



Faculty of Science



Kan genmodificering bidrage til en mere bæredygtig konventionel og økologisk landbrugsproduktion?

Michael Broberg Palmgren

Professor ved Institut for Plante- og Miljøvidenskab
Københavns Universitet



Feasibility of new breeding techniques for organic farming

Martin Marchman Andersen¹, Xavier Landes¹, Wen Xiang², Artem Anyshchenko², Janus Falhof³, Jeppe Thulin Østerberg³, Lene Irene Olsen³, Anna Kristina Edenbrandt⁴, Suzanne Elizabeth Vedel⁴, Bo Jellesmark Thorsen^{4,5}, Peter Sandøe^{4,6}, Christian Gamborg⁴, Klemens Kappel¹, and Michael G. Palmgren³

¹Department of Media, Cognition and Communication, University of Copenhagen, Karen Blixens Vej 4, DK-2300 Copenhagen S, Denmark

²Centre for Public Regulation and Administration, Faculty of Law, University of Copenhagen, Studiestræde 6, DK-1455 Copenhagen K, Denmark

³Center for Membrane Pumps in Cells and Disease - PUMPKIN, Danish National Research Foundation, Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

⁴Department of Food and Resource Economics, University of Copenhagen, Rolighedsvej 23, DK-1958 Frederiksberg C, Denmark

⁵Center for Macroecology, Evolution and Climate, University of Copenhagen, Rolighedsvej 23, DK-1958 Frederiksberg C, Denmark

⁶Department of Large Animal Sciences, University of Copenhagen, DK-1870 Frederiksberg C, Denmark

Organic farming is based on the concept of working 'with nature' instead of against it; however, compared with conventional farming, organic farming reportedly has lower productivity. Ideally, the goal should be to narrow this yield gap. In this review, we specifically discuss the feasibility of new breeding techniques (NBTs) for rewilding, a process involving the reintroduction of properties from the wild relatives of crops, as a method to close the productivity gap. The most efficient

prohibited in organic production. As a consequence, productivity is often lower in organic than in conventional agriculture [1–4], and several strategies have been suggested to close this yield gap between high- and low-performing conventional systems [5–7]. A plea for merging organic agriculture and genetic engineering approaches has previously been published [8]. Here, we discuss the feasibility, in a broad sense, of introducing new methods of plant biotechnology to enable the sustainable intensifica-

Bioteknologi og planter

Hele vores jordbrug og ernæring
baserer sig på **mutanter**

**Traditionel forædling er et
meget stærkt værktøj**

**Det baserer sig på at skabe
genetisk variation (mutanter)
og derefter vælge blandt
afkommet**

**I traditional forædling opstår
variationen ved tilfældigheder.**

**CRISPR er et værktøj, der præcist og
målrettet skaber den variation, som
forædleren ønsker**

Nogle eksempler på 'traditionelle' mutanter

Brassica oleracea
(kål var. vild kål)

Brassica oleracea
(kål var. blomkål)

En mutant, som har svært ved at blomstre

Et gen kaldet *CAL* sørger for at danne blomster i vild kål, men det er gået i stykker halvvejs i blomkål. Det gør den store forskel.

CAL i vild kål	MGRGRVEMKRIENRINRQVTF SKRRAGLLKKAHEISILCDAEVSLIVFSE	50
CAL i blomkål	MGRGRVEMKRIENRINRQVTF SKRRAGLLKKAHEISILCDAEVSLIVFSE	50
CAL i vild kål	KGKLF EYSS ESCMEKVL EHYERYSYAEKQLKVPDSHVNAQTNWSVEYSRL	100
CAL i blomkål	KGKLF EYSS ESCMEKVL ERYERYSYAEKQLKAPDSHVNAQTNWSMEYSRL	100
CAL i vild kål	KAKIELLERNRHYLGEDLESISIKELQNLEQQQDTS LKHIRSRKNQLMH	150
CAL i blomkål	KAKIELWERNQRHYLGEDLESISIKELQNLEQQQDTALKHIRSRKNQLMH	150
CAL i vild kål	ESLNHLQRKEKEILEENSVLAKQIRERESILRTHQNQSEQQNRSHH VAPQ	200
CAL i blomkål		
CAL i vild kål	PQF.....PYMASSPFLNMGGMYQGEYPTAVRRNRLDLTLEPIYNCNLGYFAA	251
CAL i blomkål		

Mutation: E151→STOP

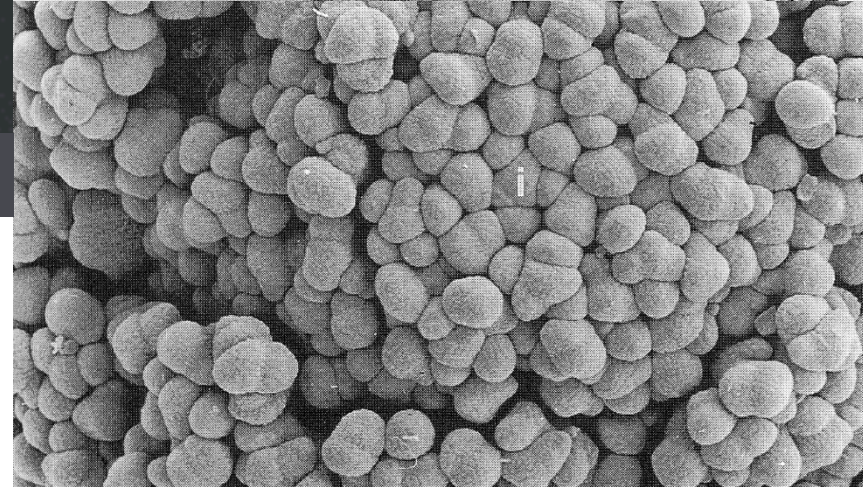
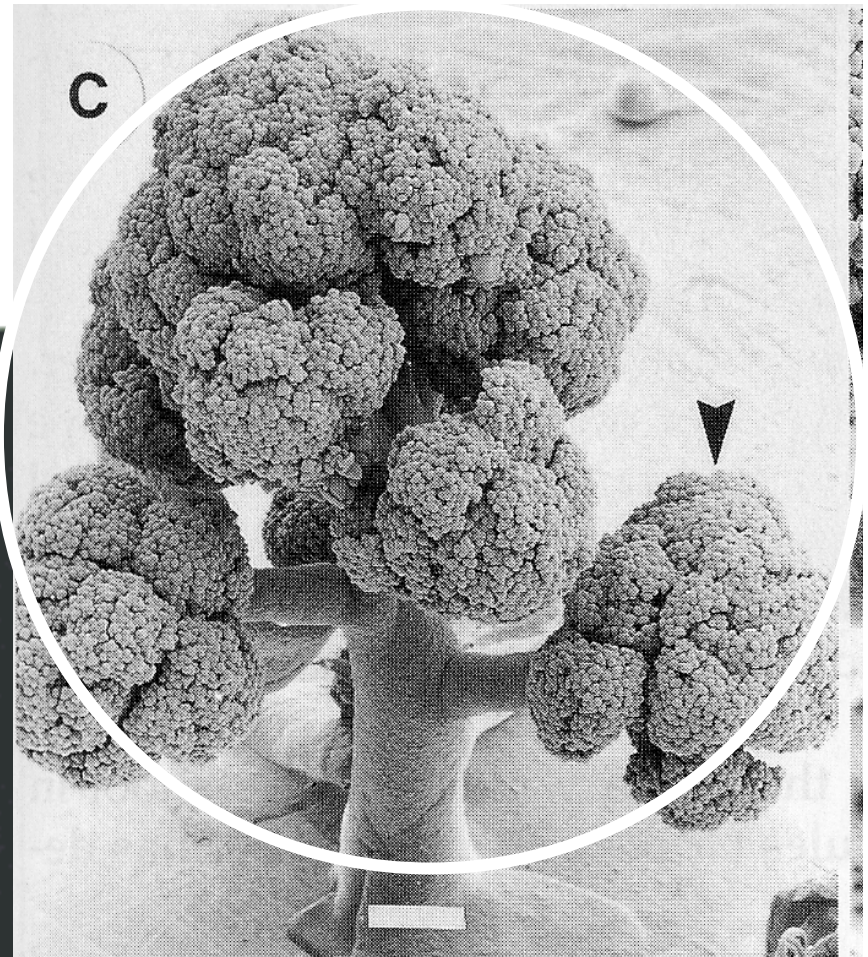
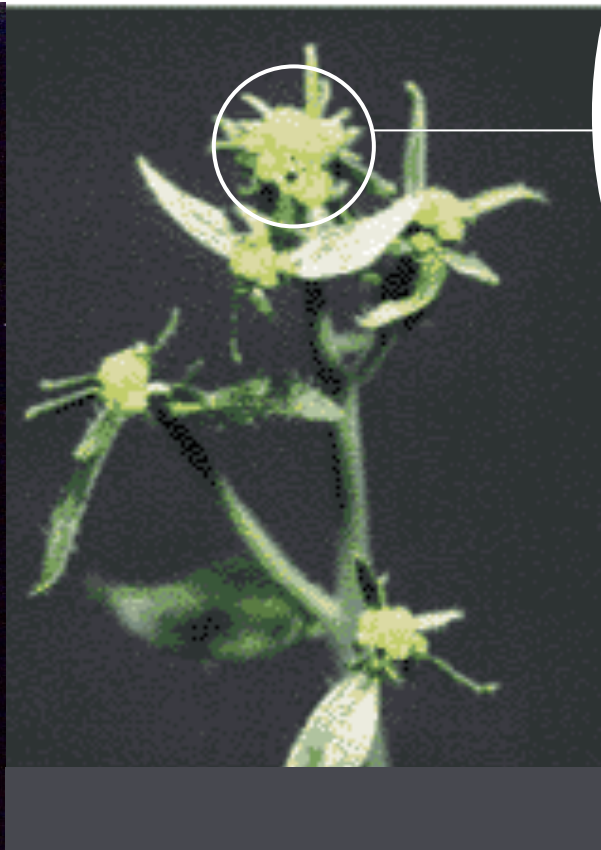
I blomkål er *CAL* genet muteret: Som et resultat mangler *CAL* proteinet de sidste 151 aminosyrer

Gåsemad

wildtype



cal, apt1 mutant

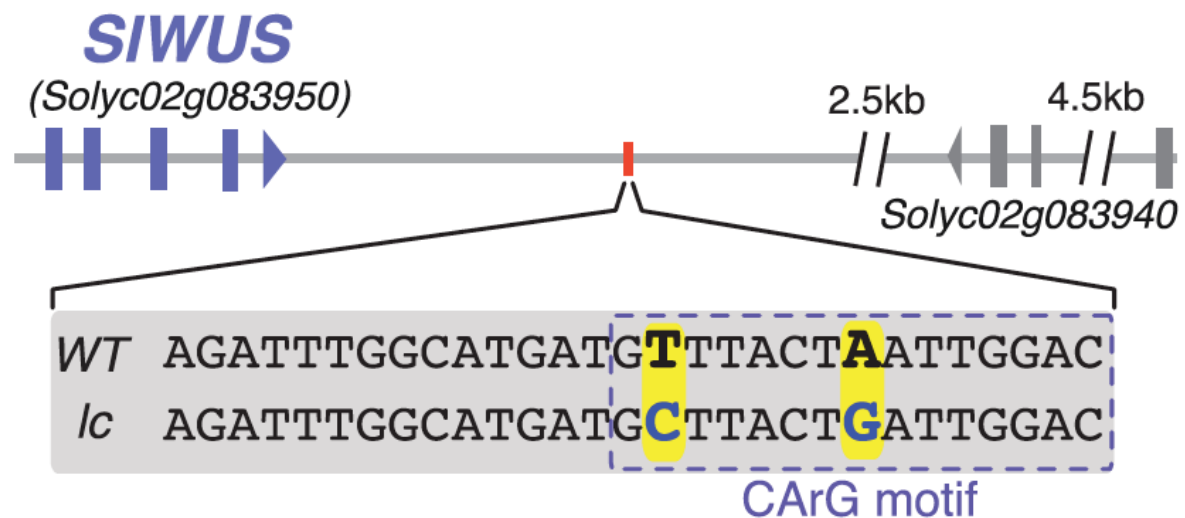
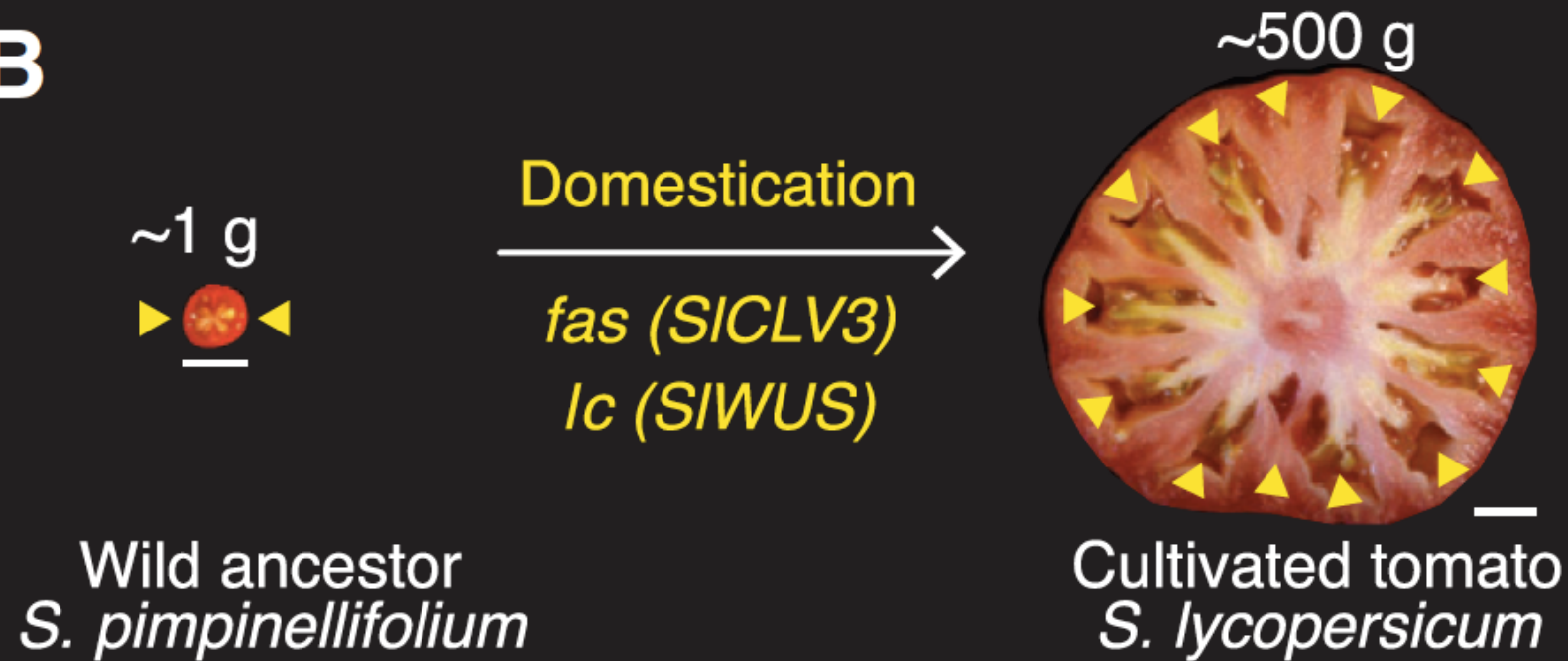


Ødelægges det tilsvarende *CAL* gen i en beslægtet plante, udvikler den en blomkålslignende struktur

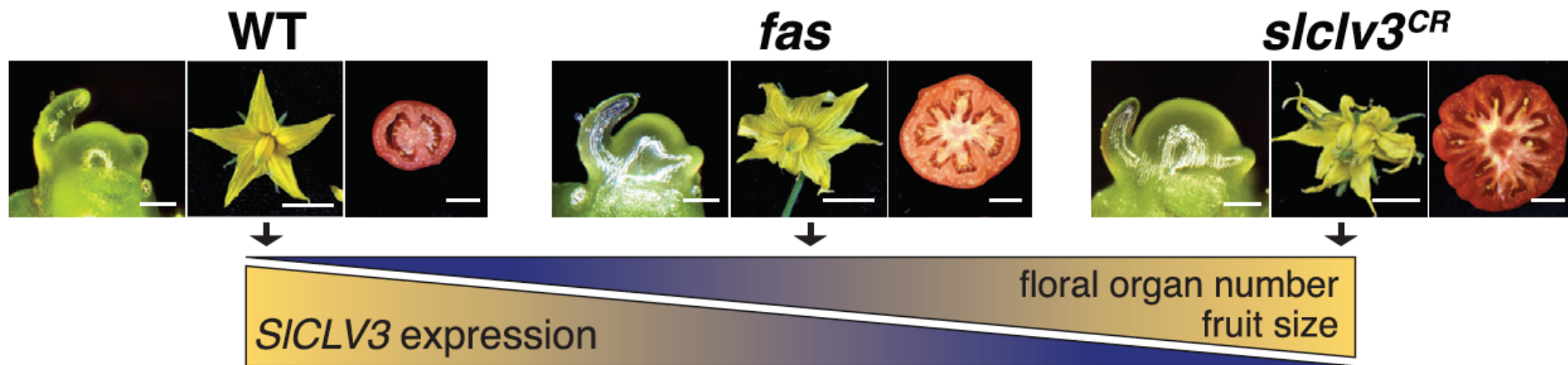
Bowman *et al.* (1993) *Development* 119:721-743

Spontane mutationer i to gener førte til den tomat vi kender i dag

B



Rodríguez-Leal *et al.*
(2017) *Cell* 171: 470-480.



Meget SICLV3 protein:

Små frugter

Få rum

Mangel på SICLV3 protein:

Store frugter

Mange rum

Rodríguez-Leal *et al.*
(2017) *Cell* 171: 470-480.

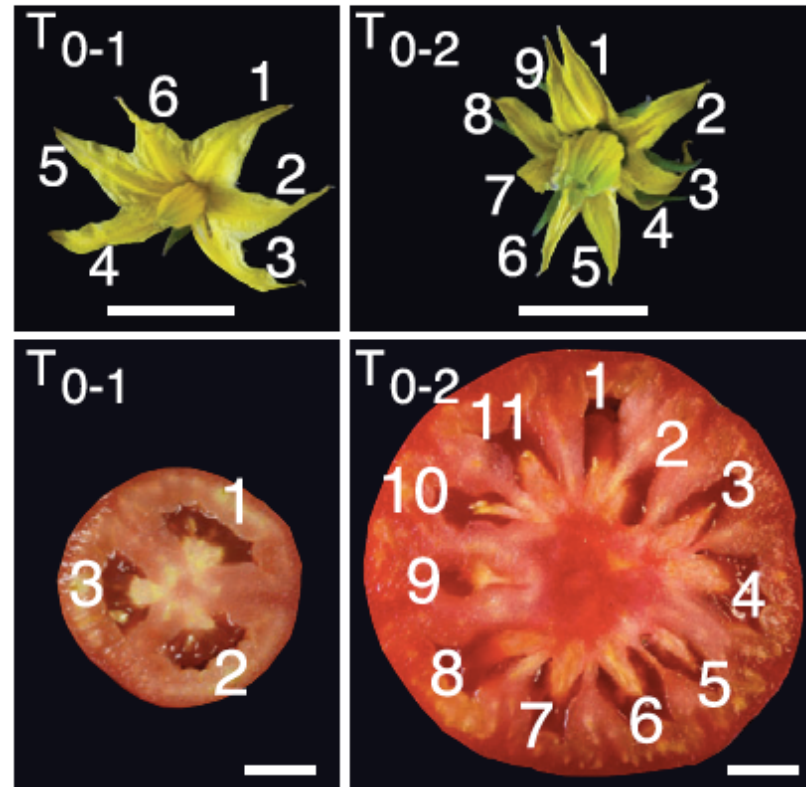
- Mange egenskaber, som var afgørende for vores afgrøders kultivering, skyldes tilfældige mutationer, der har givet tab af funktion
- Tab af funktion skyldes at et gen er blevet helt eller delvist ødelagt

**CRISPR er et værktøj, der meget præcist
skaber den variation, som forædleren ønsker**

CRISPR finder vej
Cas9 er saksen

CRISPR kan gå direkte ind og klippe et gen over,
og dermed kopiere en mutation, der giver tab af funktion

To forskellige mutanter af den vilde tomat lavet med CRISPR



CRISPR klipning kan
efterligne de mutationer
der førte til kultivering af
tomat

Rodríguez-Leal *et al.* (2017) Engineering
quantitative trait variation for crop
improvement by genome editing.
Cell 171: 470-480.

Et eksempel på, hvordan tab af
funktion ved hjælp af nye
forædlingsteknikker har gjort en
afgrøde mindre afhængig af
bekæmpelsesmidler

Gåsemad angrebet af svampen meldug (efter 10 dage)

Gåsemad angrebet af svampen meldug (efter 30 dage)

Meldug på hvede

Blumeria graminis

Meldugsvampen smager sig frem efter
MLO proteiner på plantecellens overflade



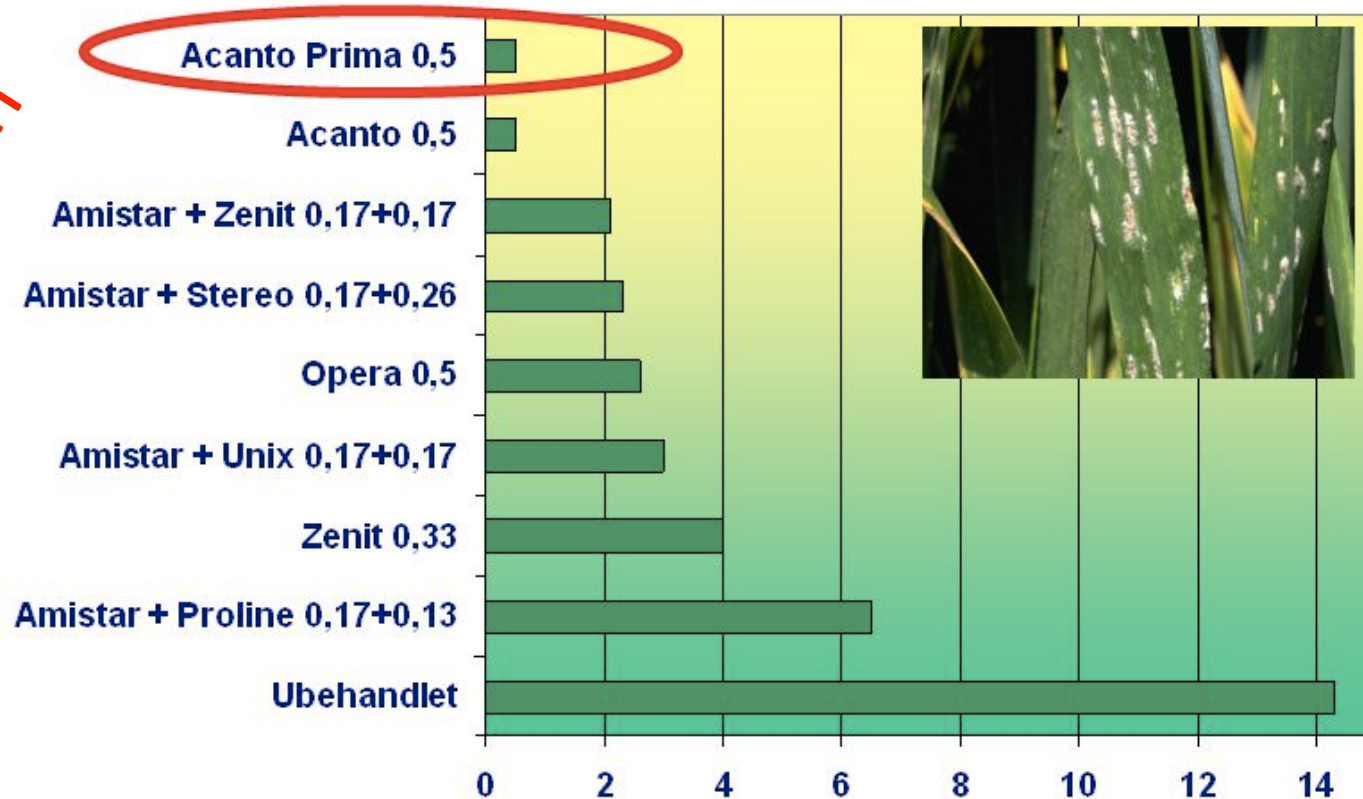
Hvede har tre *MLO* gener, der hver koder for et MLO
protein, som svampen kan genkende

I konventionelt jordbrug bekæmpes angreb af meldug med sprøjtemidler



Høj effekt mod meldug i byg

REKLAME



Vårbyg 1 fsg. DFJ 2004 (04342)
Sebastian, spr. 28/5 st. 32

% mildug gs. 71

syngenta

2017

LandbrugsAvisen.dk | Nyhedsbreve | Om os | Annoncering | LandbrugsAvisens e-avis

 Log ind



Derfor Plus:

“ Som abonnent på Mark PLUS får du adgang til fagmagasinet Mark som e-magasin



Søg her



Nye svampemidler kan øge udbyttet i vinterhvede med 7-10 hkg pr. hektar

Resistente svampe giver store tab, da de midler, der er til rådighed på det danske marked, har for ringe effekt.

26. jan 2017 | 00:00 | 

Skrevet af [Lars Kelstrup](#) | 



Seneste fra konsulenterne



Forstå dine efterafgrøder på fem minutter

23. apr | 10:50 | Konsulent 

2014: Hvede fik klippet alle sine
MLO gener i stykkerSimultaneous editing of three homoeoalleles in
hexaploid bread wheat confers heritable resistance
to powdery mildewYanpeng Wang^{1,3}, Xi Cheng^{2,3}, Qiwei Shan¹, Yi Zhang¹, Jinxing Liu¹, Caixia Gao¹ & Jin-Long Qiu²

Sequence-specific nucleases have been applied to engineer targeted modifications in polyploid genomes¹, but simultaneous modification of multiple homoeoalleles has not been reported. Here we use transcription activator–like effector nuclease (TALEN)^{2,3} and clustered, regularly interspaced, short palindromic repeats (CRISPR)-Cas9 (refs. 4, 5) technologies in hexaploid bread wheat to introduce targeted mutations in the three homoeoalleles that encode MILDEW-RESISTANCE LOCUS (MLO) proteins⁶. Genetic redundancy has prevented evaluation of whether mutation of all three *MLO* alleles in bread wheat might confer resistance to powdery mildew, a trait not found in natural populations⁷. We show that TALEN-induced mutation of all three *TaMLO* homoeologs in the same plant confers heritable broad-spectrum resistance to powdery mildew. We further use CRISPR-Cas9 technology to generate transgenic wheat plants that carry mutations in the *TaMLO-A1* allele. We also demonstrate the feasibility of engineering targeted DNA insertion in bread wheat through nonhomologous end joining of the double-strand breaks caused by TALENs. Our findings provide a methodological framework to improve polyploid crops.

breaks, which are then repaired mainly by either error-prone non-homologous end joining (NHEJ) or high-fidelity homologous recombination¹¹. All three types of SSN have been used to create targeted gene knockouts in various plant species¹². Although heritable gene modification has been demonstrated in *Arabidopsis*^{13–15} and rice¹⁶ using the SSNs, germline transmission of a mutation created by such genome editing strategies has not yet been achieved in hexaploid bread wheat. We have previously demonstrated genome editing in bread wheat using a transient protoplast expression system¹⁷. Here we report the use of TALENs and the CRISPR-Cas9 system to modify an endogenous wheat gene and produce newly introduced, stably transmitted genetic traits. Our findings underscore the potential of SSNs as efficient tools for wheat research and breeding.

We chose to target three *MLO* loci, which encode proteins that were shown to repress defenses against powdery mildew diseases in other plants¹⁸. Loss-of-function *mlo* alleles in barley¹⁹, *Arabidopsis*²⁰ and tomato²¹ lead to broad-spectrum and durable resistance to the fungal pathogens that cause powdery mildew in these species. In wheat, powdery mildew is caused by *Blumeria graminis* f. sp. *tritici* (Bgt), which is one of the most destructive plant pathogens worldwide. Modification of *MLO* genes in wheat may provide the opportunity to breed varieties

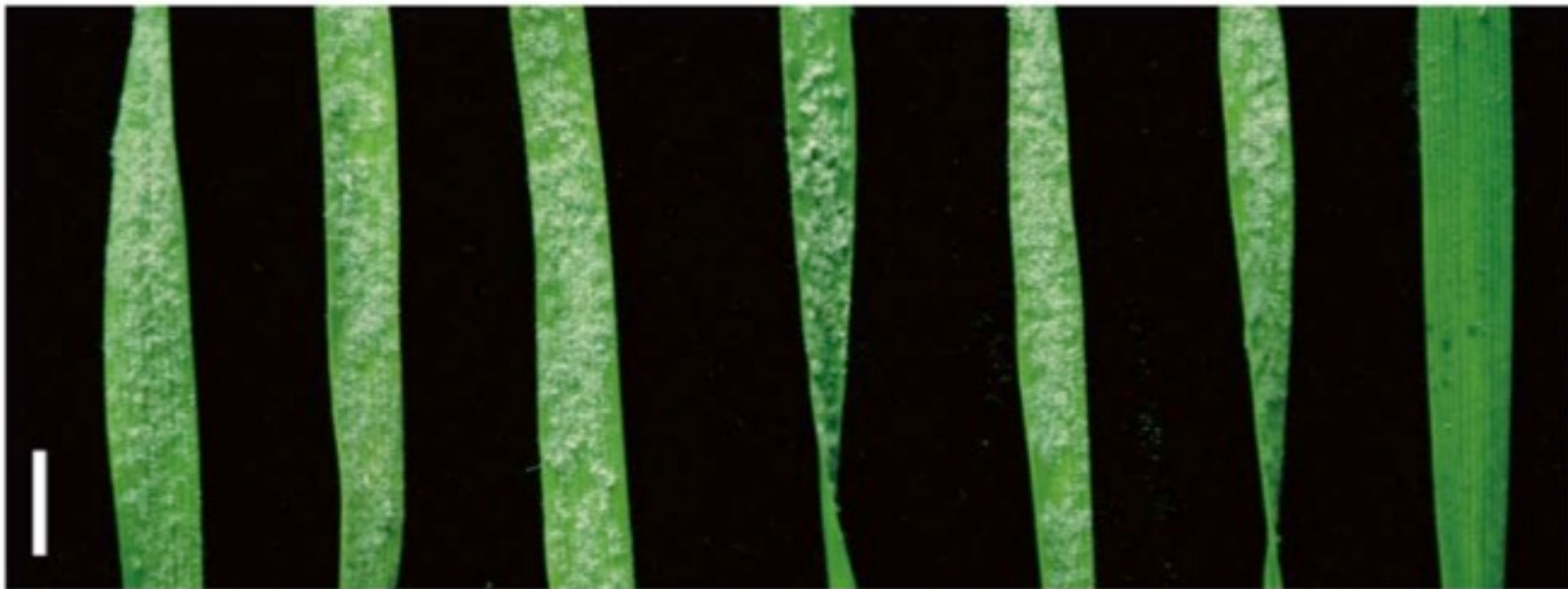
Mutationer i et af *MLO* generne fra hvede udført ved hjælp af CRISPR/Cas9

MLO-A1: TCGCTGCTGCTCGCCGTCACGCAGGACCCAATCTCCGGATATGCATCTCCCA
M3: TCGCTGCTGCTCGCCGTCA...AGGACCCAATCTCCGGATATGCATCTCCCA -3
M6: TCGCTGCTGCTCGCCGTCA.GCAGGACCCAATCTCCGGATATGCATCTCCCA -1
M11: TCGCTGCTGCTCGCCGTCA...GGACCCAATCTCCGGATATGCATCTCCCA -4
M13: TCGCTGCTGCTCGCCGTCA^TCGCAGGACCCAATCTCCGGATATGCATCTCCCA +1



Hvede med 3 klippede *MLO* gener – *A*, *B* og *D* - er resistent overfor meldug og behøver ikke sprøjtes

	kun <i>A</i>	kun <i>B</i>	kun <i>D</i>	<i>A</i> og <i>B</i>	<i>A</i> og <i>D</i>	alle tre
WT	klippet	klippet	klippet	klippet	klippet	klippet



En historie om roser

Hvordan kan
fremtidig
kultivering af roser
gøres bæredygtig?

Vild rose (*Rosa hybrida*)

Mutant rose (*Rosa hybrida*)

Fem kronblade;
mange støvblade

Mange kronblade;
få eller ingen støvblade

Tinkering with the C-Function: A Molecular Frame for the Selection of Double Flowers in Cultivated Roses

Annick Dubois¹, Olivier Raymond¹, Marion Maene¹, Sylvie Baudino², Nicolas B. Langlade³, Véronique Boltz¹, Philippe Vergne¹, Mohammed Bendahmane^{1*}

¹ Repro
Médic

tiques et

Den kultiverede rose bærer på en defekt i genet *AGAMOUS*, og som et resultat bliver støvblade til kronblade

raits
tals
hat

Methodology/Principal Findings: We have analyzed the expression of several candidate genes known to be involved in floral organ identity determination in roses from similar genetic backgrounds but exhibiting contrasting petal numbers per flower. We show that the rose ortholog of *AGAMOUS* (*RhAG*) is differentially expressed in double flowers as compared to simple flowers. In situ hybridization experiments confirm the differential expression of *RhAG* and demonstrate that in the double-flower roses, the expression domain of *RhAG* is restricted toward the center of the flower. Conversely, in simple-flower roses, *RhAG* expression domain is wider. We further show that the border of *RhAG* expression domain is labile, which allows the selection of rose flowers with increased petal number. Double-flower roses were selected independently in the two major regions for domestication, China and the peri-Mediterranean areas. Comparison of *RhAG* expression in the wild-type ancestors of cultivated roses and their descendants both in the European and Chinese lineages corroborates the correlation between the degree of restriction of *RhAG* expression domain and the number of petals. Our data suggests that a restriction of *RhAG* expression domain is the basis for selection of double flowers in both the Chinese and peri-Mediterranean centers of domestication.

Conclusions/Significance: We demonstrate that a shift in *RhAG* expression domain boundary occurred in rose hybrids, causing double-flower phenotype. This molecular event was selected independently during rose domestication in Europe/Middle East and in China.

Citation: Dubois A, Raymond O, Maene M, Baudino S, Langlade NB, et al. (2010) Tinkering with the C-Function: A Molecular Frame for the Selection of Double Flowers in Cultivated Roses. PLoS ONE 5(2): e9288. doi:10.1371/journal.pone.0009288

Editor: Dorian Q. Fuller, University College London, United Kingdom

Hvis det tilsvarende gen ødelægges i modelplanten
gåsemand, bliver blomsterne også fyldte

Vildtype



agamous



Agamos (på græsk): ugift

Diplocarpon rosae er en svamp der forårsager
rosensygdommen **stråleplet**



A photograph of a wild rose plant with green, serrated leaves and a few small, reddish buds. The plant is growing against a light-colored, textured wall.

Vild rose

A photograph of a cultivated rose plant with many thin, dark, thorny stems. Some stems have small, yellowish-orange flowers. The plant is growing in a garden bed with a wooden fence and a stone wall in the background.

Kultiveret rose

Stråleplet angriber kun den kultiverede rose – ikke den vilde



Produkter / Baymat Ultra Rosensprøjtemiddel

Like 2 Tweet Gem

Baymat® Ultra – Rosensprøjtemiddel 100ml

Baymat Ultra Rosensprøjtemiddel anvendes til bekæmpelse af meldug, rust og stråleplet på roser i væksthuse og på friland.

Påsprøjtes ved begyndende angreb og har både helbredende og forebyggende virkning. Anvend havesprøjte. Baymat Ultra Rosensprøjtemiddel er normalt skånsomt over for de fleste planter.

Fordele

Blandes med vand og sprøjtes ud.

Registreringsinformation

Registreringsnavn: Baymat Ultra Rosensprøjtemiddel

Registreringsnummer: 18-403



Hvornår kan produktet anvendes



● Optimal anvendelse

● Mulig anvendelse

Hvor kan produktet anvendes

- Terrasse
- Buske
- Blomsterbed

Sammensætning

- Syntetisk

Kategori

- Svampemiddel

Formål

- Bekæmpe
- Forhindre
- Beskytte

Type

- Flydende
- Koncentrat

Indeholder/Antal

- 100 ml

Aktive stoffer

- Tebuconazole 43g/l

Du kan også være interesseret i disse produkter



8. Table 2. List of active substances identified in Sweden to meet the “cut-off” criteria in Annex II 3.6-3.7 adopted by the Council and may therefore not be approved

Active substance	Status Annex I 91/414/	Stage	“cut-off criteria” CMR/ED/PBT/vPvB without a “negligible exposure”	#
<i>Herbicides</i>				
Amitrol	Annex I	1	ED	1
Ioxynil	Annex I	1	ED	2
Glufosinate	Annex I	2	CMR	3
Linuron	Annex I	1	CMR/ED	4
Molinate	Annex I	1	ED	5
Pendimethalin	Annex I	1	PBT	6
Tepraloxym	Annex I	New	ED	7
Tralkoxydim	Annex I	3	ED	8
<i>Fungicides</i>				
Carbendazim	Annex I	1	CMR	9
Dinocap	Annex I	1	CMR	10
Epoxiconazole	Annex I	3	ED	11
Flumioxazin	Annex I	New	CMR	12
Flusilazole	Annex I	1	CMR/ED	13
Iprodion	Annex I	1	ED	14
Mancozeb	Annex I	1	ED	15
Maneb	Annex I	1	ED	16
Metconazole	Annex I	3	ED	17
Quinoxifen	Annex I	New	vPvB/POP?	18
Tebuconazole	Pending	3	ED	19

ED – The EU Commission has defined endocrine disruptors ED as: “Endocrine disruptors are exogenous substances that alter function(s) of the endocrine system and consequently cause adverse health effects in an intact organism, or its progeny, or (sub)populations.”²

Hvorfor er den kultiverede rose så
modtagelig for stråleplet?

Hvorfor er den vilde rose så
modstandsdygtig?

Naturen giver forklaringen

Original Article

The TNL gene *Rdr1* confers broad-spectrum resistance to *Diplocarpon rosae*

Ina Menz, Jannis Straube, Marcus Linde, Thomas Debener Accepted manuscript online: 5 August 2017 [Full publication history](#)DOI: 10.1111/mpp.12589 [View/save citation](#)Cited by (CrossRef): 0 articles  [Check for updates](#)  [Citation tools](#) ▼

Summary

Black spot disease, which is caused by the ascomycete *Diplocarpon rosae*, is the most severe disease in field-grown roses in temperate regions and has been distributed worldwide, likely along with commercial cultivars. Here, we present data supporting that *muRdr1A* is the active *Rdr1* gene, a single-dominant TIR-NBS-LRR (TNL) type resistance gene against black spot disease that acts against

Den vilde rose har et gen, der beskytter den mod stråleplet. Vi formoder at det er ødelagt i den kultiverede rose.

progeny and do not show any sign of overexpression. *Rdr1* provides resistance to 13 different single-spore isolates belonging to six different races and broad field mixtures of conidia; thus far, *Rdr1* is only overcome by two races. The

- Fremtidsperspektivet er at den vilde modstandsdygtige rose kan kultiveres forfra ved hjælp af præcisionsmutationer i *AGAMOUS* (og sikkert også andre gener, der bestemmer form og farve)
- Et andet perspektiv er at det bliver muligt at reparere resistensgenet *rdr1* i den kultiverede rose
- I begge tilfælde mindskes behovet for sprøjtemidler

Kernza

(flerårig)

(en bæredygtig
kornsort som
endnu ikke er
forædlet)

Flerårige
græsser skal ikke
sås hvert år, og
tager næring og
vand mere
effektivt til sig

Hvede

(enårig)

Snart vil vi kunne forædle flerårige kornsorter.
 Vores nuværende enårige kornsorter udviklede sig fra vilde planter
 som resultat af mutationer, der giver tab af funktion, og kan
 efterlignes i flerårige med CRISPR

Crop	Gene	Function	Trait	Mutation	Refs
Barley (<i>Hordeum vulgare</i>)	<i>Btr1/2</i>	Unknown	Brittle rachis	Shortened transcript ^a	[83]
	<i>Vrs1</i>	Transcription factor, HD-ZIP	Inflorescence structure	Shortened transcript	[84]
Maize (<i>Zea mays</i>)	<i>Tga1</i>	Transcription factor, SBP	Inflorescence structure	Amino acid change ^b	[85]
	<i>ZmSh1-1/1-5</i>	Transcription factor, YABBY-like	Seed shattering	Structural change/regulatory change ^{c,d}	[86]
Rice (<i>Oryza sativa</i>)	<i>LABA1</i>	Cytokinin-activating enzyme secreted peptide	Inflorescence structure	Shortened transcript	[26]
	<i>RAE2</i>	EPF/EPFL family	Inflorescence structure	Shortened transcript	[27]
	<i>sh1</i>	Transcription factor, YABBY-like	Seed shattering	Regulatory change ^d	[86]
	<i>sh4</i>	Transcription factor, Myb3	Seed shattering	Amino acid change ^d	[87]
	<i>qSH1</i>	Transcription factor, BEL1-type	Seed shattering	Regulatory change	[88]
	<i>PROG1</i>	Transcription factor, C2H2-type	Plant structure	Amino acid change	[89,90]

University of Copenhagen
Excellence Programme
"Plants for a changing world"

Deltagende fakulteter:
SCIENCE, SUND, HUM, JUR

Prof. Michael Palmgren
Dr. Anja Fuglsang
Prof. Peter Pagh
Prof. Søren Brøgger Christensen
Prof. John Nielsen
Prof. Klemens Kappel
Prof. Bo Jellesmark Thorsen
Dr. Christian Gamborg

