Ornamental Breeding @ Plant Breeding

Overview of research

Paul Arens

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WUR Plant Breeding

- Combined group
- ~200 researchers/postdocs/technicians/students (including ~70 PhD's and 30 MSc)
- International composition (>30 nationalities)
- Three professors
- Ten Research group leaders

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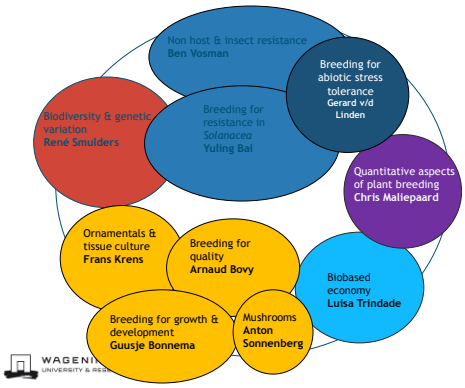

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Wageningen UR Plant Breeding

Mission: High yielding crops with excellent quality which give stable yields under different conditions in a sustainable fashion

Understand and unravel mechanisms behind and genetics of:

- Yield stability
- Yield adaptation
- Resistance
- Quality
- Sustainability

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Ornamental Breeding

- Pre-breeding projects (lily, tulip, hydrangea)
  - Interspecific crosses (lily, tulip, hydrangea, helleborus)
    - Pollination techniques, embryo rescue, polyploidisation
- Breeding tools and genetics of traits in diploid and polyploid species (chrysanthemum, lily, tulip, gerbera, rose, phalaenopsis, alstroemeria and begonia)
  - Breeding for complex traits by MAS
    - Mainly disease resistances
    - More recently also post-harvest and ornamental traits

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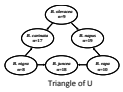
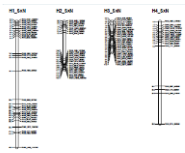
Importance interspecific crosses?



Triangle of U

- In most if not all major crops present or past improvements on disease resistance, performance shapes and colours have relied on interspecific crosses
- Many polyploids results from interspecific crosses eg phalaenopsis, rose, chrysanthemum etc

- Interspecific crossing
  - Dealing with crossing barriers
  - Much used, little known
  - Hear say



Rose haplogroups (Maliepaard)

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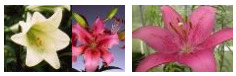
Importance of interspecific crosses?

- In ornamentals for new flower shapes and colours

Intergeneric cross
Amaryllis belladona
x Nerine bowdenii


- Improved agronomic traits

Interspecific cross
Longiflorum x Asiatic lily hybrids



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Overcoming crossability barriers

- Pre-fertilisation barriers
  - Flowering time > pollen storage
  - Pollen inhibition > pollination tricks & methods
- Post-fertilisation barriers
  - Embryo abortion methods > embryo rescue
  - Plastome-genome inc. > plastid inheritance
  - Overcoming F1-sterility > polyploidisation
  - Introgression > GISH, MAS techniques

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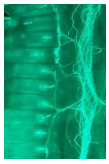
Overcoming interspecific hybridization barriers

Pollination

Inhibition of pollen tube growth in the style

Ovary with ovules

Style manipulations: cut-style, grafted style



(Janson, 1992)

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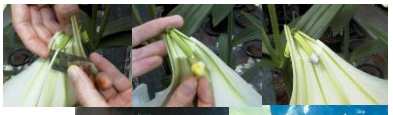
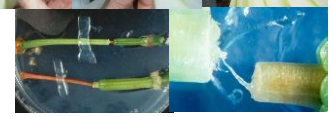
How to overcome hybridization barriers?

- Pre-fertilization e.g.:
  - Cut style



  - Grafted style



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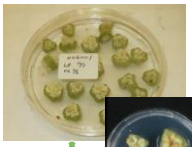
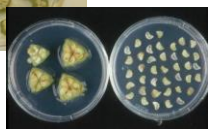
How to overcome hybridization barriers?

- Post-fertilization, e.g.



  - Ovary culture

Lily
Tulip



  - Embryo rescue

Stage = important

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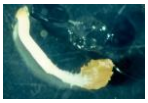
Embryo to flowering hybrid, 1-3 year

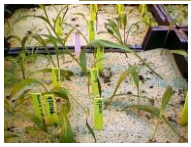


cross

vitro

vitro

greenhouse



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Overcoming F_1 sterility interspecific hybrids

- Through mitotic polyploidization
  - Somatic chromosome doubling
  - Colchicine or Oryzalin
  - Overcoming F1-sterility
  - No homoeologous recombination
- Through meiotic polyploidization
  - 2n-gametes by FDR, SDR and IMR
  - Spontaneous (abiotic stresses), induced by laughing gas
  - Homoeologous recombinations

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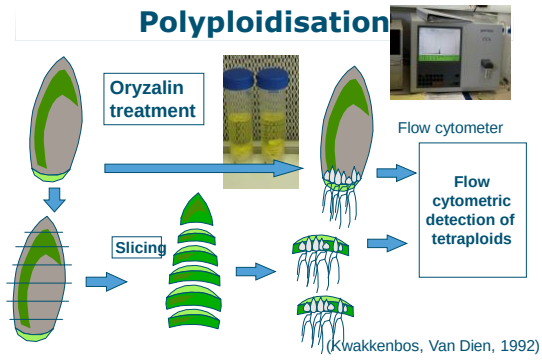
Polyploidisation



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Mitotic

Meiotic

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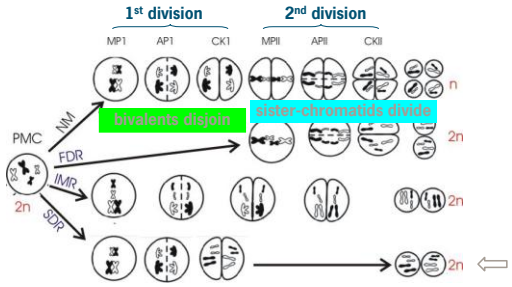
Meiotic – Nuclear restitution mechanisms



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Recombination depending

Induction of 2n gametes to overcome hybrid infertility

- Mitotic polyploidisation mainly used
- Meiotic polyploidisation gives recombination!
  - Spontaneous 2n production is rare
  - Induction methods

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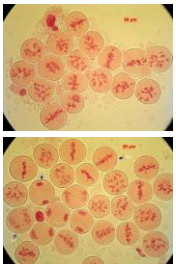
Polyploidisation induction by N_2O

Correlation between bud size and meiotic stage in pollen mother cells in Lilium

Genotypes	Bud length	Meiotic stage
'Gluhwein' & 'Shocking'	<23	Interphase
	23-29	Prophase I
	30-33	Metaphase I-Telophase II
	>36	Pollen
'Nymph' & 'Yelloween'	<26	Interphase
	26-32	Prophase I
	33-36	Metaphase I-Telophase II
	>40	Pollen



- PMC meiotic stages at bud lengths 30 and 31mm

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Inducing methods

Comparison with other methods

Inducing methods	Genotypes	Treating time	No. of treated flowers	No. and % of fertile flower	Germination (%)
N_2O treatment	'Gluhwein'	0 h	51	0	0
		24 h	45	5(11.1%)	5-90% (32.0%)
		48 h	41	6(14.6%)	45-95% (72.5%)
		72 h*	31	0	0
	'Nymph'	0 h	63	0	0
		24 h	74	4(5.4%)	5-50% (18.8%)
		48 h	50	8(16.0%)	5-90% (32.5%)
		72 h*	31	0	0
	'Yelloween'	0 h	67	0	0
		24 h	60	0	0
		48 h	46	2(4.3%)	10-90% (50%)
		72 h*	30	0	0
	'Shocking'	0 h	45	0	0
		24 h	49	0	0
		48 h	48	0	0
		72 h*	25	0	0
Low temperature	'Gluhwein'	96 h	57	0	0
	'Nymph'	96 h	61	0	0
	'Yelloween'	96 h	58	0	0
	'Shocking'	96 h	51	0	0
Temperature fluctuation	'Gluhwein'	-	65	1(1.5%)	5%
	'Nymph'	-	70	1(1.4%)	5%
	'Yelloween'	-	75	1(1.3%)	5%
	'Shocking'	-	57	0	0

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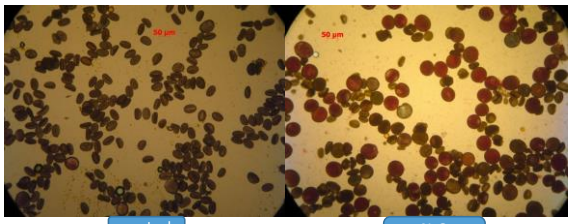
Fertility of pollen



control

N_2O treated

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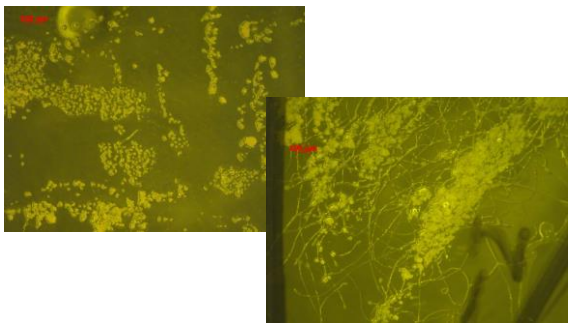
Fertility of pollen



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Results

Formation of viable 2n pollen at N_2O treatment in different meiotic stages in Lilium

Cultivars	Meiotic stage	No. of treated flowers	No. of fertile flower	% of fertile flower	Range of fertility	Mean fertility
'Nymph'	Prophase I	54	12	22.2%	5-90%	27.9%
	Metaphase I	31	0	0	0	0
	T I ---T II*	42	0	0	0	0
'Gluhwein'	Prophase I	63	9	14.3%	5-95%	50.5%
	Metaphase I	10	2	20.0%	70-90%	80%
	T I ---T II	17	0	0		0
'Yelloween'	Prophase I	65	2	3.1%	10-90%	50%
	Metaphase I	25	0	0	0	0
	T I ---T II	23	0	0	0	0

* T I ---T II: Telophase I –Telophase II

N_20 can induce 2n pollen at high frequencies in sterile OT-hybrids

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Conclusions

- Much hear say generalisations, often really tested combinations few
- Very genotype and combination dependent
- Stepwise approach if little is know
  - Pollen quality (germination, viability, *in vivo*, …)
  - Pollen tube behaviour
  - Presence embryo and endosperm
  - Systematic investigation *in vitro* culture (ovary and ovule rescue)
- Several methods of polyploïdisation exist
- Reward of success can be very high

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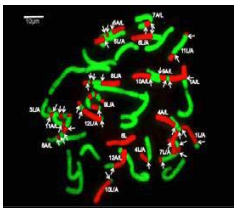
Cytogenetic research meiosis in hybrids

- Nadeem Khan PhD – Analytic breeding in LA hybrids
- Songlin Xie PhD – Chromosomal abberations in meiosis



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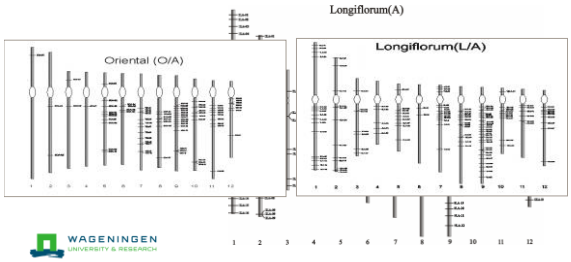
Recombination map LA hybrids

- Fertile diploid LA hybrids
- Analytic breeding possible
- Recombination differences!



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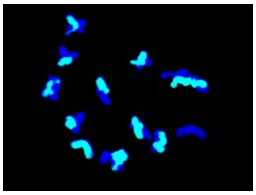
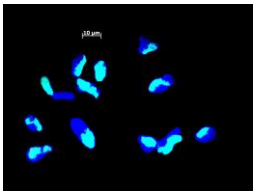
Association failure

Bivalents & univalents

12 bivalents





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Resistentie onderzoek

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Duurzamere rassen voor de sierteelt

- Duurzame rassen dwz rassen met een resistentie of verminderde vatbaarheid tegen ziekten en plagen

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Wat kan veredeling?

- Met gerichte veredeling op weerbaarheid/resistentie genen kan de weerbaarheid van planten worden verhoogd
- Voorwaarden:
  - Resistentie in het gewas aanwezig

  OF
  - Resistentie in kruisbare verwant van het gewas

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Wat is resistentie?

- Verschillende typen resistentie:
  - Gen om gen resistentie
  - Multi-gen weerstand
  - Vatbaarheid gen
- Vaak variatie in resistentiegenen en in pathogenen
  - Vooral van belang voor gen om gen resistentie

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Hoe kun je resistente/weerbare rassen gebruiken?

- Liefst combineren van verschillende types van resistentie
- Integreren in totale pakket van gewasbeschermingsmiddelen
- Afhankelijk van gewas en pathogen wisselen van rassen

Situatie (glas)-groente veredeling

- Sterke focus op resistentie veredeling
- Rassen zonder resistenties tegen hele serie ziektes komen niet op professionele markt
- Belangrijke drivers:
  - Voedselveiligheid (residuen)
  - Buitenteelten in andere landen
  - Milieu
- Chemische gewasbescherming in sommige teelten slechts sporadisch nodig

Situatie sierteelt

- Resistentie of verminderde vatbaarheid is geen startpunt voor veredeling maar zit vaak aan het eind van een veredelingstraject
  - Focus op nieuwheid, schoonheid
- In kruisen resistenties in uitkruisende gewassen kost meer tijd en geld
- Chemische gewasbescherming in aantal teelten nog heel belangrijk (roos, lelie)

Is er een noodzaak voor meer focus op resistentie veredeling?

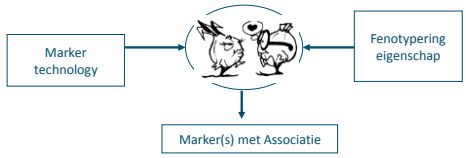
- Wegvallen van Chemische middelen
  - Resistentie pathogenen
  - Milieu overwegingen
  - Marktoverwegingen producenten
- Behandelingen (oogst en verwerking)
- Klimaat

Doel merker onderzoek

- Merkers waarmee je eigenschappen kunt volgen door koppeling op hetzelfde chromosoom
  - Merkers zijn willekeurig verdeeld en kunnen onderscheid maken tussen de twee chromosomen
  - Overerving naar nakomelingen zichtbaar te maken, zoeken naar associatie tussen merker en eigenschap
- Selectie in veredeling door merkers
- Voor gecompliceerde eigenschappen en/of eigenschappen die lastig zijn vast te stellen

Eigenschappen: ziekte resistenties

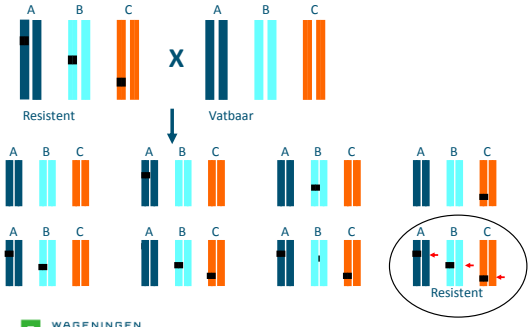
Concept van Marker Assisted Breeding



Selectie niet op eigenschap maar op markers (sequentie verschillen) in veredelingsprogramma

Indirecte Selectie

Veredelen voor resistentie (Dominant, 3 genen)



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Resistance breeding in cut-tulips

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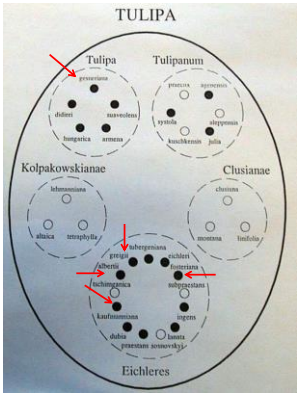
Tulipa section

- *T. Gesneriana*
  - Cut flower
- Other mainly garden use
- Crossable to *T. Gesneriana*



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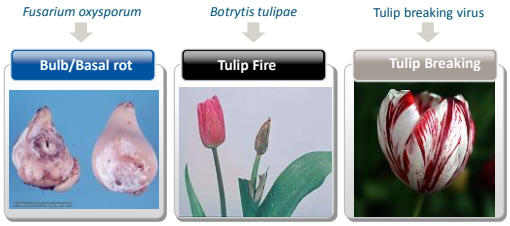
Tulip Breeding

- Pre-breeding in tulip: three major diseases



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Disease resistance presence in crossable species

Species	*Botrytis*	*Fusarium*	TBV
T. *albertii*	+	+	+
T. *eichleri*	-	+	+
T. *greigii*	+	+	-
T. *fosteriana*	+	+	+
T. *kaufmanniana*	+	+	-
Fosteriana hybriden	*	*	+
T. *gesneriana*	+	+	-

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TBV resistance

- Selection for TBV resistance
- T. fosteriana – high level of resistance

Cultivar	% Healthy
- Juan	24
- Madame Lefeber	79
- Princeps	100
- Cantata	100

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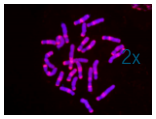
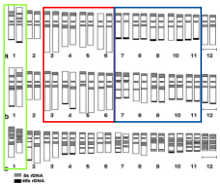
Chromosome analysis

- Tulip 2n = 2x = 24 chromosomes
  - Karyotypes similar in shape
  - Secondary constriction not visible
  - Chromosome pairing difficult
  - *T. gesneriana* chromosomes longer then *T. fosteriana*





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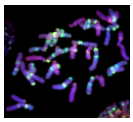
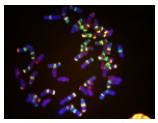
Interspecific hybridization –F1 hybrids

- Interspecific hybridization
  - *T. gesneriana* x *T. fosteriana* - Darwin hybrids
  - 2n-gametes
    - Triploid Darwin-hybrids, Apeldoorn
    - Tetraploid Darwin-hybrids, Judith Leyster
  - n-gametes Fertility? Breakthrough!
    - > Recombination? > GISH



2n= 3x = 36



2n= 4x = 48

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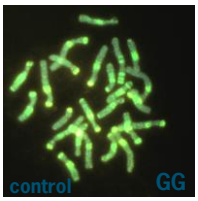
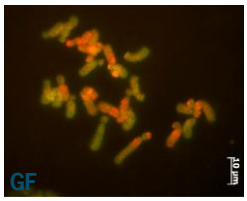
GISH analysis in Darwin-hybrids – F1 population





2n = 2x = 24

Verification of hybrids

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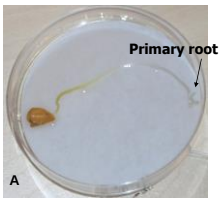
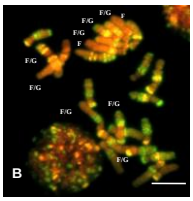
GISH analysis in Darwin-hybrids – BC1 population -> Recombination!





Marasek et al. (2012) Plant Syst Evol.

2n = 2x = 24

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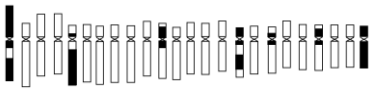
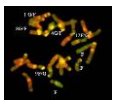
Tulip: Introgression breeding

- Interspecific cross T. gesneriana x T. fosteriana
  - Darwin hybrids F1 sterility, triploids
- Breakthrough: Fertility in some F1 hybrids
  - Obtained set of F1 hybrids with fertility
  - BC1 and BC2 progeny i.e. backcrossing to T. gesneriana (GG/F and GGG/F with 1-12% F genome)





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Next step in breeding

- Introgression breeding on diploid level feasible
- Starting breeding with good *T. gesneriana* cultivars and GF hybrids
- Start with molecular markers in population
- Phenotyping of disease resistance TMV, Fusarium, Botrytis
- Multiple years of testing
- Destructive testing in replications
- Reproduction of bulbs (up 10-12) is a limiting factor
- 2012 start breeding based on known resistances

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Mapping population

Maternal

T. gesneriana

'Kees Nelis'

Paternal

T. fosteriana

'Cantata'

Cross pollinated population

125 F1 progenies

Molecular markers

- Genotyping started in 2000
  - AFLPs
- Later NBS-profiling,
- EST-SNPs and EST-SSRs markers to broaden applicability
- Parental maps, no integrated map yet.

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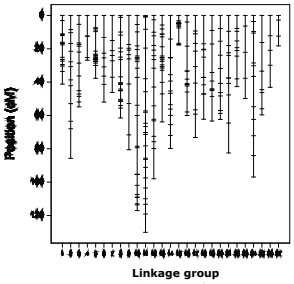
KN map – Maternal



Maternal map

- Genome length 1707 cM
- Number of mapped markers 342
- Median distance between markers 3.9 cM
- 95% percentile of distances 16.2 cM

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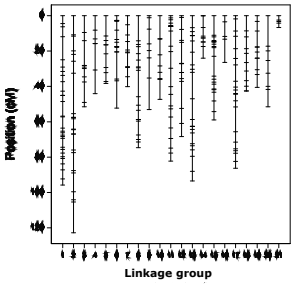
CA map - Paternal



Paternal map

- Genome length 1201 cM
- Number of mapped markers 300
- Median distance between markers 3.1 cM
- 95% percentile of distances 13.3 cM

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Phenotyping – TBV resistance

- TBV (Potyviridae) - aphid inoculation
- Field trial – visual observation:

  Two forms of symptoms in flower:
  - Mild – darkening of colour (can be pre-stadium)
  - Severe - Breaking of colour

  Influence of duration of infection and flower age.
- Leaf symptoms difficult unless severe infection
- Elisa for white and yellow flowered genotypes
- TBV mapped in population

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Fusarium resistance test – Visual

- Soil infection (2011 & 2012)
  - Three *Fusarium oxysporum f. sp. Tulipae* isolates (Tu47, Tu58, Tu67) were incubated separately and well mixed with substrate before use
  - Five replicate bulbs for each progeny
  - Each bulb was put into a separate mesh bag with *Fusarium* contaminated substrate, placed in a crate and covered by plastic bag to preserve moisture
  - Bulbs were kept in greenhouse (18-20℃) for 8 weeks

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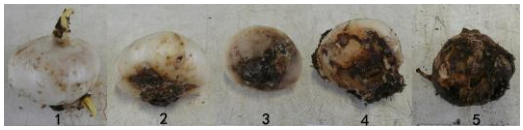
Fusarium resistance test - Visual

- Disease infection degree was scored on scaling 1-5:



Healthy | Slightly infected | Moderately infected | Heavily infected | Totally decayed

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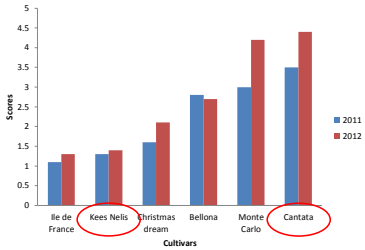
Reference cultivars evaluated by visual test



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Continuous distribution and clear separation of parents

- Visual evaluation results of mapping population

Average score of Parents

	2011	2012
KN	1.3 (±0.6)	1.4 (±0.7)
CA	3.5 (±1.5)	4.4 (±1.1)

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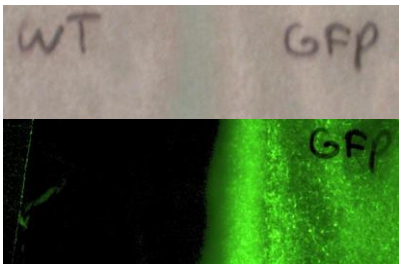
Fusarium testing in Tulip

Fusarium oxysporum transformed with GFP



Daylight

FusariumScreen

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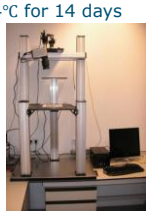
Fusarium resistance test – GFP imaging

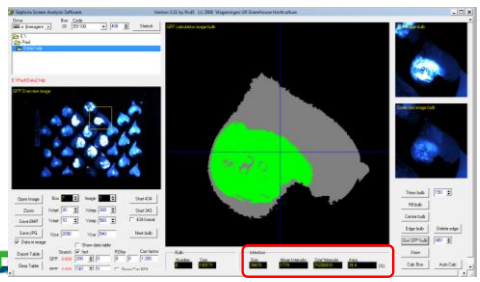
- GFP-transformed *Fusarium oxysporum f.sp. tulipae* isolate Tu67 was used for inoculation
- Healthy bulbs were wounded by three 2 mm deep punctures
- Each bulb was inoculated by $5x10^5$ spores at the wounds
- Bulbs were incubated at >90% RH and 24℃ for 14 days





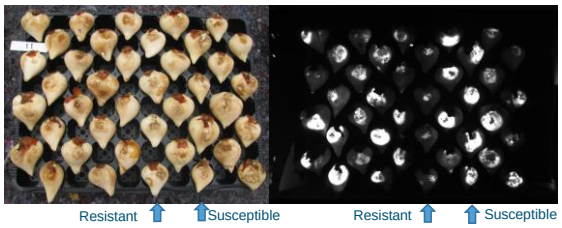
Quantification of *Fusarium* by GFP imaging

- Quantified parameters: Infection size, Mean intensity, Total intensity and Infection area (%) used for population KN x Ca



Fusarium oxysporum

1 week later



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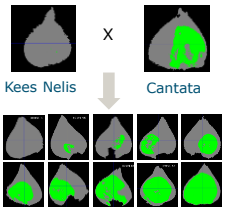
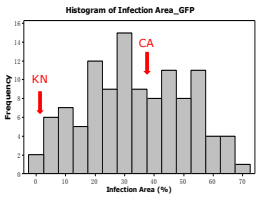
Fusarium resistance is Quantitative





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Fusarium results

QTL	LG	Flanking loci		Test	Sig.	GW	LOD peak	% Variance Exp
QTL1	KN5	KN_19786	KN_5253	GFP	******	3.4	3.2	12.5
QTL2	KN12	KN_36745	KN_23151	Visual2011	*******	3.3	1.7	7.1
				Visual2012	*******	3.5	4.4	18.5
				GFP	**	3.4	2.4	14.5
QTL3	KN23	KN_12084C	P31M54-26	Visual2011	******	3.3	3.4	14.9
				Visual2012	**	3.5	2.8	12.4
				GFP	****	3.4	2.1	8.4
QTL4	KN26	Ca_14945B	KN_20195	GFP	*****	3.4	3.4	20.7
QTL5	CA8	Ca_11976	Ca_15446	GFP	******	2.9	4.3	16.0
QTL6	CA17	Ca_13387C	Ca_12006	Visual2011	******	3.2	3.1	12.2
				Visual2012	******	3.2	3.4	13.6
				GFP	*****	2.9	2.7	10.7

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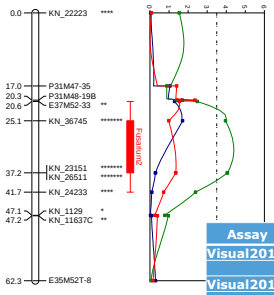
Tang et al. 2015 Molecular Breeding

QTL2 – LG KN18



Assay	LOD	%Exp.	Flanking loci	KW
Visual2011	1.71	7.1	KN_36745	****
			KN_23151	*******
Visual2012	4.4	18.5	KN_36745	*******
			KN_23151	*******
GFP	2.36	14.5	P31M48-19B	- (bridge)
			KN_36745	****

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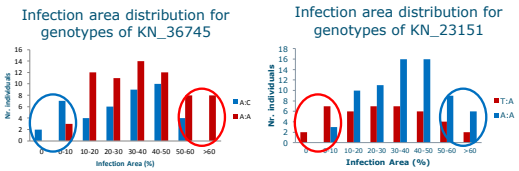
Infection area distribution for genotypes of flanking markers



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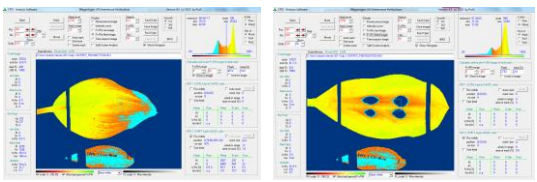
Botrytis tulipae



Resistant

Susceptible

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Pending

Breeding selection in backcross program

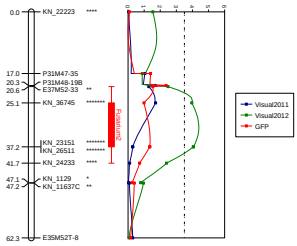
- Selection of GF lines that are TBV resistant and have Fusarium score below 2-2.5
- Selection of *T. gesneriana* cultivars with Fusarium score below 1.6
- Selecting of lines with field resistance to Botrytis and Fusarium score below 1.6 for crosses this season
- Selection based on cut-flower ornamental properties
- Waiting for next step in MAS
  - More markers/density
  - Haplotypes

Mapping of traits: How to continue

- Validation QTLs is a problem (no inbreeding)
- Size of QTL
- Dominant genes:
  - Flanking markers
  - No IBD

Solutions:

- Second population
  - GWAS like
- Co-localisation of CG
- Metabolites



Candidate gene approach - Gerbera

Cuticular defects lead to full immunity to a major plant pathogen

Céline Chassot*, Christiane Nawrath† and Jean-Pierre Métraux*

Constitutive expression of *ETHYLENE-RESPONSE-FACTOR1* in *Arabidopsis* confers resistance to several necrotrophic fungi

Disease resistance results from foreign phytoalexin expression in a novel plant

Rüdiger Hain*, Hans-Jörg Reif*, Elvira Krause*, Ruth Langebartels†, Helmut Kindl†, Barbara Vornam‡§, Wilfried Wiese‡, Elmon Schmelzer‡, Peter H. Schreier*, Ronald H. Stöcker* & Klaus Stenzel*

ORIGINAL ARTICLE

Polyketide derivatives active against *Botrytis cinerea* in *Gerbera hybrida*

Satu Koskela · Päivi P. Söderholm · Mila Ainasoja · Tero Wennberg · Karel D. Klika · Vladimir V. Ovcharenko · Irene Kylänlahti · Tiina Auerma · Jari Yli-Kauhaluoma · Kalevi Pihlaja · Pia M. Vuorela · Teemu H. Teeri

CGs approach procedure

1. Find the homologous gene in gerbera

Query= gi|13276208|emb|Z38097.2| Gerbera hybrida mRNA for 2-pyrone synthase

```
Query= gi|13276208|emb|Z38097.2| Gerbera hybrida mRNA for 2-pyrone synthase

Length=1555

                                                          Score     E
Sequences producing significant alignments:               (Bits)  Value

→ Contig11873                                              2710    0.0
  Contig29243                                               544    1e-153
  Contig13289                                               503    2e-141
```


2. Look for SNP in gerbera homolog CG
   - develop SNP marker

	6086	6637	6727	6728
PS	G (27)	G/C (19/8)	A/C (18/5)	T/G (18/5)
CK	A (37)	T (36)	C (24)	G (23)
KK	A/G (17/14)	T/C (19/8)	C/A (14/14)	T/G (14/14)
LL	G (31)	T (35)	C (12)	G (12)

3. Mapping CGs in gerbera populations

4. Look for co-localisation

5. CGs found to co-localise with QTLs

Breeding in ornamentals

With linked makers from bi-parental population difficult to extrapolate to breeding pool

- Multiple populations help – Lily
- Inbreeding sometimes possible – Gerbera
- Candidate genes very useful – Gerbera, difficult in tulip
- Transcriptome sequencing – Lily, chrysanthemum

Acknowledgements

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Paul Bijman
Sjaak van Heusden
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