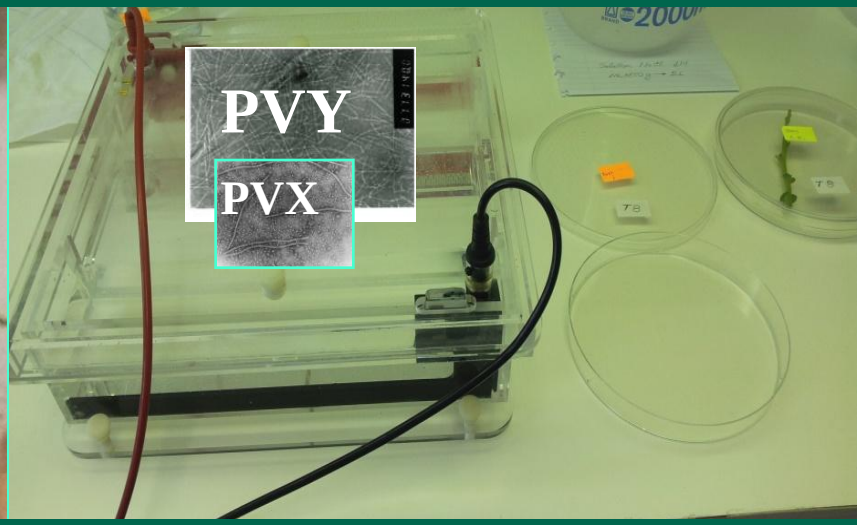
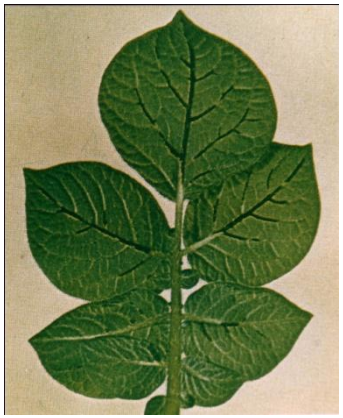
National Institute of Research and Development for Potato and Sugar Beet
Braşov, Romania

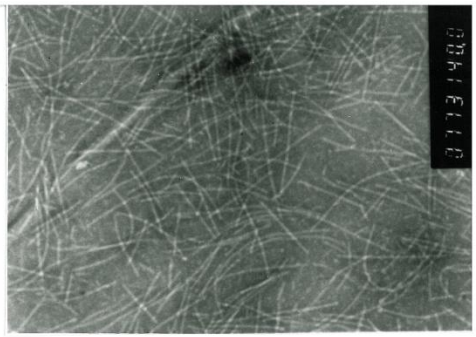


EFFECT OF SOME CHEMO- AND ELECTROTHERAPIES ON POTATO VIRUS Y AND X INFECTED *Solanum tuberosum L.* PLANTLETS (cv. ROCLAS)

Carmen Liliana BĂDĂRĂU,
Nicoleta CHIRU, Ionela Cătălina GUŢĂ



Potato virus Y (PVY) (*Potyviride*)

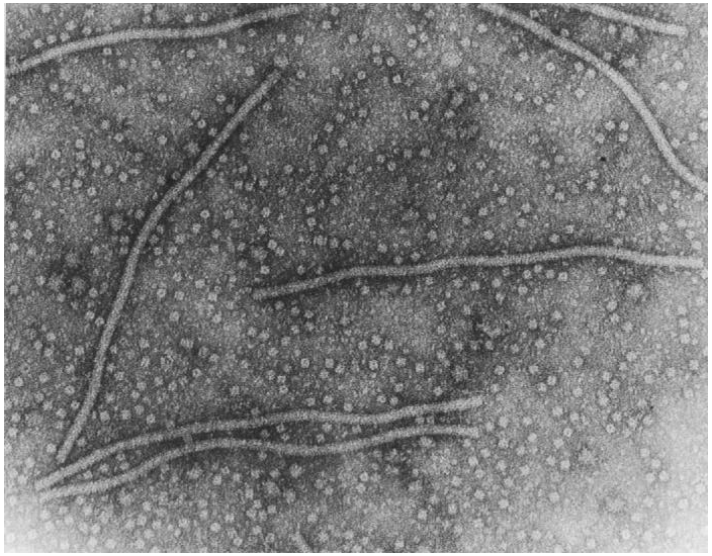


CAN CAUSE
stand loss, reduced yields,
undersized tubers
reduced quality

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

EFFORTS to **ameliorate**
PVY effects
= **essential** for potato production

HOW?
1 chemotherapy?
2 essential oils?
3 electrotherapy?



Potato virus X (PVX) (*Potexvirus*)

Occurs throughout commercial stocks of most varieties

Is responsible for many of the uncertainties and difficulties encountered in field inspections.

When Potato virus Y is present, synergy between these two viruses causes severe symptoms in potatoes

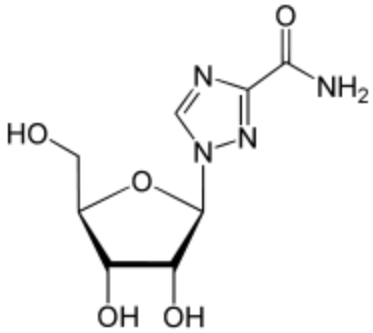
Elimination PVX from potato supply = important for potato production

HOW?
chemotherapy?
essential oils?
electrotherapy?

1. WHY CHEMOTHERAPY?

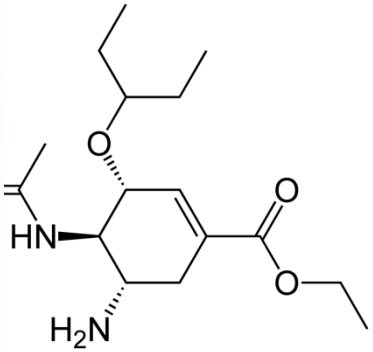
RIBAVIRIN (RBV)
(1,ß-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide)

- Broad spectrum **anti-viral activities**,
- RBV5'-phosphate = **inhibitor of inosine monophosphate (IMP) dehydrogenase** [1]



OSELTAMIVIR (OSMV) (Tamiflu)
[ethyl (3R,4R,5S)-5-amino-4-acetamido-3-(pentan-3-yloxy)-cyclohex-1-ene-1-carboxylate]

- an **antiviral prodrug**
- **used to slow the spread of flu virus** (influenza A and B) by **stopping** from chemically **cutting with its host cell.**
- produced from shikimic acid, **an inhibitor of neuraminidase** [2]



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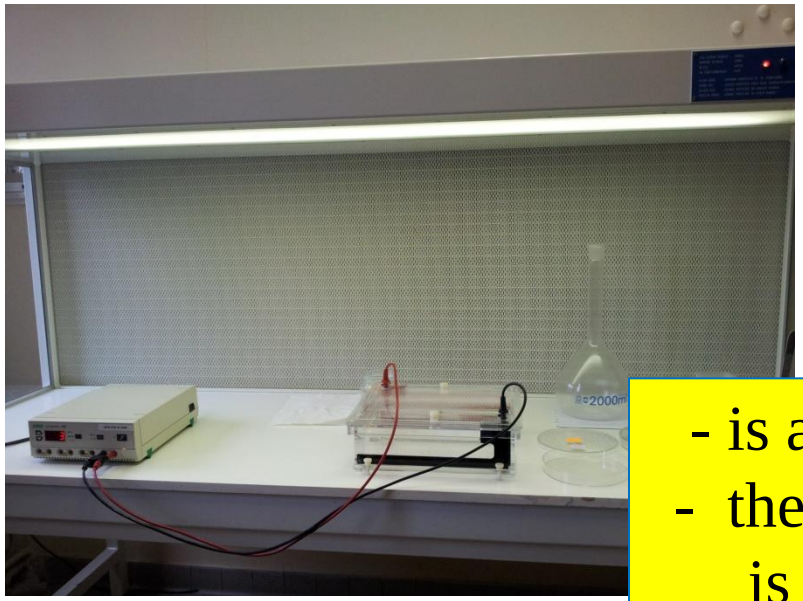
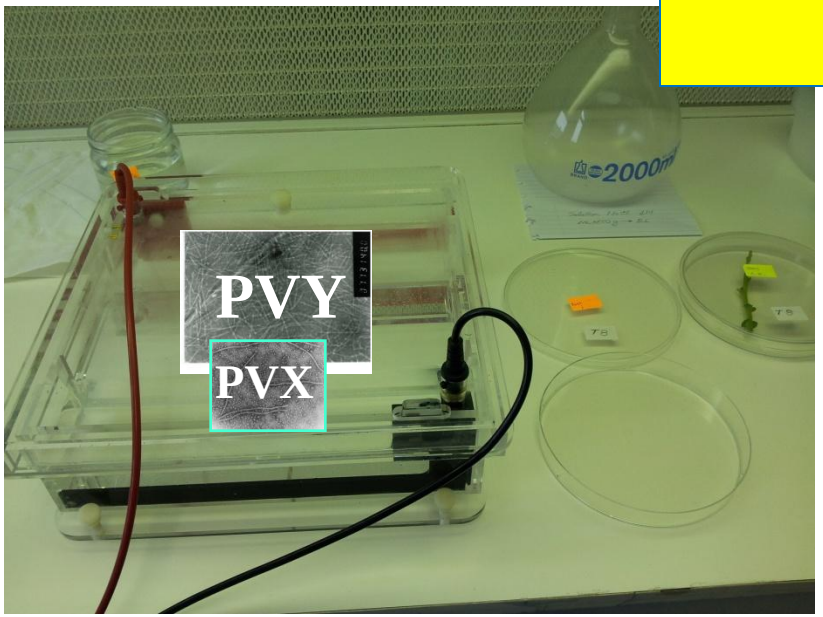
[1] Cassel, A. C. 1987. In vitro induction of virus-free potatoes by chemotherapy. In: Biotechnology in Agriculture and Forestry, Vol. 3Potato (ed.) Y.P.S. Bajaj, pp. 40-50, Springer-Verlag, Berlin, Germany
[2] Ward, P., Small, I., Smith, J., Suter, P., Dutkowski, R. 2005. Oseltamivir (Tamiflu) and its potential for use in the event of an influenza pandemic *The Journal of antimicrobial chemotherapy* 55 (Suppl 1): 5–21

2. WHY electrotherapy?



- is a simple method
- the equipment used is not special or expensive

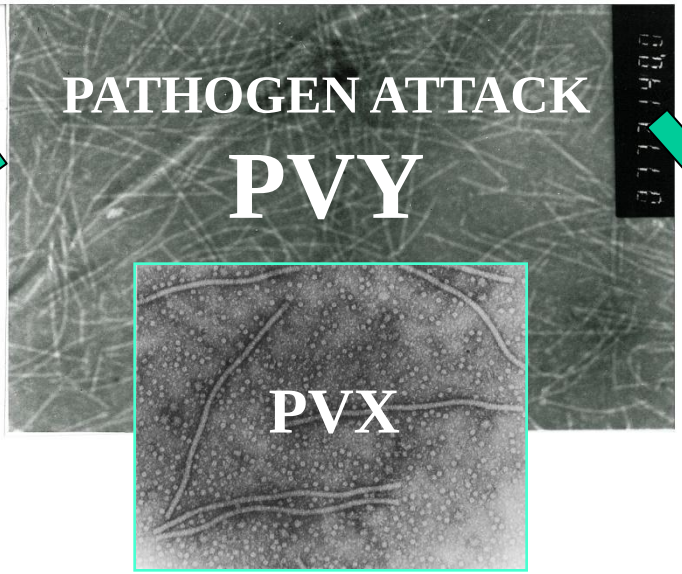


- **electric pulses** = **stimulants** on plants differentiation *in vitro*
- electric current is applied to plant tissues **for disrupt/degrade viral nucleoprotein and eliminate its virulence activity**

3. WHY hidro-distilled ESSENTIAL OILS **(EOs)** from *Satureja hortensis?*

These EOs = a potential source of **antimicrobial active compounds** ?

3. WHY treatments with HYDROGEN PEROXIDE and ASCORBIC ACID ?



STRESS

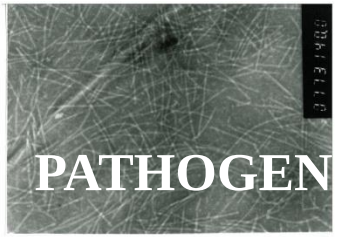
ANTIOXIDANTS

INCREASE
REACTIVE OXYGEN SPECIES (ROS)

plant **cells** have
DEFENSIVE RESPONSES
+
METABOLISM CHANGES

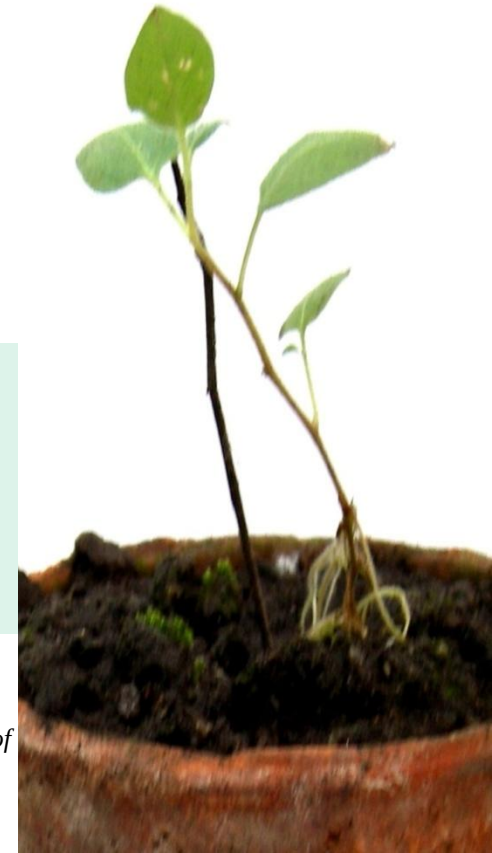
Bibliography Hammerschmidt, R., 2005. Antioxidants and the regulation of defense. *Physiological and Molecular Plant Pathology*, 66: 211–212

HYDROGEN PEROXIDE



PATHOGEN ATTACK

Is believed to play **two distinct roles**
-involves the **oxidative burst** in the **hypersensitive response**, which **restricts pathogen growth**,
-activates plant defense responses, including
induction of phytoalexins



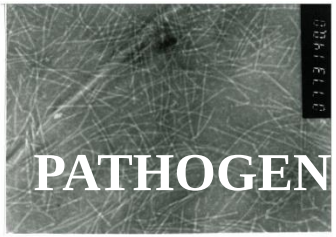
H_2O_2 produced in
- **excess** is **harmful**,
- **LOWER** concentrations=**BENEFICIAL**

Bibliography

López-Delgado,H.,H.A.Zavaleta-Mancera, M.E. Mora-Herrera, M.Vázquez-Rivera, F.X. Flores-Gutiérrez, and I.M. Scott, 2005.Hydrogen peroxide increases potato tuber and stem starch content, stem diameter and stem lignin content. *American Journal of Potato Research*, 82: 279–285.

Quan, L.J., Zhang, B., W.W. Shi, and Li, H.Y., 2008. Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. *Journal of Integrative Plant Biology*, 50: 2–18.

ASCORBIC ACID (AA)



-Participates in response to **both biotic and abiotic stresses**

-Acts as an **antioxidant**, **protecting** the cell against **oxidative stress** caused by environmental factors and **pathogens**.

-Changes in AA content can modulate systemic acquired resistance, acting as a signal transducing molecule

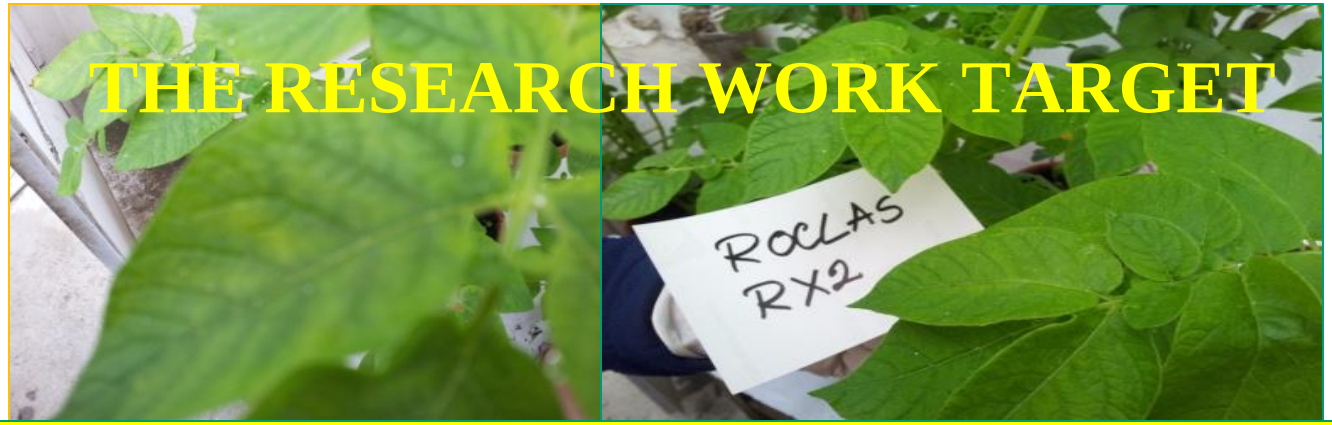
AA as a direct scavenger of ROS is the **major redox buffer**

AA is a cofactor of ascorbate peroxidase, which converts violaxanthin de-epoxidase

Bibliography

Hammerschmidt, R., 2005. Antioxidants and the regulation of defense. *Physiological and Molecular Plant Pathology*, 66: 211–212

THE RESEARCH WORK TARGET

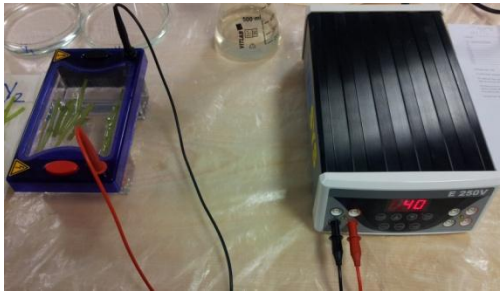


DECREASE the PVY and PVX infection level using:

- **antiviral compounds (ribavirin +oseltamivir) in tissue culture**

- **several treatments (*Satureja hortensis EOs*, H_2O_2 and vitamine C) applied to microplants acclimatisated in green house**

- **electrotherapy**



MATERIAL AND METHODS

Essential oils

EXTRACTION of *EOs by* **water vapours distillation**

The main volatils compounds

EOs from Satureja hortensis

Name	RT	Area%
Pinene α	**4.68**	**11.27**
Phellandrene α	4.80	0.46
Camphene	**5.98**	**4.56**
Pinene β	**7.85**	**9.23**
Myrcene β	11.85	1.09
CINEOL	**14.00**	**47.01**
Terpinene γ	15.45	0.62
Cymene P	16.49	2.31
Camphor	**24.11**	**10.66**
Linalool β	25.25	0.89
Bornyl Ac.	25.83	0.76
Cariophyllene α	**26.21**	**4.67**
Terpineol α	26.46	1.02
Cariophyllene β	27.90	0.48
Borneol	**28.78**	**4.48**
Cadinene	29.99	0.49

MATERIAL AND METHODS

Biologic material

-PVY inoculated plants (using a secondary infected source - cv. Record)
-PVX inoculated plants (using a secondary infected source - cv. Bintje)

Before inoculation

Plants PVY positif

Before inoculation

Plants PVX positif

MATERIAL AND METHODS

1. Chemotherapy + treatments with *EOs* and antioxidants

Nodal cuttings with a single axillary bud

Inoculated plants

Single node cuttings

Transfer in tissue culture in medium MS + viricides
S1
(RBV+OSMV)

ELISA*
after 26 days

Transfer in tissue culture in medium MS + viricides
S2
(RBV+OSMV)

ELISA*
after 30 days

Transfer in tissue culture in medium Morashige Shoog
S3
(MS)

ELISA*
after 28 days

Transfer plantlets in the green house

Treated with EOs and antioxidants

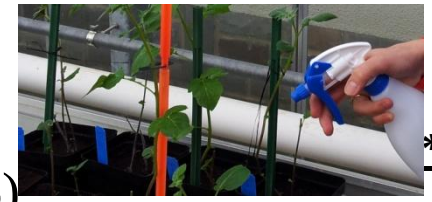
ELISA*
After 45 days

Untreated with EOs and antioxidants

ELISA*
After 45 days

Special treatments

7 days later from the EOs injection, the plants acclimatised were sprayed twice weekly with **5 mL / plant** of either *Satureja hortensis* **EOs** (1/1000) 1 mM H_2O_2 or 3 mM **AA** (pH 5.6)



***DAS ELISA used for material testing**
- protocole Clark and Adams (1977)

MATERIAL AND METHODS

1. Chemotherapy – medium's variants for the steps S1 and S2

Medium variants for S1 and S2

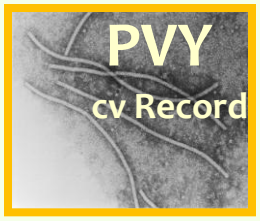
- **V1 Murashige and Skoog medium (MS) +Ribavirine (20mg/L) + Oseltamivir (40mg/L)**
- **V2 Murashige and Skoog medium (MS) +Ribavirine (40mg/L) + Oseltamivir (40mg/L)**
- **V3 Murashige and Skoog medium (MS) +Ribavirine (20mg/L) + Oseltamivir (80mg/L)**

Single node cuttings were propagated in test tubes on Murashige and Skoog medium, at 20±1°C under a 16 h photoperiod (fluorescent lights, 400–700 nm)

MATERIAL AND METHODS

2. Electrotherapy

Mechanical inoculation



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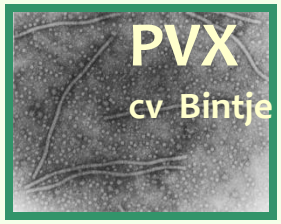
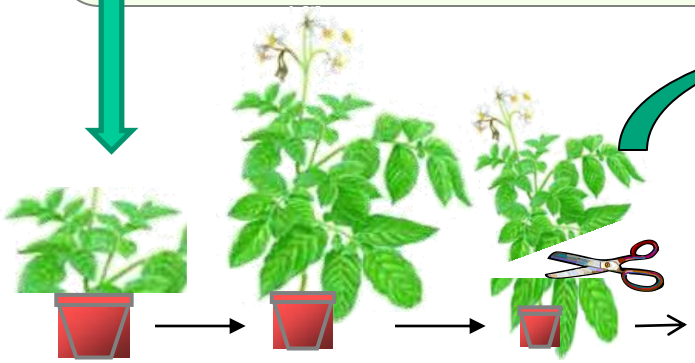


variety Roclas

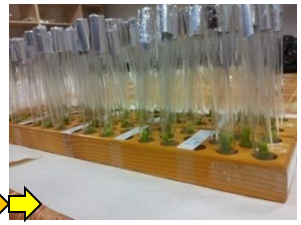


ELISA

Nodal cuttings transfer on Murashige Skoog medium

ELISA after 40 days for PVY, 36 days for PVX inoculated plants

Electrotherapy variants V1-V9



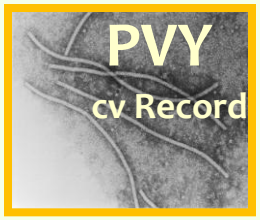
Single node transfer on MS after desinfection+wash

ELISA after 42 days for PVY, 38 days for PVX inoculated plants

2. Electrotherapy

MATERIAL AND METHODS

Mechanical inoculation



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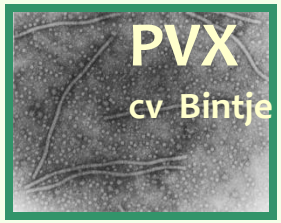
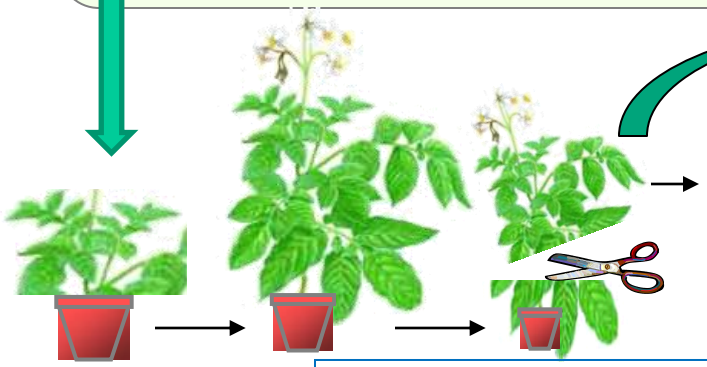


variety Roclas



V0 Controls +

V1 40 mA / 5 minutes
V2 40 mA / 10 minutes
V3 40 mA / 20 minutes

The therapy efficiency (TE)
-Lozoya- Saldaña *et al.* (1996)
% TE = % plant regenerated x % virus-free plants / 100

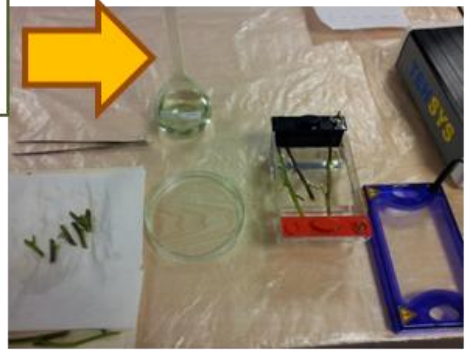
Variants of treatments with electric current

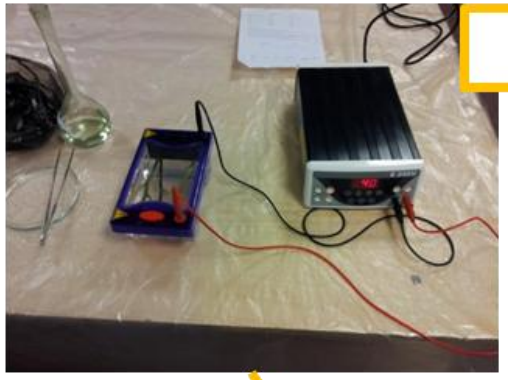
V4 50 mA / 5 minutes
V5 50 mA / 10 minutes
V6 50 mA / 20 minutes

V7 100 mA / 5 minutes
V8 100 mA / 10 minutes
V9 100 mA / 20 minutes

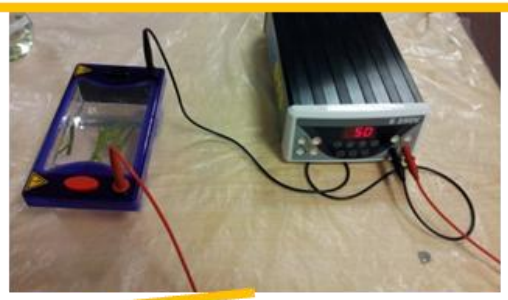
Sample prelevation

Cutting the stem segments





Electrotherapy variants



Desinfection

Alcool 96º
30seconds

Hipoclorite solution
0.1%, 1 minute

Wash three times

Tissue culture
MS medium

RESULTS

1. Chemotherapy + treatments with *EOs* +AO

A. Effects of the treatments for PVY elimination

Plants acclimatised untreated

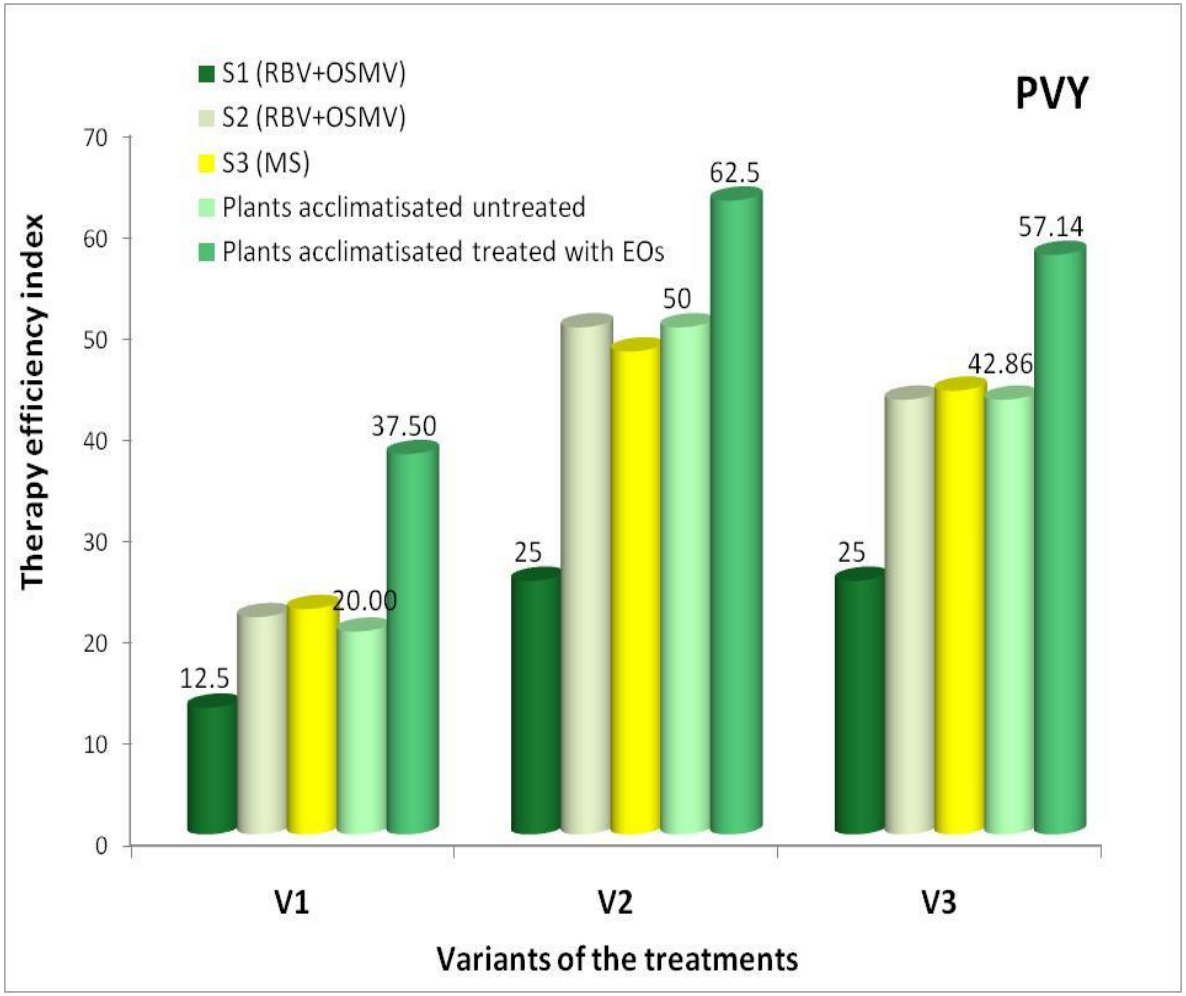
Plants acclimatised and untreated with *EOs*+AO suffered significantly harmful effects

Plants acclimatised treated with EOs +antioxidants

Treated acclimatisated plants treated with *EOs*+AO The effects were reduced by the treatments

RESULTS

Chemotherapy applied on material infected with potato virus Y (PVY) - THERAPY EFFICIENCY INDEX



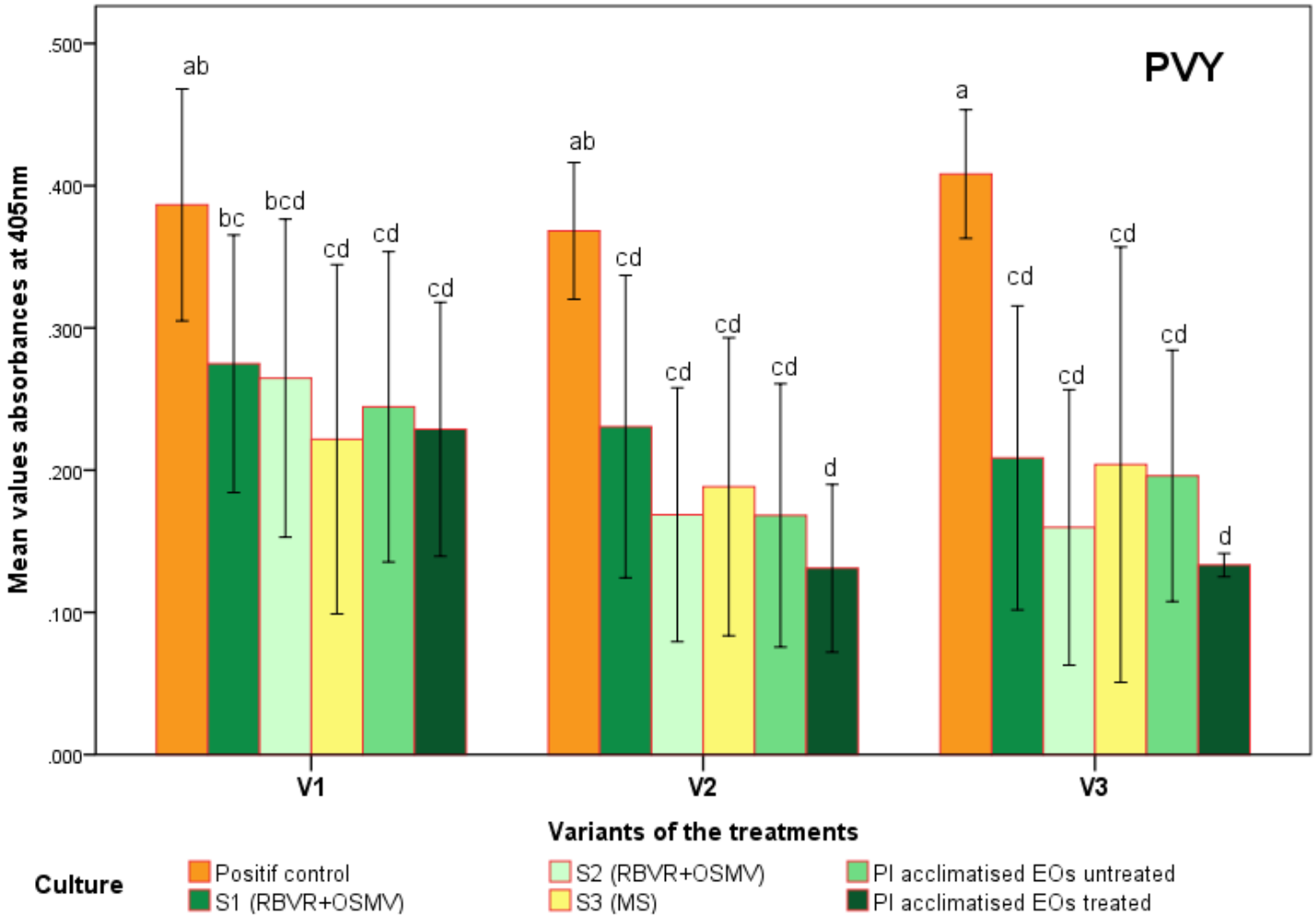
Variant of the treatment		Regeneration rate		Virus elimination rate	
		NPT/ NPM	%	NPFV NPM	%
V 1	S1	7/8	87.5	1/7	14.3
	S2	11/14	78.6	3/11	27.3
	S3	13/18	72.2	4/13	30.7
	PAUT	6/5	60	2/6	33.3
	PAT EOs+AO	7/8	**87.5**	3/7	**42.9**
V 2	S1	5/8	62.5	2/5	40.0
	S2	10/14	71.4	7/10	70.0
	S3 (MS)	16/21	76.2	10/16	62.5
	PAUT	6/8	75	4/6	66.7
	PAT EOs+AO	6/8	**75.0**	5/6	**83.3**
V 3	S1	3/8	37.5	2/3	66.7
	S2	7/14	50.0	6/7	85.7
	S3 (MS)	9/16	56.2	7/9	77.8
	PAUT	5/7	71.4	3/5	60.0
	PAT EOs+AO	4/7	**57.1**	4/4	**100.0**

V1= MS +RBV(20mg/L) + OSMV(40mg/L)
V2 = MS +RBV(40mg/L) + OSMV(40mg/L)
V3= MS +RBV(20mg/L) + OSMV(80mg/L)
MS =Murashige and Skoog
RBV=Ribavirine; OSMV+ Oseltamivir
NTP = number of tested plants (plants that survived) NMP = number multiplied plants
NPFV = number of plants free of virus
PAUT= plants acclimatised untreated

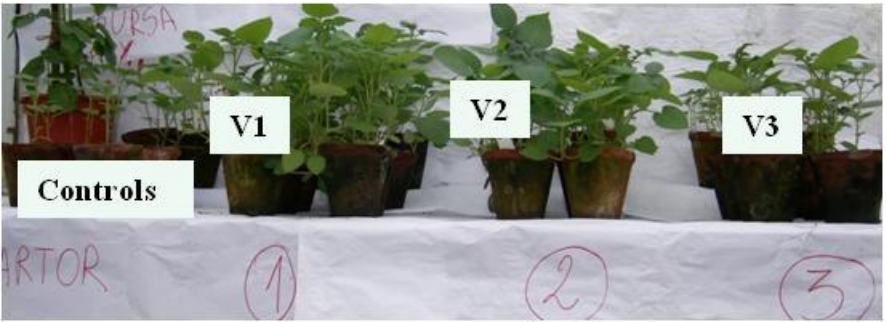
RESULTS

Effects of chemotherapy on the PVY infected plants

Mean absorbances values at 405nm



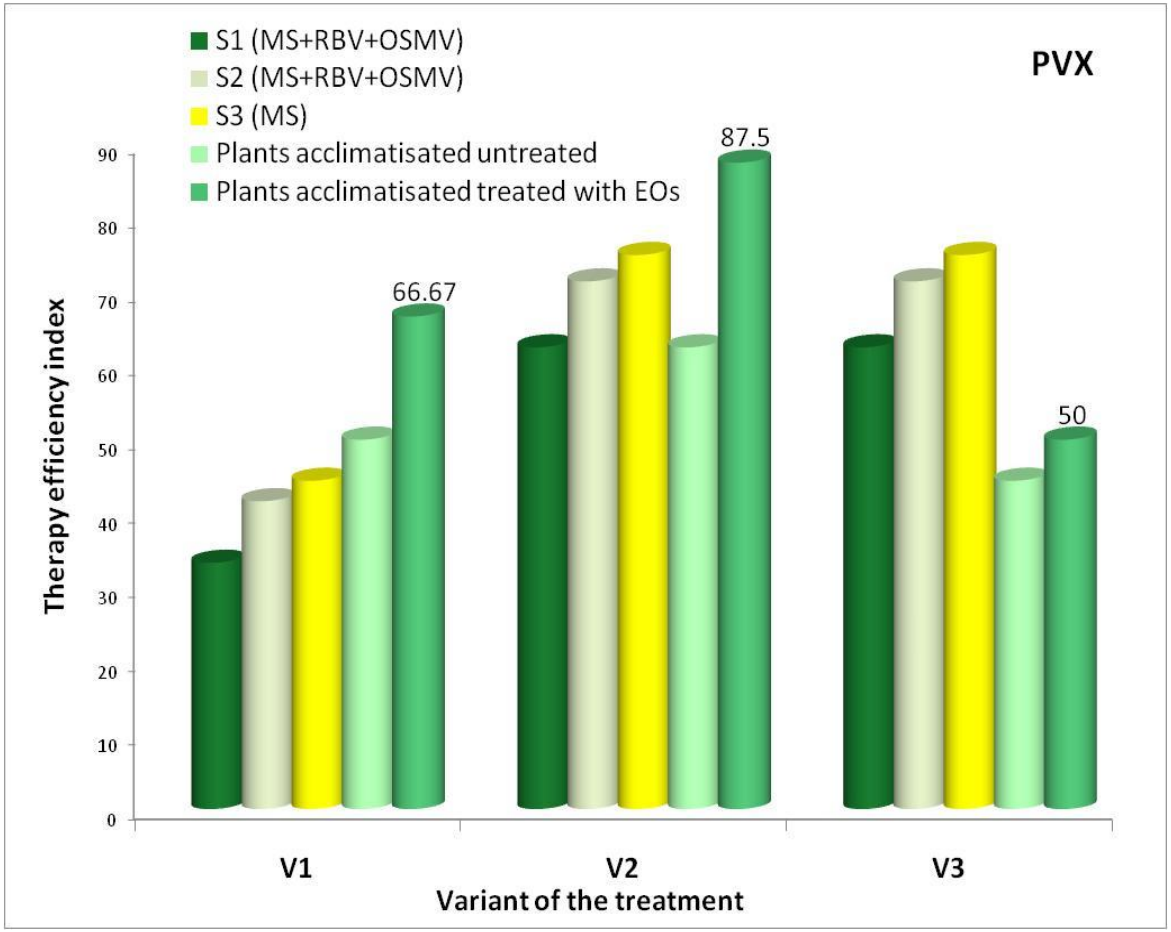
Plants acclimatised untreated with EOs+AO



V1= medium MS +**RBV**(20mg/L) + OSMV(40mg/L)
V2 = medium MS +**RBV**(40mg/L) + OSMV(40mg/L)
V3= medium MS +**RBV**(20mg/L) + OSMV(80mg/L)
MS =Murashige and Skoog
RBV=Ribavirine
OSMV+ Oseltamivir

RESULTS

Chemotherapy applied on material infected with potato virus X (PVX) - THERAPY EFFICIENCY INDEX



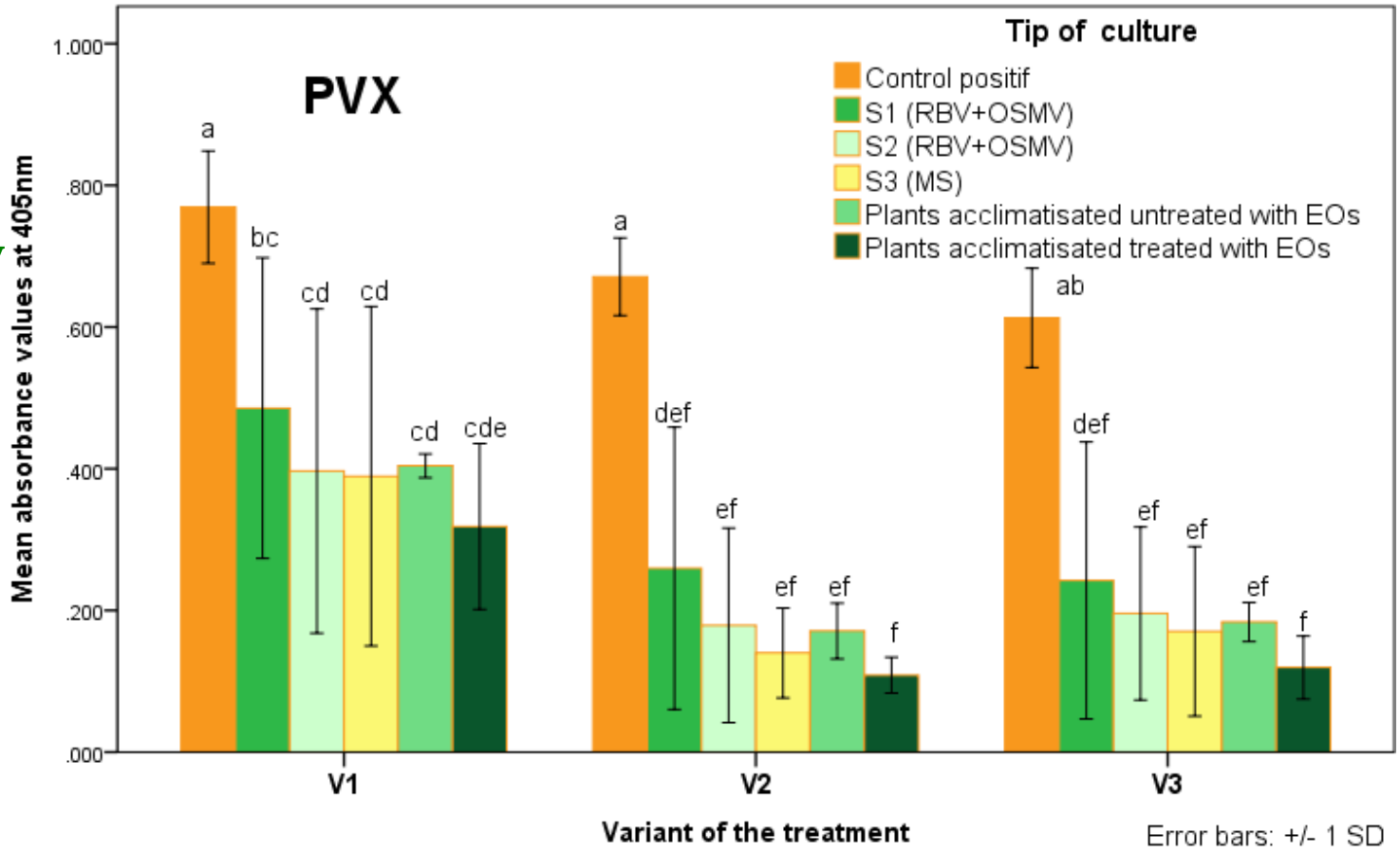
Variant of the treatment		Regeneration rate		Virus elimination rate	
		NPT/ NPM	%	NPFV NPM	%
V 1	S1	5/6	83.3	1/5	40
	S2	10/12	83.3	5/10	50
	S3	16/18	88.9	8/16	50.0
	PAUT	6/6	100	3/6	50
	PAT EOs+AO	5/6	**83.3**	3/5	**60**
V 2	S1	7/8	87.5	5/7	71.4
	S2	12/14	85.7	10/12	83.3
	S3 (MS)	22/24	91.7	18/22	81.8
	PAUT	7/8	87.5	5/7	71.4
	PAT EOs+AO	7/8	**87.5**	7/7	**100**
V 3	S1	5/8	62.5	4/5	80
	S2	7/10	70.0	6/7	87.5
	S3 (MS)	10/16	62.5	9/10	90
	PAUT	4/6	66.7	3/4	66.67
	PAT EOs+AO	3/6	**50.0**	3/3	**100**

V1= MS +**RBV**(20mg/L) + OSMV(40mg/L)
V2 = MS +**RBV**(40mg/L) + OSMV(40mg/L)
V3= MS +**RBV**(20mg/L) + OSMV(80mg/L)
MS =Murashige and Skoog
RBV=Ribavirine; OSMV= Oseltamivir
NTP = number of tested plants (plants that survived) NMP = number multiplied plants
NPFV = number of plants virus free
PAUT= plants acclimatised untreated

RESULTS

Effects of chemotherapy on the PVX infected plants

Mean absorbances values



Plants acclimatised untreated with EOs+AO

V1= MS +**RBV**(20mg/L) + OSMV(40mg/L)
V2 = MS +**RBV**(40mg/L) + OSMV(40mg/L)
V3= MS +**RBV**(20mg/L) + OSMV(80mg/L)
MS =Murashige and Skoog
RBV=Ribavirin
OSMV= Oseltamivir

2. Electrotherapy

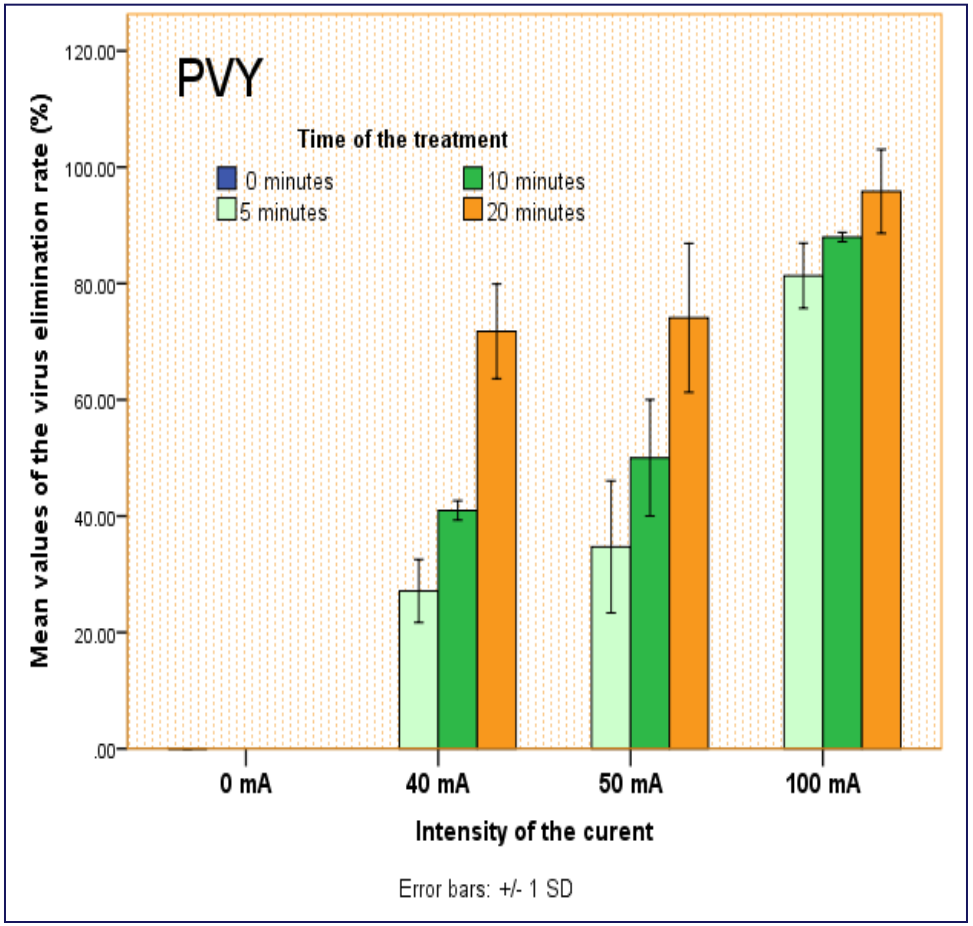
A. Effects of electrotherapy treatments of PVY infected plantlets cv Roclas

Regeneration rate

Variant	Treatment mA/min	Regeneration rate		
		Regenerated [a]/ treated[b]	%	±STDEV
V0	0/0	5/24	20.8	±4.194
V1	40/5	37/48	77.1	±13.38
V2	40/10	27/40	67.5	±15.12
V3	40/20	25/48	52.1	±13.19
V4	50/5	26/35	74.3	±9.311
V5	50/10	30/41	73.2	±12.43
V6	50/20	30/48	62.5	±2.887
V7	100/5	21/40	52.5	±1.925
V8	**100/10**	**25/42**	**59.5**	**±13.57**
V9	100/20	24/48	50.0	±12.72

[a] number of regenerated plantlets;
[b] number of explants treated

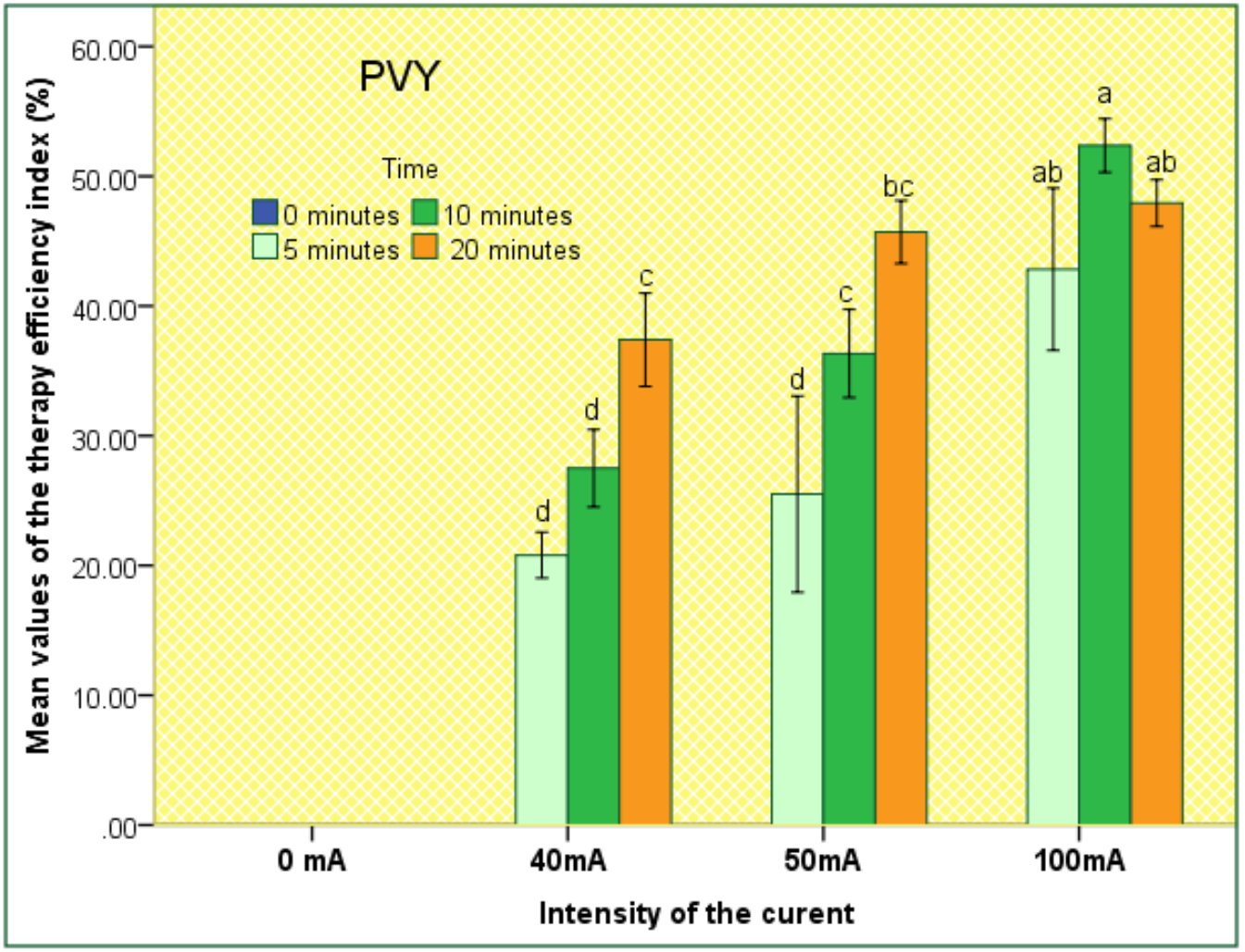
Virus elimination rate



Results are the mean of 3 experiments.

RESULTS

Effects of the ELECTROTHERAPY at microplants infected with potato virus Y (PVY) -THERAPY EFFICIENCY

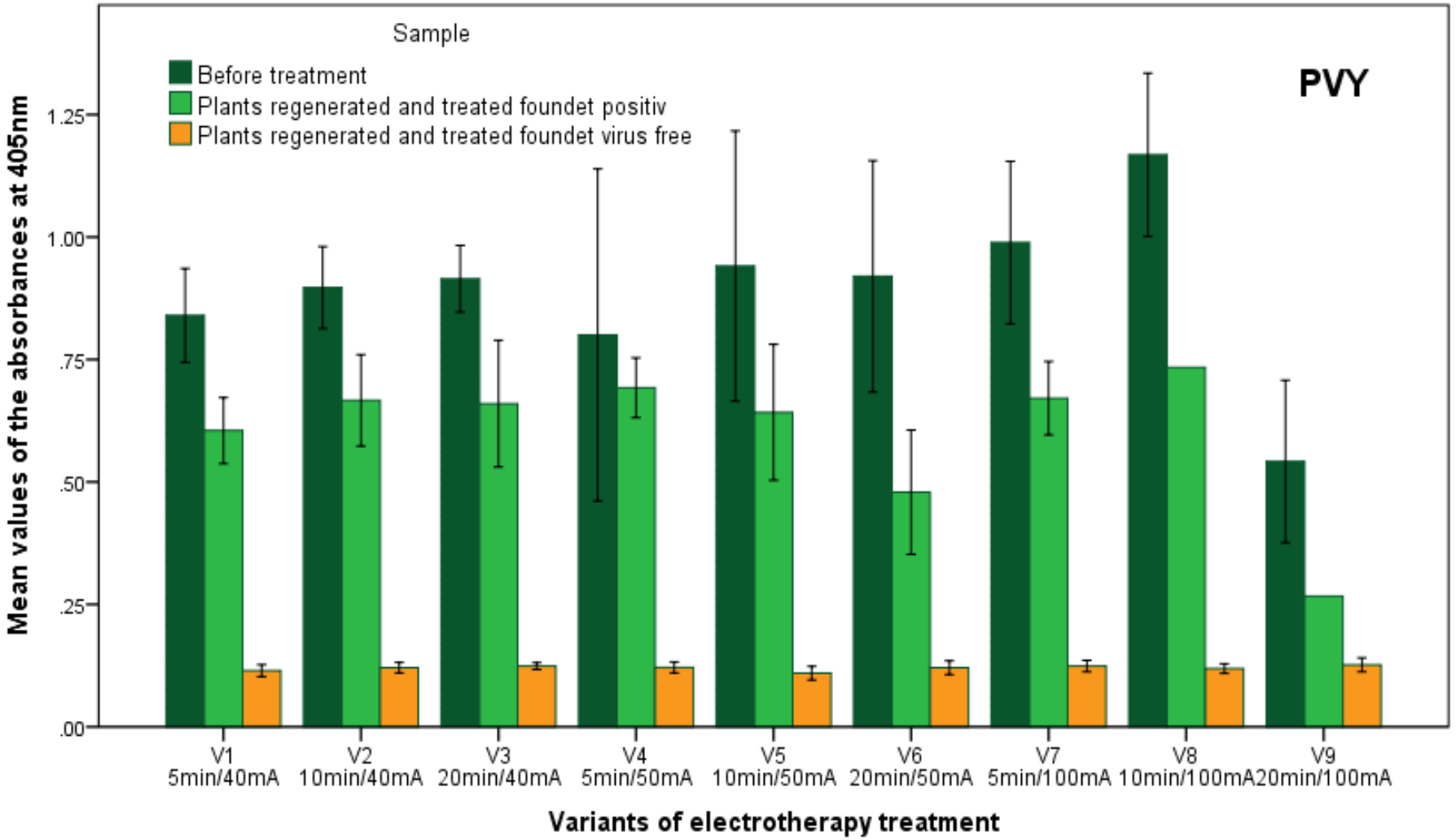


Bars with different letters differ significantly Duncan's test ($P<0.05$)

Results are the mean of 3 experiments.

RESULTS

Effects of the ELECTROTHERAPY at microplants infected with potato virus Y (PVY) –Mean absorbances values



The treatments lead up to an decreasement of OD to PVY infected plants

Results are the mean of 3 experiments.

2. Electrotherapy

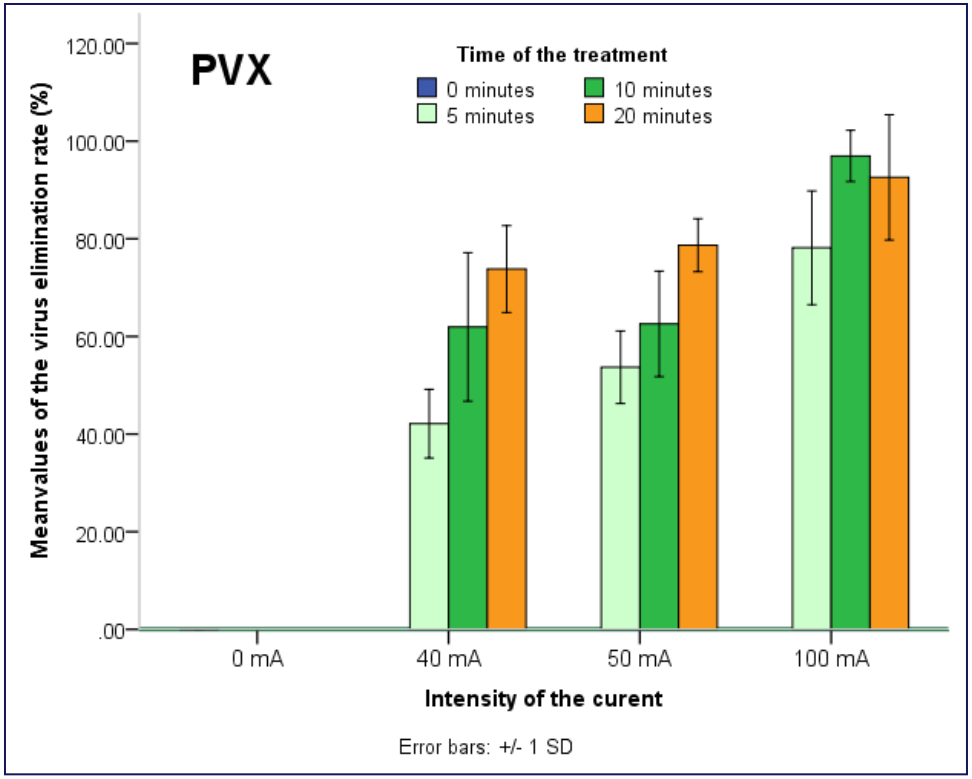
B. Effects of electrotherapy treatments of PVX infected plantlets (cv Roclas)

Regeneration rate

Variant	Treatment mA/min	Regeneration rate		
		Regenerated [a]/ treated[b]	%	± STDV
V0	0/0	4/21	19.0	± 8.3
V1	40/5	23/41	56.1	± 11.7
V2	40/10	26/38	68.4	± 17.1
V3	40/20	30/48	62.5	± 5.5
V4	50/5	30/40	75.0	± 12.7
V5	50/10	24/35	68.6	± 12.3
V6	50/20	33/51	64.7	± 7.1
V7	100/5	31/42	73.8	± 6.7
V8	**100/10**	**27/35**	**77.1**	± 13.0
V9	100/20	26/39	66.7	± 7.0

[a] number of regenerated plantlets;
[b] number of explants treated

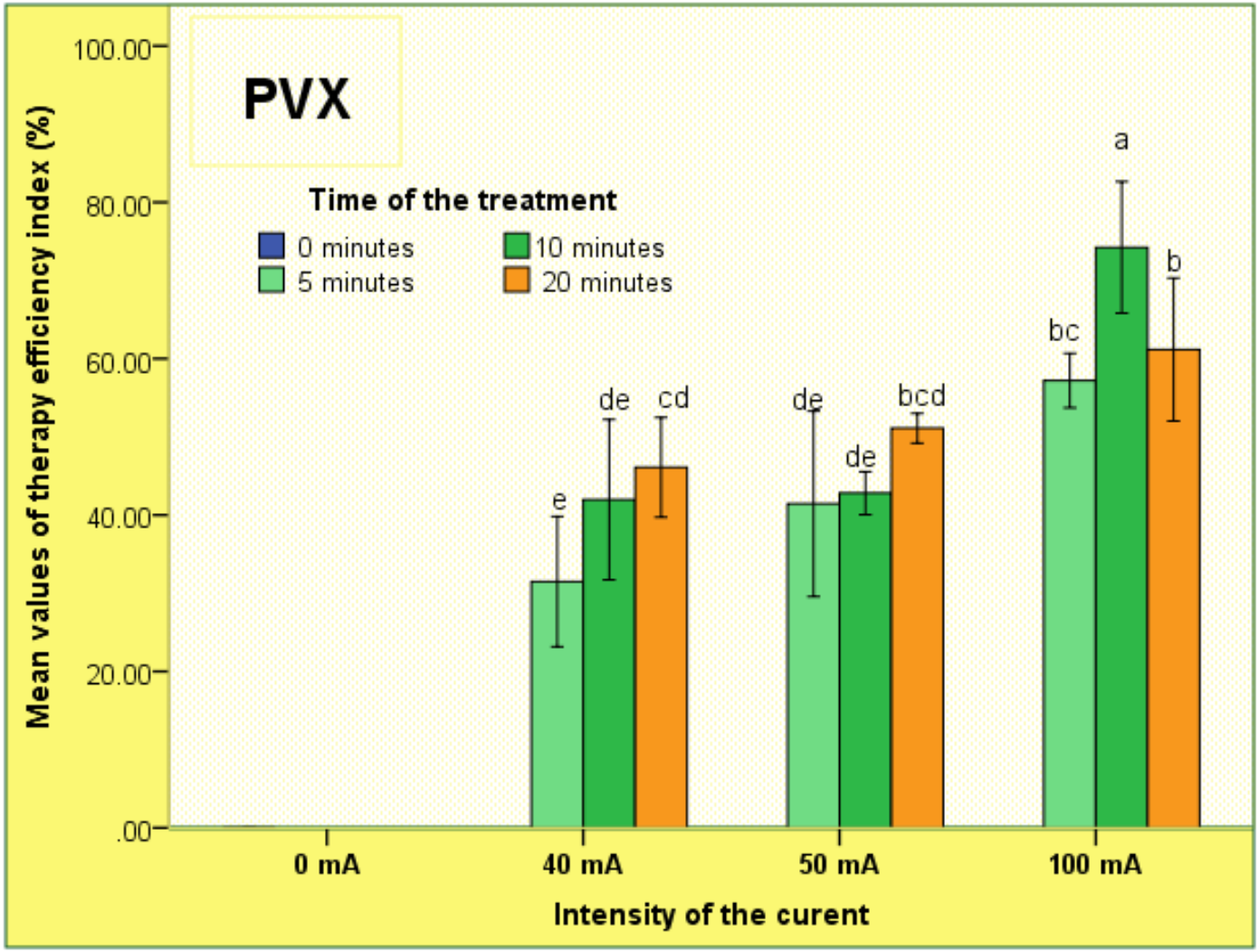
Virus Elimination rate



Results are the mean of 3 experiments.

RESULTS

Effects of the ELECTROTHERAPY at plants infected with potato virus X (PVX) -THERAPY EFFICIENCY

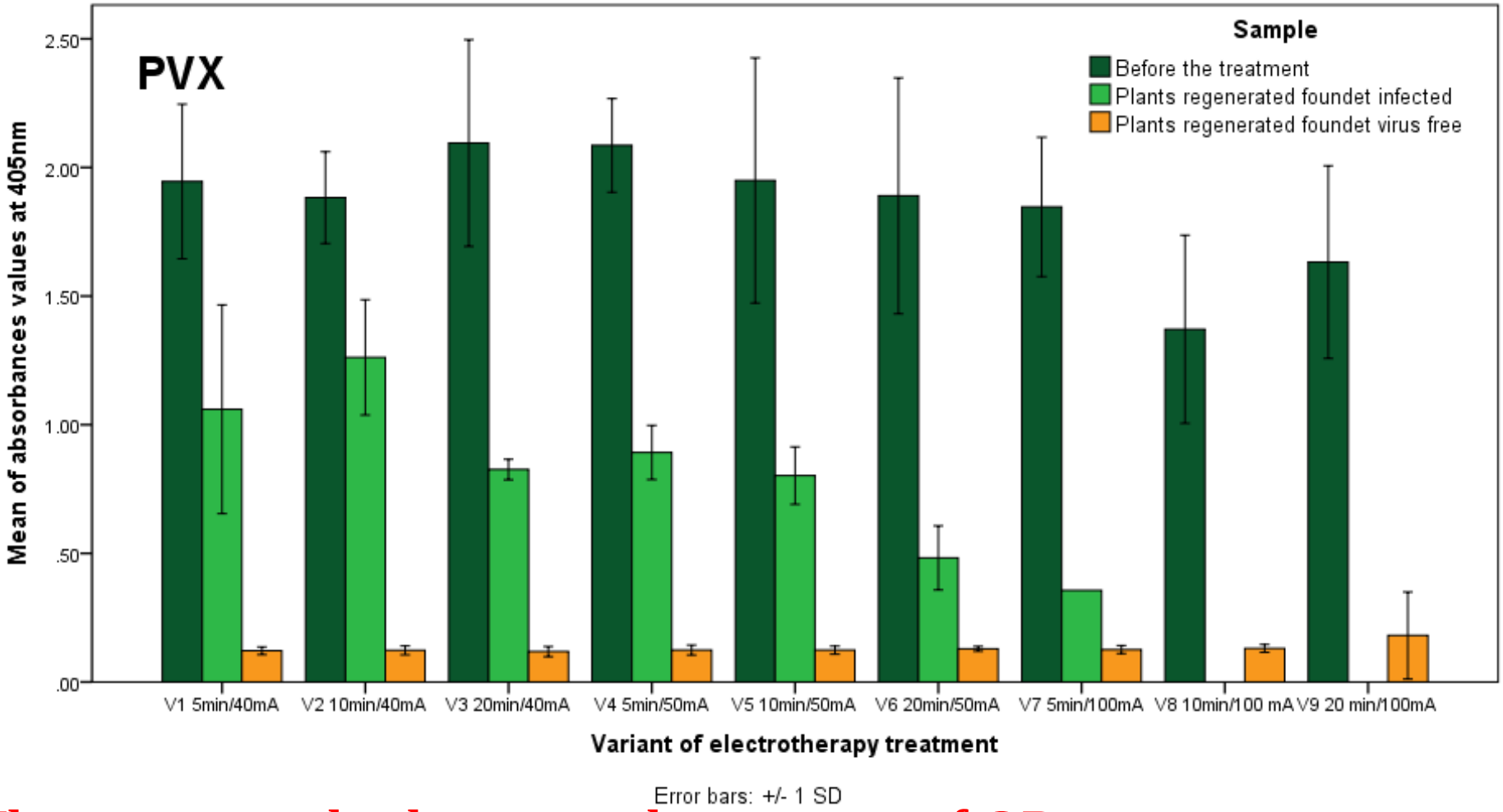


Error bars: +/- 1 SD

Bars with different letters differ significantly Duncan's test ($P<0.05$)

RESULTS

Effects of the ELECTROTHERAPY at plants infected with potato virus X (PVX) –Mean absorbances values



The treatments lead up to an decresement of OD to PVX infected plants compared to their control

Results are the mean of 3 experiments.

CONCLUSIONS

- Combined **chemotherapy** (V2: **RBV 40mg/l + OSMV 40mg/l**) + treatments (*EOs*+AO) of acclimatisated plants (cv Roclas), have led to:
  - 83.7% PVY free plants and 100% PVX free plants
  - the higher values of the therapy efficiency index (TEI): 62.5 for PVY infected plants and 87.5 for PVX

- V 3 (RBV 20mg/l +OSMV 80mg/l) + treatments *EOs* +AO have led to the highest values for viruses elimination rate (100%), but decrease the regeneration rate (57% for PVY and 50% for PVX) ⟶ TEI had lower values than in variant V2.

-EOs **TREATMENTS** and hydrogen peroxide / ascorbic acid of acclimatised plants increase the TEI in all the variants .

***Satureja hortensis* oils + H_2O_2 (1mM) or AA (3mM)**

BENEFICIAL EFFECTS on the plants obtained by chemotherapy from PVY and PVX infected POTATO sources

CONCLUSIONS

ELECTROTHERAPY

- The greatest value for therapy efficiency index (TEI) was obtained when the explants were exposed to **100mA for 10 minutes** :

  - **52.4** (53.3; 50; 53.8) for PVY
  - **74.3** (71.4; 66.7; 86.3) for PVX

-The most severe exposure at 100mA for 20 minutes resulted in 47.9% TEI for PVY and 61.5% TEI for PVX

- High values of regeneration rate but few virus free microplants for the lowest values of current intensity (40mA, 5 minutes) (77.1% and 27% for PVY ; 73.3% and 43.3% for PVX)

- Electrotherapy have led to an decreasement of OD to all PVY and PVX regenerated plants obtained from infected sources

CONCLUSIONS

But……..

Some elements remain to be tested and/or improved

- the treatments success is cultivar dependent !

- the phytotoxicity of the treatments ?

- there are many other *EOs* that could be used!

- to define the efficiency of the treatments with bulked samples!

- to combine chemotherapy + electrotherapy + treatments with *EOs* and AO !

« Il ne faut jamais renoncer à la récolte ***des plantes aromathiques….*** *Pour ceci,* ***penchez-vous*** *jusqu'à la* ***terre*** *et* ***érigez-vous*** *jusqu'aux* ***ciels*** *! »*

Maurice Messeque