GENETICALLY TRANSFORMED *ORNITHOGALUM* PLANTS EXPRESSING THE ANTIMICROBIAL PEPTIDE TACHYPLESIN I DISPLAY RESISTANCE TO SOFT ROT

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Soft rot disease caused by *Pectobacterium* in *Ornithogalum*

- ***Ornithogalum*** **is a monocot flower bulb belonging to the family Asparagaceae**
- **Israel is a major producer of *Ornithogalum* in the form of cut flowers, potted plants and as dry bulbs**
- **The export is mainly to the EU but also to the US market (~15 million € yearly)**
- **Soft rot damage caused by *Pectoacterium* spp. in this crop in Israel may reach 30-60% of the yield**

Soft rot symptoms: in pot plants and in the greenhouse

Ornithogalum F1 interspecific hybrids

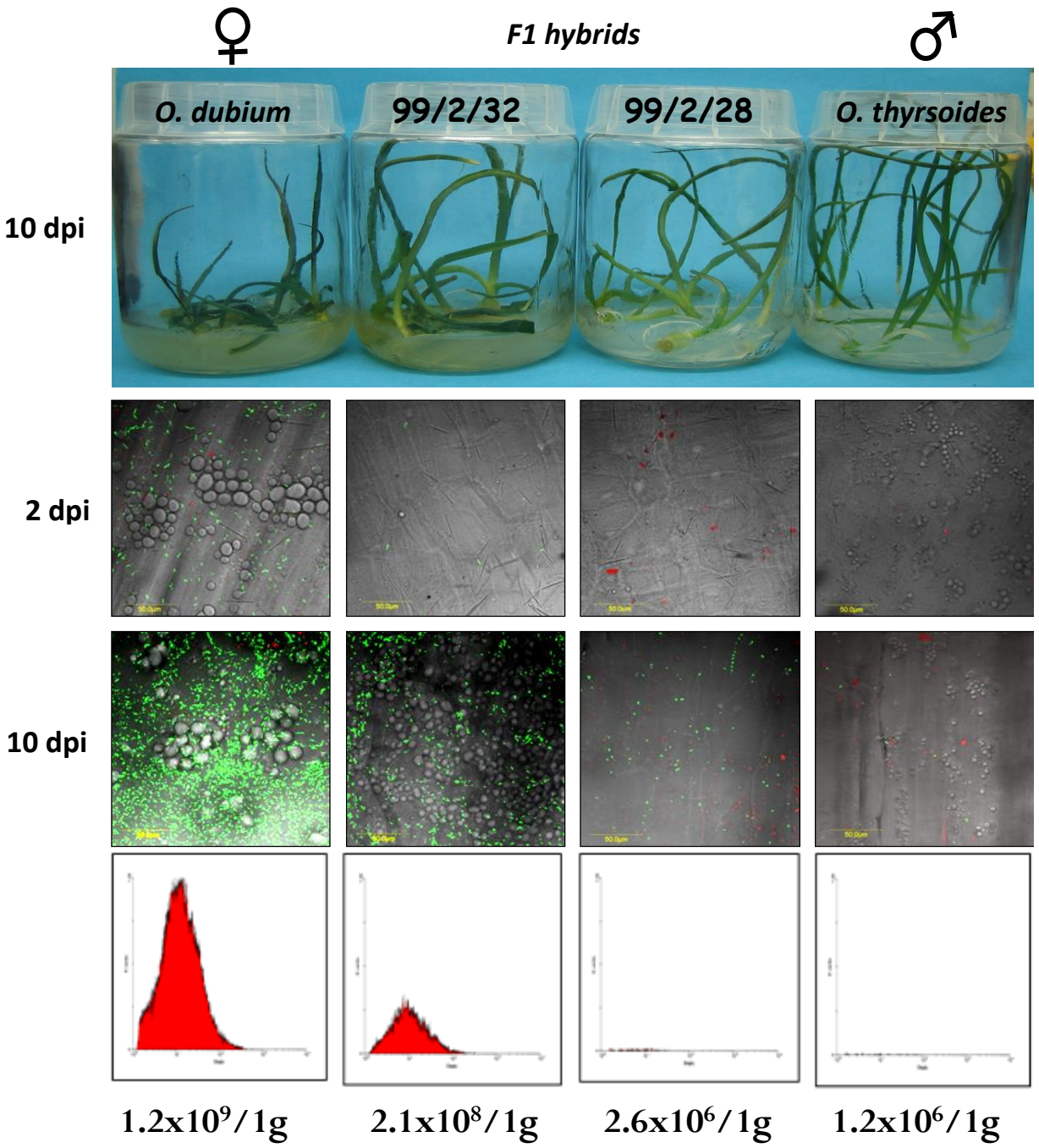
♀ ♂

95/49/60	99/2/32	99/2/28	95/36/1







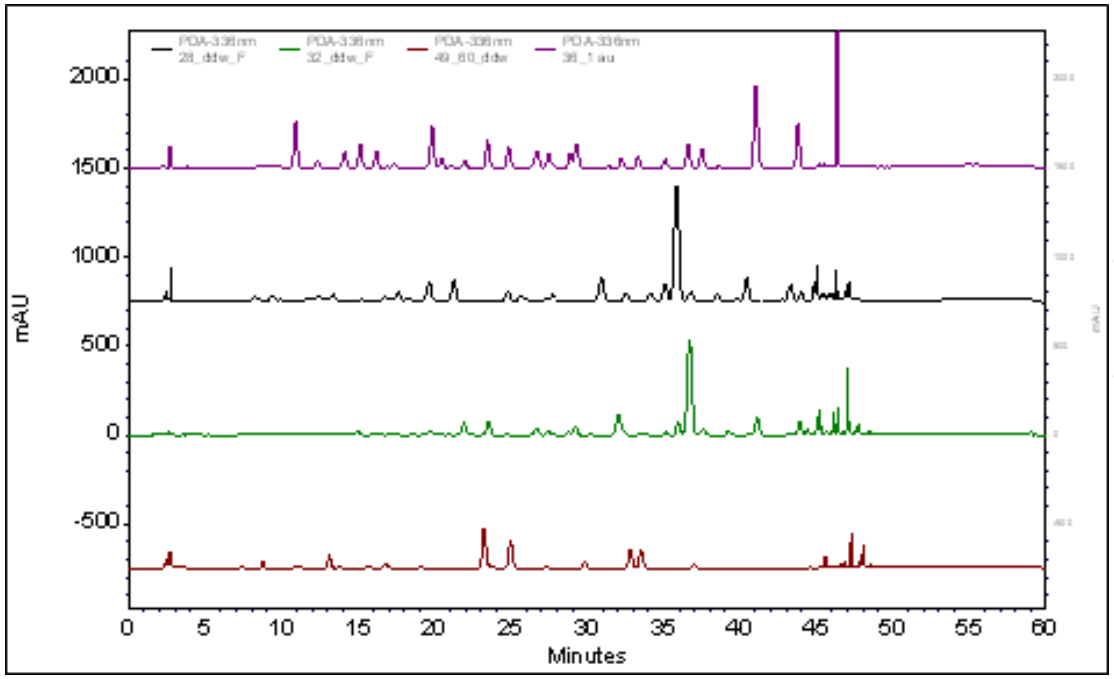


Interspecific breeding

A cross between *O. dubium* (highly sensitive) and *O. thyrsoides* (relatively resistant)

Bacterial cells (labeled with GFP) were quantified using FACS and visualized using confocal microscopy

Characterization of Polyphenolic profiles of *Ornithogalum* clones



O. thyrsoides

99/2/28

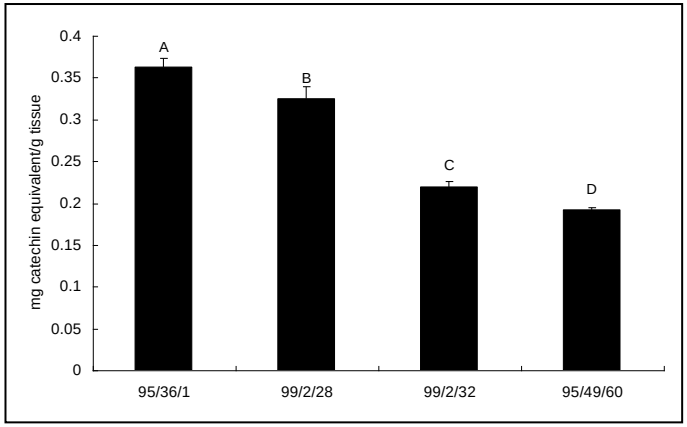
99/2/32

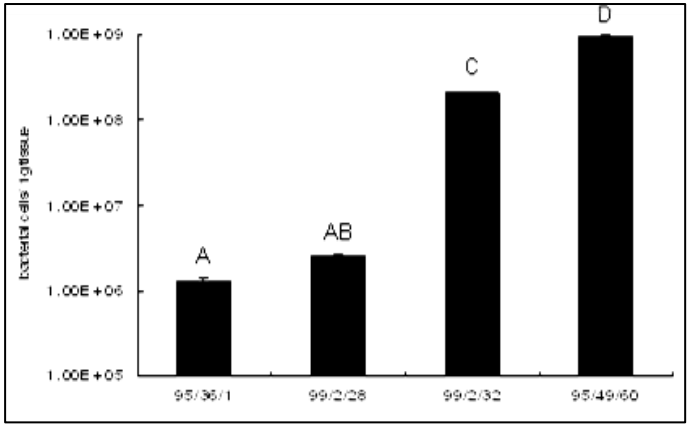
O. dubium

95/49/45

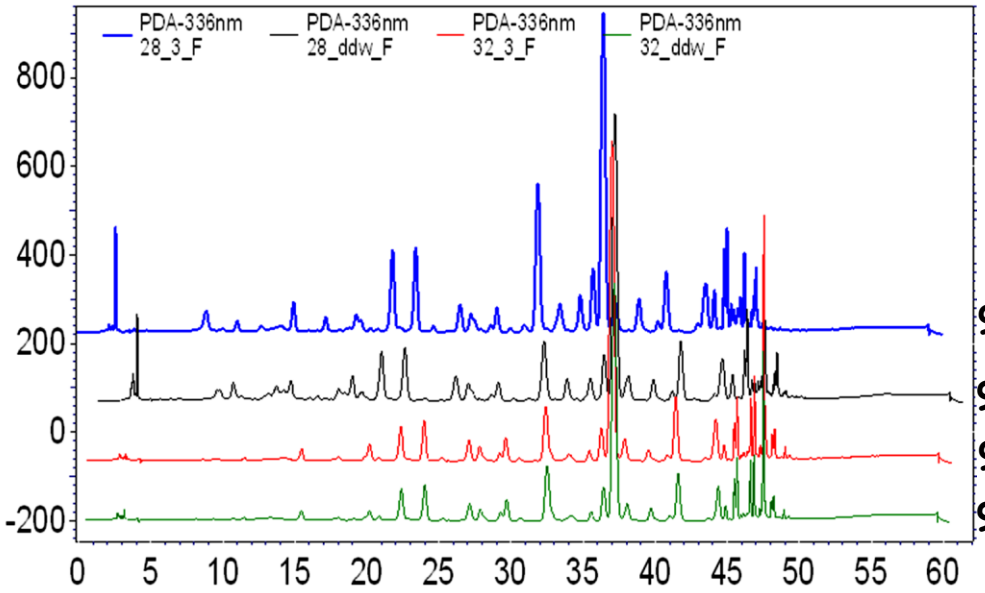
00/36

Total phenolics content in the accessions and F1 hybrids





Polyphenolic profiles of *ornithogalum* F1 hybrids following infection with Pcc



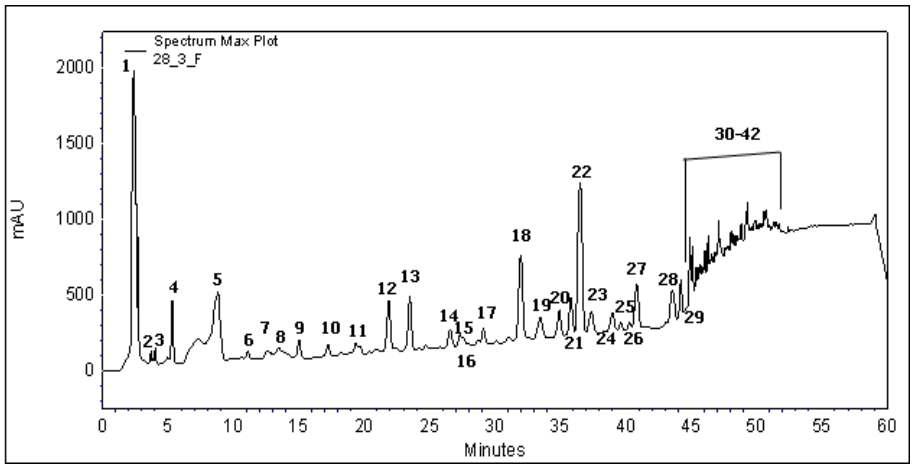
99/2/28 Pc+

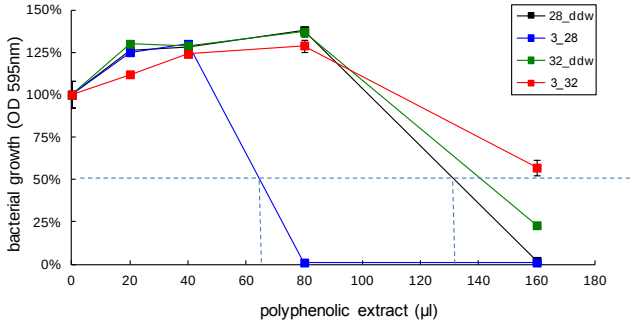
99/2/28

99/2/32 Pc+

99/2/32

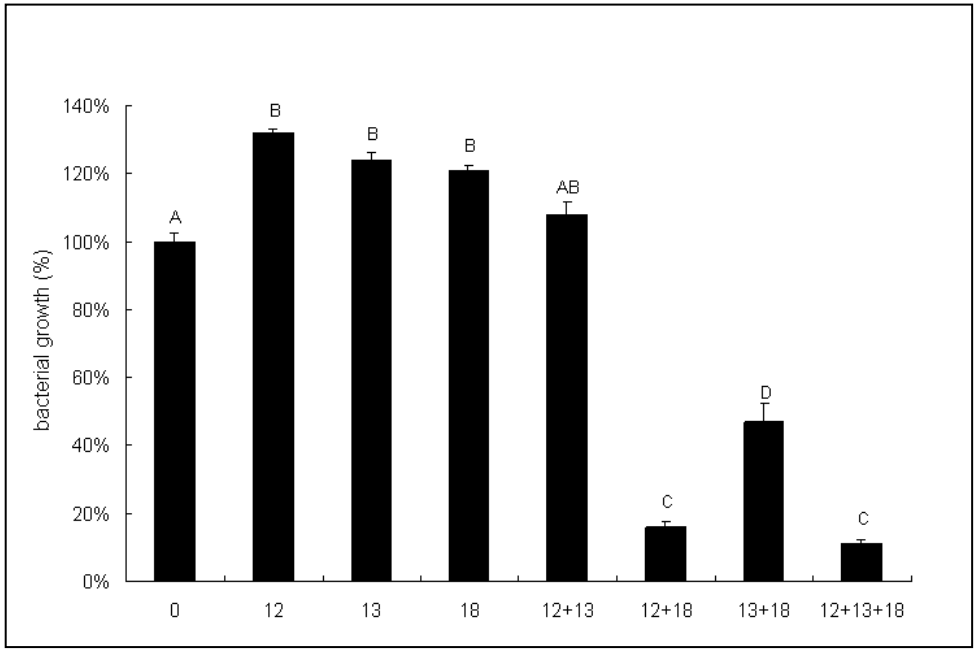
Following infection, only the Pc tolerant F1 hybrid 2/28 displayed elevated levels of polyphenolics





Activity ratio at 50% bacterial growth ~ 2.5 folds

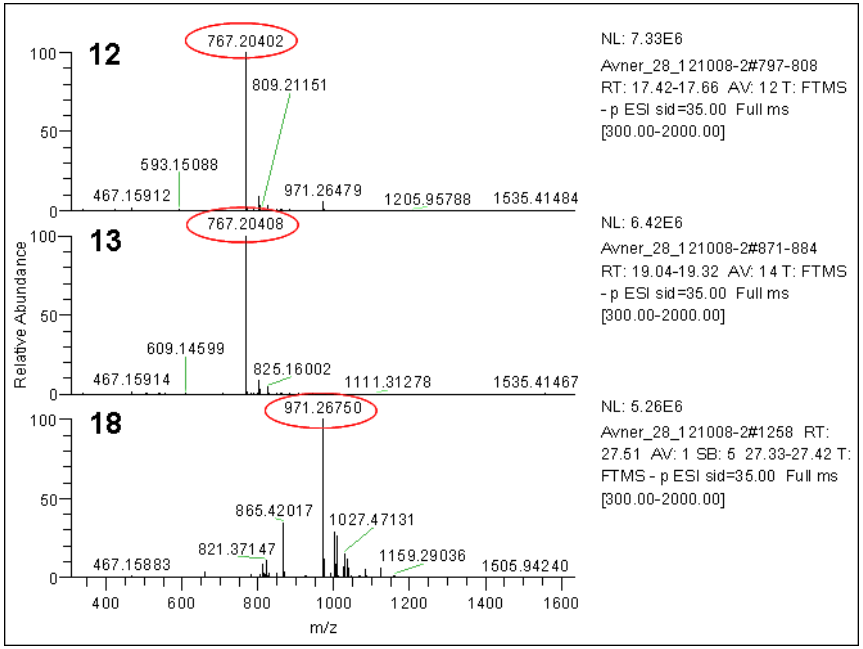
Antimicrobial activity of compounds # 12, 13, 18



Compounds 12, 13 have an identical molecular mass = 767.2 mu

Compound 18 = 971.267 mu

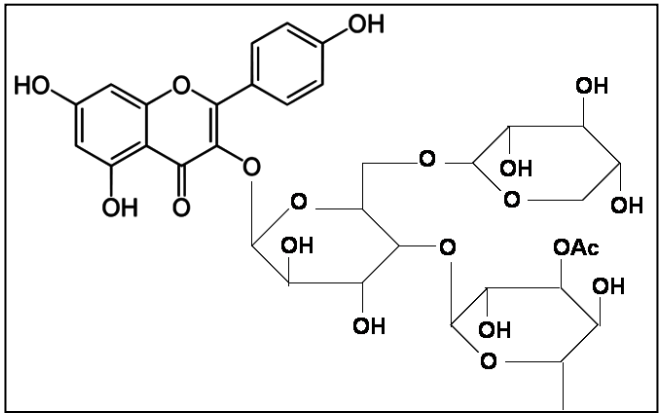
Mass Spectrometry (LC-MS/MS) analysis



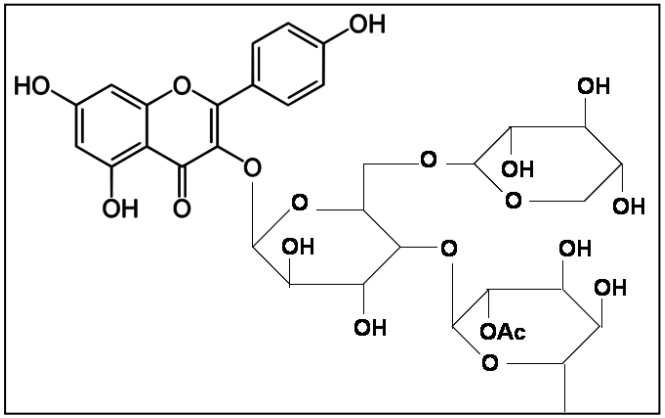
NMR and MS analysis of compounds 12, 13, 18

- The compounds were identified as isomers of flavonoid –o-glycosides.
- A Kaempferol aglycon is connected directly to glucose which is linked to xylose and rhamnose with an additional group of acetate.
- All compounds are new to science.

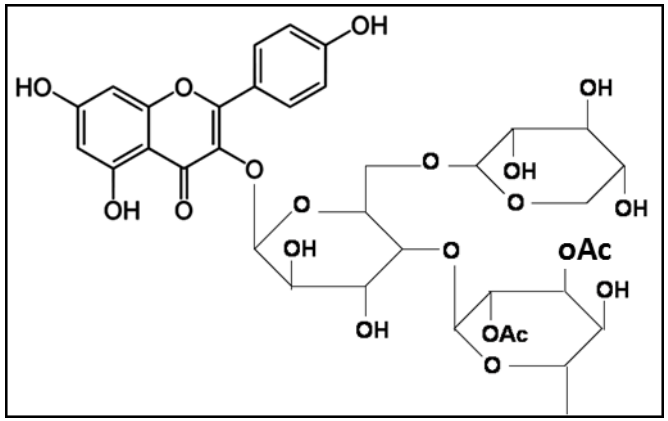
Compound 12



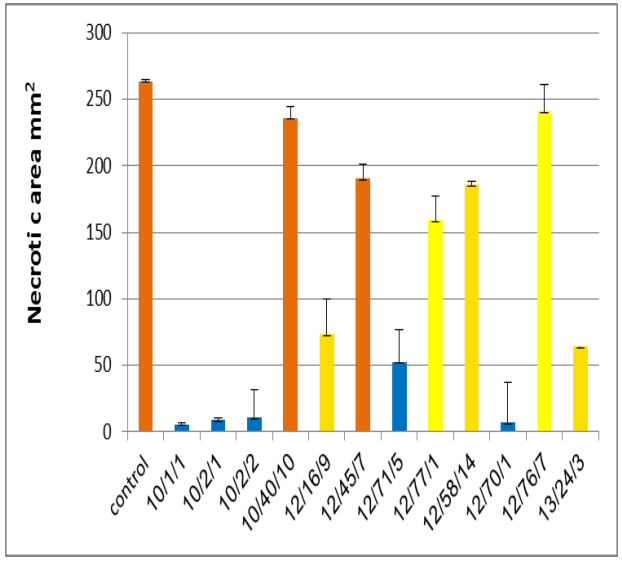
Compound 13



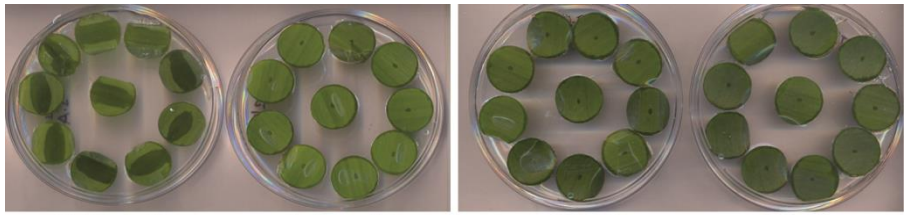
Compound 18



Interspecific breeding lines with higher resistance to soft rot

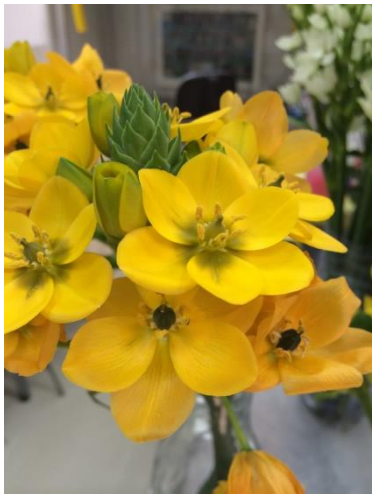


The interspecific crossings generated resistant new phenotypes with larger flowers and new colors



Ornithogalum new phenotypes: improved resistance to soft rot and desirable flower traits

Commercial



13/24/3

12/58/14

12/45/7

12/76/7

Commercial

10/1/5

Commer vs 10/1/5

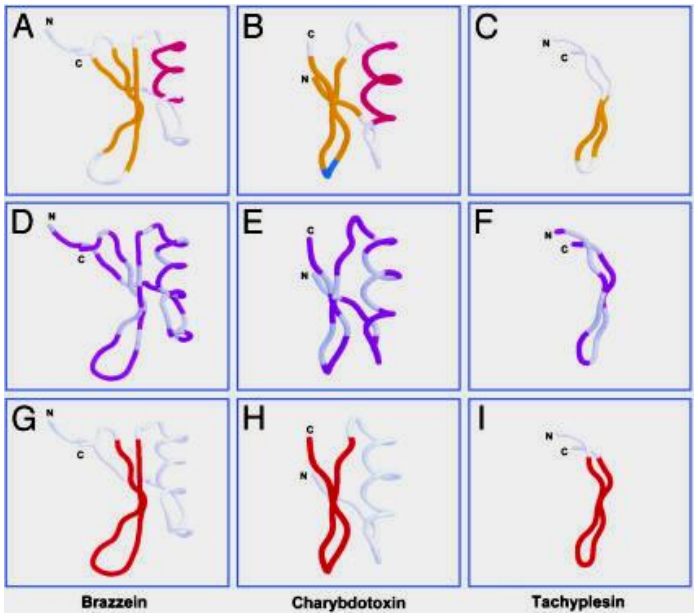
10/2/2

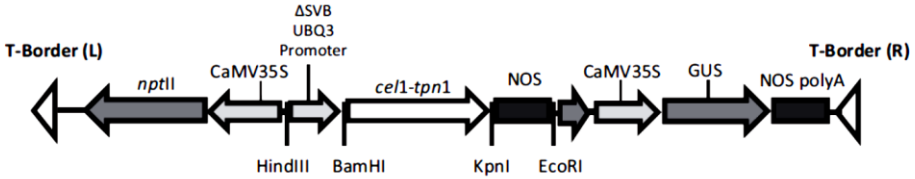
12/58/12

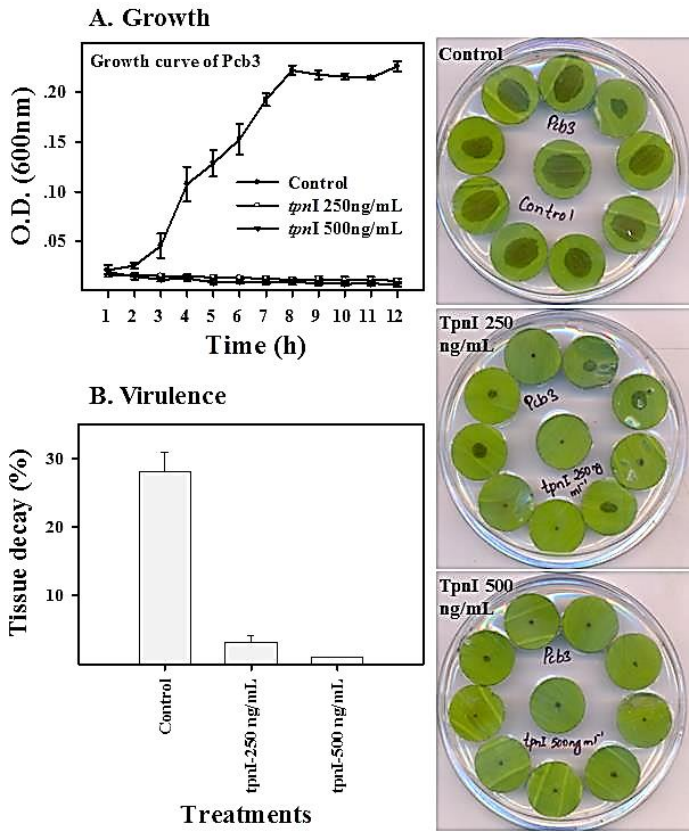
Genetic transformation of *Ornithogalum* with an antimicrobial peptide:

Production of soft rot resistant plants

Tachyplesins are cysteine-containing antimicrobial peptides from a horseshoe crab (*Tachypleus*): (*i*) small size, <10 kDa; (*ii*) cationic charge, from +2 to +7 (pH 7); and (*iii*) affecting membrane integrity.

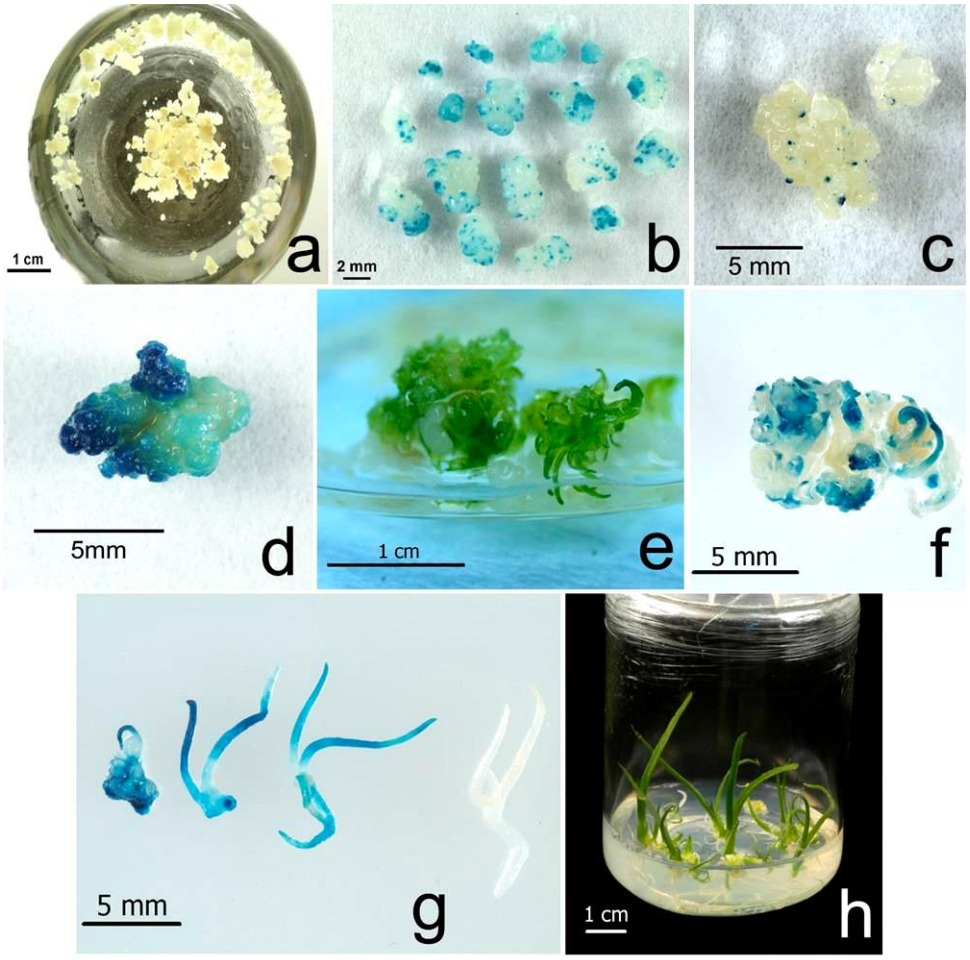






Nannette et al., 2004. *Proc Natl Acad Sci U S A.* 101:7368–7363

Transformation and regeneration system for *Ornithogalum*



a. Cell clusters of *Ornithogalum* grown in the dark

b. Transient GUS expression in competent cells following bombardment (pUBQ3genGUS).

c. Stable expression of the reporter gene in selection medium containing 80mg/l kanamycin

d. Organized transgenic meristematic centers

e. Plantlet regeneration in the light (80 mg/l kanamycin).

f. GUS expression in regenerating cell clumps

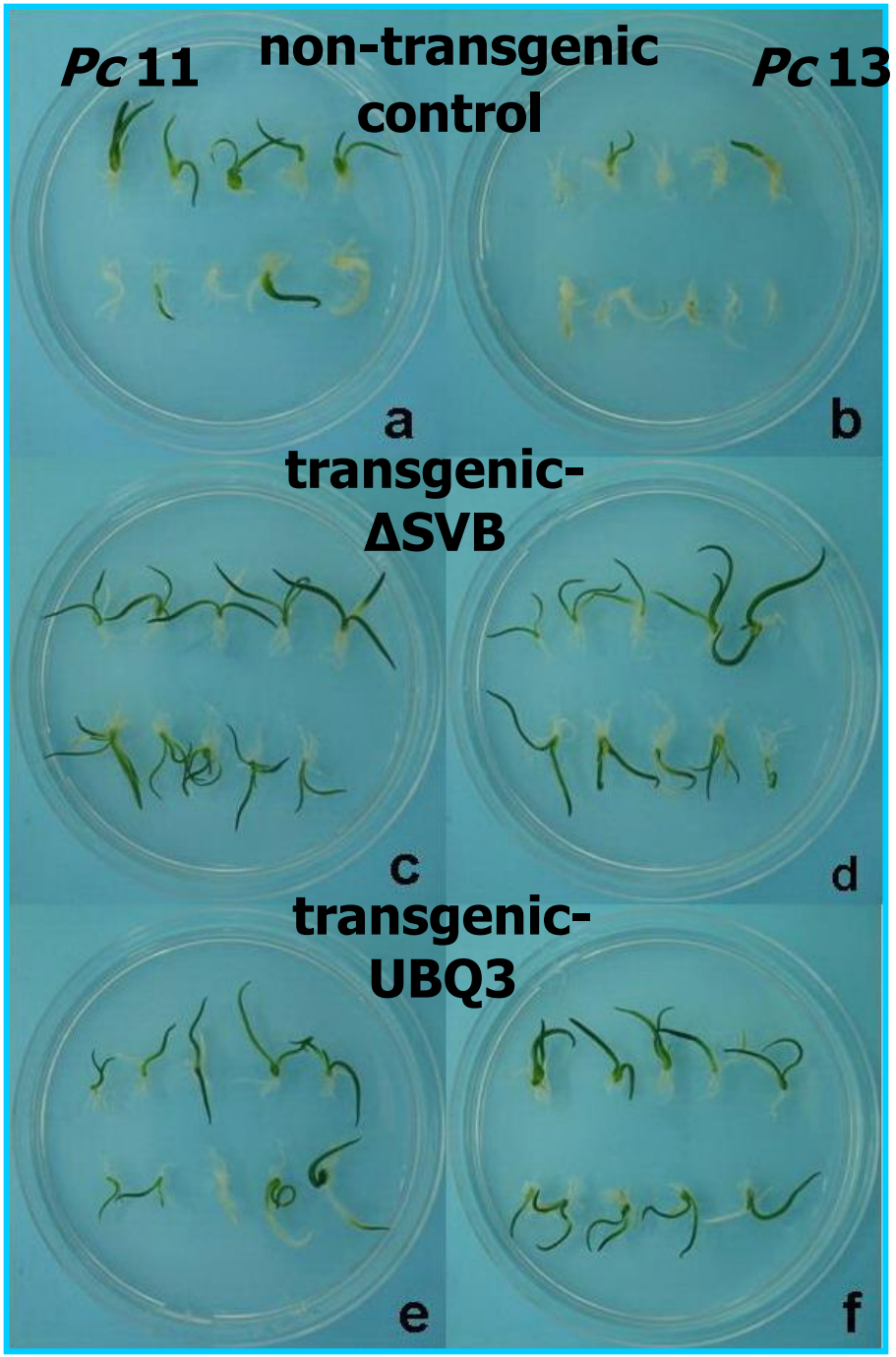
g. GUS expression in transgenic plantlets

h. Transgenic *Ornithogalum* plants in MS agar (50mg/l kanamycin).

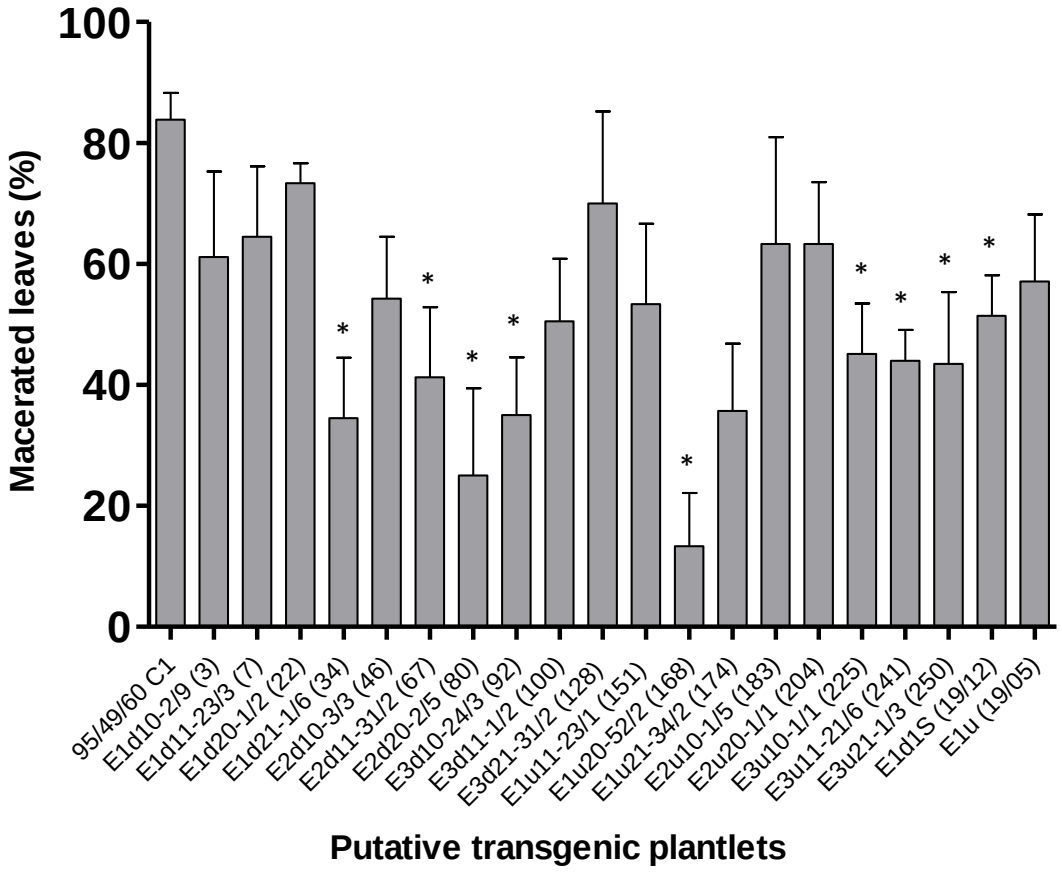
Lipsky et al., Plant Science. 228: 150-158

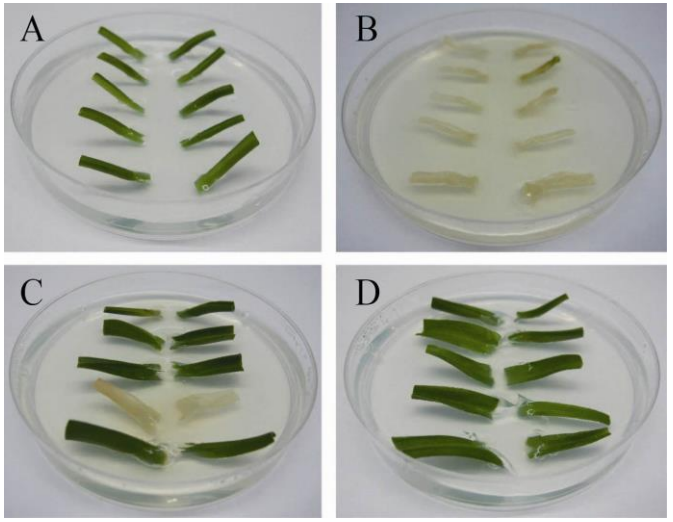
Screening for resistance to *Pc* at early development

- Plantlets challenged with two bacterial isolates: a virulent *Pc*13 (calla lily) and a mildly –virulent *Pc* 11 (tomato)
- Non-transgenic control plantlets did not survive more than 10 days
- 50% of transgenic plantlets survived for more than 100 days post infection
- No promoter-specific effect was observed



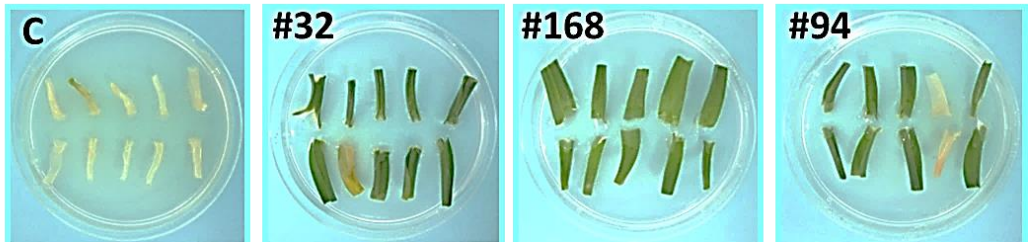
Screening for resistance in putative transgenic plantlets infected with *P. carotovorum*



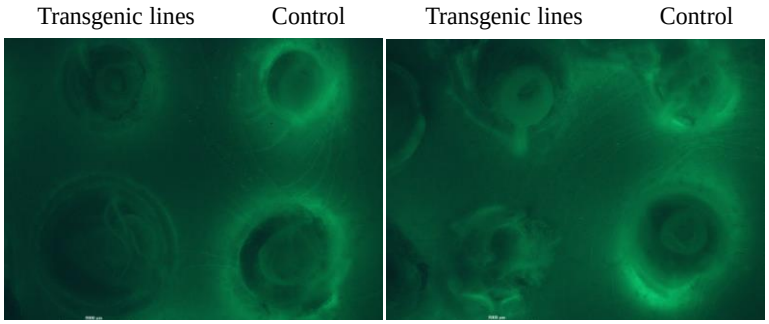


Lipsky et al., Plant Science 2014

Bacterial colonization (Pc-GFP) of *O. dubium* transgenic lines



A



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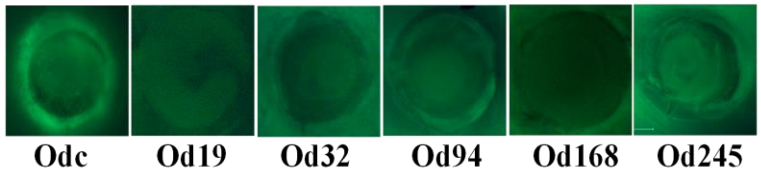
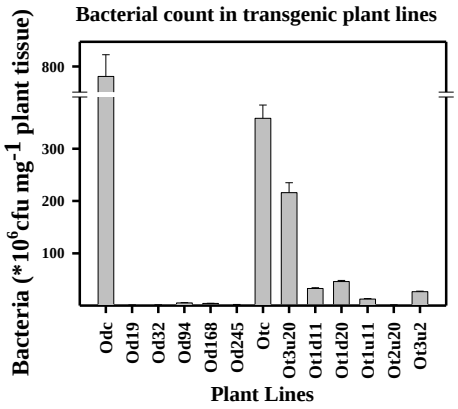
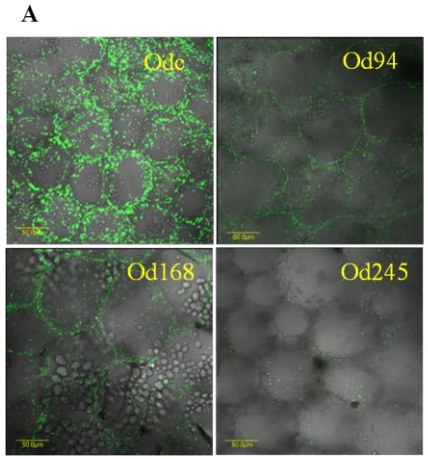
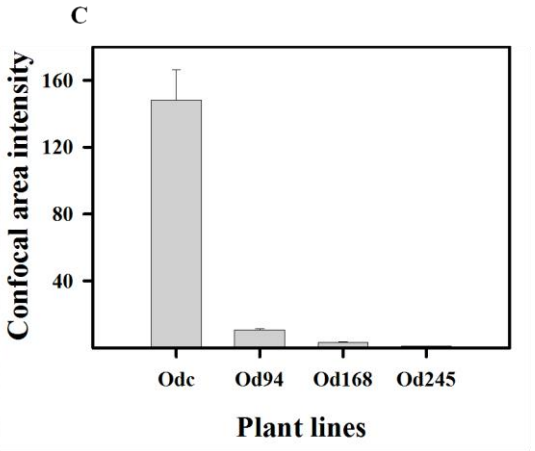
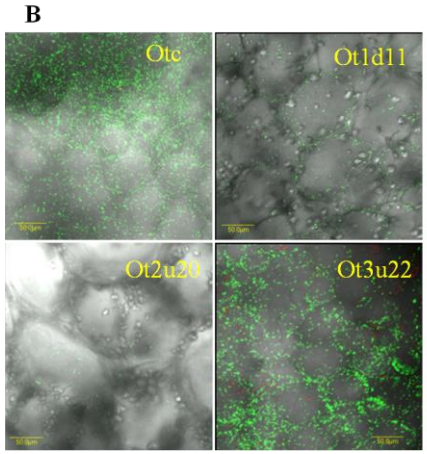
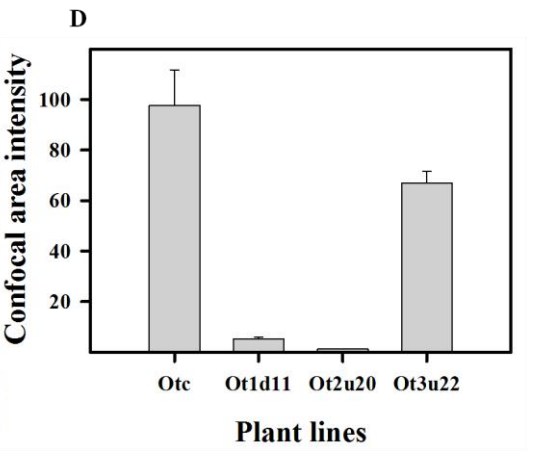




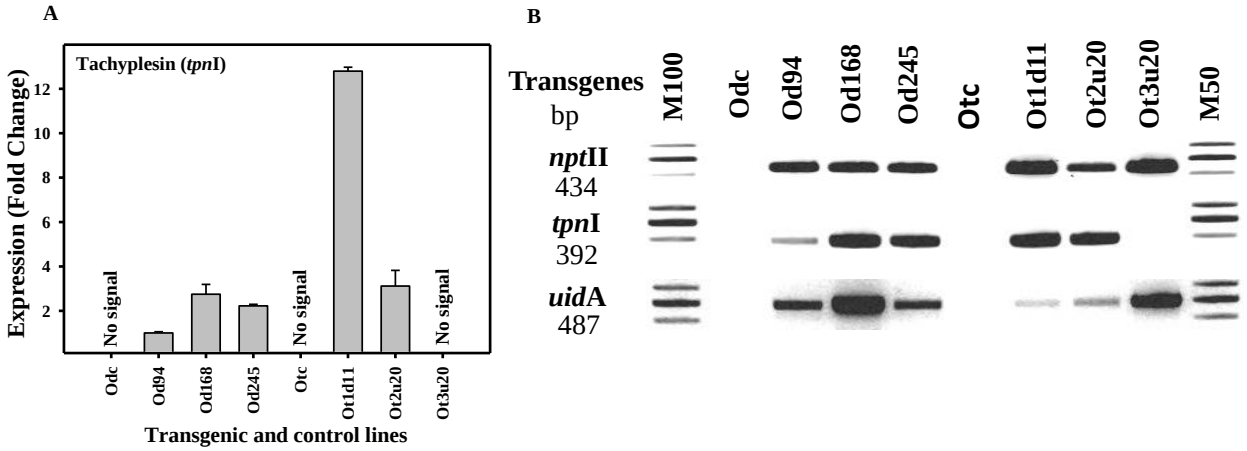


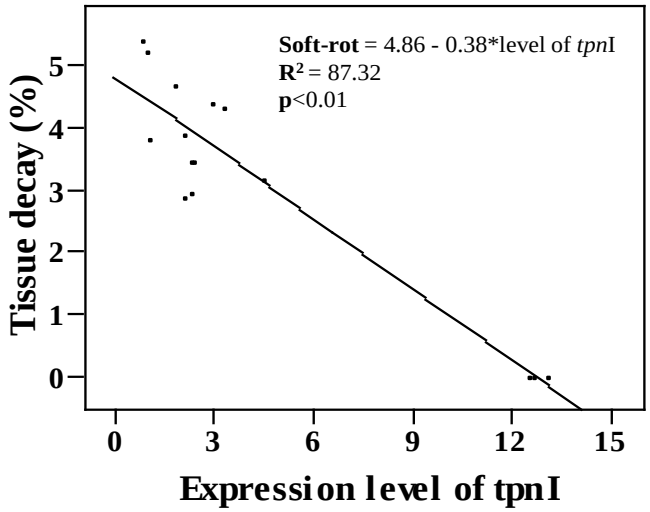






Expression level of *tpn*I is associated with soft rot resistance

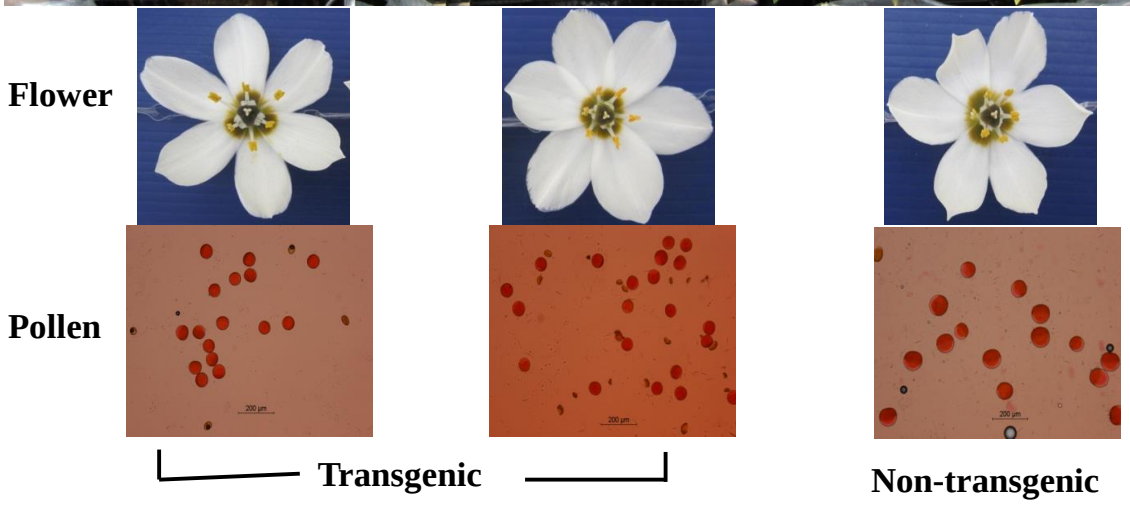




Conclusions:

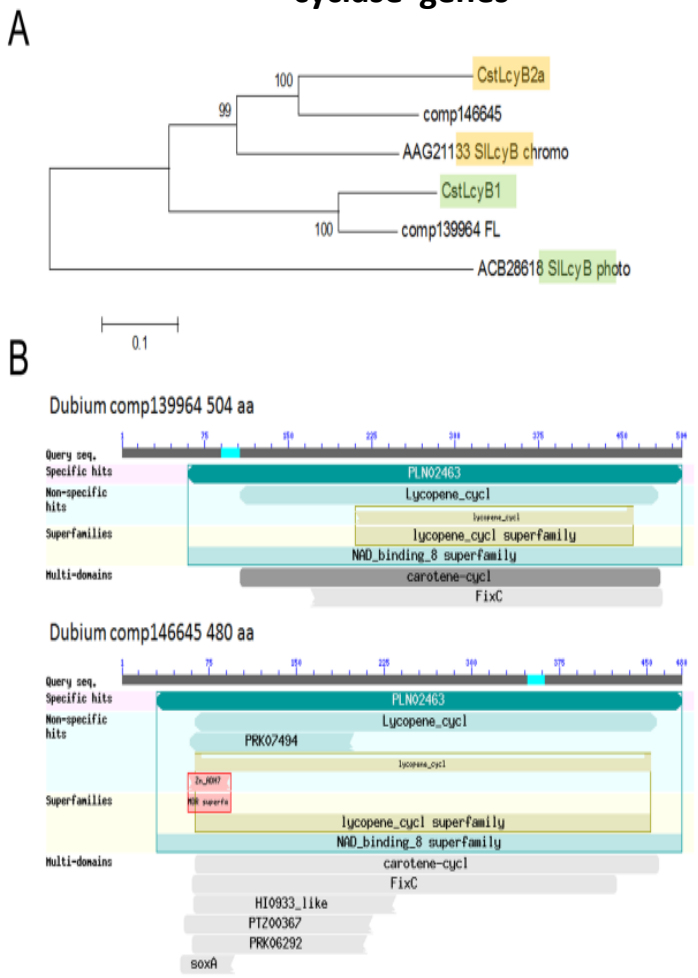
1. Tachyplesin is expressed in the transgenic lines
2. The expression level is related to symptoms development and bacterial colonization of the transgenic lines.

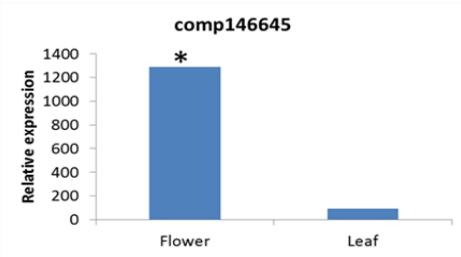
Transgenic vs non-transgenic *O. thyrsoides* in the greenhouse



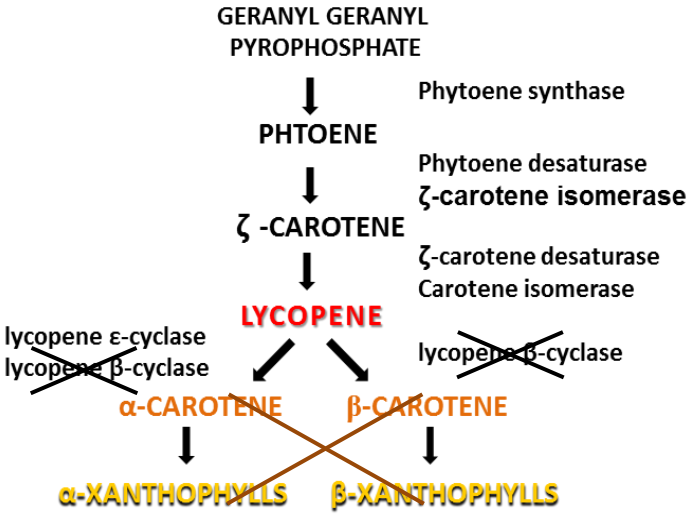
Gene editing a new approach to design desirable traits, our aim - Red *Ornithogalum*

RNAseq transcriptomic analysis yielded two Lycopen cyclase genes



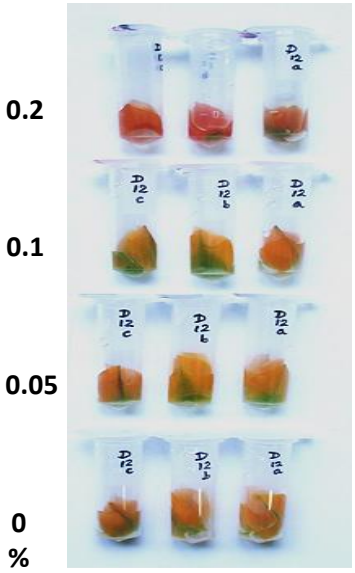


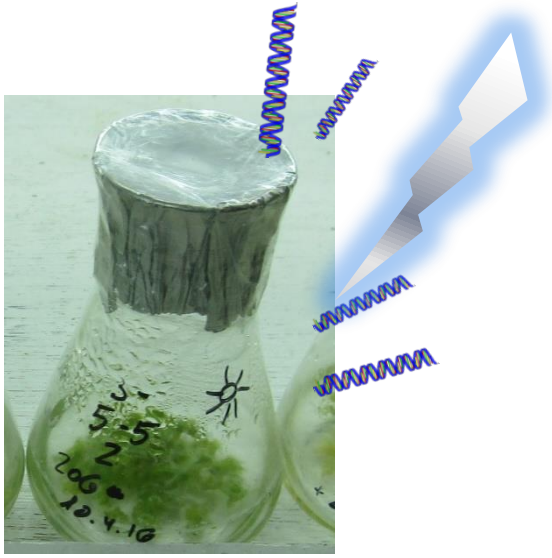
3. Quantitative expression of chromoplastic lycopene cyclase in the petioles and the leaves

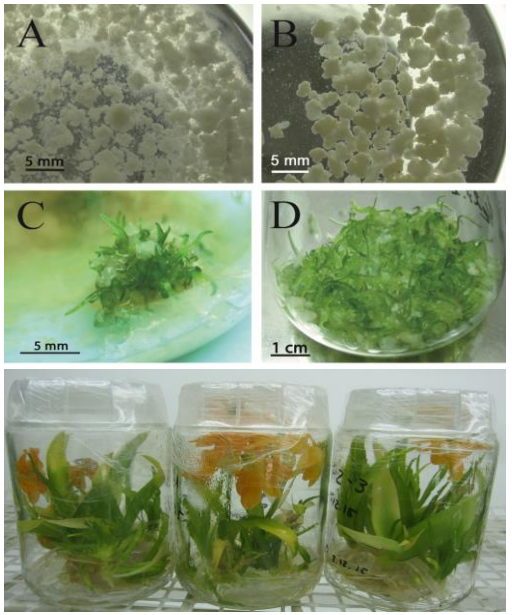


2. Carotenoids biosynthesis pathway

Gene editing scheme for red *Ornithogalum*

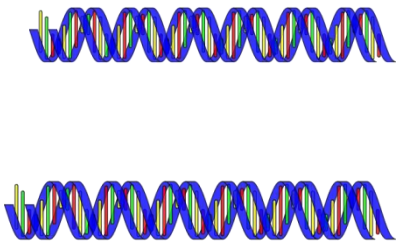


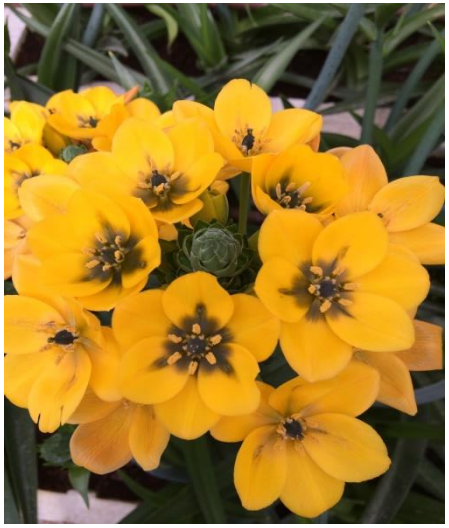




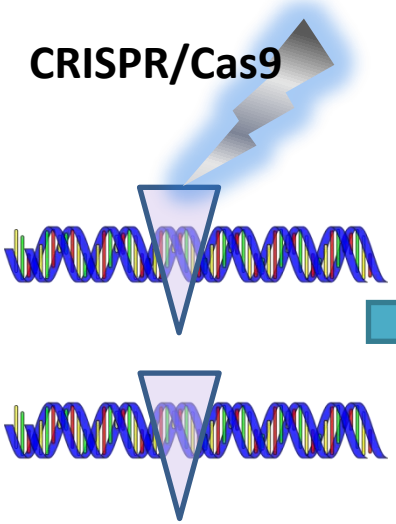
Increasing concentrations of CPTA – Lycopen Cyclase inhibitor – red tissue

LcyB





CRISPR/Cas9




Thank you
Alexander Lipsky
Avner Cohen
Nofar Hod
Janak Raj Joshi