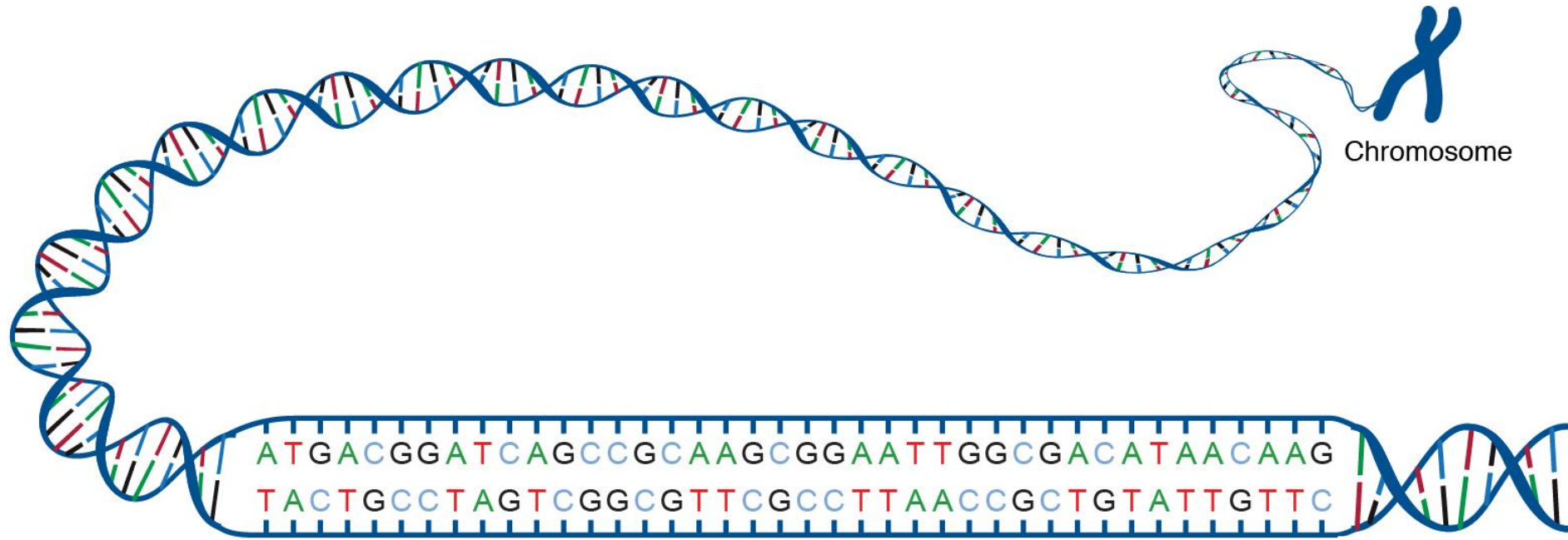


Editování genetické informace

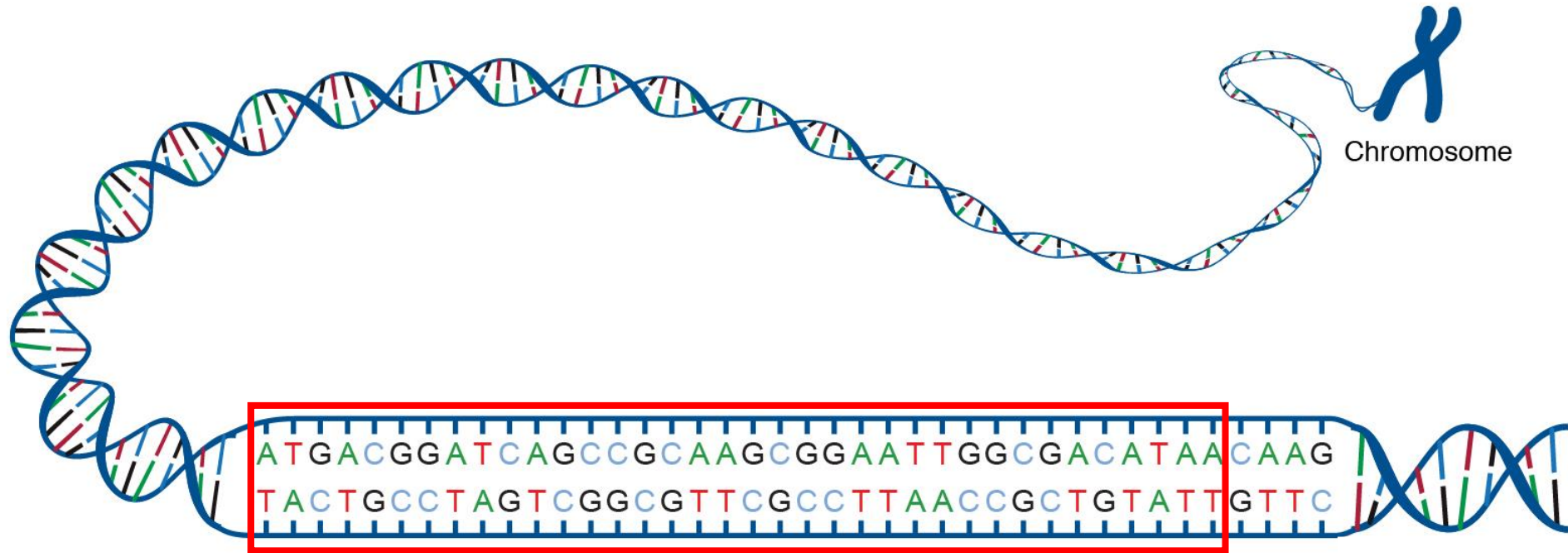


Vojta Hudzieczek
Biofyzikální ústav AV ČR, Brno

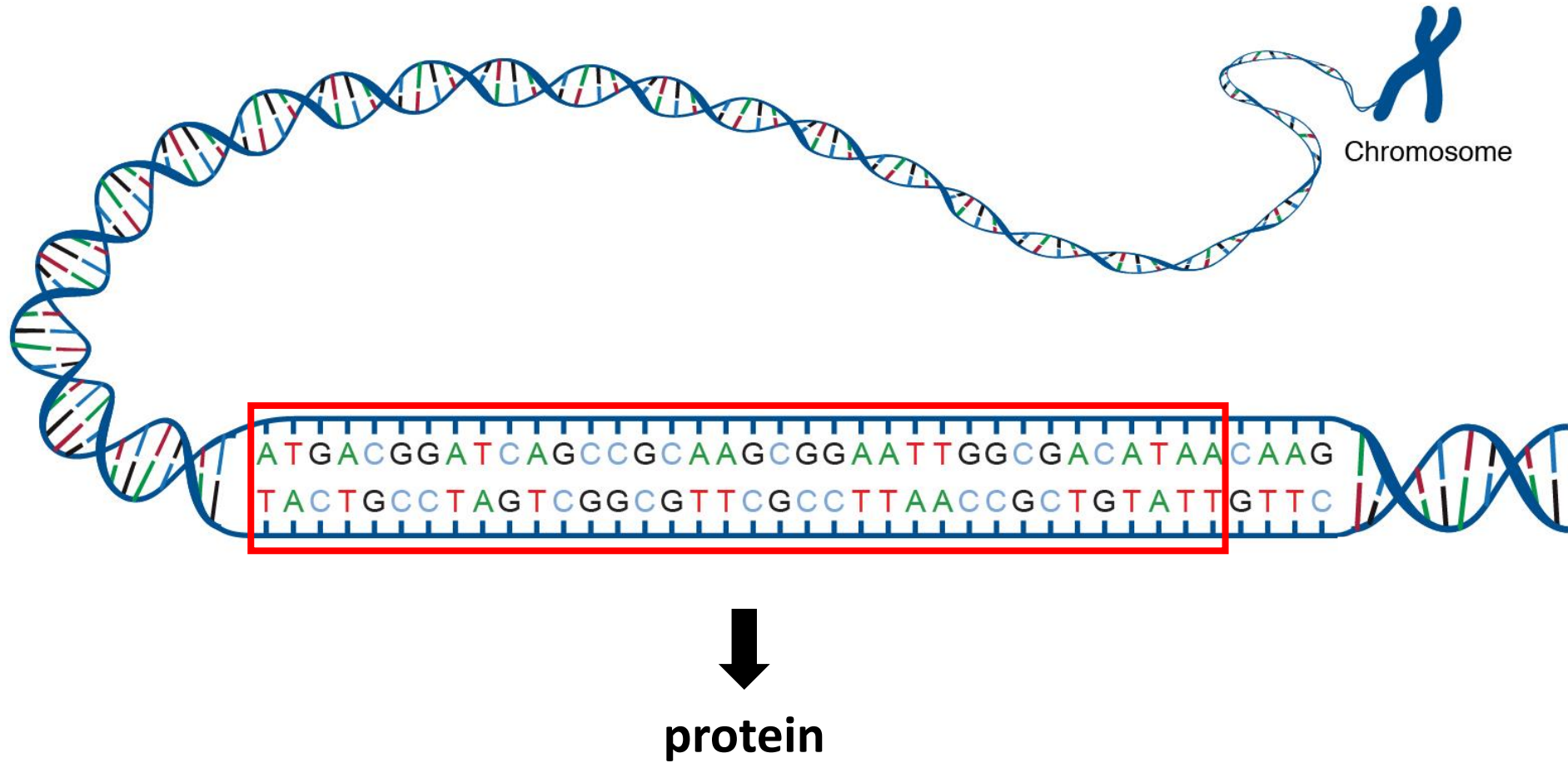
DNA a genetická informace



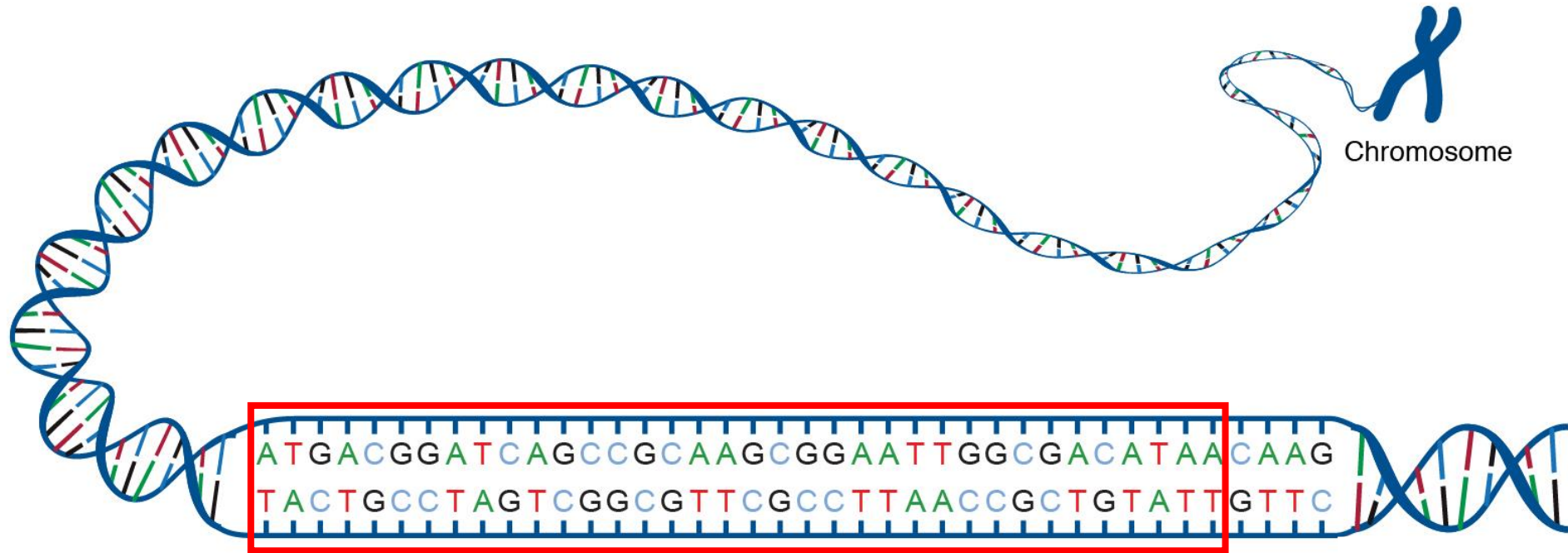
DNA a genetická informace



DNA a genetická informace



DNA a genetická informace

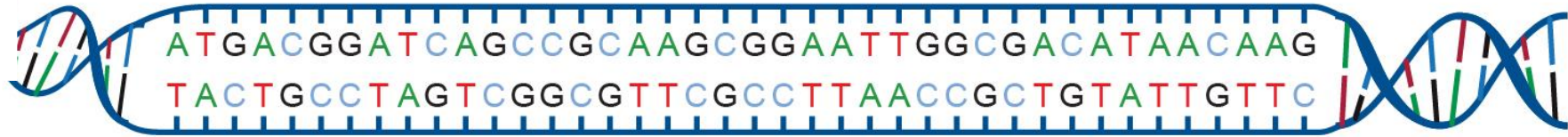


protein

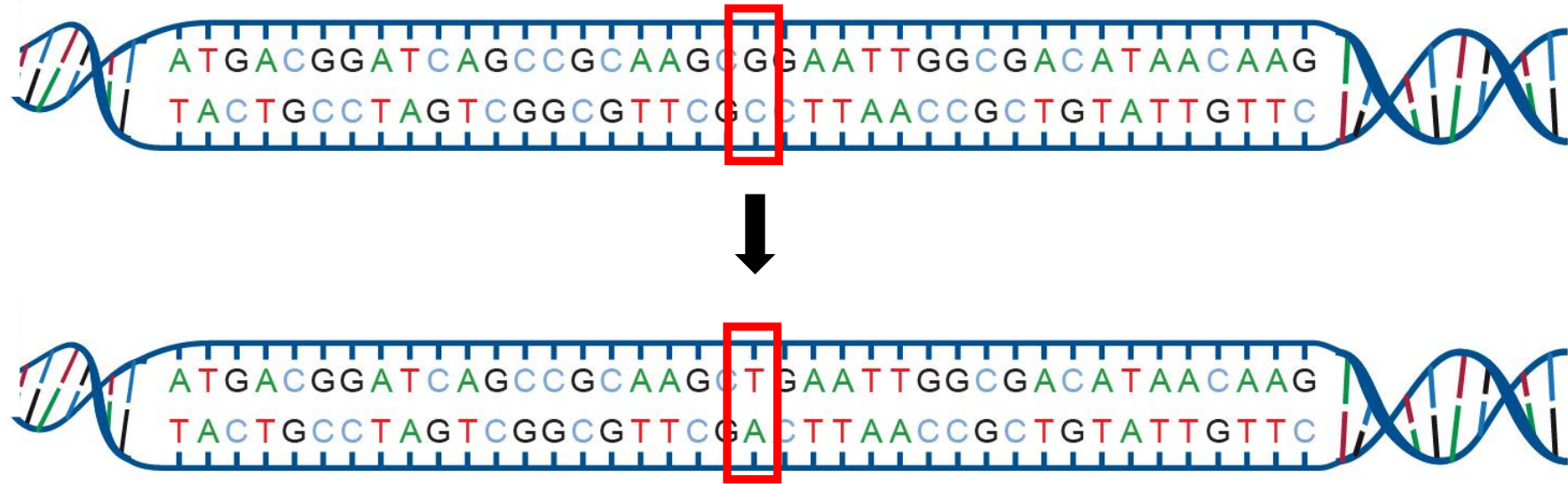


fenotyp

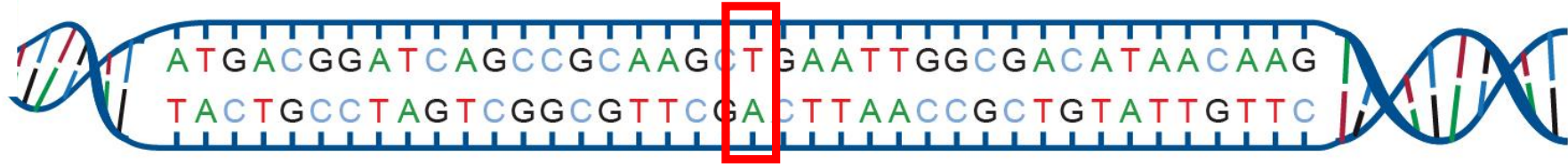
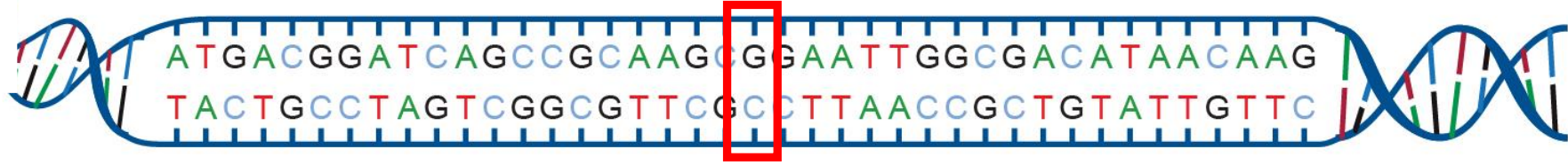
Změna genetické informace - mutace



Změna genetické informace - mutace



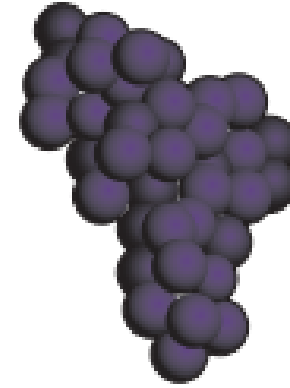
Změna genetické informace - mutace



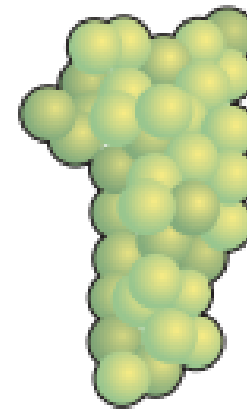
Mutace mohou vznikat spontánně či indukovaně a mohou mít různý rozsah i následky.

Změna genetické informace - mutace

Cabernet



Chardonnay

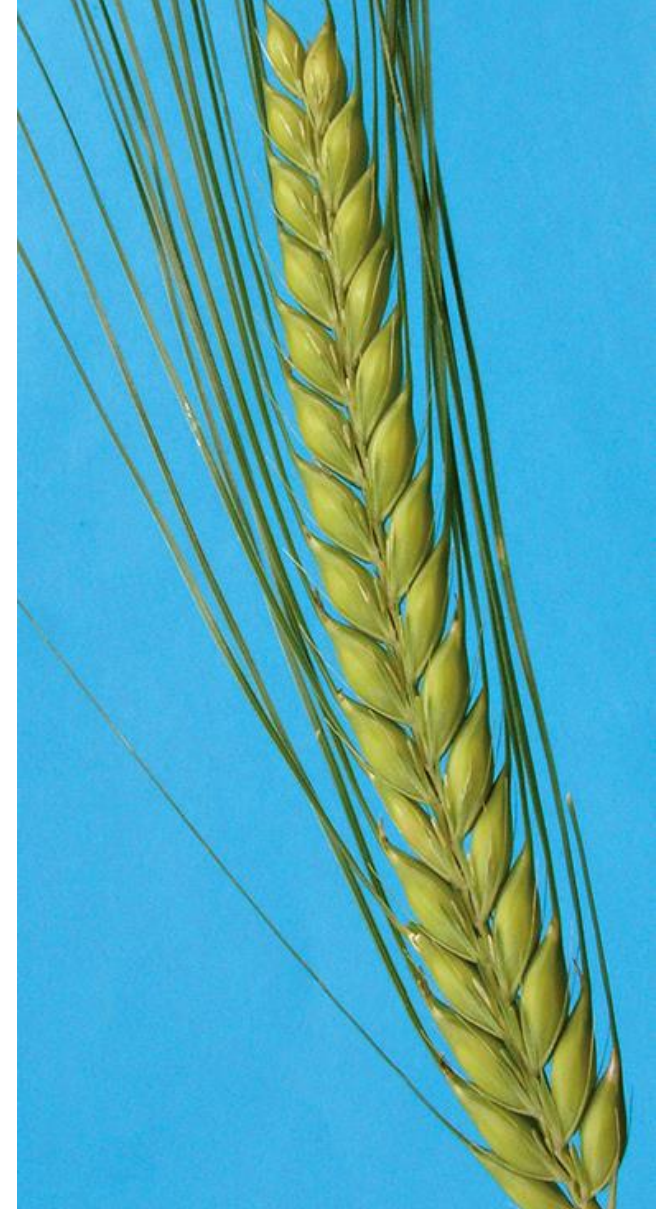


Bílé víno vzniklo před 7 tis. lety náhodnou mutací genu pro antokyan u původě červeného vína.

Šlechtění a genetická variabilita - mutageneze

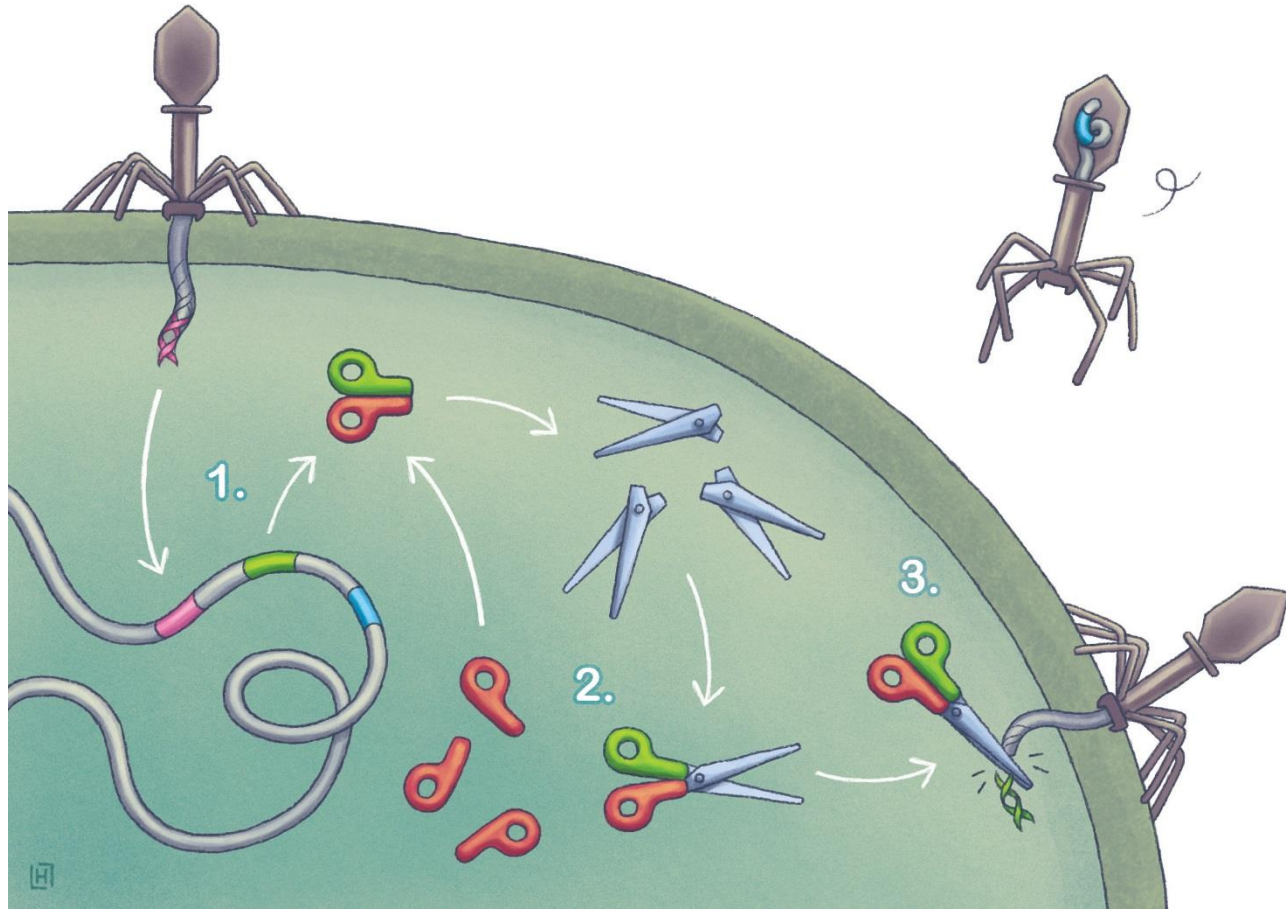


Šlechtění a genetická variabilita - mutageneze

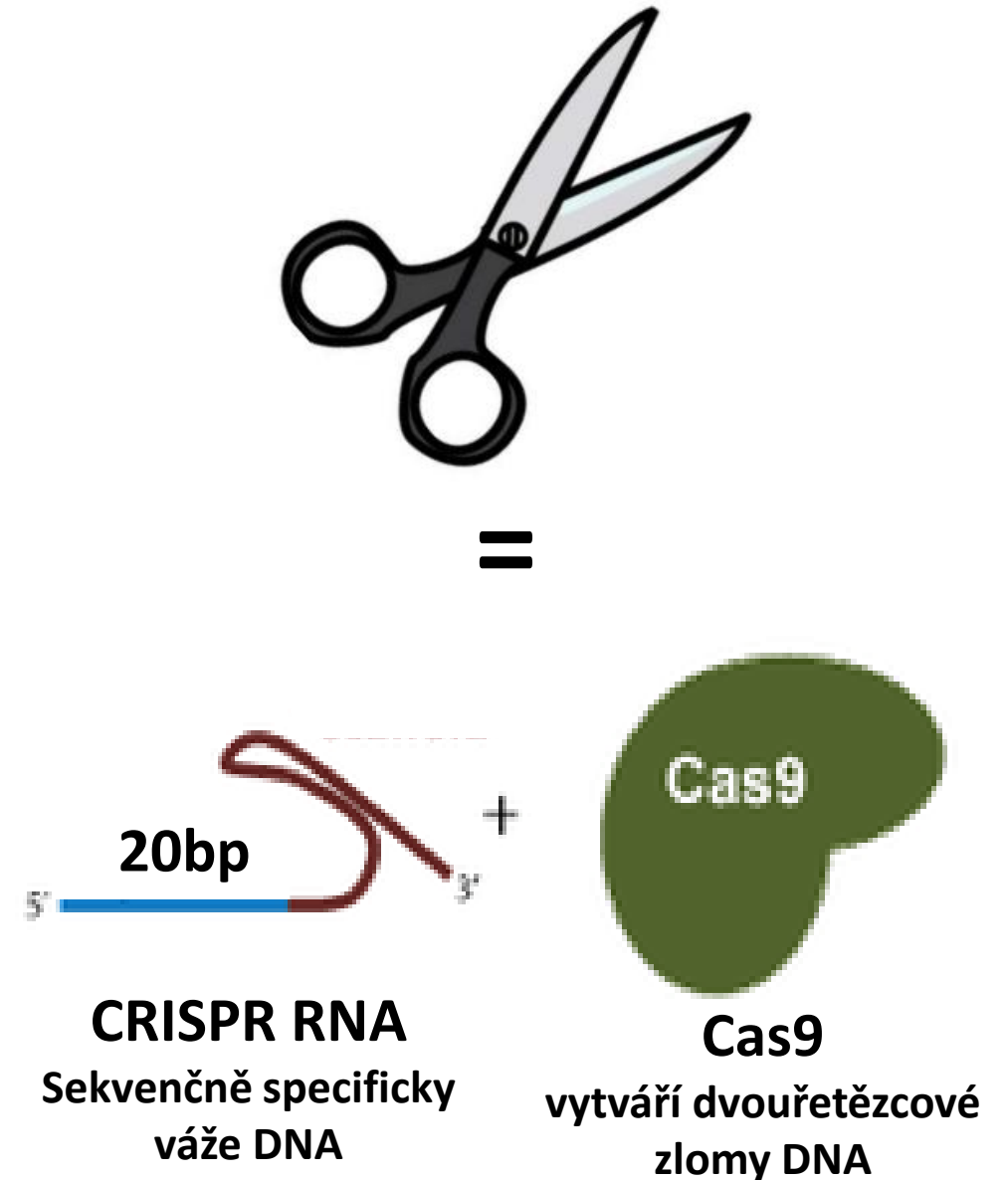
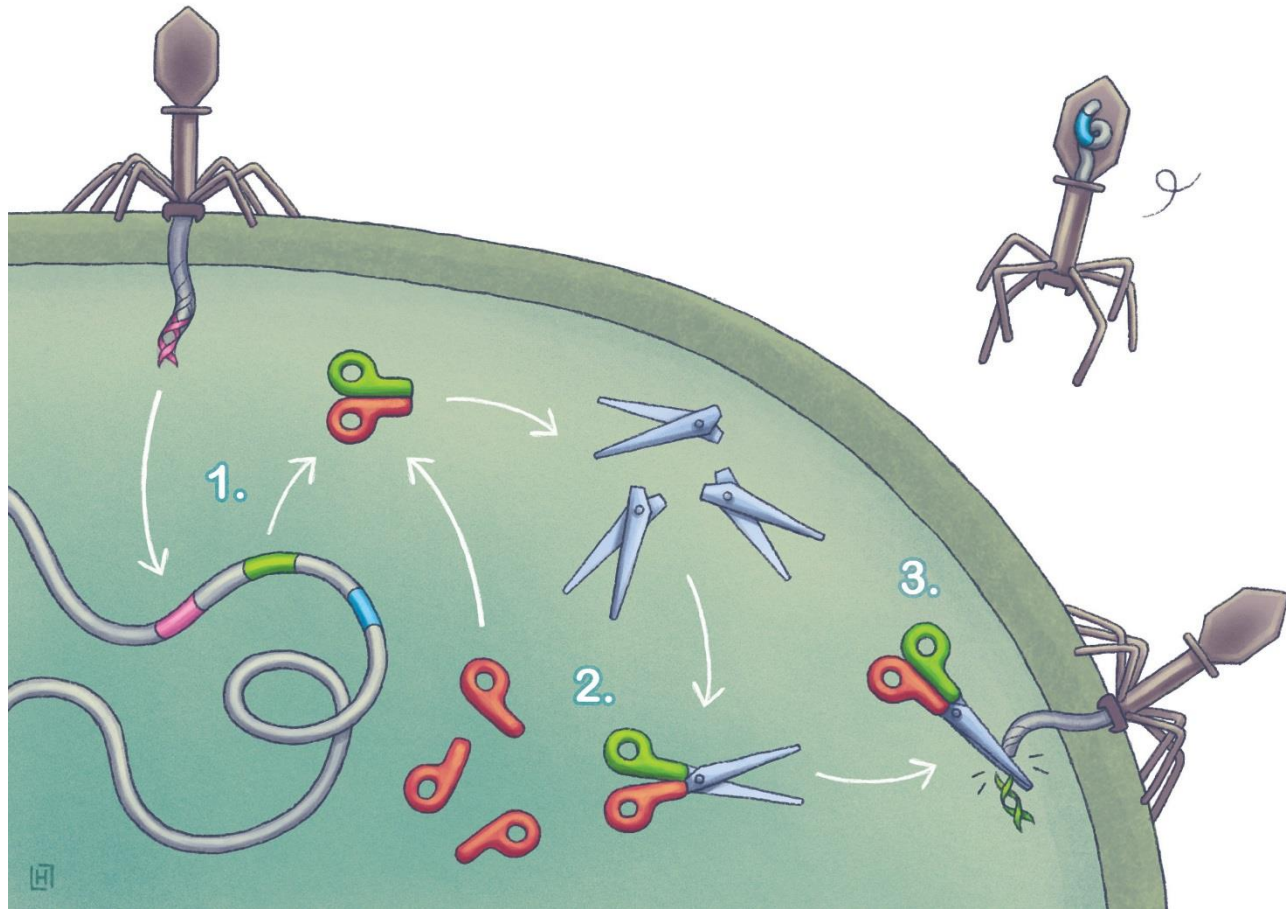


Cílená mutageneze - editace genomu

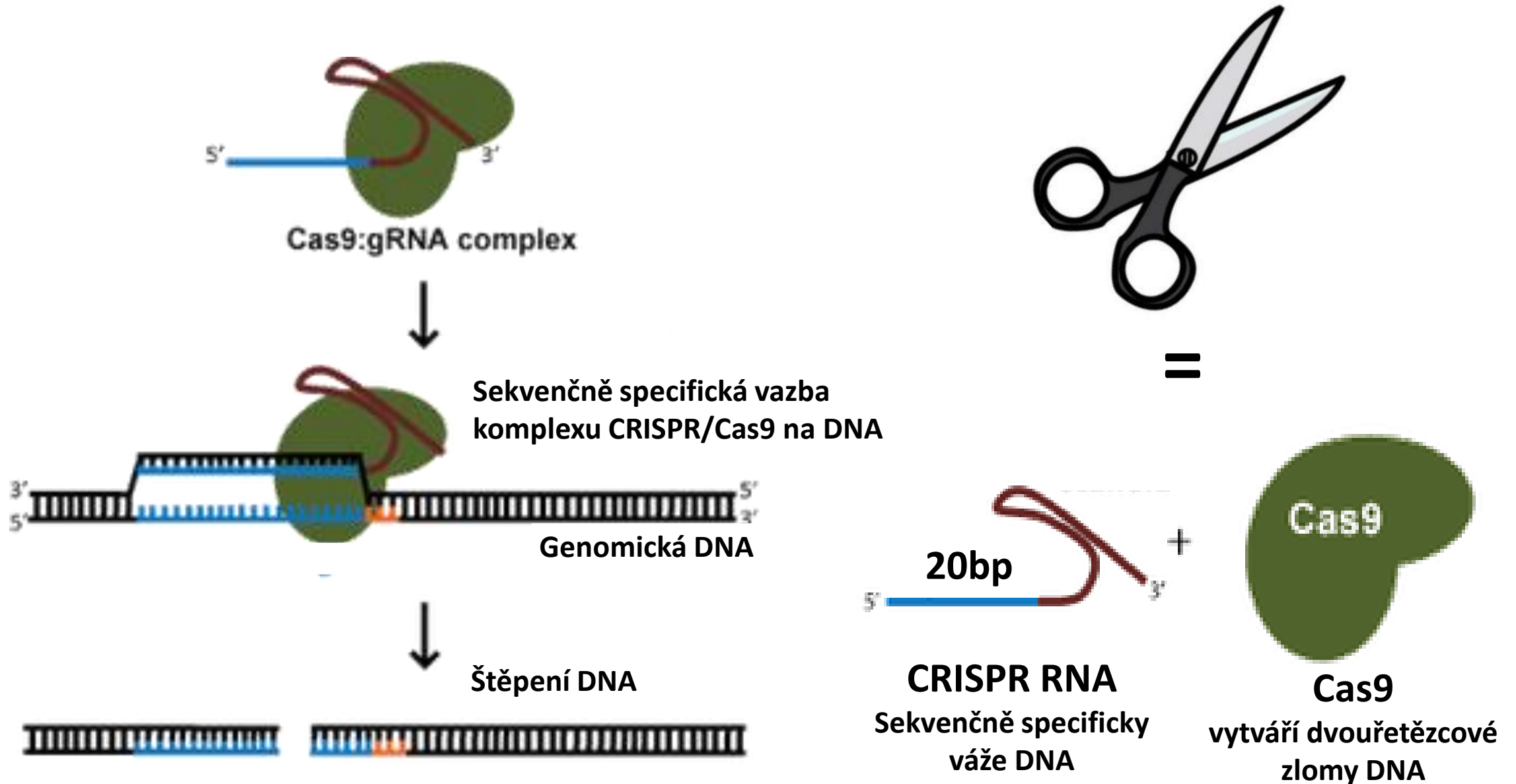
CRISPR/Cas9 nukleáza - "molekulární nůžky"



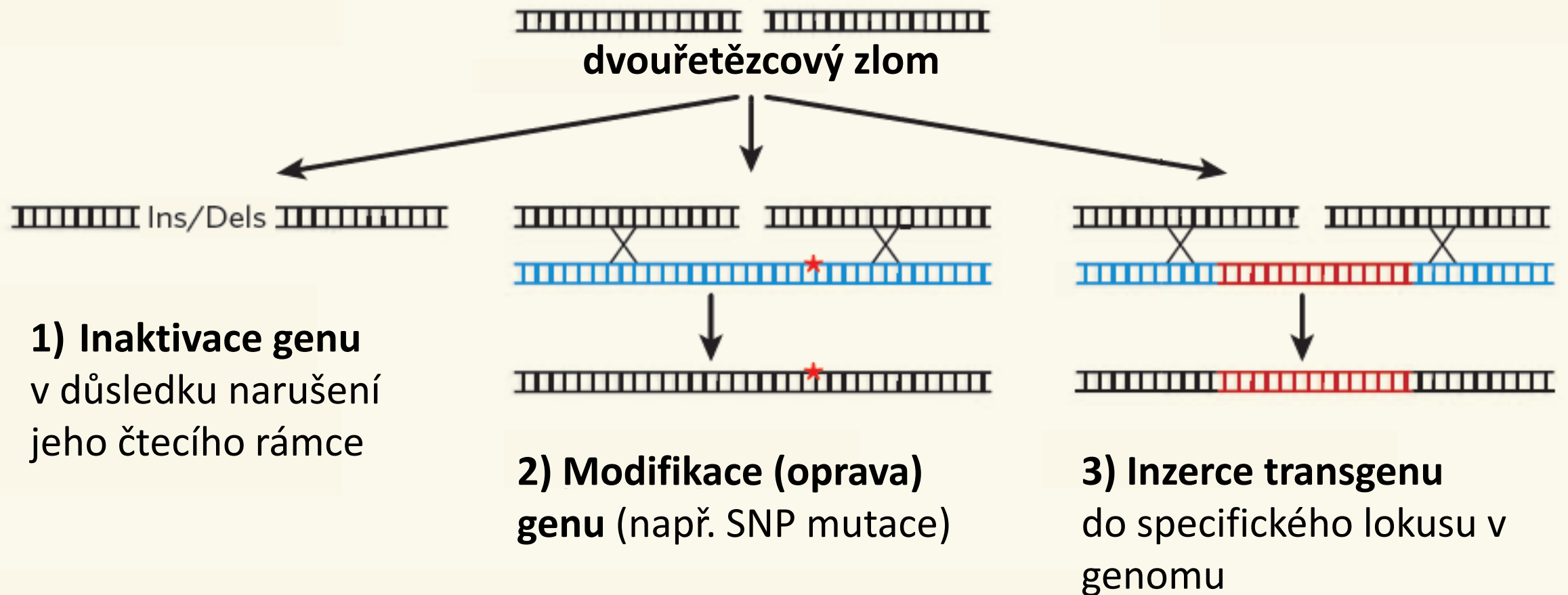
CRISPR/Cas9 nukleáza - "molekulární nůžky"



CRISPR/Cas9 nukleáza - "molekulární nůžky"



Cílená editace genomu rostlin



K čemu slouží editace genů?

1) Funkční analýzy – zjištění/ověření funkce genu

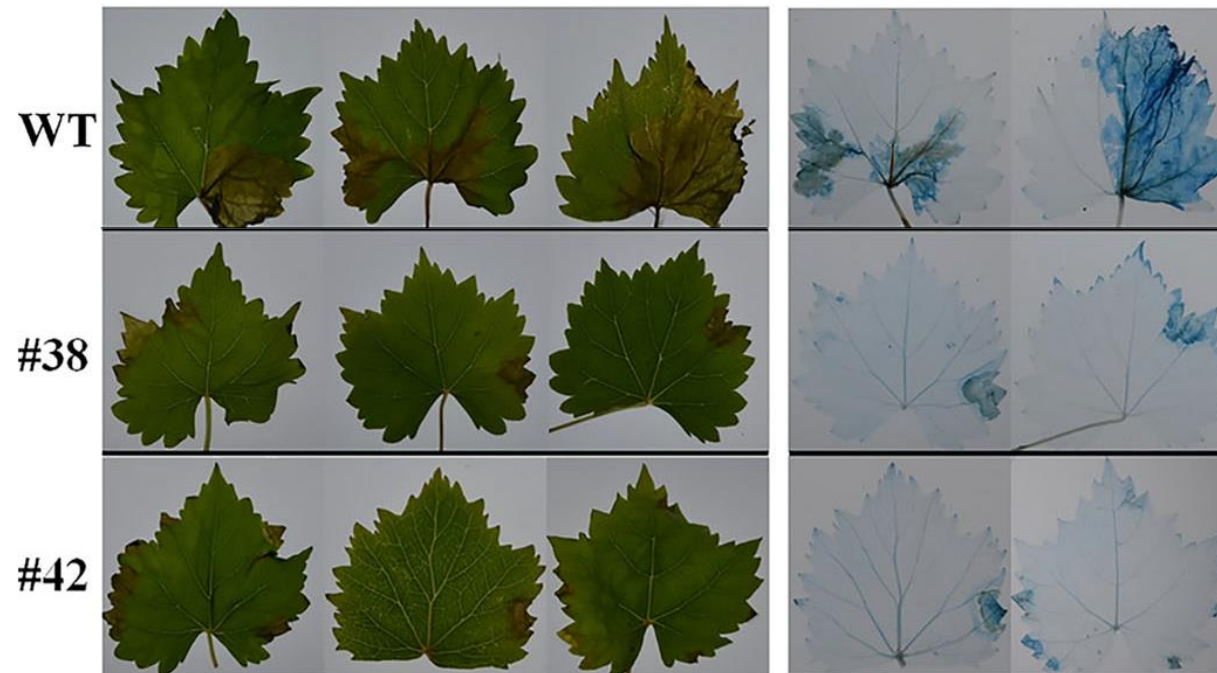
2) Tvorba genetické variability

(znaky využitelné pro šlechtění)

Indukce rezistence vůči rostlinným patogenům

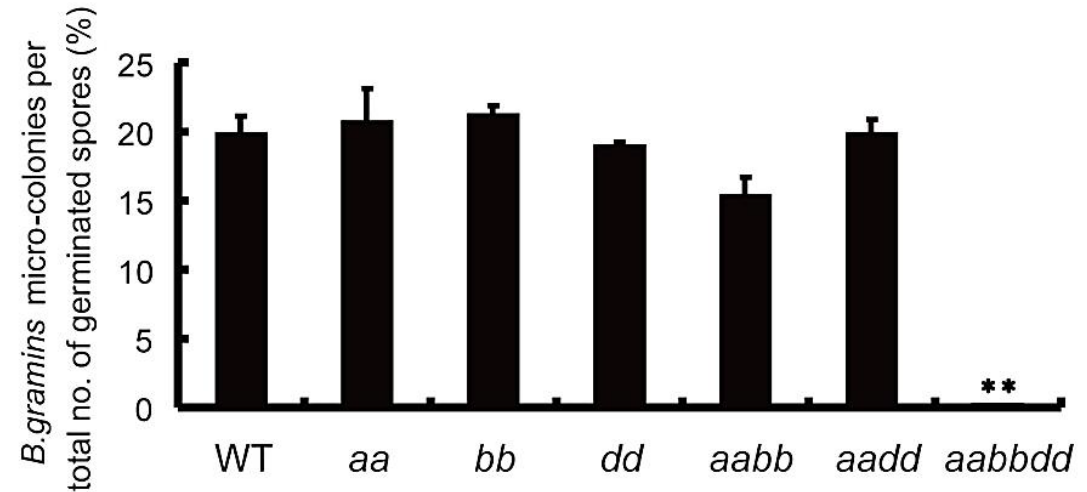
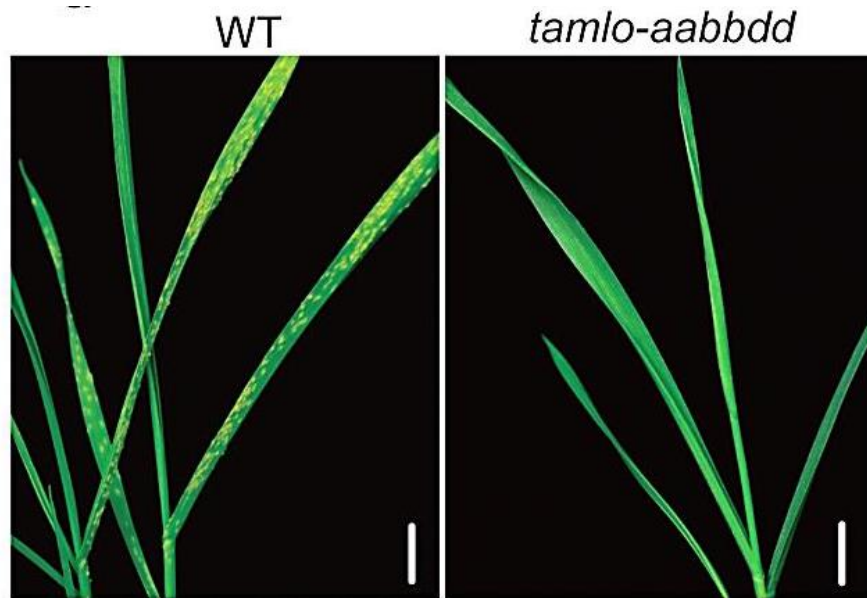
Indukce rezistence vůči houbovým patogenům

- vypnutí transkripčního faktoru *VvWRKY52* indukuje rezistenci vůči plísní šedé (*Botrytis cinerea*)



Indukce rezistence vůči houbovým patogenům

- mutace všech 6 alel *MLO* (MILDREW RESISTANCE LOCUS) u pšenice vede k rezistenci k patogenu *Blumeria graminis* (padlí travní).



nature
biotechnology

LETTERS

Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew

Yanpeng Wang^{1,3}, Xi Cheng^{2,3}, Qiwei Shan¹, Yi Zhang¹, Jinxing Liu¹, Caixia Gao¹ & Jin-Long Qiu²

Indukce rezistence vůči bakteriálním patogenům

- mutace genu *CsLOB1* vede ke zvýšené rezistenci vůči bakterii *Xanthomonas citri* u citrusu.



WT

6 dpi

12 dpi



S2-5



S2-6



Plant Biotechnology
Journal

aab SEB
Association of Agricultural Biologists Society for
Experimental Biology

Plant Biotechnology Journal (2017) 15, pp. 1509–1519

doi: 10.1111/pbi.12733

Engineering canker-resistant plants through CRISPR/ Cas9-targeted editing of the susceptibility gene *CsLOB1* promoter in citrus

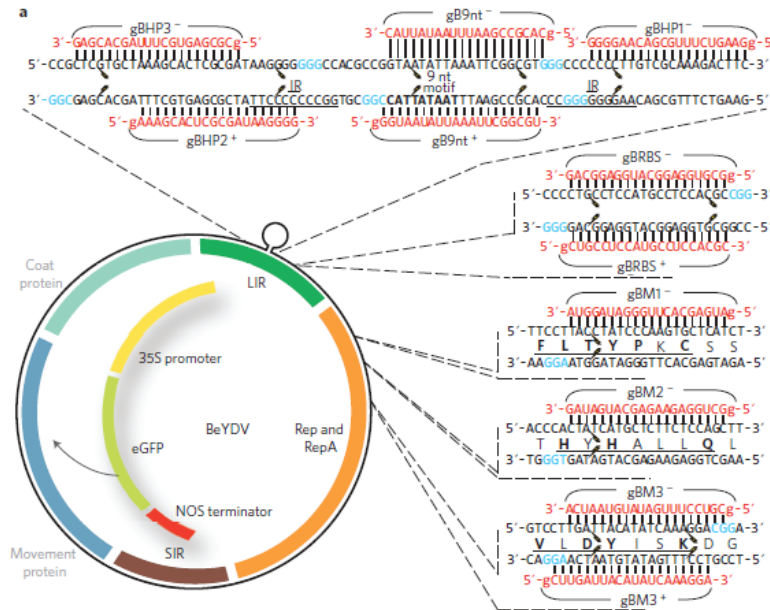
Aihong Peng^{1,2,†}, Shanchun Chen^{1,2,†}, Tiangang Lei^{1,2}, Lanzhen Xu^{1,2}, Yongrui He^{1,2}, Liu Wu², Lixiao Yao^{1,2} and Xiuping Zou^{1,2,*}

¹Citrus Research Institute, Chinese Academy of Agricultural Sciences and National Center for Citrus Variety Improvement, Chongqing, China

²Citrus Research Institute, Southwest University, Chongqing, China

CRISPR/Cas9 indukovaná virová rezistence

Systém CRISPR/Cas9 lze designovat tak, aby štěpil virovou DNA např. rodu *Geminivirus*.



Cas9 only



Cas9 with gBM3⁺

nature
plants

BRIEF COMMUNICATION

PUBLISHED: 28 SEPTEMBER 2015 | ARTICLE NUMBER: 15145 | DOI: 10.1038/NPLANTS.2015.145

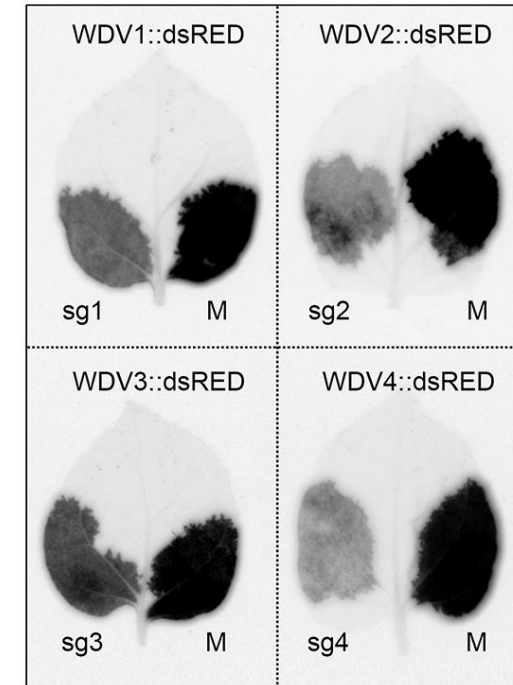
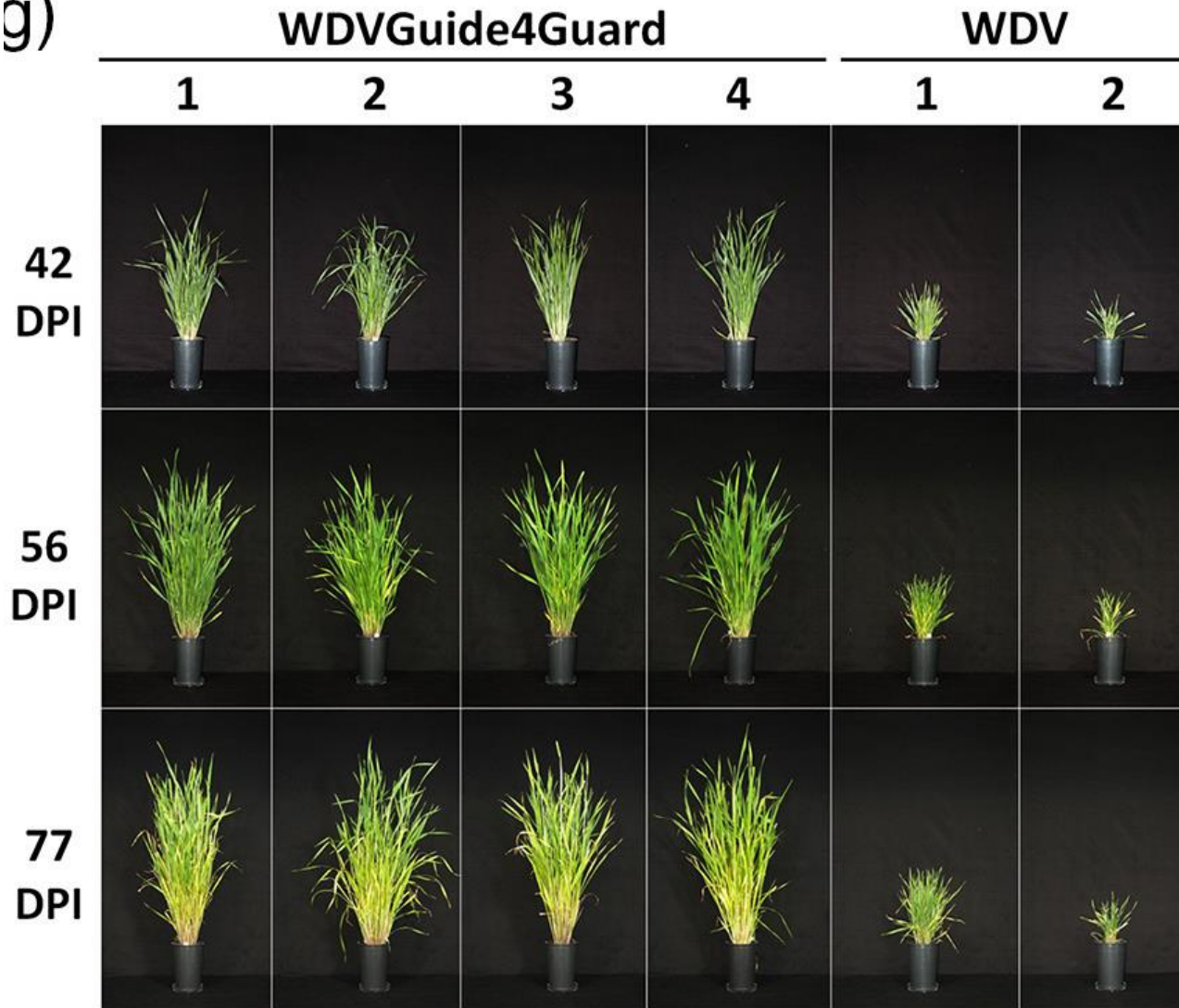
Conferring resistance to geminiviruses with the CRISPR-Cas prokaryotic immune system

Nicholas J. Baltes^{1,2†}, Aaron W. Hummel^{1,2†}, Eva Konecna^{1,2}, Radim Cegan³, Aaron N. Bruns⁴, David M. Bisaro⁴ and Daniel F. Voytas^{1,2*}

CRISPR/Cas9 indukovaná virová rezistence

Cílená degradace DNA *Wheat dwarf virus* pomocí CRISPR/Cas9 u ječmene.

g)



Plant Biotechnology
Journal

aab
Society for
Experimental Biology

Plant Biotechnology Journal (2019) 17, pp. 1004–1006

doi: 10.1111/pbi.13077

Letter

Creating highly efficient resistance against wheat dwarf virus in barley by employing CRISPR/Cas9 system

András Kis¹, Éva Hamar^{1,2}, Gergely Tholt^{3,4}, Rita Bán⁵ and Zoltán Havelda^{1,*}

¹National Agricultural Research and Innovation Centre, Agricultural Biotechnology Institute, Gödöllő, Hungary

²Georgikon Faculty, Fesetics Doctoral School, University of Pannonia, Keszthely, Hungary

³Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

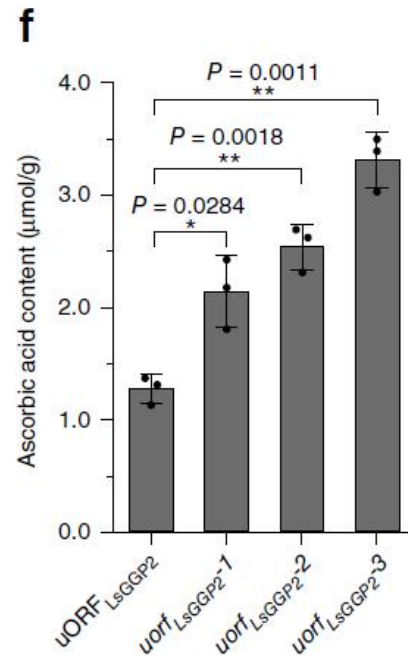
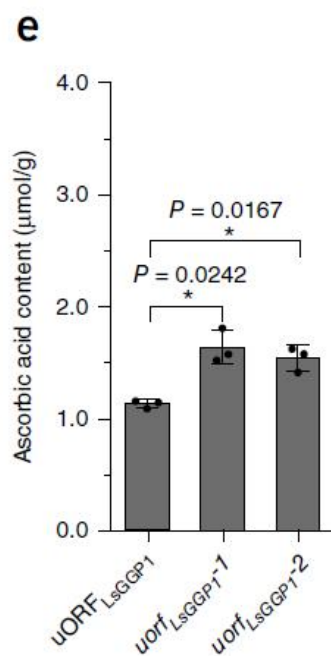
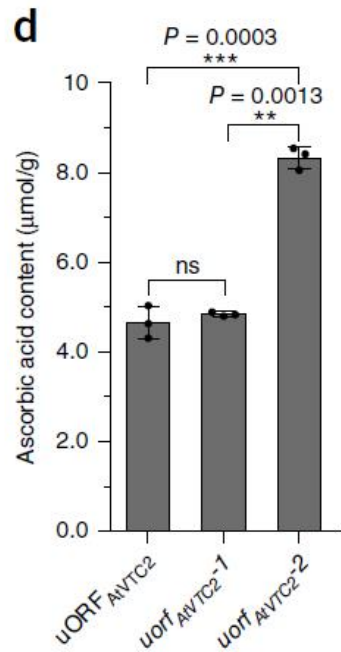
⁴Department of Systematic Biology and Ecology, Faculty of Science, Institute of Biology, Eötvös Loránd University, Budapest, Hungary

⁵Plant Protection Institute, Faculty of Agricultural and Environmental Sciences, Szent István University, Gödöllő, Hungary

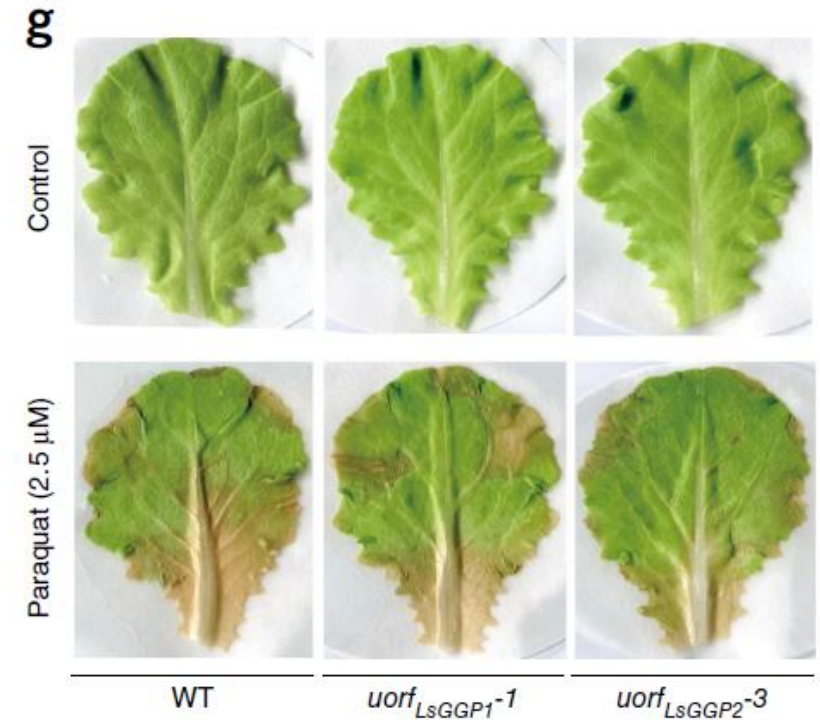
Modifikace obsahu látek

Modifikace obsahu látek

- Zvýšení koncentrace vitamínu C v salátu modifikací promotoru genu *LsGGP2*, zlepšení trvanlivosti.



nature
biotechnology

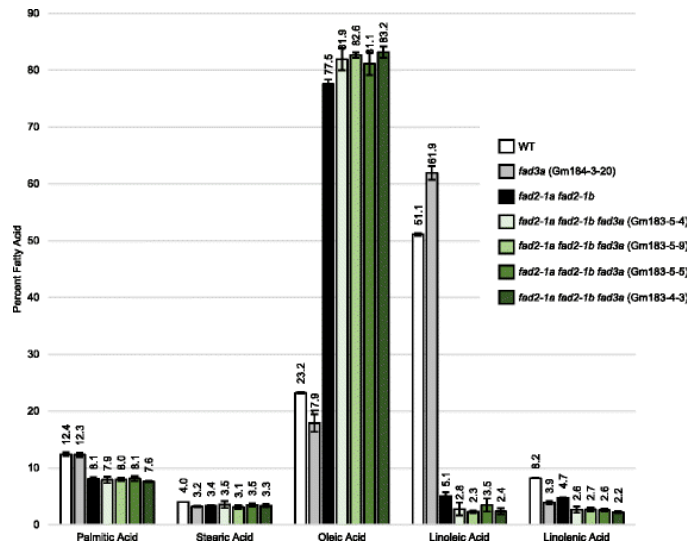


Genome editing of upstream open reading frames enables translational control in plants

Huawei Zhang^{1,4}, Xiaomin Si^{1,2,4}, Xiang Ji^{1,2,4}, Rong Fan^{2,3}, Jinxing Liu¹, Kunling Chen¹, Daowen Wang^{1,2} & Caixia Gao^{1,2}

Modifikace obsahu látek

Mutace 3 genů pro *fatty acid desaturase* (*FAD2-1A*, *FAD2-1B*, *FAD3A*) u sóji – modifikace obsahu mastných kyselin



vyšší obsah olejové kyseliny – lepší stabilita

RESEARCH ARTICLE

Open Access

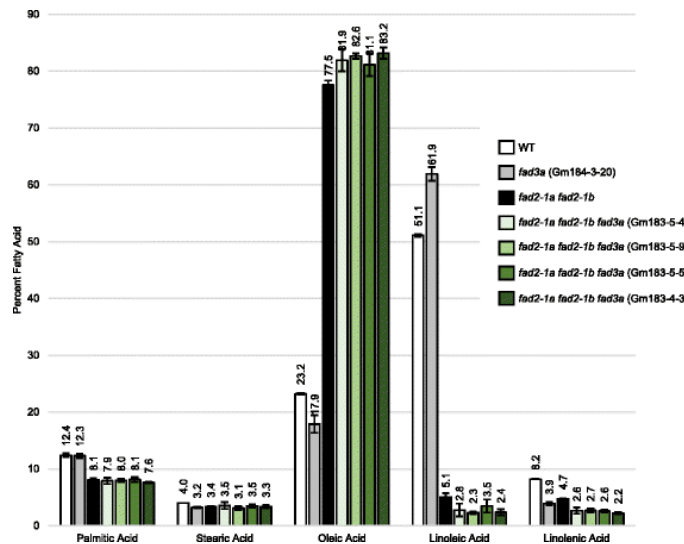


Direct stacking of sequence-specific nuclease-induced mutations to produce high oleic and low linolenic soybean oil

Zachary L. Demorest, Andrew Coffman, Nicholas J. Baltes, Thomas J. Stoddard, Benjamin M. Clasen, Song Luo, Adam Retterath, Ann Yabandith, Maria Elena Gamro, Jeff Bissen, Luc Mathis, Daniel F. Voytas and Feng Zhang*

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vyšší obsah olejové kyseliny – lepší stabilita

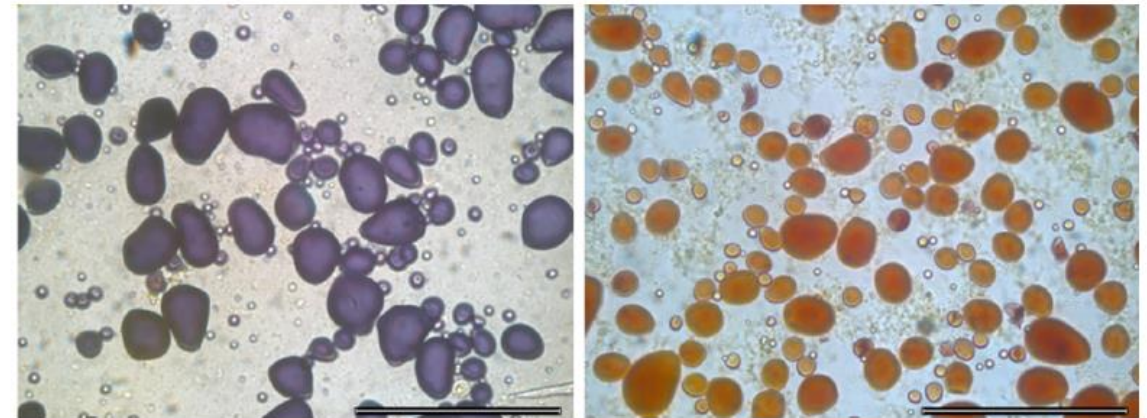
RESEARCH ARTICLE

Open Access

Direct stacking of sequence-specific nuclease-induced mutations to produce high oleic and low linolenic soybean oil

Zachary L. Demorest, Andrew Coffman, Nicholas J. Baltes, Thomas J. Stoddard, Benjamin M. Clasen, Song Luo, Adam Retterath, Ann Yabandith, Maria Elena Gamio, Jeff Bissen, Luc Mathis, Daniel F. Voytas and Feng Zhang*

modifikace obsahu škrobu u tetraploidního bramboru – mutace *granule-bound starch synthase* (*GBSS*)



Plant Cell Rep (2017) 36:117–128
DOI 10.1007/s00299-016-2062-3



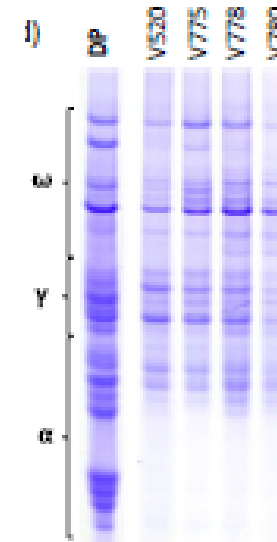
ORIGINAL ARTICLE

Efficient targeted multiallelic mutagenesis in tetraploid potato (*Solanum tuberosum*) by transient CRISPR-Cas9 expression in protoplasts

Mariette Andersson¹ · Helle Turesson¹ · Alessandro Nicolai² · Ann-Sofie Fält¹ · Mathias Samuelsson³ · Per Hofvander¹

Modifikace obsahu látek

„Gluten-free pšenice“ – snížení imunoreaktivity o 80% (delece bloku alfa-gliadinů).



Plant Biotechnology
Journal

aab
SOCIETY FOR
AGRICULTURAL BIOLOGY

SEB
SOCIETY FOR
EXPERIMENTAL BIOLOGY

Plant Biotechnology Journal (2018) 16, pp. 902–910

doi: 10.1111/pbi.12837

Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9

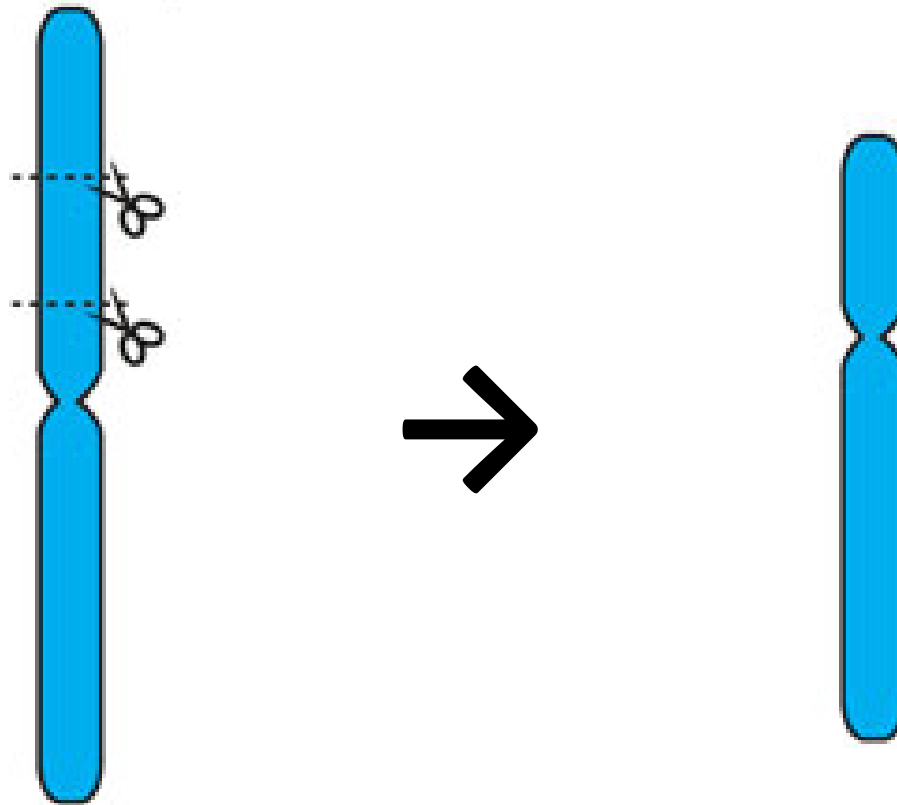
Susana Sánchez-León^{1,*}, Javier Gil-Humanes^{2,*}, Carmen V. Ozuna¹, María J. Giménez¹, Carolina Sousa³, Daniel F. Voytas² and Francisco Barro^{1,*}

¹Departamento de Mejora Genética Vegetal, Instituto de Agricultura Sostenible (IAS-CSIC), Córdoba, Spain

²Department of Genetics, Cell Biology, and Development, Center for Genome Engineering, University of Minnesota, Minneapolis, MN, USA

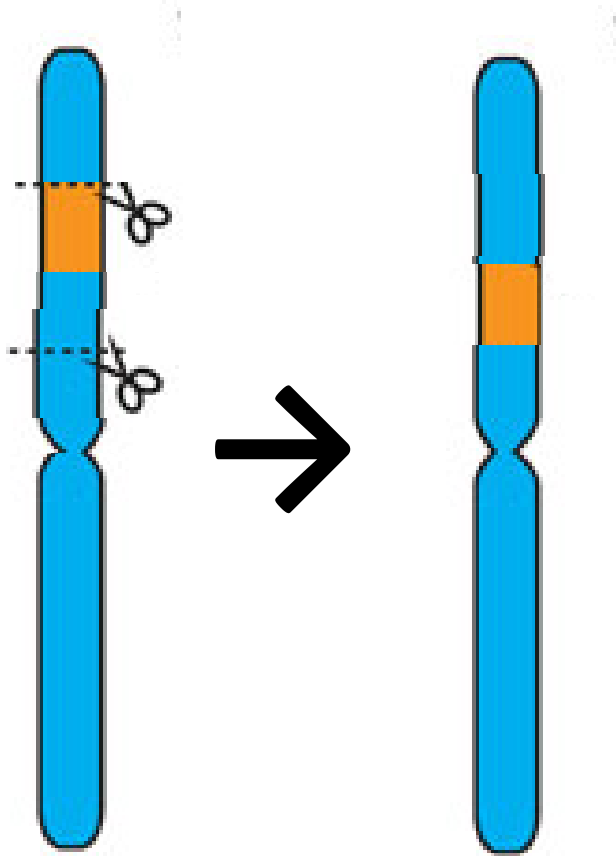
³Departamento de Microbiología y Parasitología, Facultad de Farmacia, Universidad de Sevilla, Sevilla, Spain

Chromozomální aberace - delece

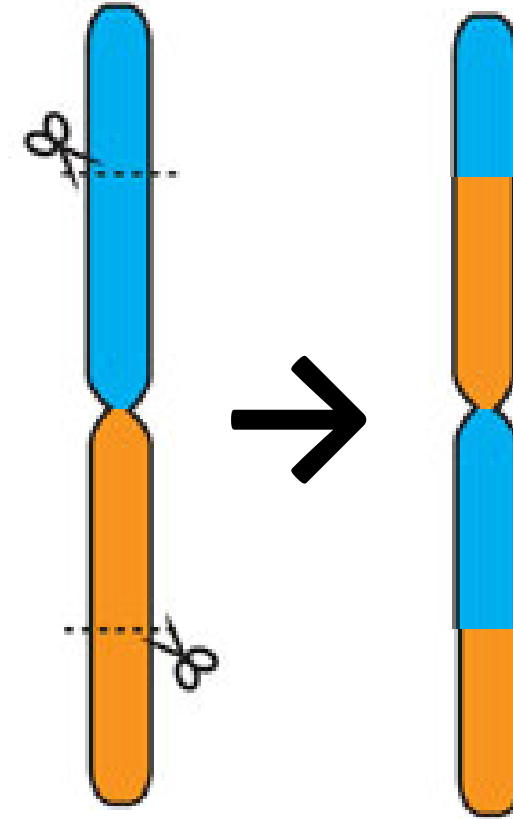


Delece v rozmezí cca 20bp – několik Mb

Chromozomální aberace - inverze

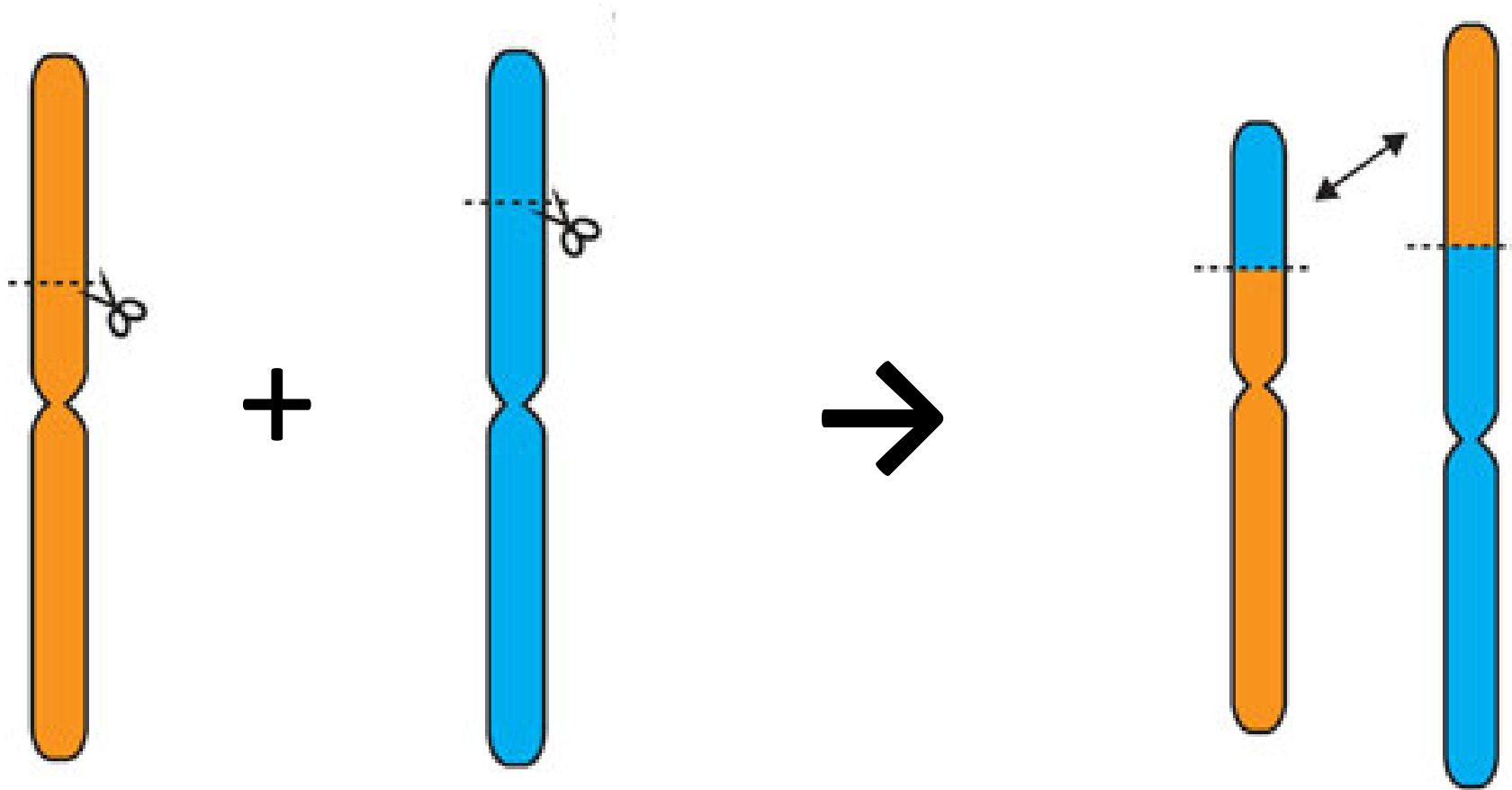


Paracentrická inverze



Pericentrická inverze

Chromozomální aberace - translokace

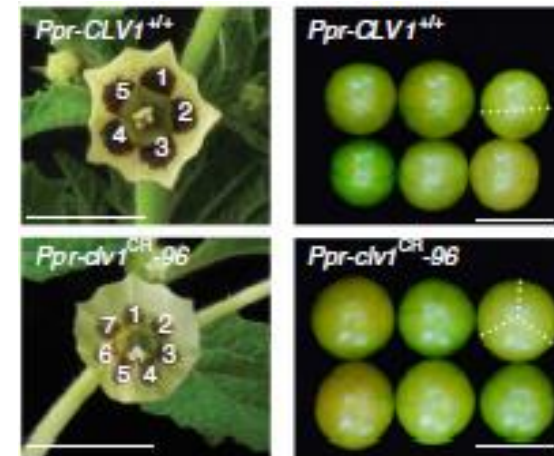
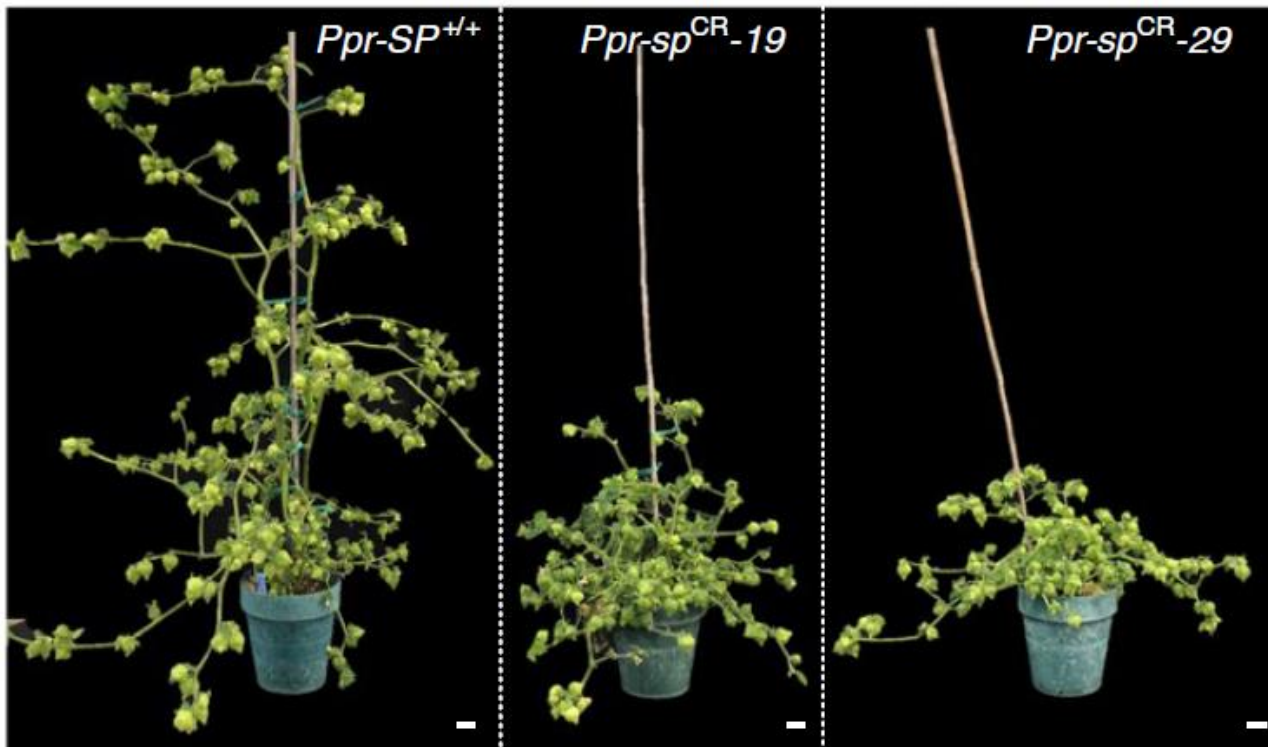


Reciproká translokace

„Domestikace“

Domestikace

- mochyně ojíňená (*Physalis pruinosa*) – mutace genů pro architekturu, tvorbu květů a velikost plodů



BRIEF COMMUNICATION

<https://doi.org/10.1038/s41477-018-0259-x>

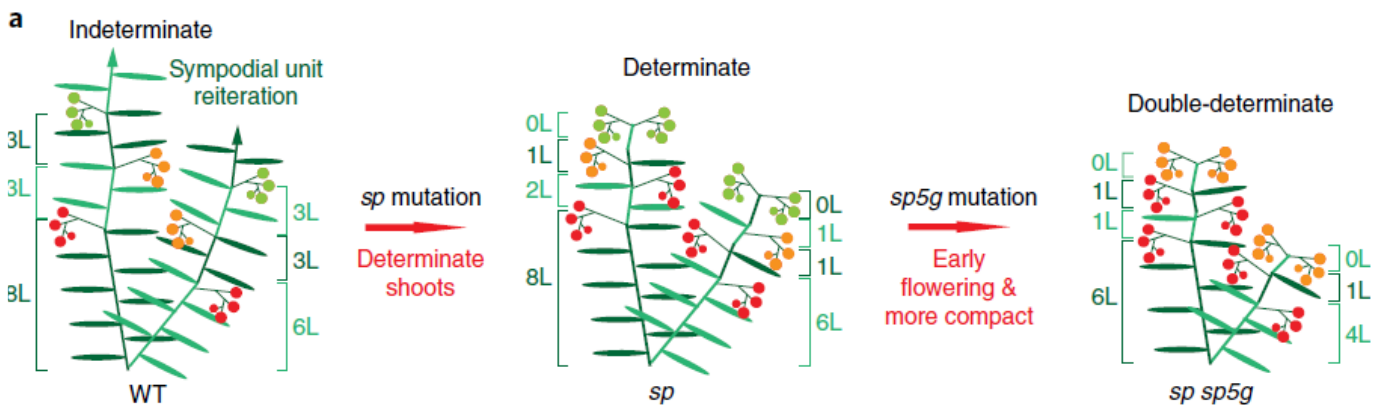
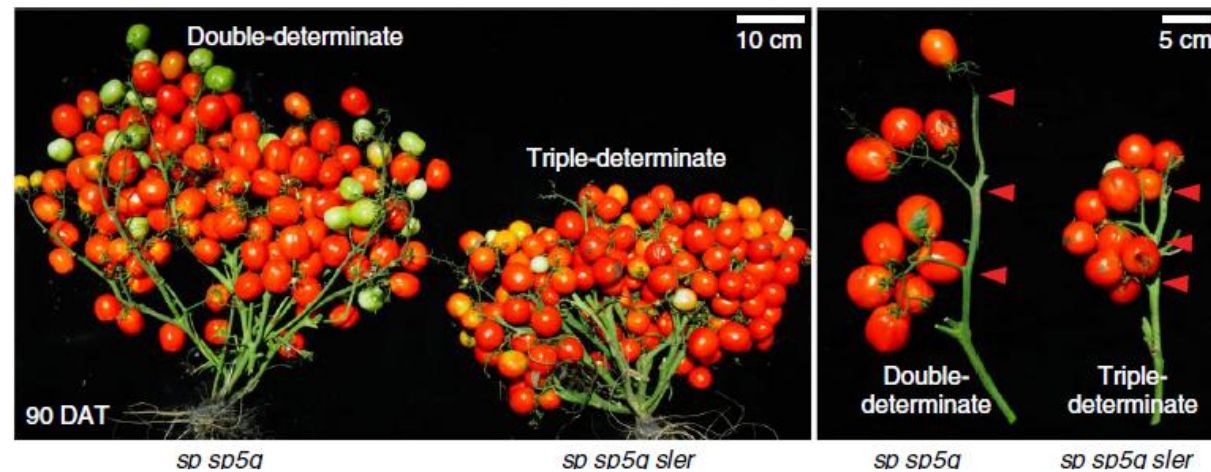
nature
plants

Rapid improvement of domestication traits in an orphan crop by genome editing

Zachary H. Lemmon¹, Nathan T. Reem^{2,5}, Justin Dalrymple^{1,5}, Sebastian Soyk^{1,5},
Kerry E. Swartwood², Daniel Rodríguez-Leal¹, Joyce Van Eck^{2,3*} and Zachary B. Lippman^{1,4*}

Domestikace

- „urban agriculture“ – snížená délka internodií, rychlejší kvetení.

nature
biotechnology

LETTERS

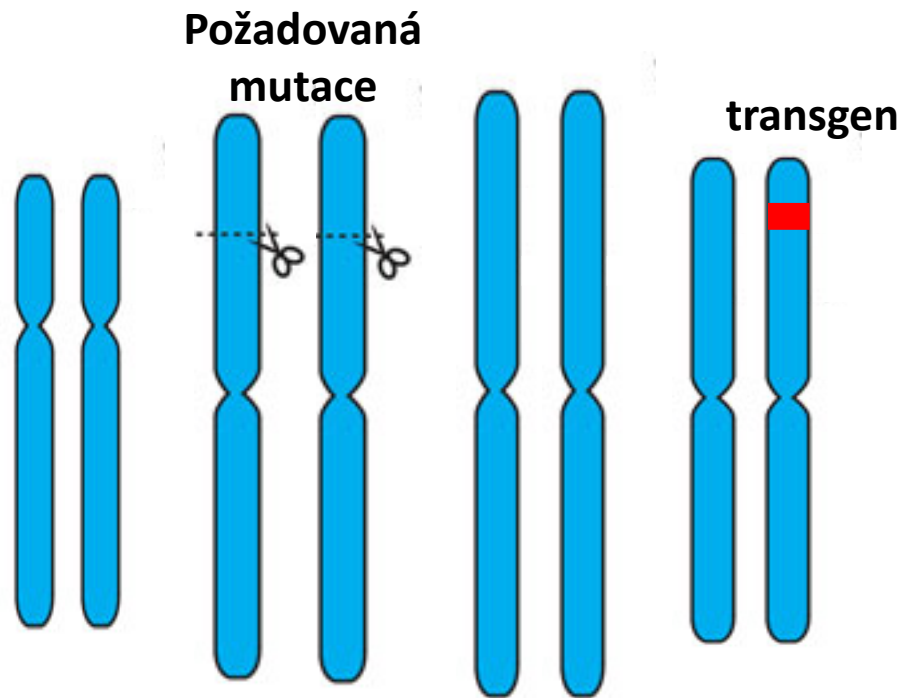
<https://doi.org/10.1038/s41587-019-0361-2>

Rapid customization of Solanaceae fruit crops for urban agriculture

Choon-Tak Kwon¹, Jung Heo², Zachary H. Lemmon^{1,8}, Yossi Capua^{3,9}, Samuel F. Hutton⁴,
Joyce Van Eck^{5,6}, Soon Ju Park² and Zachary B. Lippman^{1,7*}

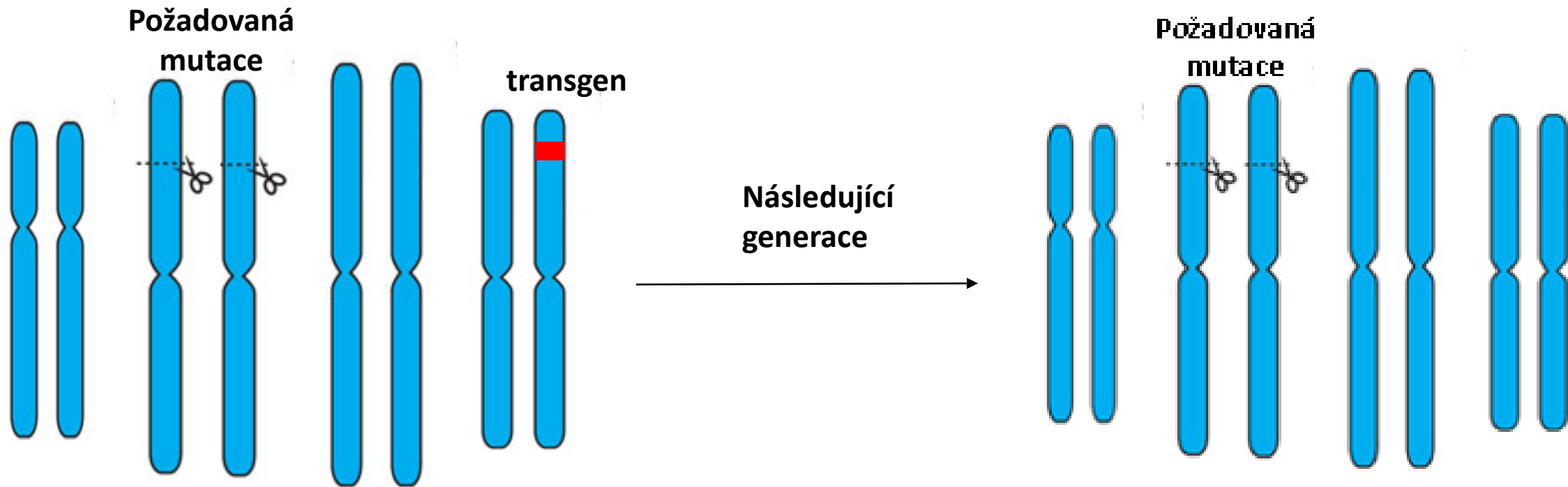
Tvorba "transgene-free" rostlin

- 1) transientní transformace protoplastů (nedochází k integraci konstruktů do rostlinného genomu)
- 2) segregace transgenů v následujících generacích



Tvorba "transgene-free" rostlin

- 1) transientní transformace protoplastů (nedochází k integraci konstruktů do rostlinného genomu)
- 2) segregace transgenů v následujících generacích



Legislative

Briefing

May 2016



Biotechnology and Biological Sciences Research Council

European Plant Science Organisation

French High Council for Biotechnology

Deutschen Akademien für Wissenschaften

Dutch Commission on Genetic Modification

New plant-breeding techniques Applicability of GM rules



In this briefing:

- The uncertain status of new plant breeding techniques
- Background
- Legal basis
- Regulating the new techniques
- European Parliament
- Further reading



"...CRISPR/Cas9 should not be subject to European GMO legislation..."

"...and suggest case by case policy."

Legislativa



Press and Information

Court of Justice of the European Union

PRESS RELEASE No 111/18

Luxembourg, 25 July 2018

Judgment in Case C-528/16

Confédération paysanne and Others v Premier ministre and Ministre de
l'Agriculture, de l'Agroalimentaire et de la Forêt

Organisms obtained by mutagenesis are GMOs and are, in principle, subject to the obligations laid down by the GMO Directive

However, organisms obtained by mutagenesis techniques which have conventionally been used in a number of applications and have a long safety record are exempt from those obligations, on the understanding that the Member States are free to subject them, in compliance with EU law, to the obligations laid down by the directive or to other obligations

Potraviny pro budoucnost



<http://www.potravinyav21.cz/>



Děkuji za pozornost