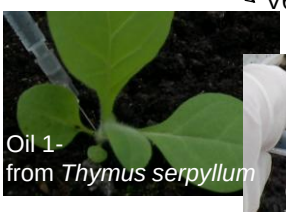


Treatments on *Nicotiana tabacum*

Variants

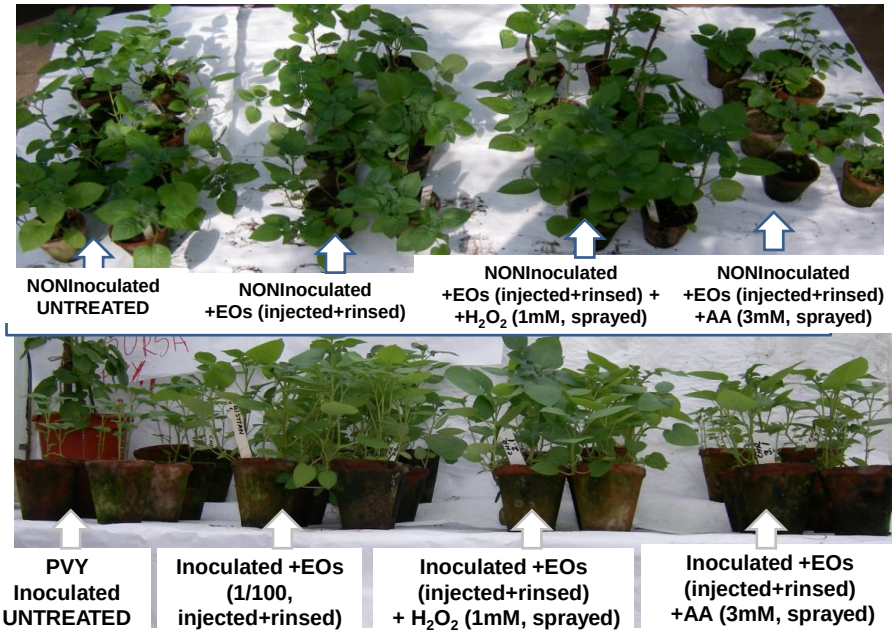
- V1 Negative CONTROLS → UNTREATED UNINFECTED
- V2 Positive CONTROLS → UNTREATED PVY INOCULATED
- V3 Oil 1 (1/100)
- V4 Oil 1 (1/1000)
- V5 Oil 2 (1/100)
- V6 Oil 2 (1/1000)

→ INJECTED + PVY INOCULATED + SPRAYED+ RINSED



Oil 1- from *Thymus serpyllum*

Oil 2- from *Lavandula officinalis*



B. Identification of treatments with EOs that could have benefficial effects on PVY inoculated plants (improuving different quality indicators)

-WITHOUT chemical treatment, all the inoculated plants showed significant REDUCTIONS in leaf pigments content compared to uninfected controls.

For potato:

-EOs TREATMENTS and hydrogen peroxide / ascorbic acid of inoculated plants significantly reduced the number of minitubers, enhancing their weights, while leaf pigment content also increased.

For tobacco:

-*Thymus serpyllum* oils (1/100) TREATMENTS and hydrogen peroxide / ascorbic acid spraying of inoculated plants enhanced significantly their fresh weights while leaf pigment content also increased.

*EO*s + H_2O_2 (1mM) or AA (3mM)

BENEFICIAL ANTI-STRESS EFFECTS ON THE PVY INOCULATED PLANTS

Another takled problems by our work group

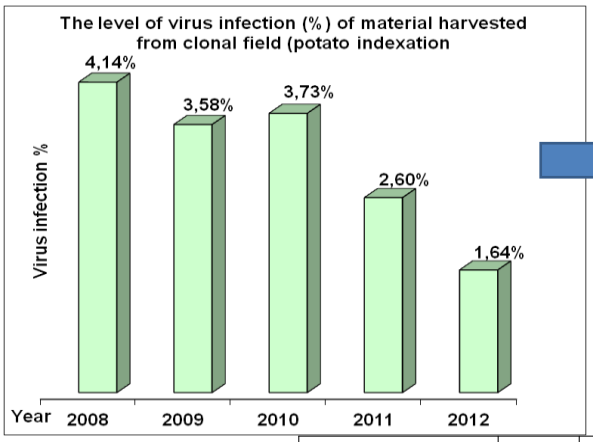
1. Testing potato viruses

-ELISA technique is used for detection potato viruses (PVX, PVS, PVY, PVM, PVA and PLRV) in seed potato, pre-basic seed potato material and other samples

-Usually, for the clonal selection, we evaluated the level of virus infections using a variant of ELISA technique, in field (on the plants) and in **post harvest (on the tuber)**.

Due to this methodology, unexpected transmissions of particular viruses in pre basic potato seed material decreased (e.g. PVS, PVA, PVM)

ELISA test of the pre-basic seed potato material (potato indexation)



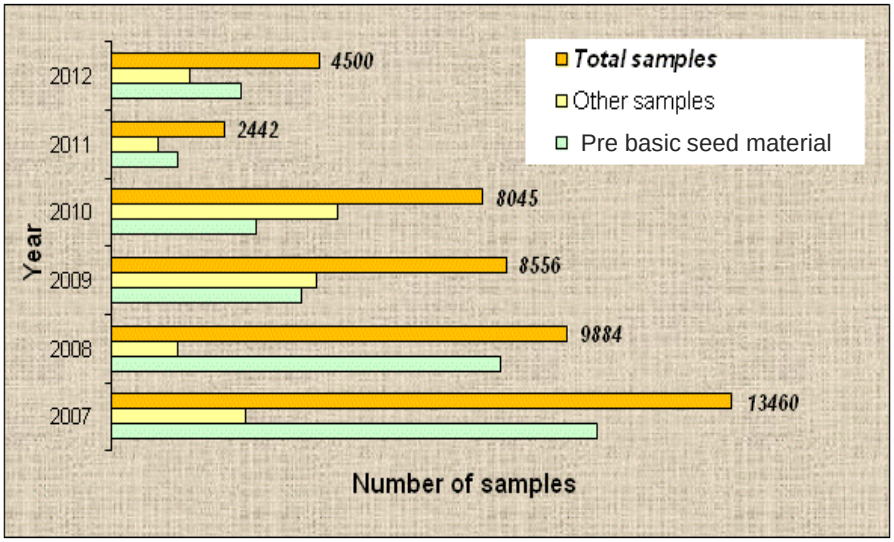
Samples from tubers and sprouts

The virus infection level **decreased**

Better biological material
The testing methodology was improved

Number/ Year	2008	2009	2010	2011	2012
PLRV - PVA	96	71	51	8	11
PVY - PVM	112	44	39	23	27
PVX - PVS	123	19	9	5	8
Positiv samples	331	134	99	36	46
Samples tested	7992	3746	2657	1384	2812

Total number of ELISA tests made in virology laboratory during 2007-2011



2. Obtaining IgG and conjugates for ELISA test

The VOLUME OF MATERIAL TESTED in the seed potato indexation and in our research work is very HIGH

Very HIGH COSTS for buying the kit from import.

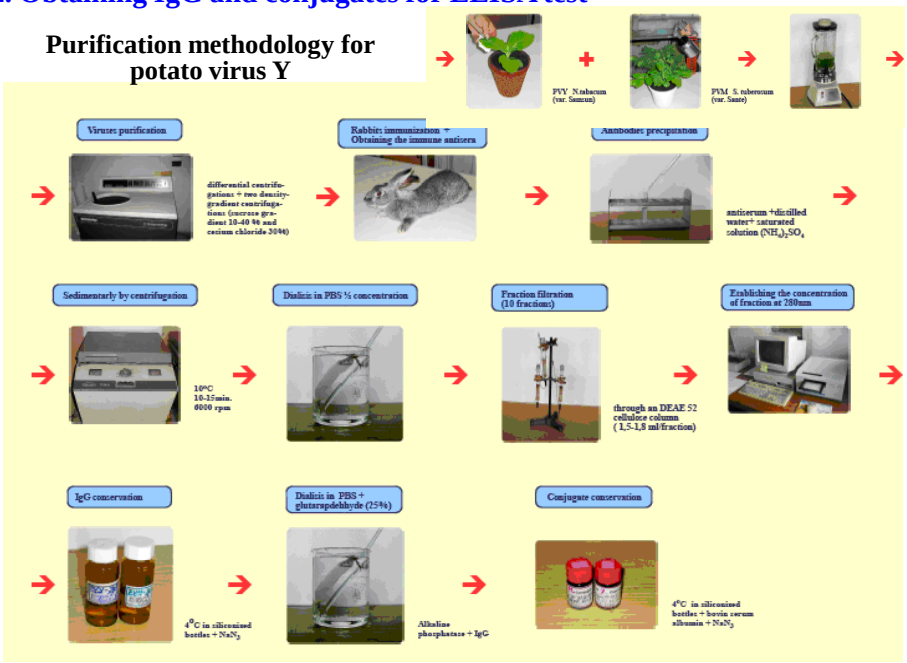
acitvity GOAL

Reducing the financial effort due to the import of IgG and conjugate

OBTAINING ELISA kit for PVX, PVS, PVA, PVM

2. Obtaining IgG and conjugates for ELISA test

Purification methodology for potato virus Y



2. Obtaining IgG and conjugates for ELISA test

PVY, PVM, PVX, PVS
IgG and conjugate preparated during 2008-2011

-Year 2008: kit for 20000 analysis
-Year 2009: kit for 20000 analysis
-Year 2010 :kit for 20000 analysis
-Year 2011: kit for 10000 analysis

Our research work RESULTS were promoted by the following achievements:

ISI articles		**2**
Articles indexed in other databases	**Journals indexed (CNCSIS B+)**	**12**
	Non-indexed journals	1
Articles in Journals indexed in other international databases		3
Book : "*Food preservatives-limits and perfomances", Transilvania University ed., 2012, ISBN978-606-19-0042-8*		**1**
National Scientific Communications		**7**
International Scientific Communications		**12**
Other publications		14
Patent demand (request) registered at SOIT (OSIM): *1. Substitution of the extraction /homogenization buffer with McIlvain buffer in order to improve safety identification of some potyviruses (potato viruses Y and potato viruses A)* *2. Buffer solutions conjugated with a modified composition used for potato viruses detection*		**2**
Articles in press		
ISI articles accepted for publication		**1**
Journals indexed (CNCSIS B+)		2

Some of our work group OBJECTIVES FOR THE NEXT YEARS:

We will be involved in new research directions, improving our efficacy. **Special attention** will be paid to:

-operating the laboratory under **quality assurance standards**.

-evaluate the effects of **Eos treatments** on viruses inoculated plants

- collection and **caracterisation potato Y isolates from different areas of seed potato production**, for settling down the distribution and evolution of PVY populations**, in order to decrease viruses spreading** in different counties of our country **(proposal project PNCDII)**

-improving and implementation of new viruses detection techniques


Thank you for your attention!