UPOV

International Union for the Protection of New Varieties of Plants

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Technical Working Party for Vegetables

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Fifty-First Session
Roelofarendsveen, Netherlands, July 3 to 7, 2017

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ADDENDUM TO NEW ISSUES ARISING FOR DUS EXAMINATION

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This document contains copies of presentations to be made at the fifty-first session of the Technical Working Party for Vegetables (TWV), as follows:

- Annex I: "Use of disease and insect resistance characteristics in DUS examination", by an expert from France;

- Annex II: "Improvement of the assessment of the Squash resistance to 3 virus and CORKYRES project" by an expert from France.

[Annexes follow]

ANNEX II

Use of disease and insect resistance characteristics in DUS examination

UPOV – 51st TWV, July 2017 in Leiden (NL)
France GEVES

GEVES
Expertise & Performance

SUMMARY

- **Context**, background
- **Satisfy UPOV** characteristics criteria
- Representation in **UPOV Test guidelines**
- Criteria, advantages and limits of the use
- **Tools** to secure the use : net system, protocols, example varieties, identified isolates
- **Challenges** : quantitative resistances, interaction with the genetic backgrounds, provide services to Examination Offices and companies,
  →progress for DUS tests

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Disease resistance tests : What for ?

Genetic ressources

Resistance bio tests

Breeding

Registration /Protection

DUS
Characteristics with criteries

Fusarium/pea

- Comparison of a candidate to resistant and susceptible controls for characterisation
- Protocols for resistance tests
  Reproducible, practical, representative

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Context and background

Breeding for ?

Since 1960's, significative investments→ **important variety traits**

Large success and progress for vegetable and several field crop species, recognised and adopted by growers.

Continuous researchs in public and private sectors

→ Explore the relationship between the bio agressors and the varieties

For Authorities in charge of Agriculture, a necessity **to adopt genetic disease and insect resistances traits**, in relation with technical methods : bio control, crop rotation, association…

→ Co-Construction **to respond to the challenge for sustainable agriculture**

→ Breeding for resistances - **A challenge for Plant Production**

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Context and background

-**Large success for fungi, bacteria, virus**

-Limited for **insect** except rice, coton (Heliothizea), eggplant (Leucinodes, leptinotaire, trialeurodes), melon (Vat gene against aphids), lettuce (Nasonovia aphid)

→ **large investments nowadays**

-**Vegetables** : 150 "host/ pest" couples

-**Field crop** : sunflower (downy mildew, rust, Orobranche), cotton (Bacterial blight, ramulose, mildew, nématodes…), sugar beet (nématodes), wheat (*Fusarium*, yellow rust), maize (*Fusarium, Helmintosporium*), lucerne (*Ditylenchus, Verticillium, Colletotrichum*…)…..

-**Fruit** : apple (scab/*Venturia*), apricot (Sharka), peach (Sharka, *Oïdium*, Aphids), banana (*Cercospora*)…

-Ornamental : not significative (complexity of genome, less pression …?)

-Forest: an other strategy through non uniform populations, not for DUS

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Satisfy UPOV DUS approach

We only consider ,

. **Traits with simple genetic control (monogenic/polygenic)**

→ **high heritability of the trait**

. **The phenotype**, in conformity with the Convention, not the genotype (except model 1)

. **Resistances** which are **significatively not dependant of the environment,**

. **High level of resistance**.

We do not consider VCU traits as tolerance, better ability….

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Satisfy UPOV criteria : as DUS characteristics

-**Reliable** → develop **bio tests** under controlled conditions with **recognised standards and protocols**

-**Easy to describe and representative of the resistance in field:**
1 (Susceptible)–9 (Resistant) or 1 (S) -2 (Intermediate R)-3 (R) or quantitative

-**Spread into the DUS collection:** satisfied as soon as the trait is sufficiently used by breeders.

-**Practical: result in a reasonable delay and cost** → Bio tests where the resistance is quickly revealed (generally 2 to 4 weeks after inoculation)

-**Published Recognised Methodology** and availability of the technologies

-Used for Description, **D, U and S**

-Not covered by Patent at any step

-Recognised by the user of the variety or any third party

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Representation in UPOV guide lines

-Since 1970's:

-Tomato : TMV, *Verticillium, Fusarium o.f.sp.l. strain* 0, Nématodes

-Bean : Virus 1, *Colletotrichum*

-Pea : *Fusarium o.f.sp.pisi strains 1, 5 and 6, Ascochyta pisi…*

-2017 :

.Tomato (11 bio pests, 6 *), Melon (8, 3*), Pepper (6, 4 *), Cucumber (7), Bean (4), Pea (3), Lettuce (4, 1*), Corn salad(1), Spinach(1)….

. Sunflower (downy mildew), lucerne (six characteristics)

.Ornamental and fruit : no one, no necessity for D

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Criteria, advantages and limits of the use

Criteria, advantages of the use

-**Structure the DUS reference collection** with important characteristics → optimise the set of close varieties → reduce the cost with an input

-**Permit to use the UPOV** option 'Marker used as a predictor of traditional characteristic' , **BM MODEL 1**

Example: Verticillium /Tomato « OK »; Nématodes/Tomato « * mind »

-**Develop harmonization, common data bases and process to use it**

Limits of the use

-Necessity to test all the collection, including susceptibles, to use them for D

-Test the Uniformity → the bio tests consider it

-Interaction with genetic background

-Quarantine status : mind for TYLCV-Tomato//possible for TSWV-tomato-pepper, Xanthomonas bean

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Tools to secure the use

Tools to secure the use : Net system to provide informations

. **Recognised protocols → Harmonize what and how ?**

.Controls (S, IR, R) : validated, available, healthy

.Reference isolates: validated, available, stable, representative of natural conditions (250 pests organisms stored at GEVES)

.Protocols, notation, scales, interpretation: validated, repeatable, reproducible

.Several initiatives :

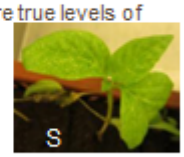
ISF WG DRT / ESA / CPPSI / France MATREF / HARMORES CPVO

→EO's develop a system with the applicants to allow:

. applicants to caliber their methodology, candidates varieties and declare true levels of resistance,

. the EO's to check DUS material with reliability,

in conformity with the DUS system.



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DRT,Disease resistance terminology – CPPSI, Collaboration for Plant Pathogen Strain Identification

Tools to secure the use

Example : Harmonization of isolates

- Pepper tobamoviruses for pepper

		Tested	Validated	Selected
Nb of strains	TMV: 0	5	2	1
	PMMoV: 1.2	5	2	1
	PMMoV: 1.2.3	4	1	1
	PVY: 0	4	1	1

1 isolate: easy notation, reproducible, validated as TMV:0

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Challenges

1-**Quantitative resistances**:

Challenge to contribute to sustainable agriculture.

Consider them if the level of resistance is significatively high,

→ **A minimum distance between S, IR and R**
→ ***Clear Distinction***
→ ***Uniformity : check the continuous expression into the variety (no discontinuity from plant to plant of the candidate)***

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Challenges

Quantitative resistances: What ?

Cumulate disease polygenic resistances, co associate them to production factors (crop rotation, grafting, conduct, elicitors, stimulants, environnent....)

- One genetique origin : Melon (Fom 1-2), Tomato (*Pyrenochaeta*), Bean (*Pseudomonas*)
- Several genetic origins: Tomato (*Clavibacter*), Bean (*Xanthomonas*)
- Different mecanisms: Pepper (*Phytophthora*, CMV, PVY...)
- 2017: Melon (*Pseudomonas, powdery mildew*, WMV, CABYV...), Tomato (TYLCV...), Cauliflower (*Plasmodiophora, club root*), Carrot (*Alternaria*), Onion (*Fusarium*...), Squash (*Powdery mildew*, ZYMV, CMV, PRSV), Strawberry (*Phytophthora cactorum, Colletotrichum*), Lettuce (virus yellowings, *Fusarium*), Pea (*Ascochyta*, PSBMV) ....

Xanthomonas

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Mycosphaerela

Challenges

2 - Interaction with the genetic background :

more and more genetic crosses → Modification of the expression of the phenotype (modificators and regulators genes..., new genes of resistances....) (Nématodes/Tomato...)

→ Adapt biotests to this challenge (concentration...)

3 - Disease resistance Test Laboratories : **it is a technology.**

Develop **easy access to these laboratories for the examination offices**

→ Cooperation – share mutualised services

DUS data bases, Markers, disease resistance tests..→ improve D

Share the impacts to progress for DUS tests

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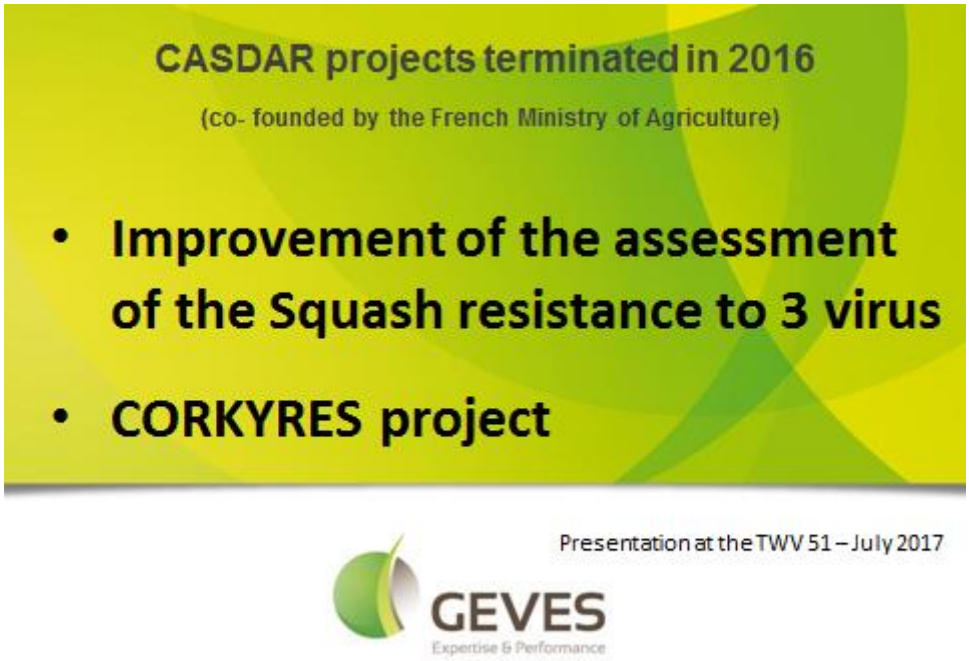
[Annex II follows]

ANNEX II


CASDAR projects terminated in 2016
(co- founded by the French Ministry of Agriculture)
• Improvement of the assessment of the Squash resistance to 3 virus
• CORKYRES project
Presentation at the TWV 51 – July 2017
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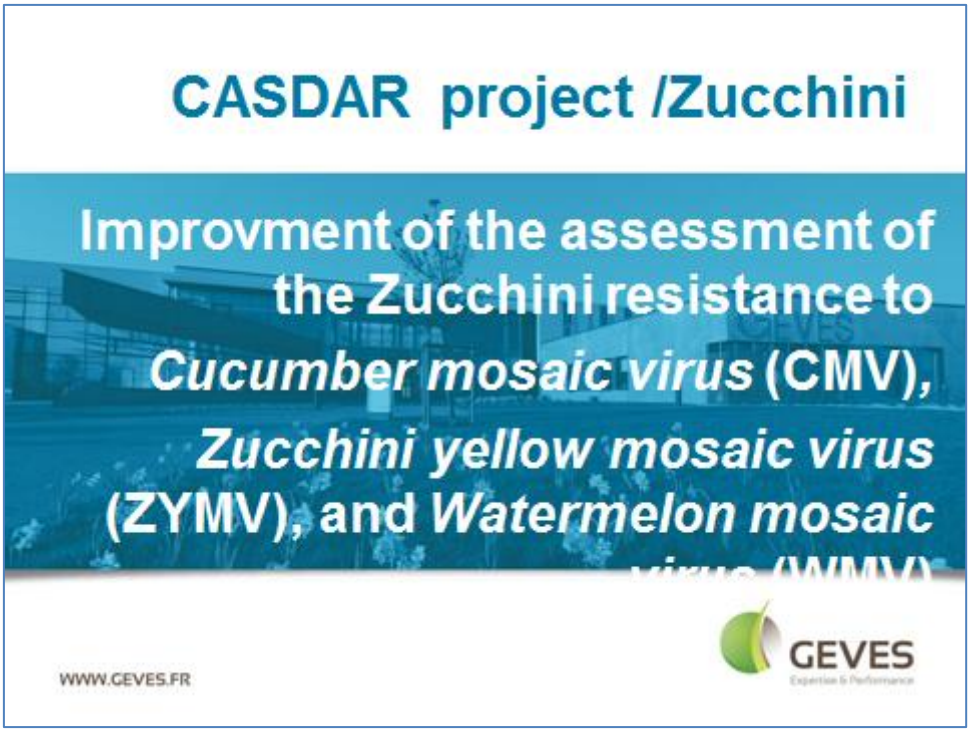
Expertise & Performance


CASDAR project /Zucchini
Improvment of the assessment of the Zucchini resistance to Cucumber mosaic virus (CMV), Zucchini yellow mosaic virus (ZYMV), and Watermelon mosaic
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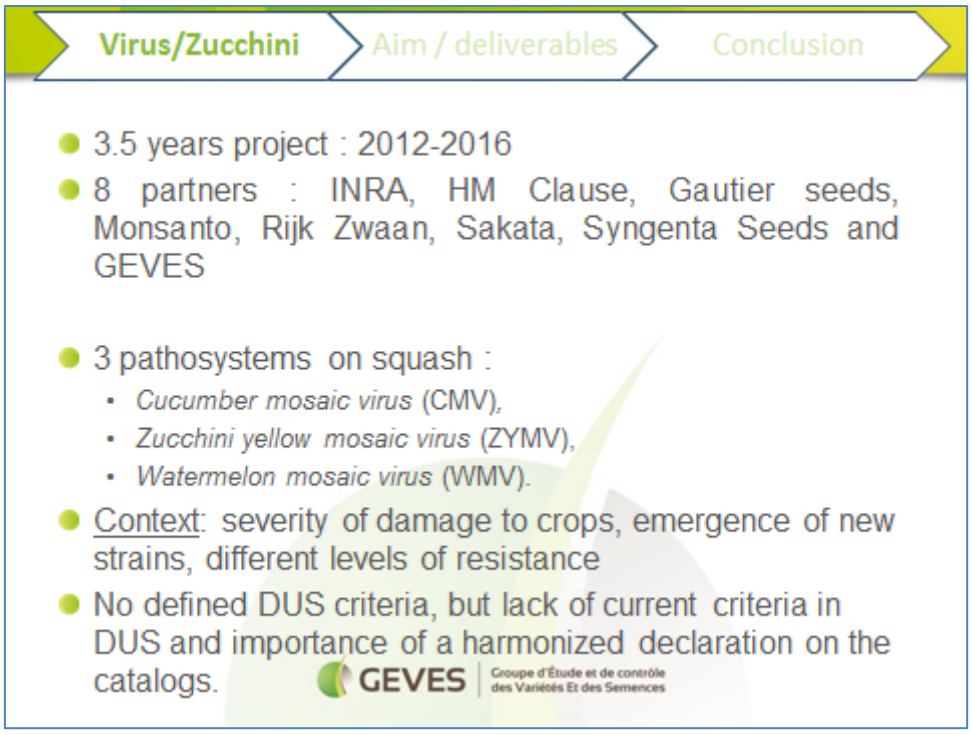
Virus/Zucchini
Aim / deliverables
Conclusion
3.5 years project : 2012-2016
8 partners : INRA, HM Clause, Gautier seeds, Monsanto, Rijk Zwaan, Sakata, Syngenta Seeds and GEVES
3 pathosystems on squash :
Cucumber mosaic virus (CMV),
Zucchini yellow mosaic virus (ZYMV),
Watermelon mosaic virus (WMV).
Context: severity of damage to crops, emergence of new strains, different levels of resistance
No defined DUS criteria, but lack of current criteria in DUS and importance of a harmonized declaration on the catalogs.
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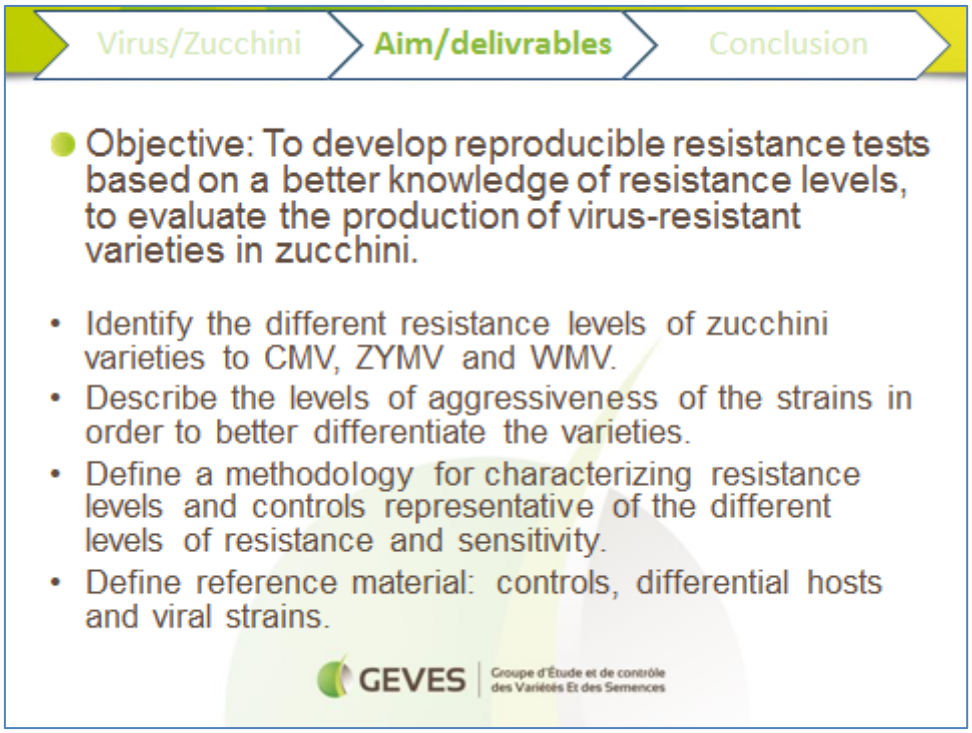
Virus/Zucchini
Aim/delivrables
Conclusion
Objective: To develop reproducible resistance tests based on a better knowledge of resistance levels, to evaluate the production of virus-resistant varieties in zucchini.
Identify the different resistance levels of zucchini varieties to CMV, ZYMV and WMV.
Describe the levels of aggressiveness of the strains in order to better differentiate the varieties.
Define a methodology for characterizing resistance levels and controls representative of the different levels of resistance and sensitivity.
Define reference material: controls, differential hosts and viral strains.
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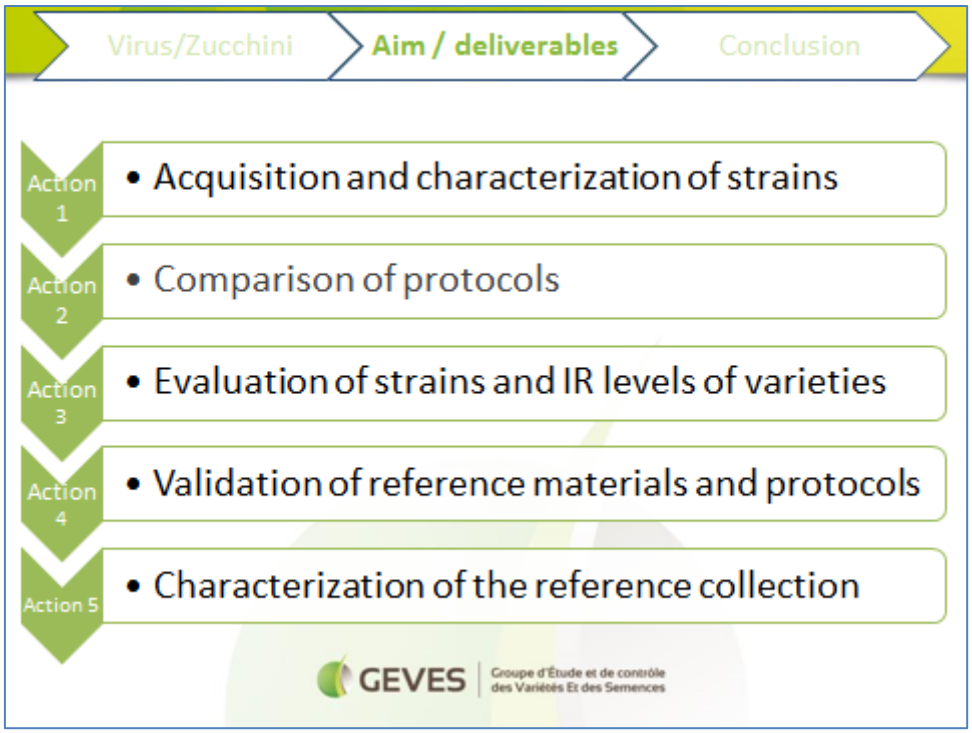
Virus/Zucchini
Aim / deliverables
Conclusion
Action 1
• Acquisition and characterization of strains
Action 2
• Comparison of protocols
Action 3
• Evaluation of strains and IR levels of varieties
Action 4
• Validation of reference materials and protocols
Action 5
• Characterization of the reference collection
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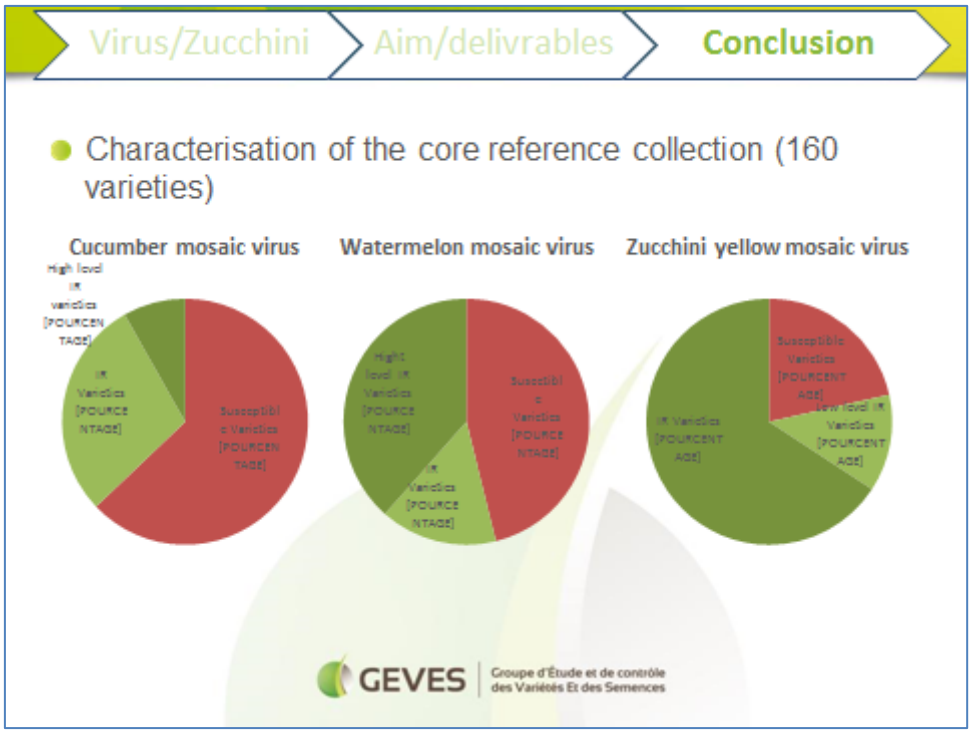
Virus/Zucchini
Aim/delivrables
Conclusion
Characterisation of the core reference collection (160 varieties)
Cucumber mosaic virus
Watermelon mosaic virus
Zucchini yellow mosaic virus
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Virus/Zucchini > Aim/delivrables > **Conclusion**

- ZYMV : ZYMV-E9 strain (regular, France)

➔**NEW proposal (National listing context): Official test for all new applications, with a claim of resistance**

(previously blind test set up by the applicant)

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Virus/Zucchini > Aim/delivrables > **Conclusion**

- CMV : CMV-14 strain (emerging, severe)

- WMV: WMV LL1A strain (emerging, severe)

➔***If, these results are confirmed after 2 tests sessions,***

- with duplicate tests (GEVES and applicant premises),
- following the defined protocol during the projet

❖ **Official test** for a new application with claim of resistance.

(previously blind test set up by applicant)

CASDAR project / Tomato – Tomato rootstock

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CORKYRES | Aim/delivrables | Results | Conclusion

- 3 years project: 2013-2016, labeled by Vegepolys
- 2 partners : GEVES and UFS (Gautier seeds, HM Clause, Rijk Zwaan, Sakata, Syngenta, Vilmorin)
- The *Pyrenochaeta lycopersici* pathosystem on :
  - Tomato
  - Tomato rootstock (RT)
- Context: increase in attacks due to the cessation of soil disinfection, severe reduction in yield, little knowledge on strains variability, laboratory conservation problems
- Present in the Tomato and RT CPVO protocols (compulsory for RT until November 2015) and in the UPOV Guideline Tomato (compulsory until June 2015): heavy field protocol to be implemented.

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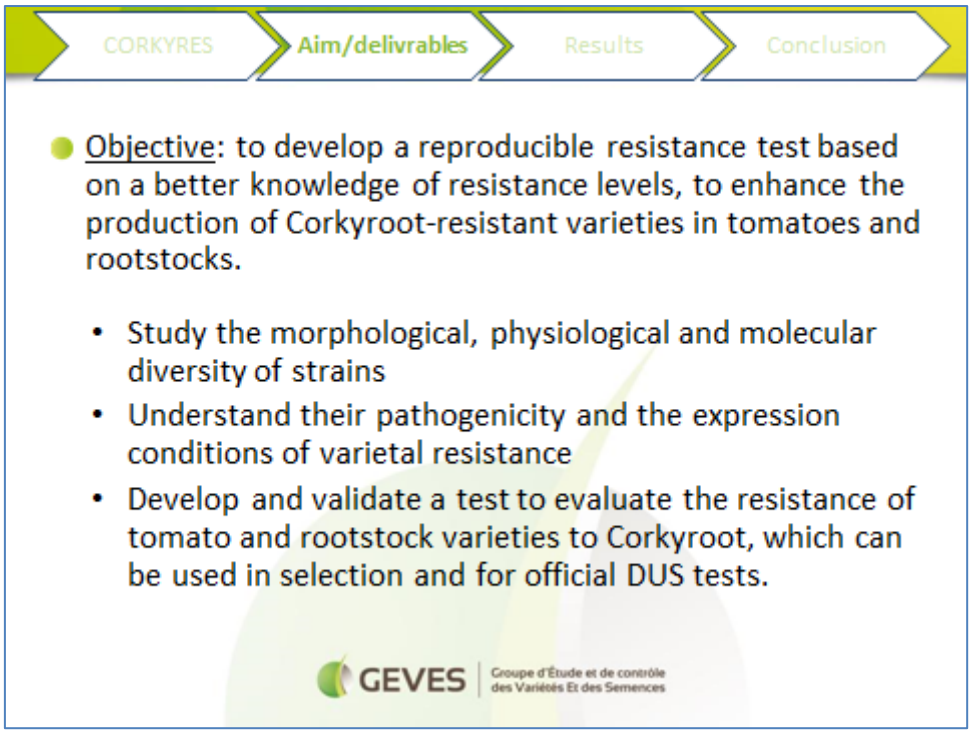
CORKYRES
Aim/delivrables
Results
Conclusion
Objective: to develop a reproducible resistance test based on a better knowledge of resistance levels, to enhance the production of Corkyroot-resistant varieties in tomatoes and rootstocks.
Study the morphological, physiological and molecular diversity of strains
Understand their pathogenicity and the expression conditions of varietal resistance
Develop and validate a test to evaluate the resistance of tomato and rootstock varieties to Corkyroot, which can be used in selection and for official DUS tests.
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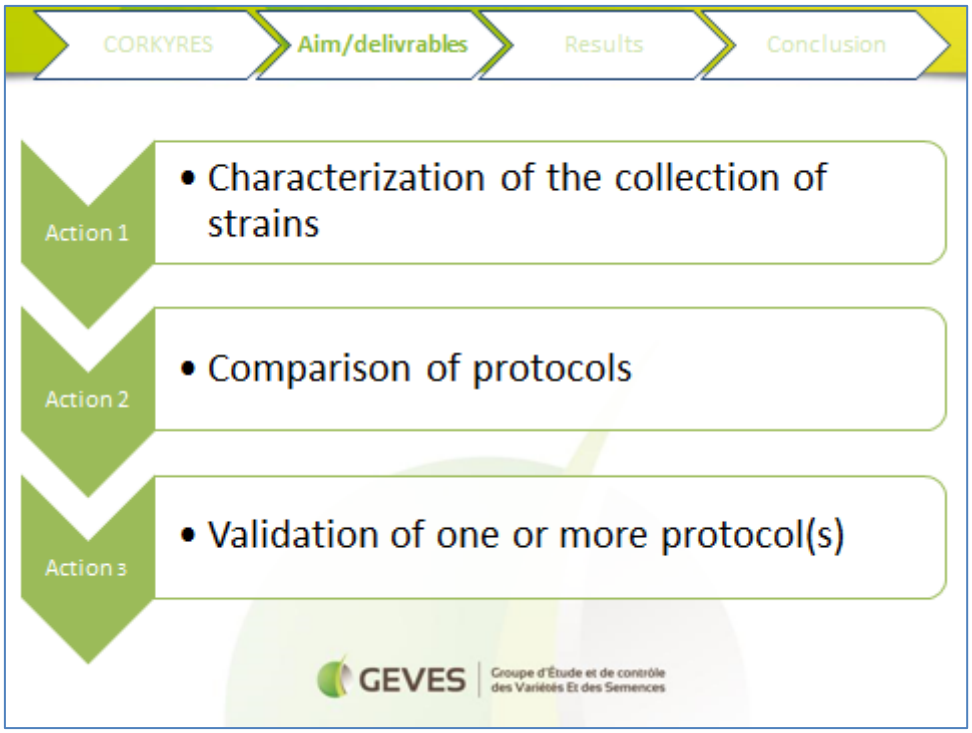
CORKYRES
Aim/delivrables
Results
Conclusion
Action 1
Characterization of the collection of strains
Action 2
Comparison of protocols
Action 3
Validation of one or more protocol(s)
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CORKYRES | Aim/delivrables | **Results** | Conclusion

- Characterization of the collection of strains
  - Construction of a 16 characterized strains collection : morphology, molecular and aggressiveness (levels)
  - Development of a strains pathogenicity protocol
  - Definition of the strains conservation conditions.
- Comparison of resistance test protocols
  - Selection of a representative strain of the field: PI21
  - Selection of tomatoes and rootstocks
  - Choice of an inoculation method: subculture in contaminated substrate
  - Definition of an observation scale and an interpretation rule.

Pathogenicity test

Symptoms in test

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CORKYRES | Aim/delivrables | Results | **Conclusion**

- **Validation of the selected protocol**
  - Common to tomato and rootstock.
  - Two differentiated levels: Susceptible and Intermediate Resistant.
  - Any variety considered to be at the same level or higher than Garance is proposed IR.

  - In GEVES 's test, proposal of 2 Intermediate Resistant levels
  - Garance's level → ≥ Garance is proposed IR Garance's level
  - Emperador's level ≥ Emperador is proposed IR Emperador's level.
  - Results are available from GEVES only, at the request of the applicant, for valorization in the commercial catalog.

→NEW proposal (National listing context): Official test for all new applications, with a claim of resistance

(previously blind test set up by the applicant)

[End of Annex II and of document]