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Agroscope

From the lab to the Protected Site: field trial with fire blight resistant cisgenic apple trees in Switzerland

Andrea Patocchi

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Content

- Fire blight
- Breeding for fire blight resistant apples
- Cisgenic apples and the line used in the field trial
- Application for a field trial
- The Protected Site
- The experiment
- Final remarks

Fire blight – E. amylovora



- Is the most important bacterial disease of apple
- First report in Switzerland 1989 (Canton SH)
- Worst year in CH: 2007 (125ha orchards and ca. 20'000 meadow trees uprooted)
- Most successful commercial apples are susceptible to fire blight
- Total costs of last 20 years (confederation + cantons): >100 mio CHF
- It is considered as a quarantine disease in Switzerland (status will change in 2020 to "regulated non-quarantine organism")
- Control: Sanitation and application of antagonists (*B. subtilis*, *A. pullulans*,...), Myko-Sin (acid clay), antibiotics (streptomycin, since 2016 no more allowed in CH), use of fire blight resistant cultivars

Classical breeding of fire blight resistant cultivars

- It is possible and it is done
- Resistances are mainly found in wild apples, but their introduction by classical breeding
 - takes many years (20-25 years)
 - leads to a new cultivar that needs to be established in the market
- That is why genetic transformation of apple is interesting (the cultivar is maintained)





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Definition of Cisgenesis



Cisgenesis is the genetic modification of a recipient plant with a natural gene from <u>a crossable—sexually compatible</u>—plant. Such a gene includes its introns and is flanked by its native promoter and terminator in the normalsense orientation. Cisgenic plants can harbour one or more cisgenes, but they do not contain any transgenes.

The borders of classical breeding are respected in cisgenic plants.

(Schouten, H. J., Krens, F. A., Jacobsen, K., Jacobsen, E., 2006. Cisgenic plants are similar to traditionally bred plants - International regulations for genetically modified organisms should be altered to exempt cisgenesis. Embo Rep. 7, 750-753.)

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C44.4.146: the first fire blight resistant cisgenic apple line

- Developed at ETHZ (Prof. Cesare Gessler)
- The linie carries *FB_MR5* that originates from *Malus* x *robusta* 5 and was identified in 2014 (ETHZ/JKI/Agroscope)
- R-Gen induces high FB-resistance
- In north America strains of *E. amylovora* able to overcome *FB_MR5* have been identified



Kost et al. 2015, PLOS ONE | DOI:10.1371/journal.pone.0143980

Opplication for a field trial with GM plants

- The documents is divided in :
 - a) General description of the experiment
 - b) Technical Dossier (sexually compatible cultures, dissemination, genetic mod., place of the experiment, measures to prevent spreading, etc.)
 - c) Results of earlier experiments
 - d) Risks assessment (identification, evaluation, measures to reduce it, final estimation): human health, dissemination, gene transfer, etc.
 - e) Control plans
 - f) Interest weighting according to Article 8 GTG
 - g) Information concept
 - h) Proof of the security responsibility
 - i) References
 - j) Annexes



87 pages

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Approval procedure cont.

If the decision is positive: permits with a list of constraints
→In general the constraints aim at the reduction of the risk of dissemination of genetically modified material from the field



Apple field trial:

- October 2015: submission of application
- 29th April 2016: field trial approved
- 10th May 2016: planting

The Protected Site: the only place in CH for field trials with GM plants

Aims:

Test the risks and benefits of GM plants which may be of interest for Swiss agriculture Perform basic research

Perform biosafety research on GM plants

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Services» of the Protected Site



ca. 3 ha land



Support to implement the biosafety measures



Agronomic assistance of the experiments



Scientific coordination of the experiments



Communication in agreement with the researchers



Schooling of persons with access authorization

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The «apple» field trial



2016

2019

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Questions and design of the experiment

- Are there morphologic differences between the trees, the flowers and the fruits of the cisgenic line and its background ,Gala Galaxy' ?
- Are there differences between the cisgenic line and ,Gala Galaxy' regarding the studied groups of omics (DNA, RNA, proteins or metabolites)?



Put the differences in the context of the variation of the same trait measured in a set of natural mutants of 'Gala' included in the study

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Final remarks

- The so-called new breeding technologies (cisgenesis, early flowering, CRISPR/Cas9,...) deliver products which are more and more similar to classically breed cultivars
- These products can be part of the solution of several current problems of our agricultural system (e.g. dependence of fungicides,...)
- Field trials with genetically modified plants are necessary
 - They are the only way to perform an extensive evaluation of the lines under natural conditions
 - Their results deliver important data also for the classical breeding
- Switzerland has a good system to authorize, regulate, perform and survey field trials with genetically modified plants
- Thank you to all the federal and cantonal offices and commissions for their constructive collaboration

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Thank you for your attention

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