CRISPR/Cas

Navegando pelo seu universo de aplicações

Disciplina: LGN5809 – Genética Molecular

Docente: Dra. Maria Carolina Quecine Verdi

Piracicaba, São Paulo, Brasil

Junho de 2023

Nosso time

Bruna Marques Moreno

Mestranda – Genética e Melhoramento de Plantas

Laboratório de Genética Molecular de Plantas Cultivadas

Camila Karen Candeira da Silva

Mestranda – Ecologia Aplicada

Laboratório de Evolução

Gabriel Luiz de Melo Sales

Doutorando – Genética e Melhoramento de Plantas

Laboratório de Genética Estatística

Gabriela Corrêa Morais

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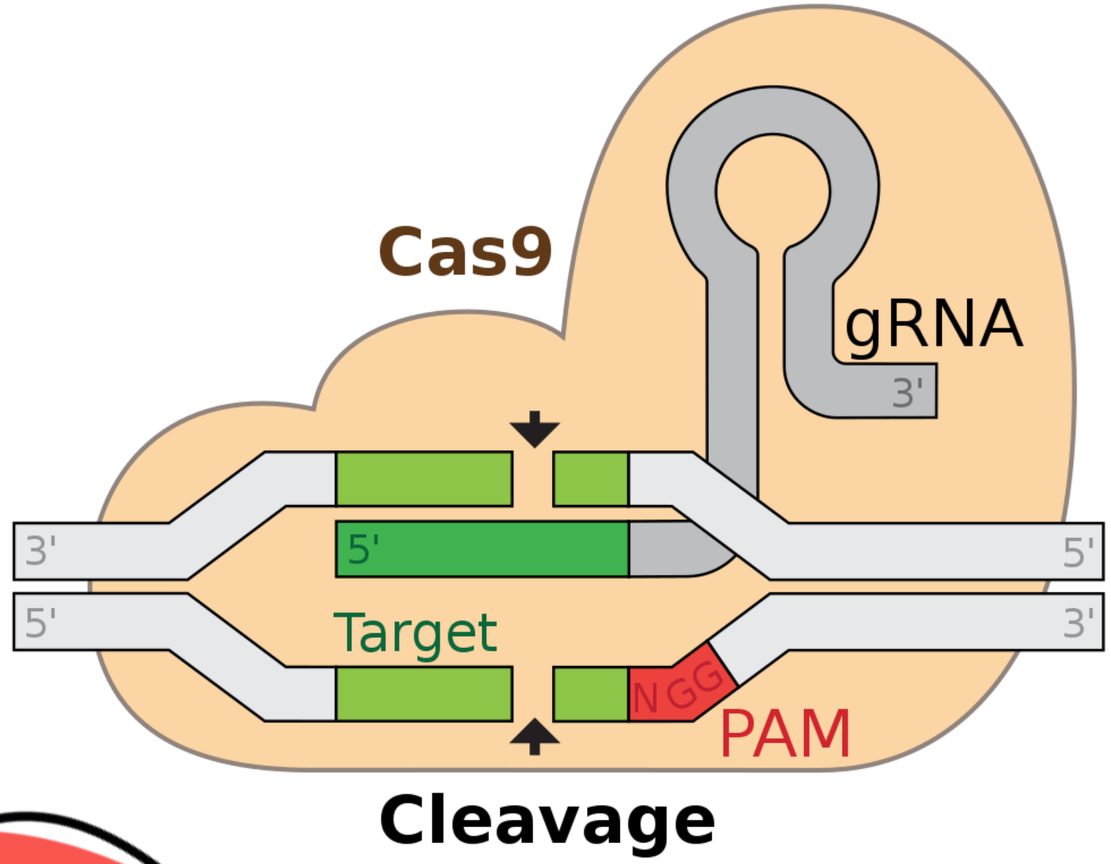
Laboratório de Evolução

Joe Abdul Vilcherrez Atoche

Doutorando – Genética e Melhoramento de Plantas

Laboratório de Citogenômica e Epigenética

Eixos de aplicações

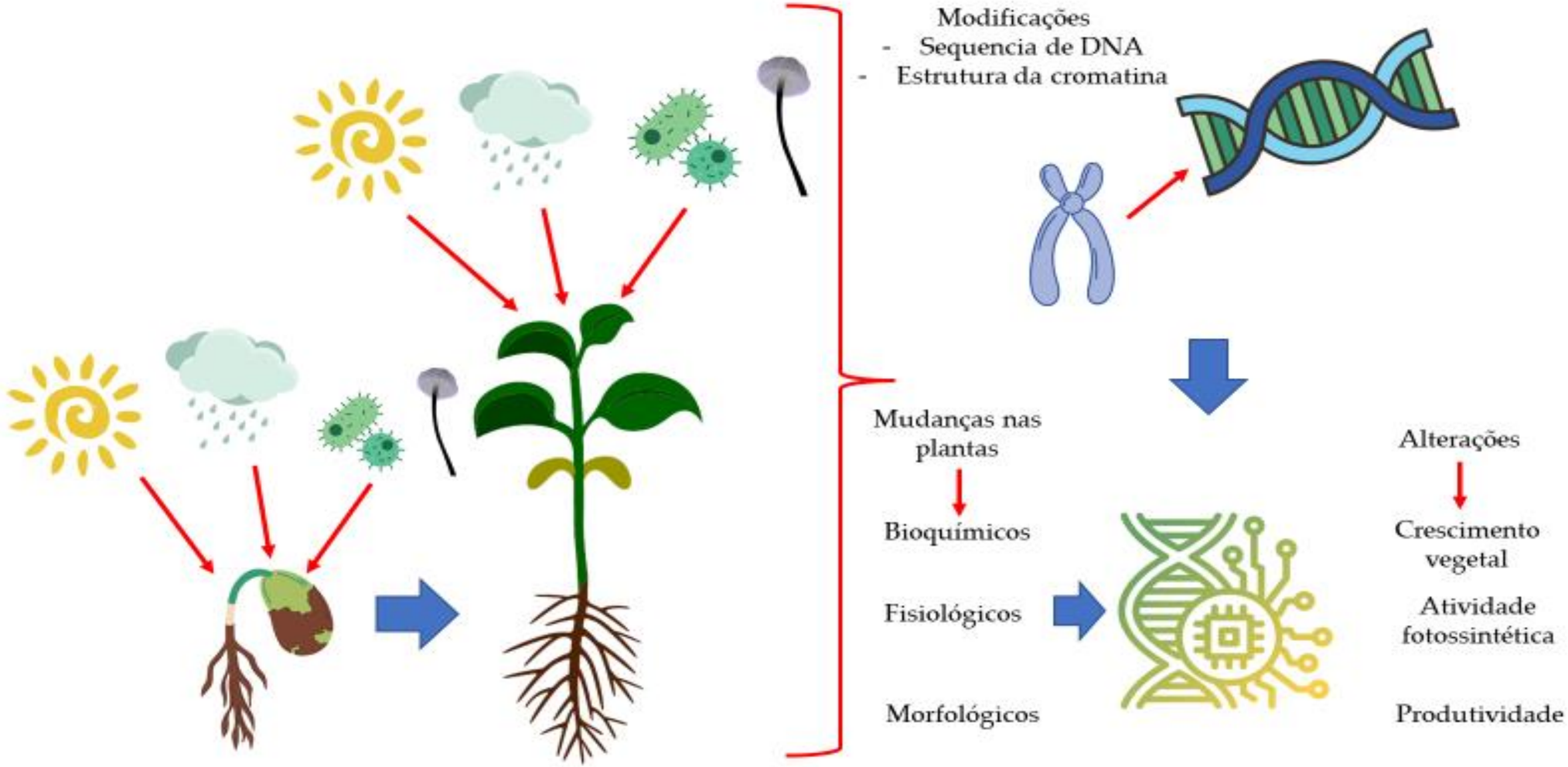


- Epigenética
- Domesticação
- Conservação
- Melhoramento

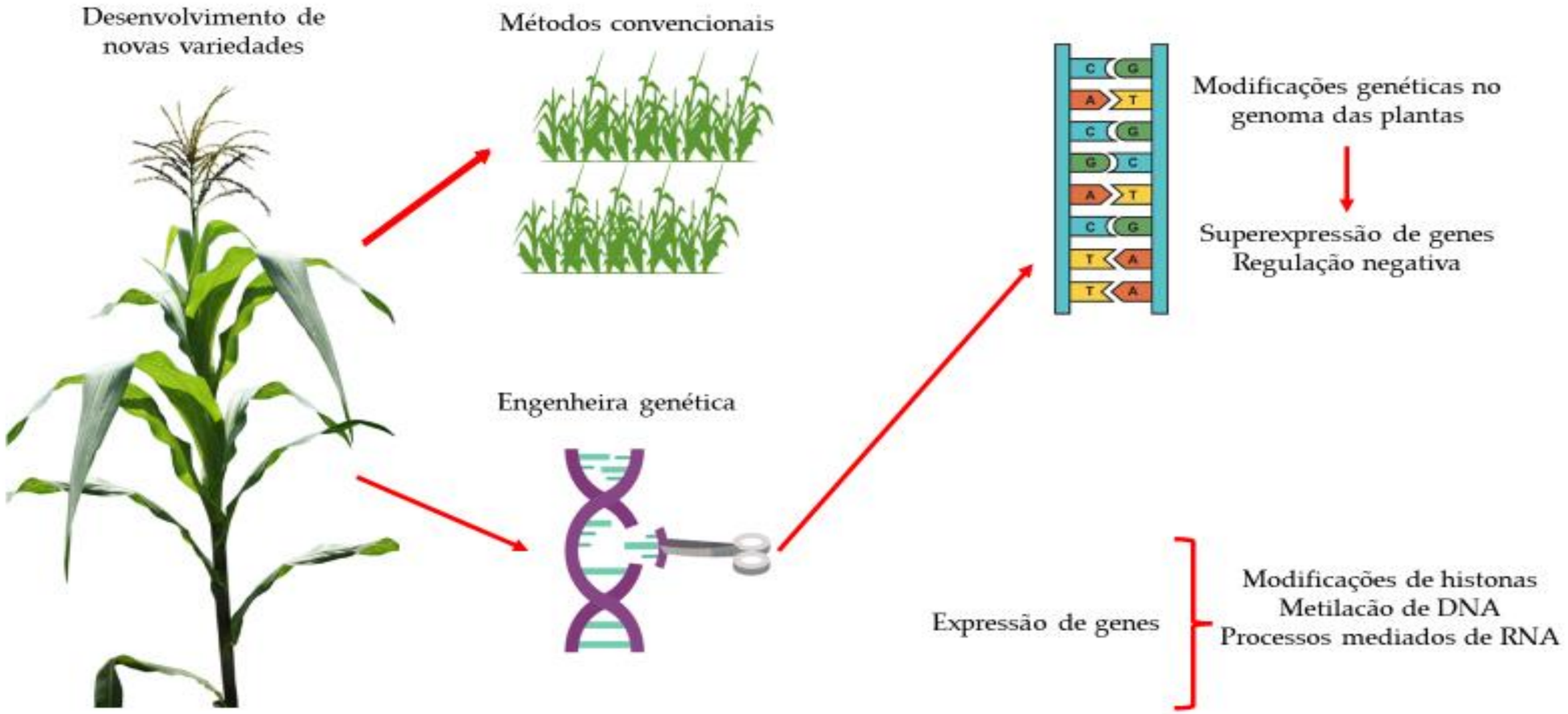
CRISPR/Cas

Aplicações em epigenética

CRISPR/Cas – Aplicações em epigenética

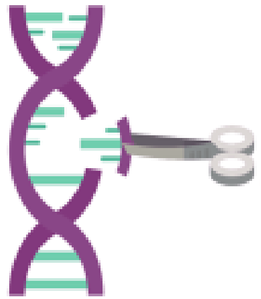


CRISPR/Cas – Aplicações em epigenética



CRISPR/Cas – Aplicações em epigenética

Engenheira epigenética



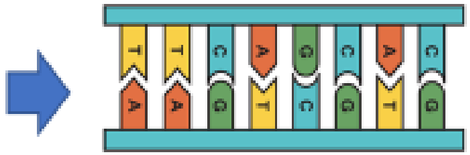
ZFN: Zinc Finger nucleases

TALEN

CRISPR-Cas

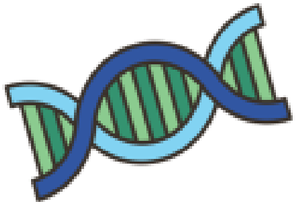
Regulação dos genes da planta

- Região promotora
- Transcrição



Regulação epigenética de planta

- Enzimas modificadoras de histonas
- RNA non-coding
- Metilacão de DNA



CRISPR/Cas – Aplicações em epigenética

ZFN: Zinc Finger nucleases

+

Fator de metilação de DNA dirigido por RNA SUVH9

↓

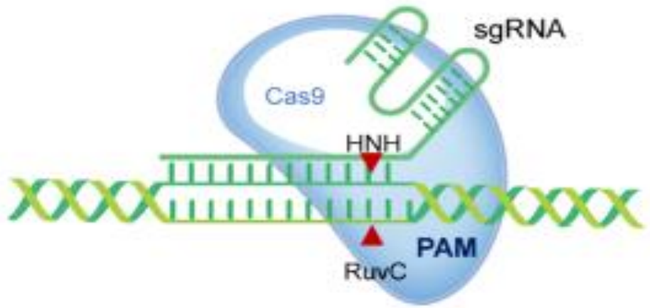
Promotor FLOWERING WAGENINGEN (FWA)

LETTER

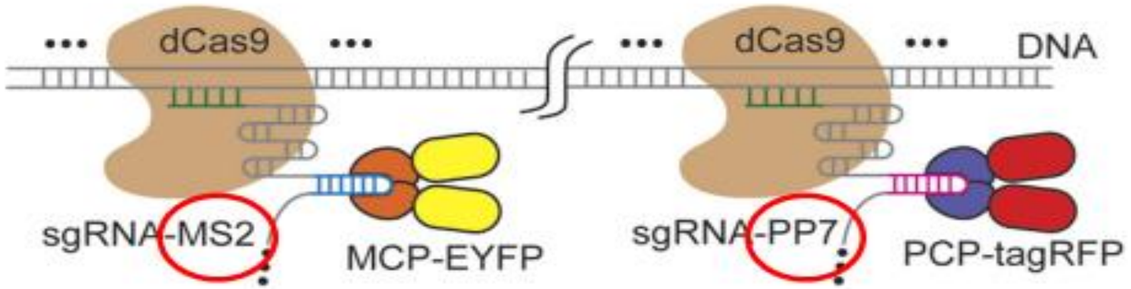
doi:10.1038/nature12931

SRA– and SET–domain–containing proteins link RNA polymerase V occupancy to DNA methylation

Lianna M. Johnson[1]*, Jiamu Du[2]*, Christopher J. Hale[3], Sylvain Bischof[4], Suhua Feng[1,3], Ramakrishna K. Chodavarapu[1], Xuehua Zhong[1]†, Giuseppe Marson[2], Matteo Pellegrini[1], David J. Segal[4], Dinshaw J. Patel[2] & Steven E. Jacobsen[1,3]



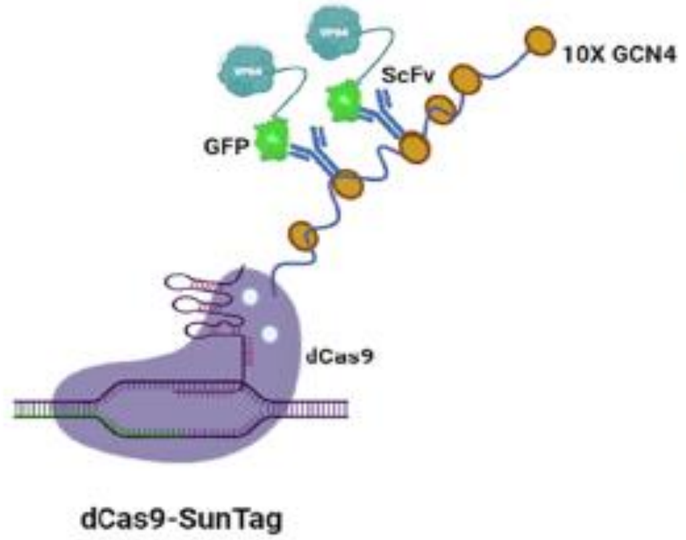
CRISPR/Cas9



Aptâmero de RNA

CRISPR/Cas – Aplicações em epigenética

Ferramentas de engenharia epigenética



dCas9-SunTag

Fig. 1. DeadCas9-SunTag system: 10× GCN4 peptides appended in array to dCas9 carboxy-terminus. 10× GCN4 peptides fused to single-chain variable fragment (scFv) antibodies. scFv has an affinity for multiple GFP and VP64. The

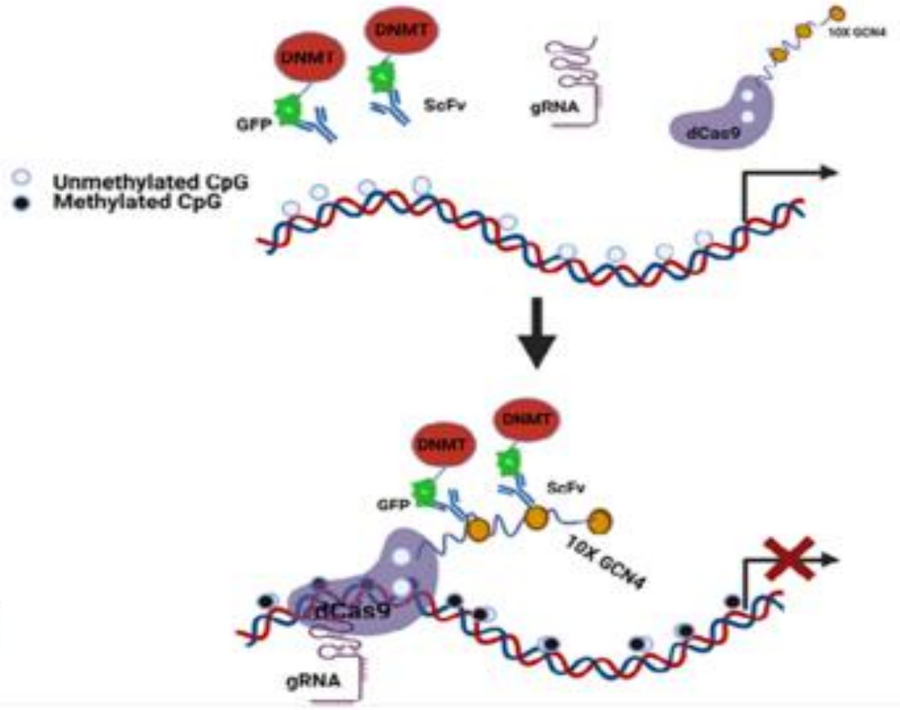


Fig. 2. DNMT or TET1 effectors tethered to dCas9 mediated epigenetic editing. DNA methylation occurs due to the collective effort of various components, i.e., gRNA/dCas9-effector complex. Here the effector includes DNMT, either attached to the dCas9 protein via a linker or repetitive peptide epitopes binding proteins, e.g., single-chain variable fragment (ScFv) antibody. gRNA to recruit the dCas9 nuclease to a targeted location on the genome. Here the effectors include DNMT, in which DNMTs add the methyl group to cytosine in DNA, resulting in a silencing effect.

dCas9 fused with DNA methyltransferase (DNMT) or demethylase (TET)

CRISPR/Cas – Aplicações em epigenética

Ferramentas de engenharia epigenética

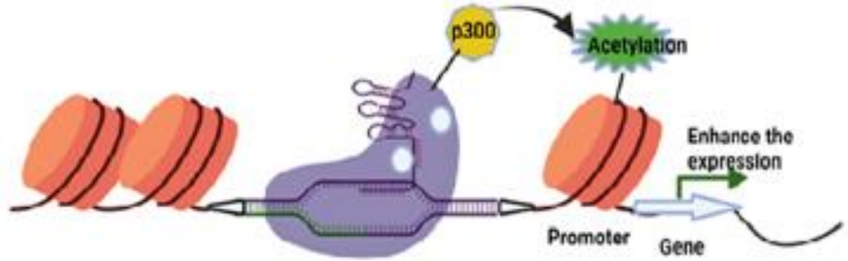


Fig. 3. dCas9-p300 chimeric protein enhances acetylation of H3K27 (histone H3 lysine 27) in the promoter regic

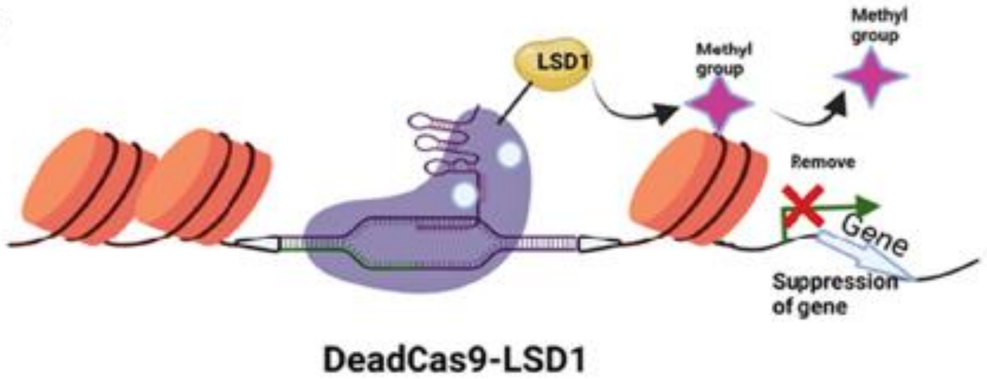


Fig. 4. dCas9-LSD1 removes methyl groups from the target site leads to the repression of the targeted gene.

CRISPR/Cas – Aplicações em epigenética

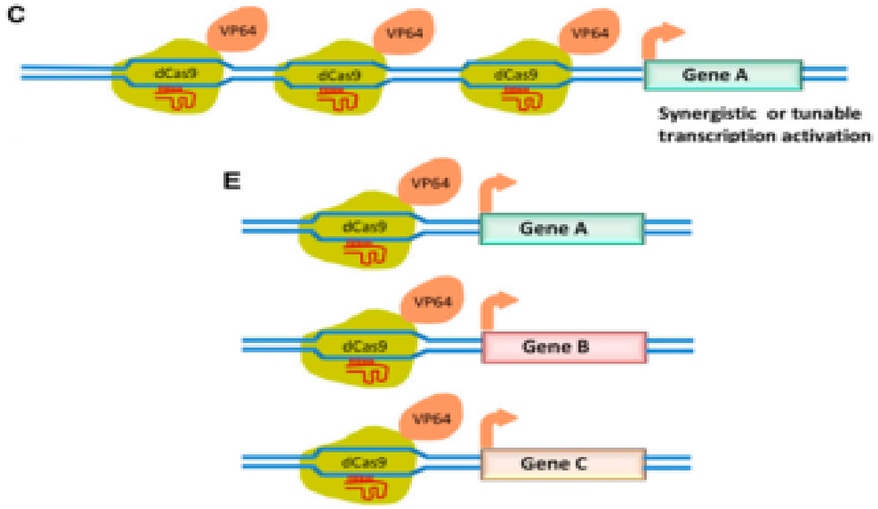
Aplicações das modificações epigenéticas usando CRISPR-Cas em plantas

Regulação transcricional : CRISPR-dCas9a

Breakthrough Technologies

A CRISPR/Cas9 Toolbox for Multiplexed Plant Genome Editing and Transcriptional Regulation[1][OPEN]

Levi G. Lowder[3], Dengwei Zhang[3], Nicholas J. Baltes, Joseph W. Paul III, Xu Tang, Xuelian Zheng, Daniel F. Voytas, Tzung-Fu Hsieh, Yong Zhang*, and Yiping Qi*



Gene 807 (2022) 145919

Contents lists available at ScienceDirect

Gene

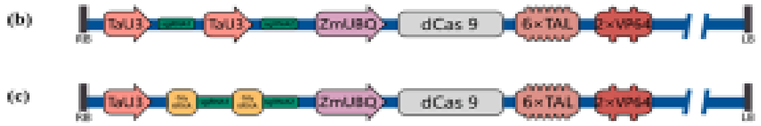
journal homepage: www.elsevier.com/locate/gene

Research paper

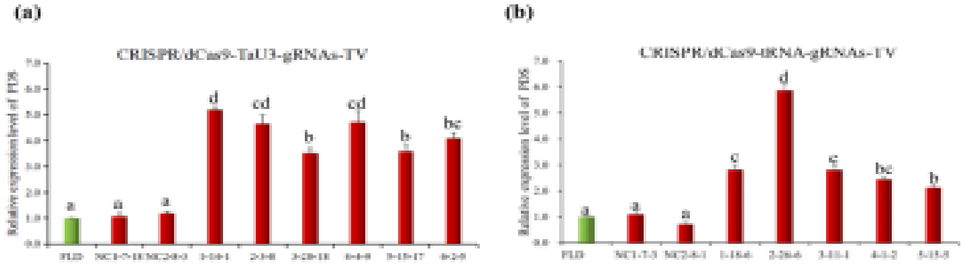
Transcriptional regulation by CRISPR/dCas9 in common wheat

Huajie Zhou[a], Lei Xu[a], Feng Li[b], Yansha Li[b,*]

[a] College of Life Sciences, Shandong Normal University, No. 88 East Wenhua Road, Lixia District, Jinan 250014, Shandong, China
[b] Shandong Shanfeng Biotechnology Co. Ltd., 11 Floor, Main Building, QiLu Innovalley Incubator, High-tech Industry Development Zone, Jinan 250000, Shandong, China



Ativação transcricional dos níveis de expressão PDS (phytoenodesnaturase)



CRISPR/Cas – Aplicações em epigenética

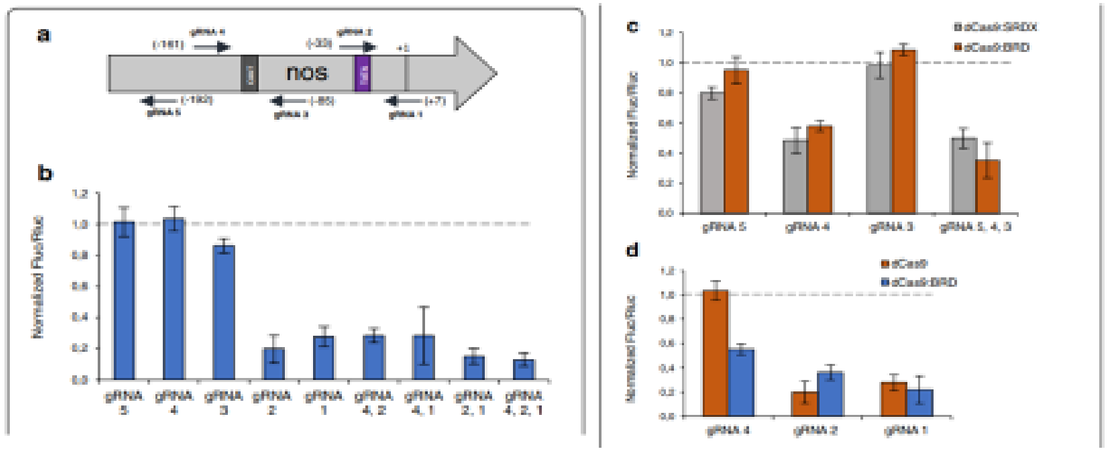
Aplicações das modificações epigenéticas usando CRISPR-Cas em plantas

Regulação transcricional: CRISPR-dCas9i

METHODOLOGY Open Access

A modular toolbox for gRNA–Cas9 genome engineering in plants based on the GoldenBraid standard

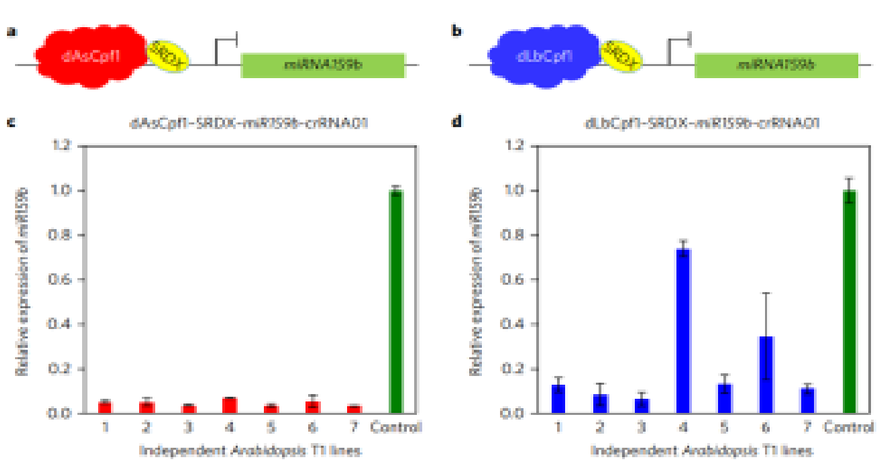
Marta Vazquez-Vilar[1], Joan Miquel Bernabé-Orts[1], Asun Fernandez-del-Carmen[1], Pello Ziarsolo[2], Jose Blanca[2], Antonio Granell[1] and Diego Orzaez[1*]



nature plants BRIEF COMMUNICATION
PUBLISHED: 17 FEBRUARY 2017 | VOLUME: 3 | ARTICLE NUMBER: 17018

A CRISPR-Cpf1 system for efficient genome editing and transcriptional repression in plants

Xu Tang[1†], Levi G. Lowder[2†], Tao Zhang[3], Aimee A. Malzahn[4], Xuelian Zheng[1], Daniel F. Voytas[5], Zhaohui Zhong[1], Yiyi Chen[1], Qiurong Ren[1], Qian Li[1], Elida R. Kirkland[2], Yong Zhang[1*] and Yiping Qi[1,4*]



CRISPR/Cas – Aplicações em epigenética

Aplicações das modificações epigenéticas usando CRISPR-Cas em plantas

Metilacão e desmetilação de DNA em plantas

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-019-08736-7 OPEN

Site-specific manipulation of *Arabidopsis* loci using CRISPR-Cas9 SunTag systems

Ashot Papikian[1,2], Wanlu Liu[1,3], Javier Gallego-Bartolomé[1] & Steven E. Jacobsen[1,4]

Catalytic domain of the *Nicotiana tabacum* DRM methyltransferase

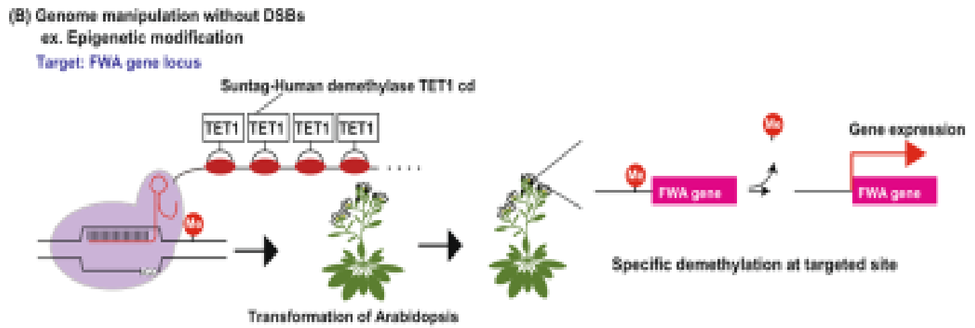
Promotor FWA

Promotor Superman

Targeted DNA demethylation of the *Arabidopsis* genome using the human TET1 catalytic domain

Javier Gallego-Bartolomé[A,1], Jason Gardiner[A,1], Wanlu Liu[A,B,1], Ashot Papikian[A,C,1], Basudev Ghoshal[D], Hsuan Yu Kuo[A], Jenny Miao-Chi Zhao[A], David J. Segal[D], and Steven E. Jacobsen[A,B,2]

FWA gene ← Catalytic domain of the human demethylase TEN-ELEVEN TRANSLOCATION1 (TET1cd) → CACTA1 loci



CRISPR/Cas – Aplicações em epigenética

Aplicações das modificações epigenéticas usando CRISPR-Cas em plantas

Acetilação de histonas em plantas

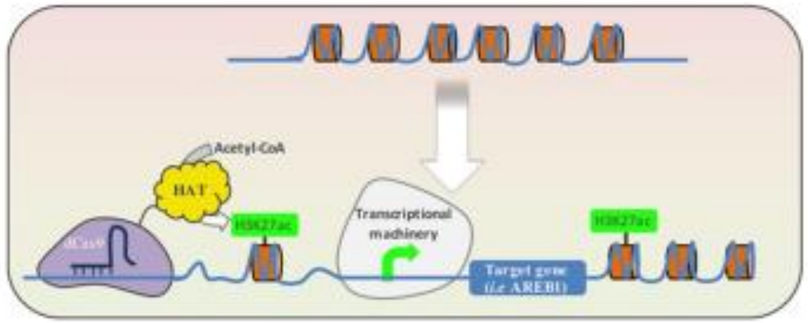
SCIENTIFIC REPORTS

OPEN

Improved drought stress tolerance in Arabidopsis by CRISPR/ dCas9 fusion with a Histone AcetylTransferase

Received: 11 September 2018
Accepted: 9 May 2019
Published online: 30 May 2019

Joaquin Felipe Roca Paixão[1,2], François-Xavier Gillet[1], Thuanne Pires Ribeiro[1], Caroline Bournaud[3], Isabela Tristan Lourenço-Tessutti[1], Daniel D. Noriega[3], Bruno Paes de Melo[1], Janice de Almeida-Engler[2] & Maria Fatima Grossi-de-Sa[1,3]

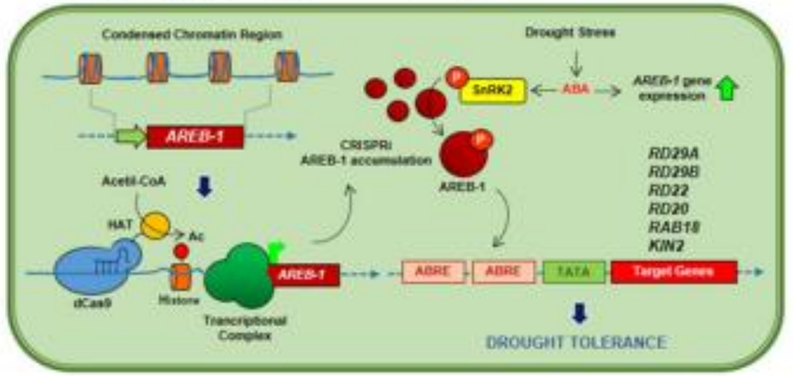


SCIENTIFIC REPORTS
nature research

OPEN

Transcriptional modulation of *AREB-1* by CRISPRa improves plant physiological performance under severe water deficit

Bruno Paes de Melo[1,2,3], Isabela Tristan Lourenço-Tessutti[1,3], Joaquin Felipe Roca Paixão[1,2], Daniel David Noriega[1,4], Maria Cristina Mattar Silva[1], Janice de Almeida-Engler[2], Elizabeth Pacheco Batista Fontes[2,3] & Maria Fatima Grossi-de-Sa[1,3,5]



CRISPR/Cas

Aplicações em domesticação

CRISPR/Cas – Aplicações em domesticação

PCP
PLANT & CELL PHYSIOLOGY

From Evolution to Revolution: Accelerating Crop Domestication through Genome Editing

Kishor Kumar[1,*], Swarupa Nanda Mandal[2,3], Bhubaneswar Pradhan[1], Pavneet Kaur[4], Karminderbir Kaur[4] and Kumari Neelam[4,*]

[1]Faculty Centre for Integrated Rural Development and Management, Ramakrishna Mission Vivekananda Educational and Research Institute, Narendrapur, Kolkata 700103, India
[2]Department of Genetics and Plant Breeding, Bidhan Chandra Krishi Viswavidyalaya, Extended Campus, Burdwan, West Bengal 713101, India
[3]Department of Plant and Soil Science, Texas Tech University, Lubbock, TX 79415, USA
[4]School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab 141004, India

*Corresponding authors: Kishor Kumar, E-mail, kishorsinha23@gmail.com; Kumari Neelam, E-mail, kneelam@pau.edu.

(Received 8 February 2022; Accepted 25 August 2022)

Crop domestication has a tremendous impact on socioeconomic conditions and human civilization. Modern cultivars were domesticated from their wild progenitors thousands of years ago by the selection of natural variation by humans. New cultivars are being developed by crossing two or more

Introduction

Agriculture plays an immense role in human civilization. Modern cultivated and horticultural crops are domesticated from their wild progenitors by the selection of natural variations

Special issue – Review

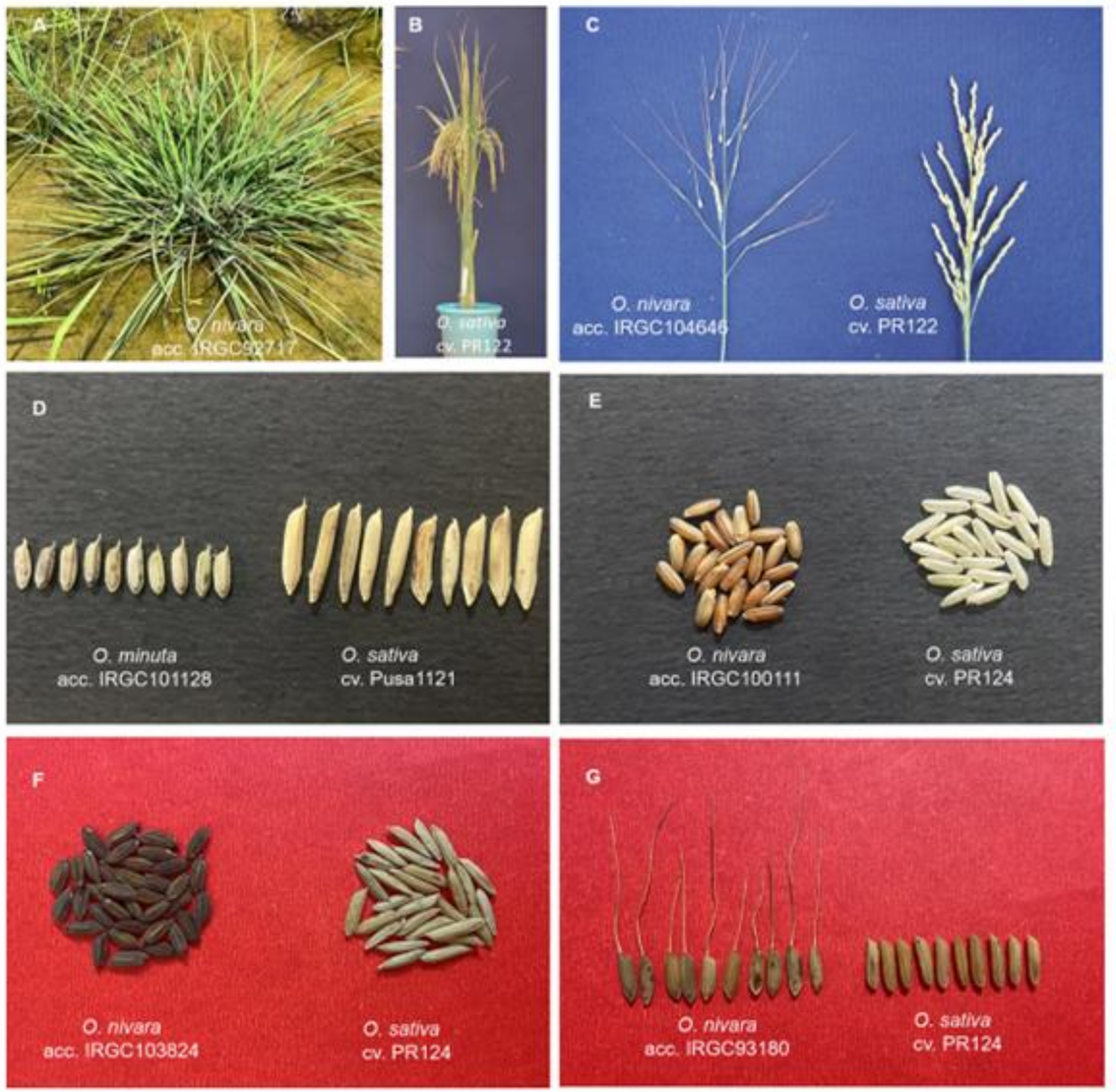
Da Revolução à Evolução – Acelerando a Domesticação de Cultura por meio de edição do Genoma

CRISPR/Cas – Aplicações em domesticação

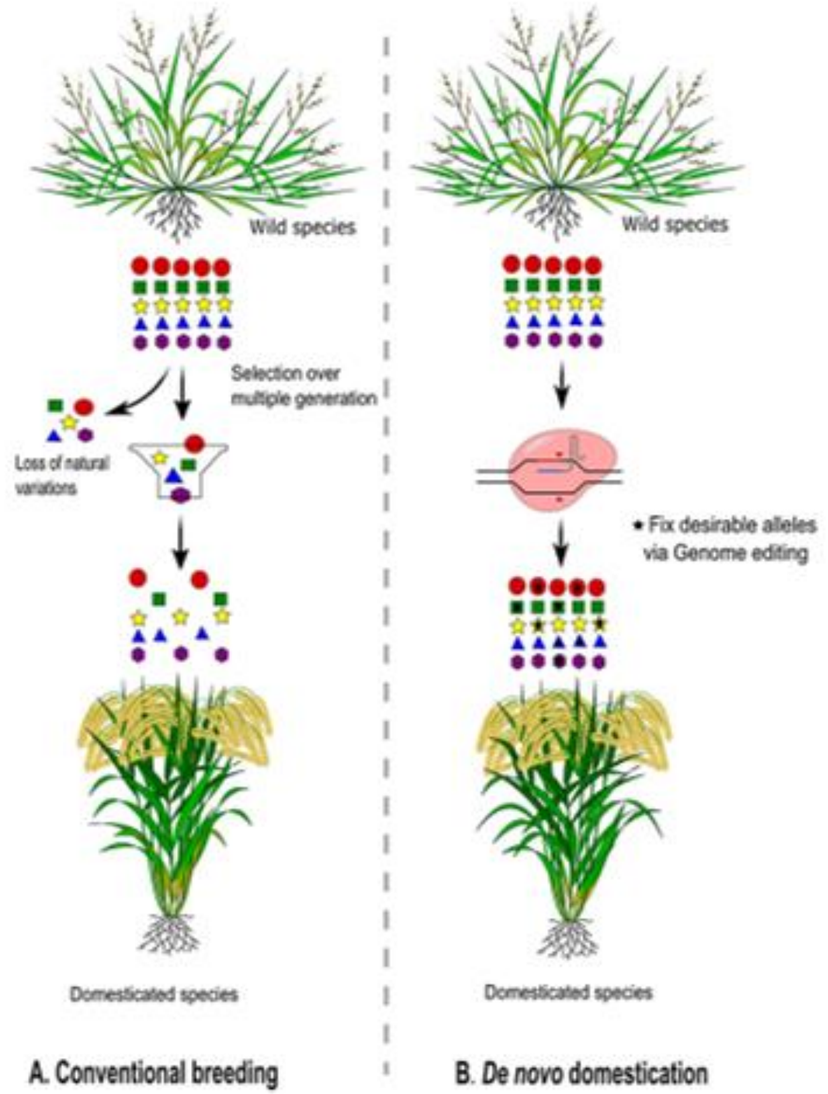
- A importância da Agricultura na civilização humana.
- Domesticação de plantas cultivadas a partir de seus progenitores selvagens.
- Os Parentes Selvagens de Culturas são valiosos recursos para características agronômicas úteis.
- Limitações da reprodução convencional e a necessidade de ferramentas de edição do genoma, como a tecnologia CRISPR/Cas.

CRISPR/Cas – Aplicações em domesticação

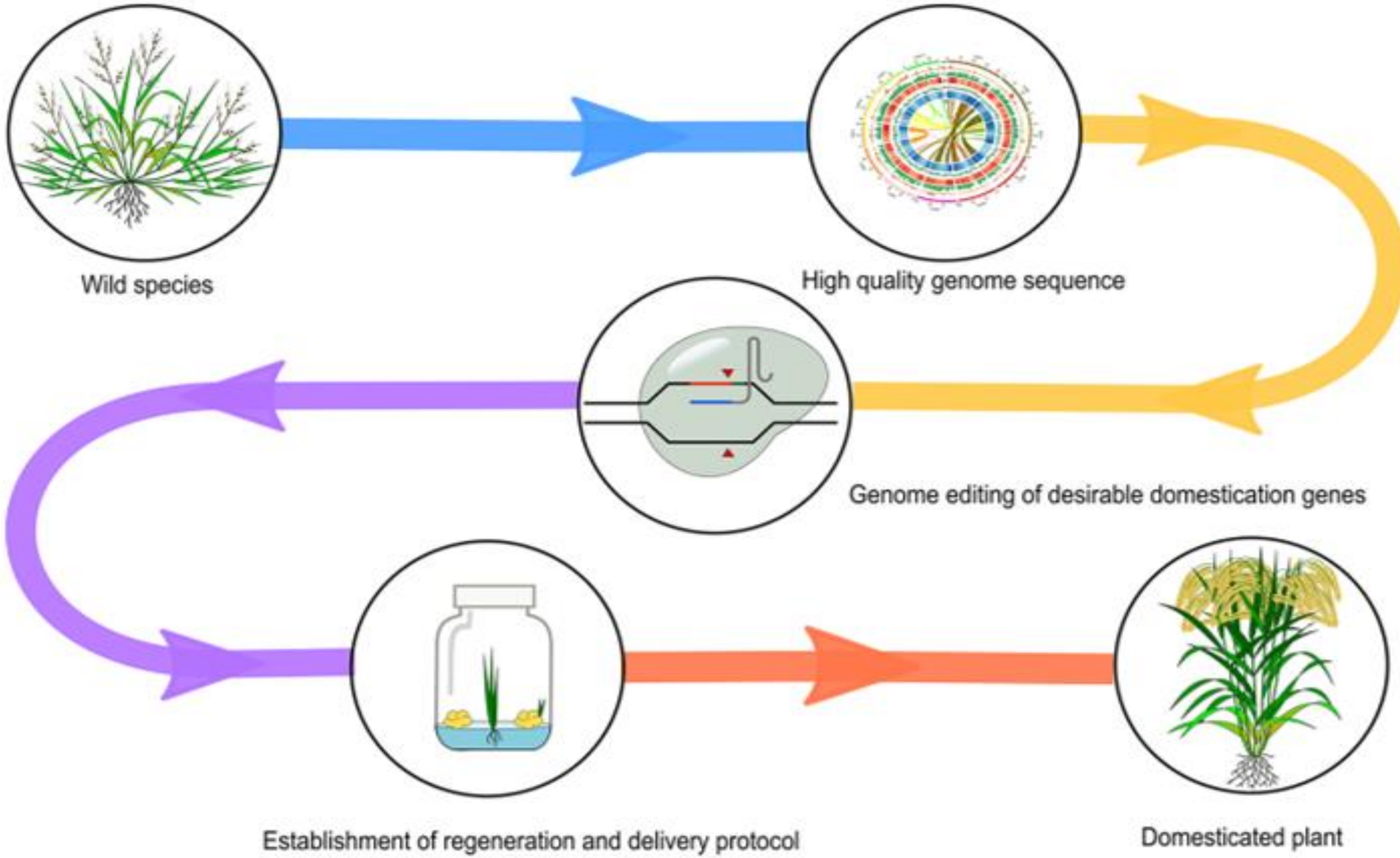
- Conceito de domesticação de culturas e seu papel na agricultura.
- Gene SH4 influencia na desarticulação dos grãos de arroz.
- O gene TUN1 afeta a removibilidade da casca dos grãos de milho.



CRISPR/Cas – Aplicações em domesticação



CRISPR/Cas – Aplicações em domesticação



CRISPR/Cas – Aplicações em domesticação

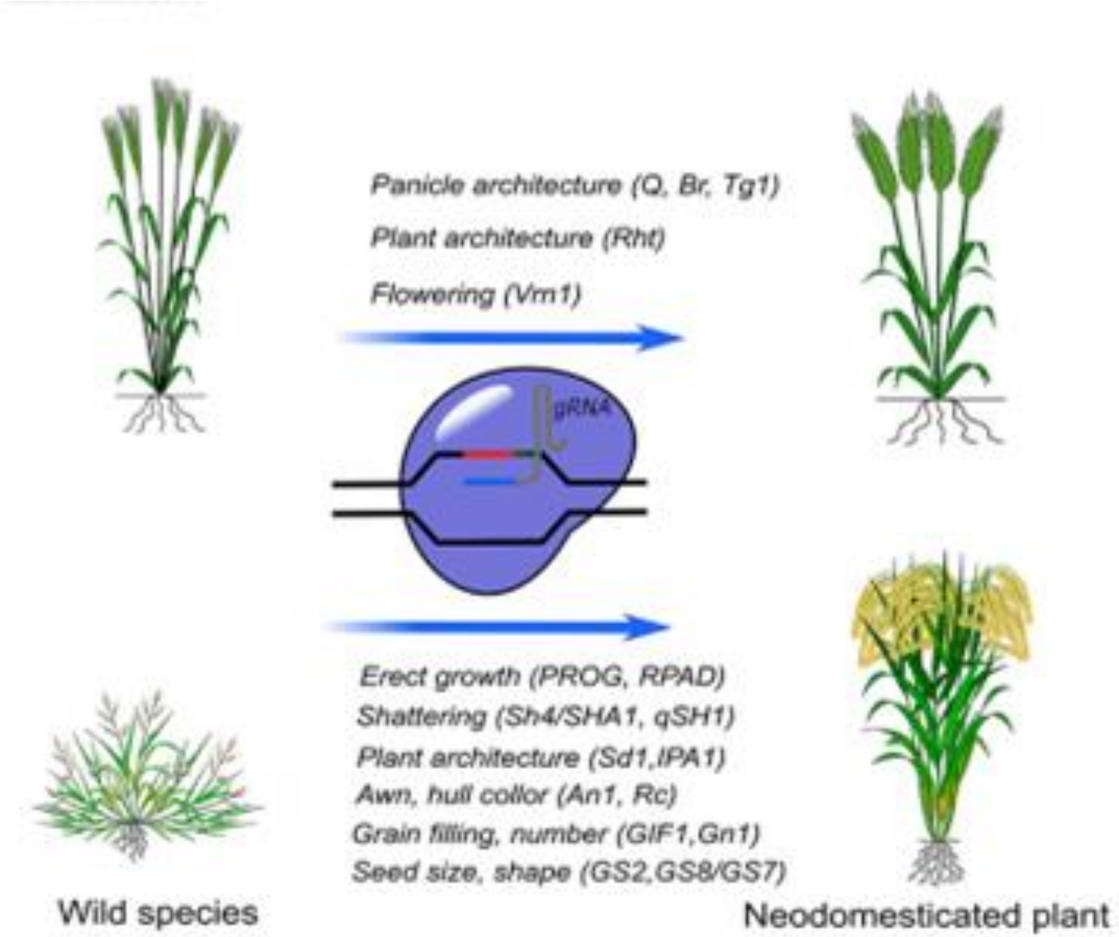


Fig. 5 De novo domestication in wheat and rice. Key domestication traits found in wild species. The precise mutagenesis in these traits using CRISPR/Cas9-mediated multiplex genome editing could accelerate the transition of wild species to domesticated crops.

CRISPR/Cas – Aplicações em domesticação

- GENE Q: Está associado à arquitetura da planta e o número de grãos em espigas. Em plantas de arroz, o gene Q regula o desenvolvimento de ramos secundários da influorescência e influencia o número de grãos produzidos.
- Gene BR, também conhecido como gene de enanismo semigloba, controla a altura da planta. Resultam em um fenótipo de plantas anãs.
- O gene TG1 está envolvido na determinação da presença ou ausência de glumas.

CRISPR/Cas – Aplicações em domesticação

Esses genes, juntamente com outros genes relacionados à domesticação e ao melhoramento de plantas, são alvos importantes para estudos de genética e biotecnologia visando o desenvolvimento de variedades de culturas com características desejáveis, como maior produtividade, resistência a doenças, adaptação a condições ambientais adversas e qualidade dos grãos. A manipulação desses genes por meio de técnicas de edição genômica, como CRISPR/Cas, pode acelerar o processo de melhoramento de plantas e contribuir para a segurança alimentar global.

CRISPR/Cas

Aplicações na conservação

CRISPR/Cas – Aplicações na conservação

SCIENCE ADVANCES | RESEARCH ARTICLE

ENVIRONMENTAL STUDIES

The direct drivers of recent global anthropogenic biodiversity loss

Pedro Jaureguiberry[1]†, Nicolas Titeux[2,3,4]†, Martin Wiemers[2,5], Diana E. Bowler[3,6,7], Luca Coscieme[8], Abigail S. Golden[9,10], Carlos A. Guerra[3,11], Ute Jacob[12,13], Yasuo Takahashi[14], Josef Settele[2,3,15], Sandra Díaz[1], Zsolt Molnár[16], Andy Purvis[17,18]*

Apesar de alguns esforços, **não seremos capazes de retardar as taxas de perda de biodiversidade**.

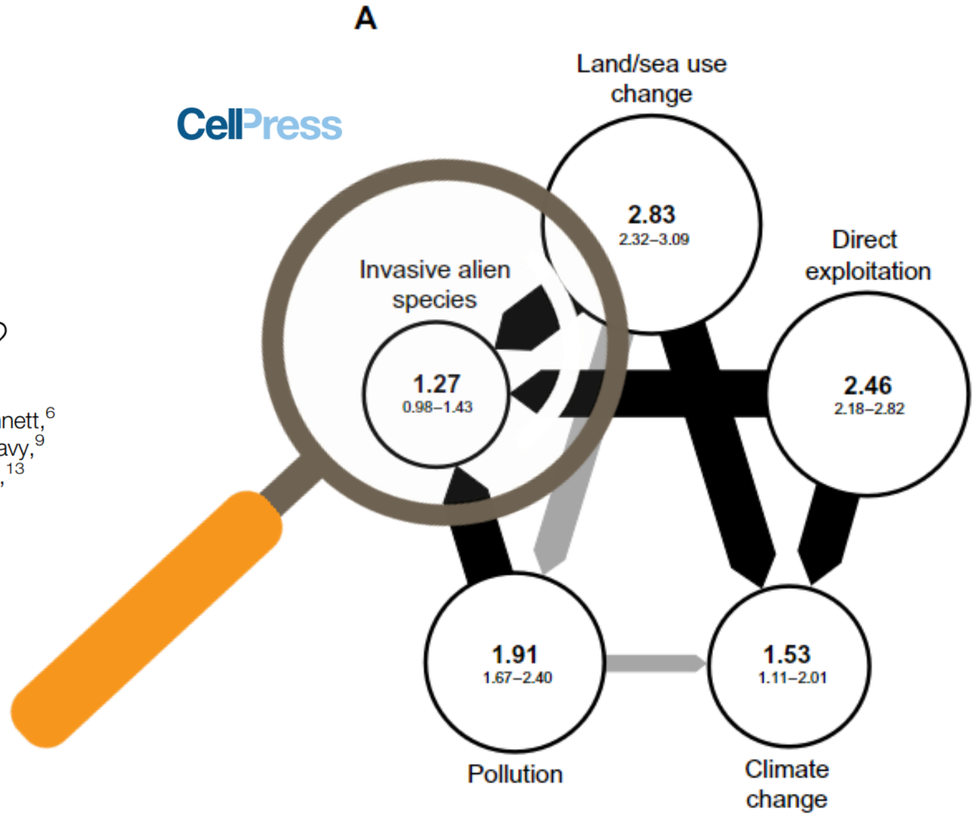
Trends in Ecology & Evolution

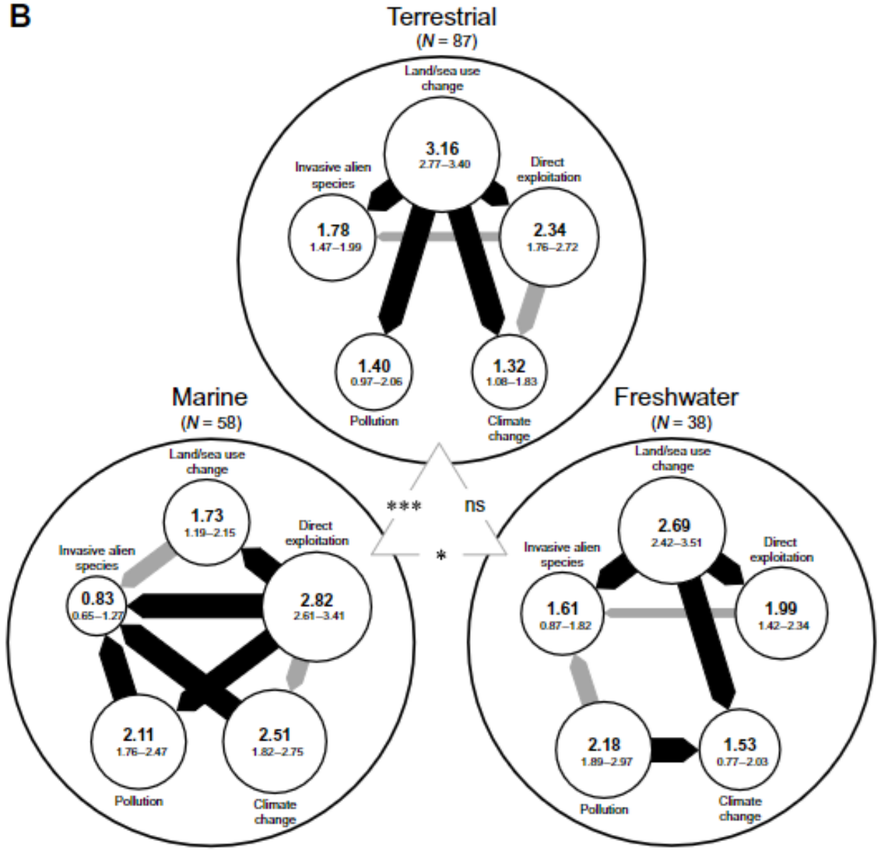
CellPress

Opinion

Is It Time for Synthetic Biodiversity Conservation?

Antoinette J. Piaggio,[1,17,*] Gernot Segelbacher,[2,17] Philip J. Seddon,[3,17] Luke Alphey,[4,5] Elizabeth L. Bennett,[6] Robert H. Carlson,[7] Robert M. Friedman,[8] Dona Kanavy,[9] Ryan Phelan,[10] Kent H. Redford,[11,12] Marina Rosales,[13] Lydia Slobodian,[14] and Keith Wheeler[15,16]





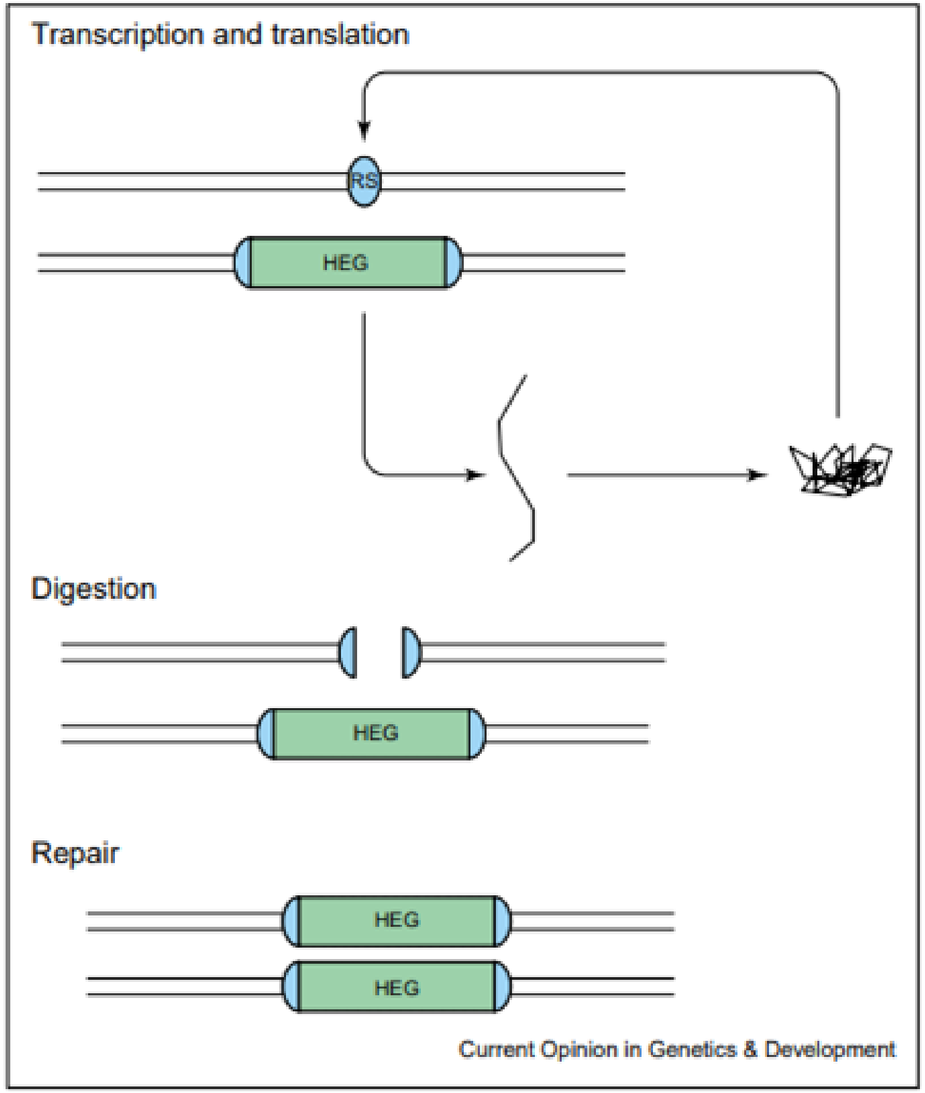
CRISPR/Cas – Aplicações na conservação

Homing endonuclease genes (HEGs) – Um exemplo de gene driver.

ELSEVIER

Homing endonuclease genes: the rise and fall and rise again of a selfish element

Austin Burt and Vassiliki Koufopanou



How homing endonuclease genes (HEGs) home. HEGs encode a protein (top) that in heterozygous cells recognises a sequence on the homologous chromosome and cleaves it (middle). The HEG is inserted into the middle of the recognition sequence (RS), and the chromosome carrying the HEG is protected from being cut. After cleavage, the recombinational repair system of the cell is stimulated, and the HEG-containing chromosome is used as a template for repair. As a consequence, the heterozygote is converted to a homozygote with two copies of the HEG (bottom).

CRISPR/Cas – Aplicações na conservação

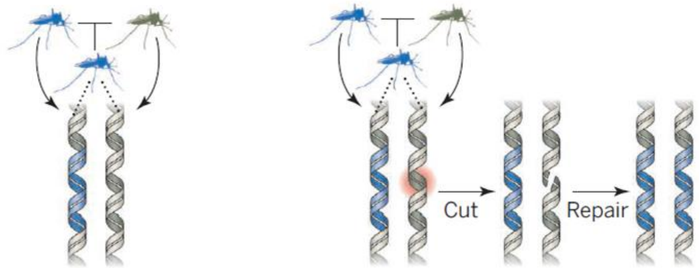
REVIEWS

Check for updates

Gene drives gaining speed

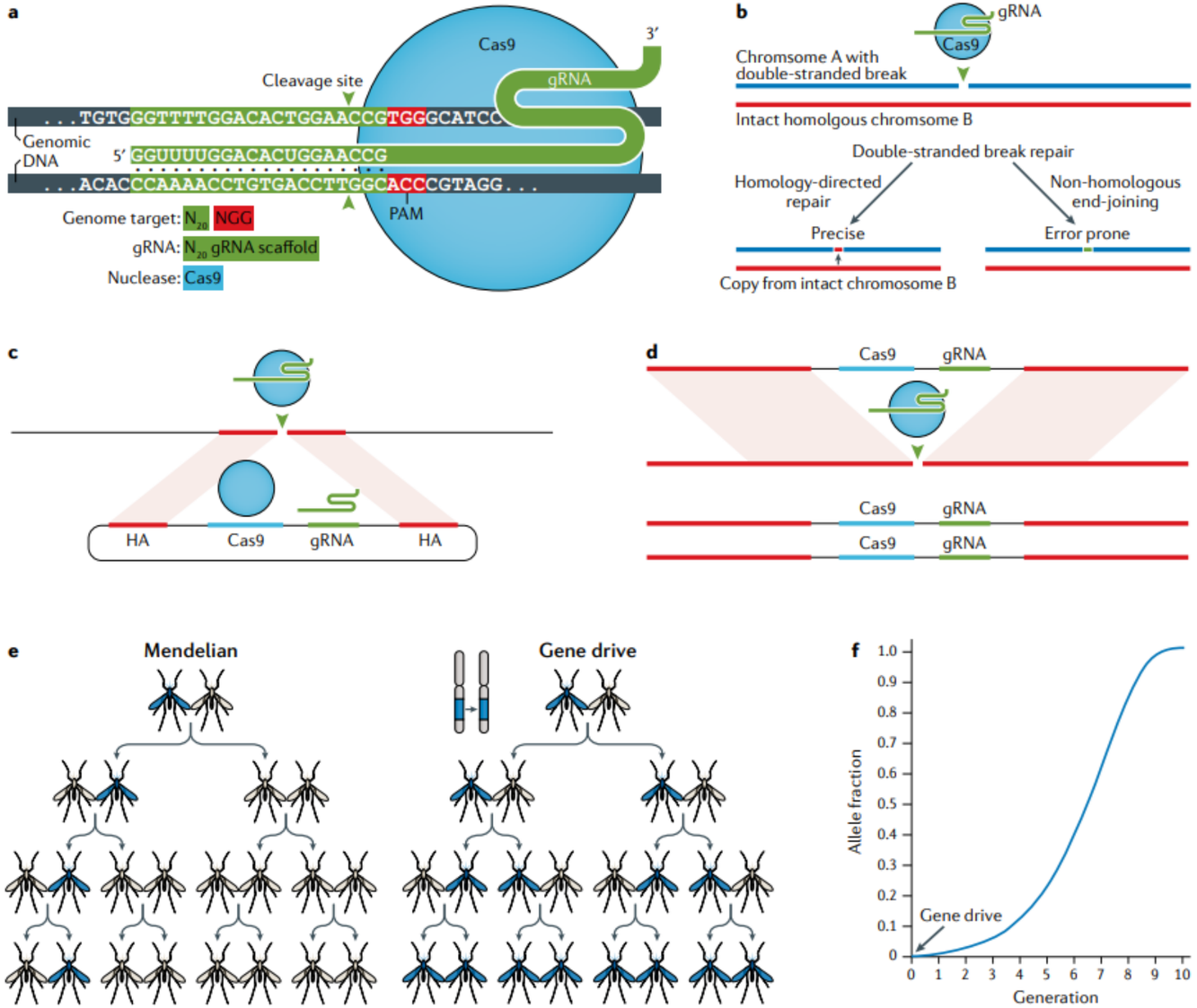
Ethan Bier

Abstract | Gene drives are selfish genetic elements that are transmitted to progeny at super-Mendelian (>50%) frequencies. Recently developed CRISPR–Cas9-based gene-drive systems are highly efficient in laboratory settings, offering the potential to reduce the prevalence of vector-borne diseases, crop pests and non-native invasive species. However, concerns have been raised regarding the potential unintended impacts of gene-drive systems. This Review summarizes the phenomenal progress in this field, focusing on optimal design features for full-drive elements (drives with linked Cas9 and guide RNA components) that either suppress target mosquito populations or modify them to prevent pathogen transmission, allelic drives for updating genetic elements, mitigating strategies including *trans*-complementing split-drives and genetic neutralizing elements, and the adaptation of drive technology to other organisms. These scientific advances, combined with ethical and social considerations, will facilitate the transparent and responsible advancement of these technologies towards field implementation.



Standard altered gene
1 copy inherited from 1 parent
50% chance of passing it on

Altered gene + gene drive
1 copy → 2 copies
100% chance of passing it on



CRISPR/Cas – Aplicações na conservação

ARTICLES

nature biotechnology

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A CRISPR–Cas9 gene drive targeting *doublesex* causes complete population suppression in caged *Anopheles gambiae* mosquitoes

Kyros Kyrou[1,2], Andrew M Hammond[1,2], Roberto Galizi[1], Nace Kranjc[1], Austin Burt[1], Andrea K Beaghton[1], Tony Nolan[1] & Andrea Crisanti[1]

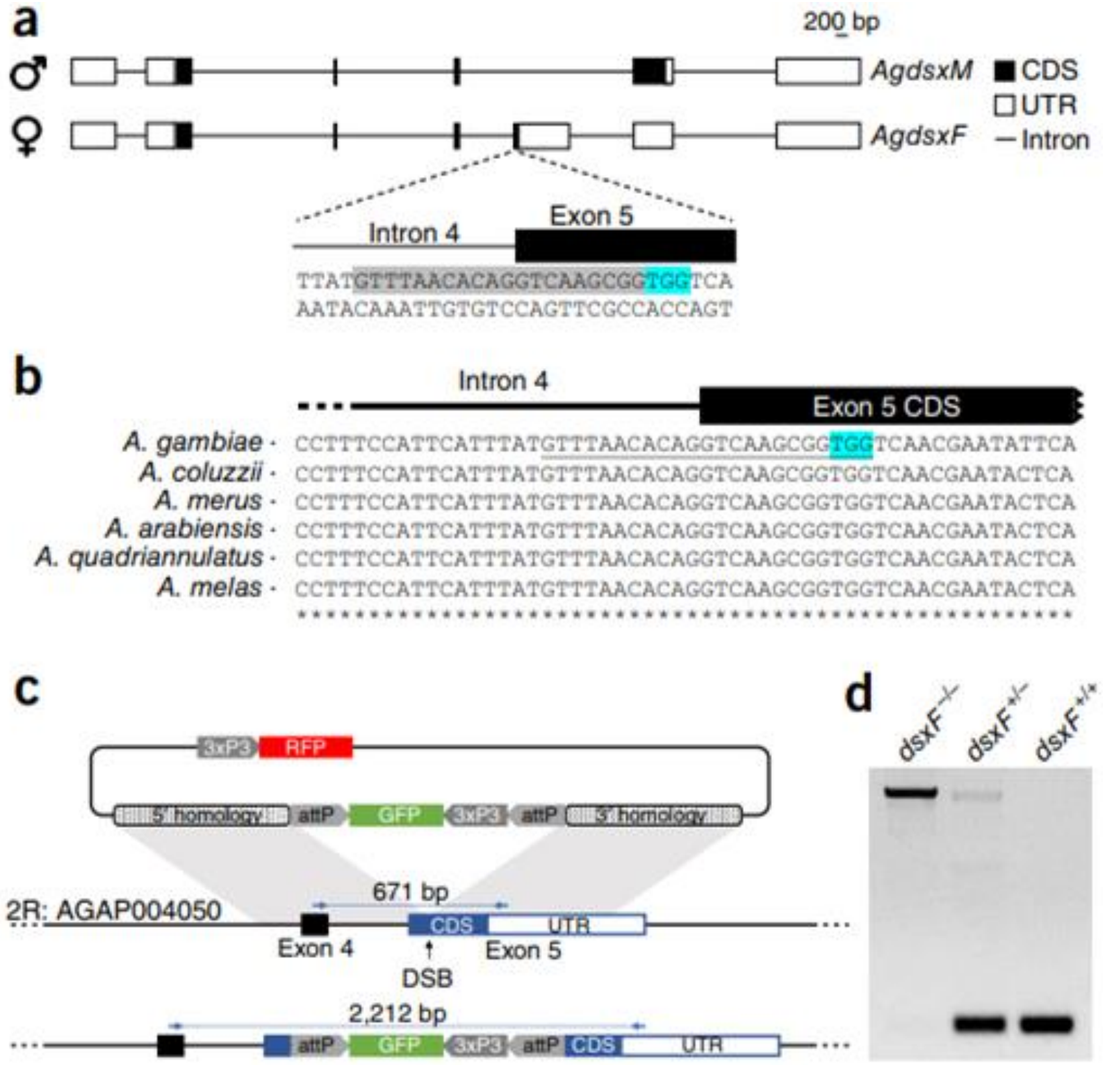

Figure 1 Targeting the female-specific isoform of *doublesex*. (**a**) Schematic representation of the male- and female-specific *dsx* transcripts and the gRNA sequence used to target the gene (shaded in gray). The gRNA spans the intron 4–exon 5 boundary. The protospacer-adjacent motif (PAM) of the gRNA is highlighted in blue. Scale bar, 200 bp. Coding regions of exons (CDS) are shaded in black, noncoding regions in white. Introns are not drawn to scale. UTR, untranslated region. (**b**) Sequence alignment of the *dsx* intron 4–exon 5 boundary in six of the species from the *A. gambiae* complex. The sequence is highly conserved within the complex suggesting tight functional constraint at this region of the *dsx* gene. The gRNA used to target the gene is underlined and the protospacer-adjacent motif is highlighted in blue. (**c**) Schematic representation of the HDR knockout construct specifically recognizing exon 5 and the corresponding target locus. DSB, double-strand break. (**d**) Diagnostic PCR using a primer set (blue arrows in **c**) to discriminate between the wild-type and *dsxF* allele in homozygous ($dsxF^{-/-}$), heterozygous ($dsxF^{+/-}$) and wild-type ($dsxF^{+/+}$) individuals.

CRISPR/Cas – Aplicações na conservação

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A sequência do gene ***dsx*** tem **potencial** para atuar como um **gene driver**.

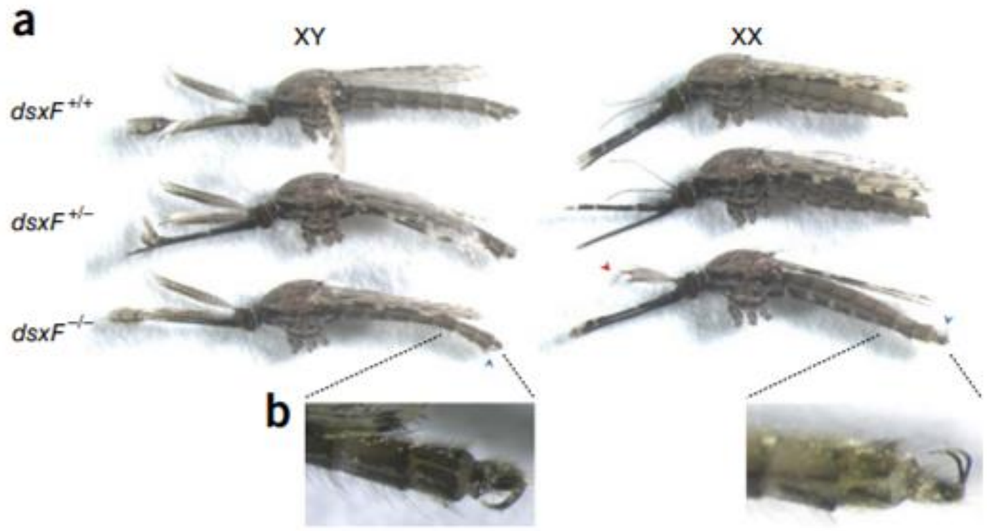

Figure 2 Morphological analysis of homozygous *dsxF*$^{-/-}$ mutants. (**a**) Morphological appearance of genetic males and females heterozygous (*dsxF*$^{+/-}$) or homozygous (*dsxF*$^{-/-}$) for the exon 5 null allele. This assay was performed in a strain containing a dominant RFP marker linked to the Y chromosome, whose presence permits unambiguous determination of male or female genotype. Anomalies in sexual morphology were observed only in *dsxF*$^{-/-}$ genetic female mosquitoes. This group of XX individuals showed male-specific traits, including a plumose antenna (red arrowhead) and claspers (blue arrowheads). This group also showed anomalies in the proboscis and accordingly they could not bite and feed on blood. Representative samples of each genotype are shown. (**b**) Magnification of the external genitalia. All *dsxF*$^{-/-}$ females carried claspers, a male-specific characteristic. The claspers were dorsally rotated rather than in the normal ventral position.

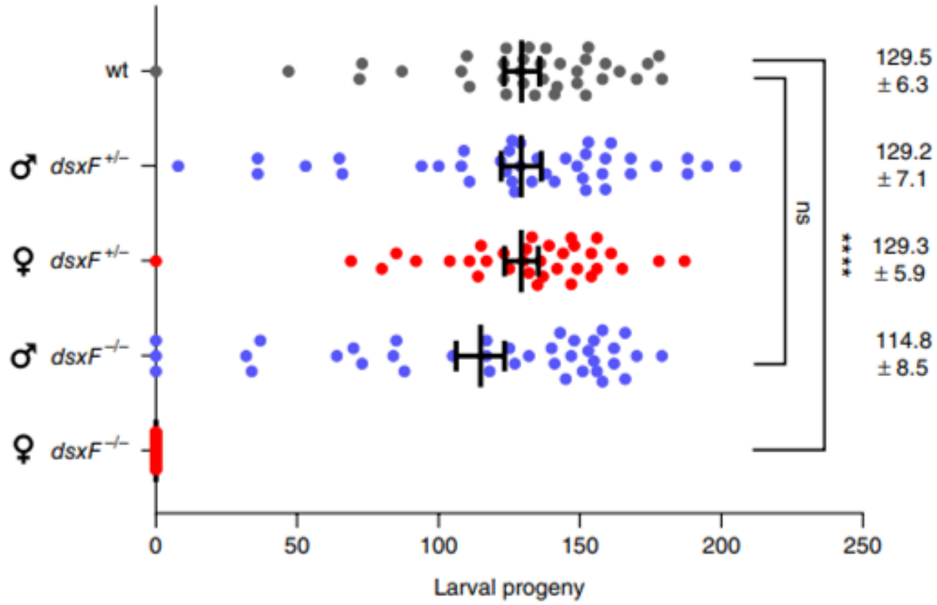

Figure 3 Reproductive phenotype of *dsxF* mutants. Male and female *dsxF*$^{-/-}$ and *dsxF*$^{+/-}$ individuals were mated with the corresponding wild-type sexes. Females were given access to a blood meal and subsequently allowed to lay individually. Fecundity was investigated by counting the number of larval progeny per lay ($n \geq 43$). Using wild type (wt) as a comparator, we saw no significant differences ('ns') in any genotype other than *dsxF*$^{-/-}$ females, which were unable to feed on blood and therefore failed to produce a single egg (**** $P < 0.0001$; Kruskal–Wallis test). Vertical bars indicate the mean and the s.e.m. Blue and red indicate the crosses of male or female *dsxF* mutants, respectively, to wild type, whereas the gray dots represent wild-type-only crosses.

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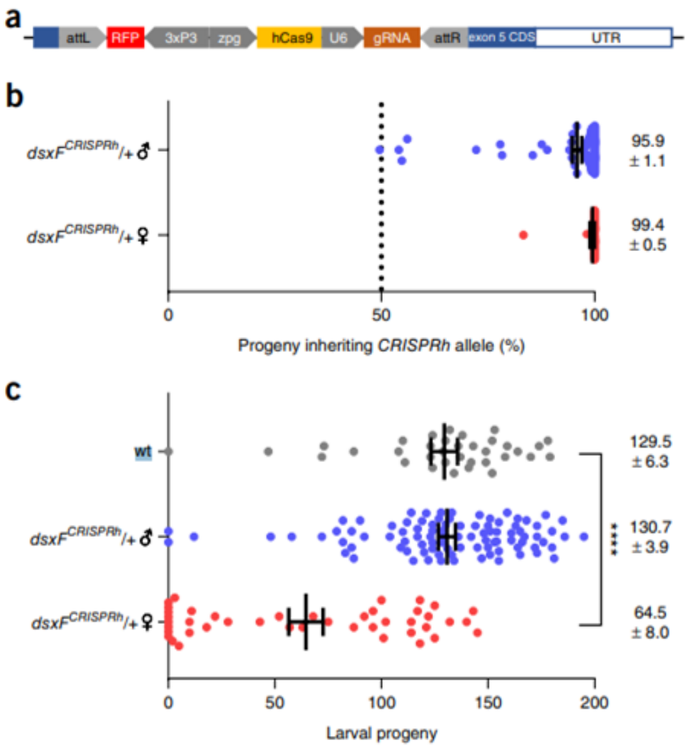


Figure 4 Transmission rate of the *dsxF*^{CRISPRh} driving allele and fecundity analysis of heterozygous male and female mosquitoes. (**a**–**c**) Male and female mosquitoes heterozygous for the *dsxF*^{CRISPRh} allele (**a**) were analyzed in crosses with wild-type mosquitoes to assess the inheritance bias of the *dsxF*^{CRISPRh} drive construct (**b**) and for the effect of the construct on their reproductive phenotype (**c**). (**b**) Scatter plot of the transgenic rate observed in the progeny of *dsxF*^{CRISPRh}/+ female or male mosquitoes that gave progeny when crossed to wild-type individuals ($n \geq 33$). Each dot represents the progeny derived from a single female. Both male and female *dsxF*^{CRISPRh}/+ showed a high transmission rate of up to 100% of the *dsxF*^{CRISPRh} allele to the progeny. The transmission rate was determined by visually scoring offspring for the RFP marker that is linked to the *dsxF*^{CRISPRh} allele. The dotted line indicates the expected Mendelian inheritance. Mean transmission rate (± s.e.m.) is shown. (**c**) Scatter plot showing the number of larvae produced by single females ($n \geq 35$) from crosses of *dsxF*^{CRISPRh}/+ mosquitoes with wild-type individuals after one blood meal. Mean progeny count (± s.e.m.) is shown (****$P < 0.0001$; Kruskal–Wallis test).

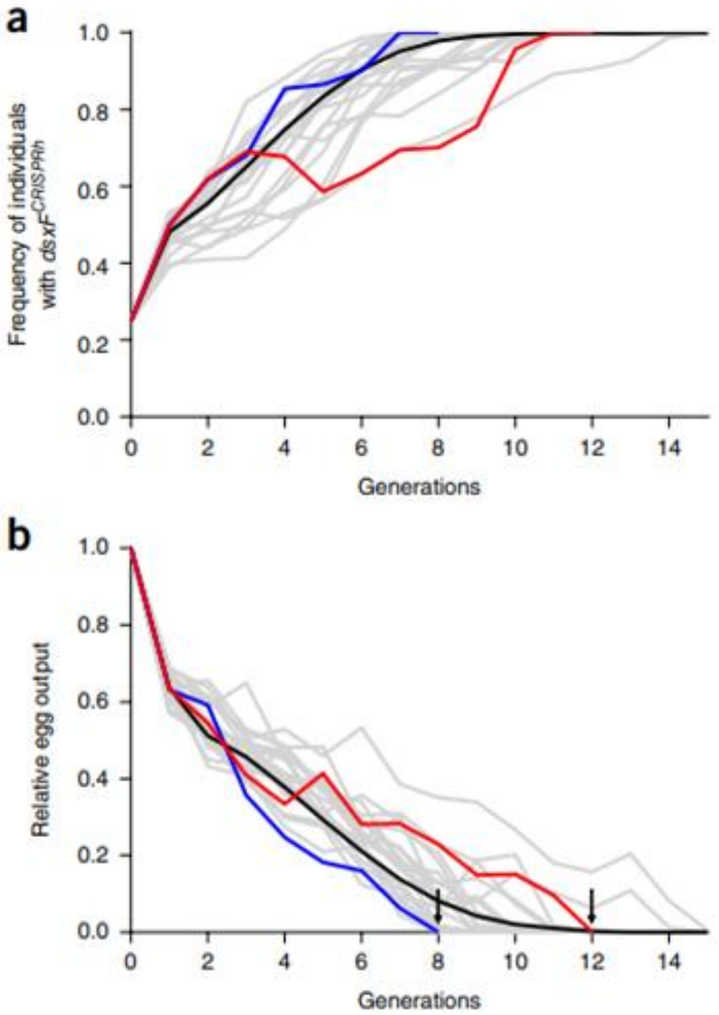


Figure 5 Dynamics of the spread of the *dsxF*^{CRISPRh} allele and effect on population reproductive capacity. Two cages were set up with a starting population of 300 wild-type females, 150 wild-type males and 150 *dsxF*^{CRISPRh}/+ males, seeding each cage with a *dsxF*^{CRISPRh} allele frequency of 12.5%. (**a**) The frequency of *dsxF*^{CRISPRh} mosquitoes was scored for each generation. The drive allele reached 100% prevalence in both cage 2 (blue) and cage 1 (red) at generation 7 and 11, respectively, in agreement with a deterministic model (black line) that takes into account the parameter values retrieved from the fecundity assays. Twenty stochastic simulations were run (gray lines) assuming a maximum population size of 650 individuals. (**b**) Total egg output deriving from each generation of the cage was measured and normalized relative to the output from the starting generation. Suppression of the reproductive output of each cage led the population to collapse completely (black arrows) by generation 8 (cage 2) or generation 12 (cage 1). Parameter estimates included in the model are provided in **Supplementary Table 5**.

CRISPR/Cas – Aplicações na conservação

RESEARCH ARTICLE

Ecology and Evolution Open Access WILEY

Trust your guts? The effect of gut section on diet composition and impact of *Mus musculus* on islands using metabarcoding

Catarina J. Pinho[1,2,3] | Evandro P. Lopes[1,2,3,4] | Joana Paupério[1,3] | Isildo Gomes[5] | Maria M. Romeiras[6] | Raquel Vasconcelos[1,3,4]

131 *taxa*

73 plantas

58 invertebrados

Nativas

Endêmicas

Importância econômica

PNAS RESEARCH ARTICLE | GENETICS OPEN ACCESS Check for updates

Leveraging a natural murine meiotic drive to suppress invasive populations

Luke Gierus[a,b,1], Aysegul Birand[c,1], Mark D. Bunting[a,b], Gelshan I. Godahewa[b,d], Sandra G. Piltz[a,b], Kevin P. Oh[e,f], Antoinette J. Piaggio[g], David W. Threadgill[h], John Godwin[i], Owain Edwards[e,j], Phillip Cassey[c], Joshua V. Ross[k], Thomas A. A. Prowse[c] and Paul Q. Thomas[a,b,2]

Edited by James Bull, University of Idaho, Moscow, ID; received August 3, 2022; accepted October 4, 2022

***t*^{w2} como gene drive** para o biocontrole genético de **ratos domésticos**.

ORIGINAL ARTICLE

Evolutionary Applications Open Access WILEY

Population genomics of invasive rodents on islands: Genetic consequences of colonization and prospects for localized synthetic gene drive

Kevin P. Oh[1,2] | Aaron B. Shiels[1] | Laura Shiels[1] | Dimitri V. Blondel[3] | Karl J. Campbell[4,5] | J. Royden Saah[4,6] | Alun L. Lloyd[6,7] | Paul Q. Thomas[8] | Fred Gould[6,9] | Zaid Abdo[2] | John R. Godwin[3,6] | Antoinette J. Piaggio[1]

CRISPR/Cas – Aplicações na conservação

iScience

CellPress
OPEN ACCESS

Review

Direct and indirect impacts of synthetic biology on biodiversity conservation

Nicholas B.W. Macfarlane,[1,*] Jonathan Adams,[2] Elizabeth L. Bennett,[3] Thomas M. Brooks,[4,5,6] Jason A. Delborne,[7,8] Hilde Eggermont,[9,10] Drew Endy,[11] Kevin M. Esvelt,[12] Bartlomiej Kolodziejczyk,[13] Todd Kuiken,[8] Maria Julia Oliva,[14] Sonia Peña Moreno,[4] Lydia Slobodian,[15] Risa B. Smith,[16] Delphine Thizy,[17,18] Daniel M. Tompkins,[19] Wei Wei,[20] and Kent H. Redford[21,22]

Limitações:

i. requer reprodução sexual.

ii. a velocidade pode ser lenta.

iii. populações não se encontram isoladas.

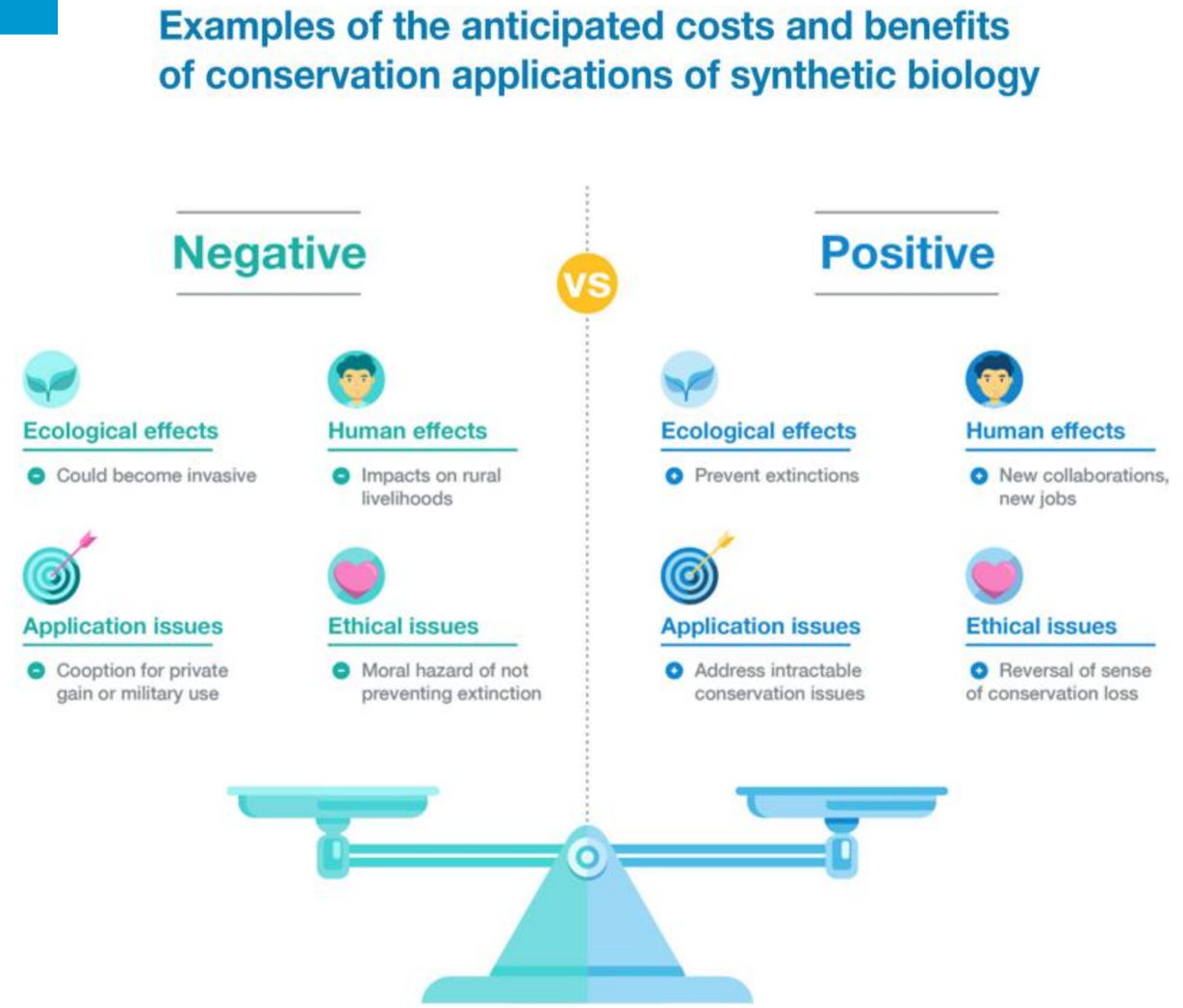


Figure 1. Examples of the anticipated costs and benefits of conservation applications of synthetic biology (adapted with permission from K.H. Redford et al., 2019)

CRISPR/Cas

Aplicações no melhoramento

CRISPR/Cas – Aplicações no melhoramento

Annual Review of Plant Biology

CRISPR/Cas Genome Editing and Precision Plant Breeding in Agriculture

Kunling Chen,[1,*] Yanpeng Wang,[1,*] Rui Zhang,[1] Huawei Zhang,[1] and Caixia Gao[1,2]

[1]State Key Laboratory of Plant Cell and Chromosome Engineering, Center for Genome Editing, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China 100101; email: cxgao@genetics.ac.cn

[2]University of Chinese Academy of Sciences, Beijing, China 100864

CRISPR/Cas systems: opportunities and challenges for crop breeding

Sukumar Biswas[1] · Dabing Zhang[1] · Jianxin Shi[1]

Received: 17 January 2021 / Accepted: 28 April 2021 / Published online: 11 May 2021

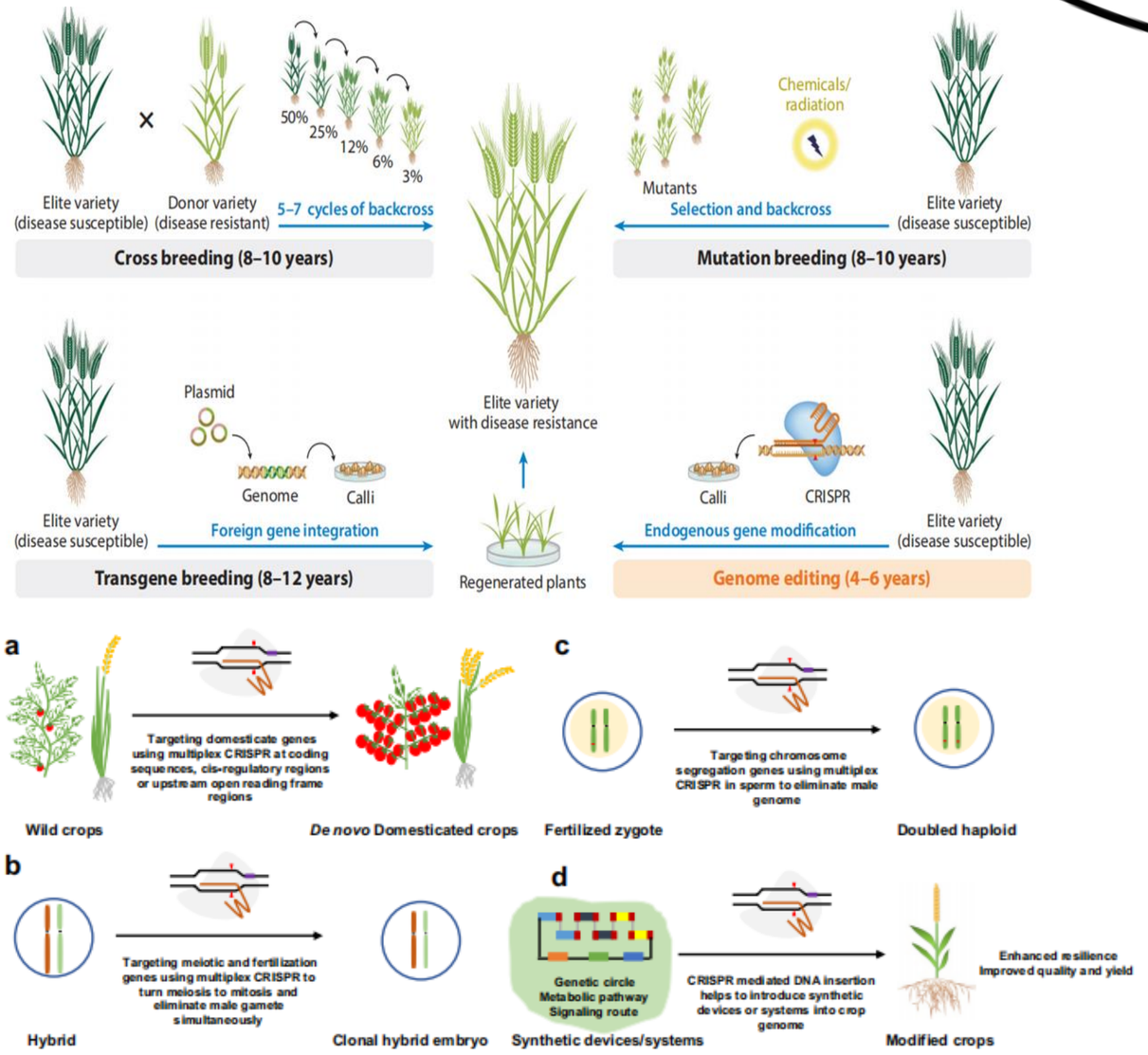


Fig. 1 Principles and methods of CRISPR systems in crops improvement. **a** Crop domestication. **b** Heterosis. **c** Haploid induction. **d** Synthetic biology

CRISPR/Cas – Aplicações no melhoramento

REVIEW

CRISPR-Cas technology based genome editing for modification of salinity stress tolerance responses in rice (*Oryza sativa* L.)

Ibrahim Khan[1] · Sikandar Khan[2] · Yong Zhang[1] · Jianping Zhou[1] · Maryam Akhoundian[3] · Sohail Ahmad Jan[4]

Received: 17 January 2021 / Accepted: 24 April 2021 / Published online: 5 May 2021

O aumento da **população** e demanda por **produção**.

Eventos extremos afetam o desenvolvimento, crescimento e a produtividade de diversas culturas.

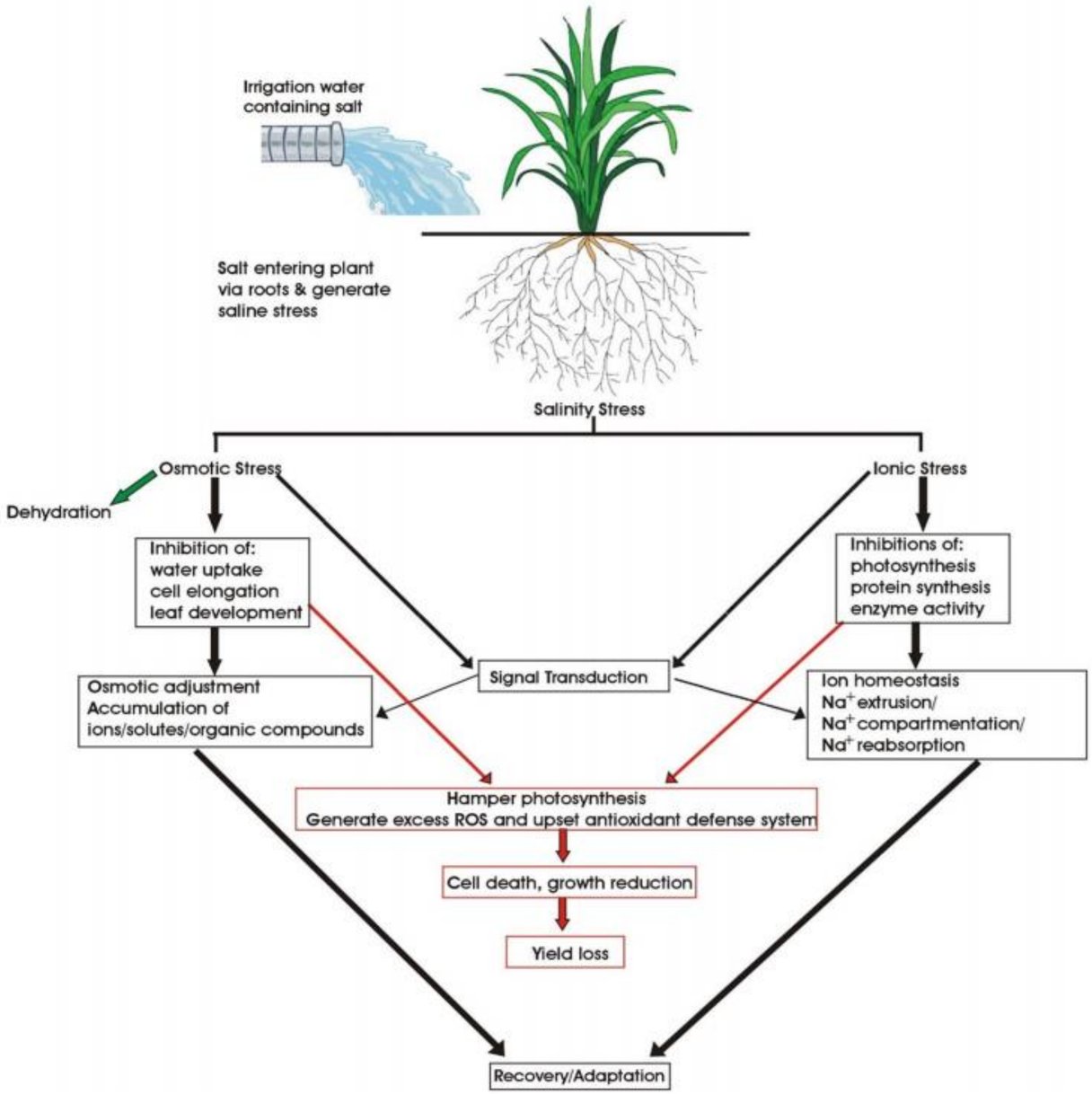
A **salinidade** — estresse osmótico e a toxicidade do acúmulo de íons.

Solução?

Plantas resistentes minimizam o estresse osmótico e os danos celulares.

CRISPR/Cas – Aplicações no melhoramento

Fig. 1 Adaptive responses of plants to salt stress. Salinitny stress induced osmotic and ionic stress in plants. Plants species with salt and droght stress resistance adjust to saline stress by regulating the inclusion and exclusion of varoius ions and organic solutes (black colored route) while in sanlity sensitive plant species high salt in growth medium hamper phtosynthesis and generate reactive oxygen (ROS) in abudnace which eventually cause cell death and hence yield loss (red colored route). (Color figure online)



CRISPR/Cas – Aplicações no melhoramento

Como chegar aos genes? **Mapeamento, marcadores, QTLs.**

CRISPR/Cas — edição **sitio-especifica** de genomas.

Dessa forma, **novas variedades** de arroz com qualidades melhoradas podem ser geradas.

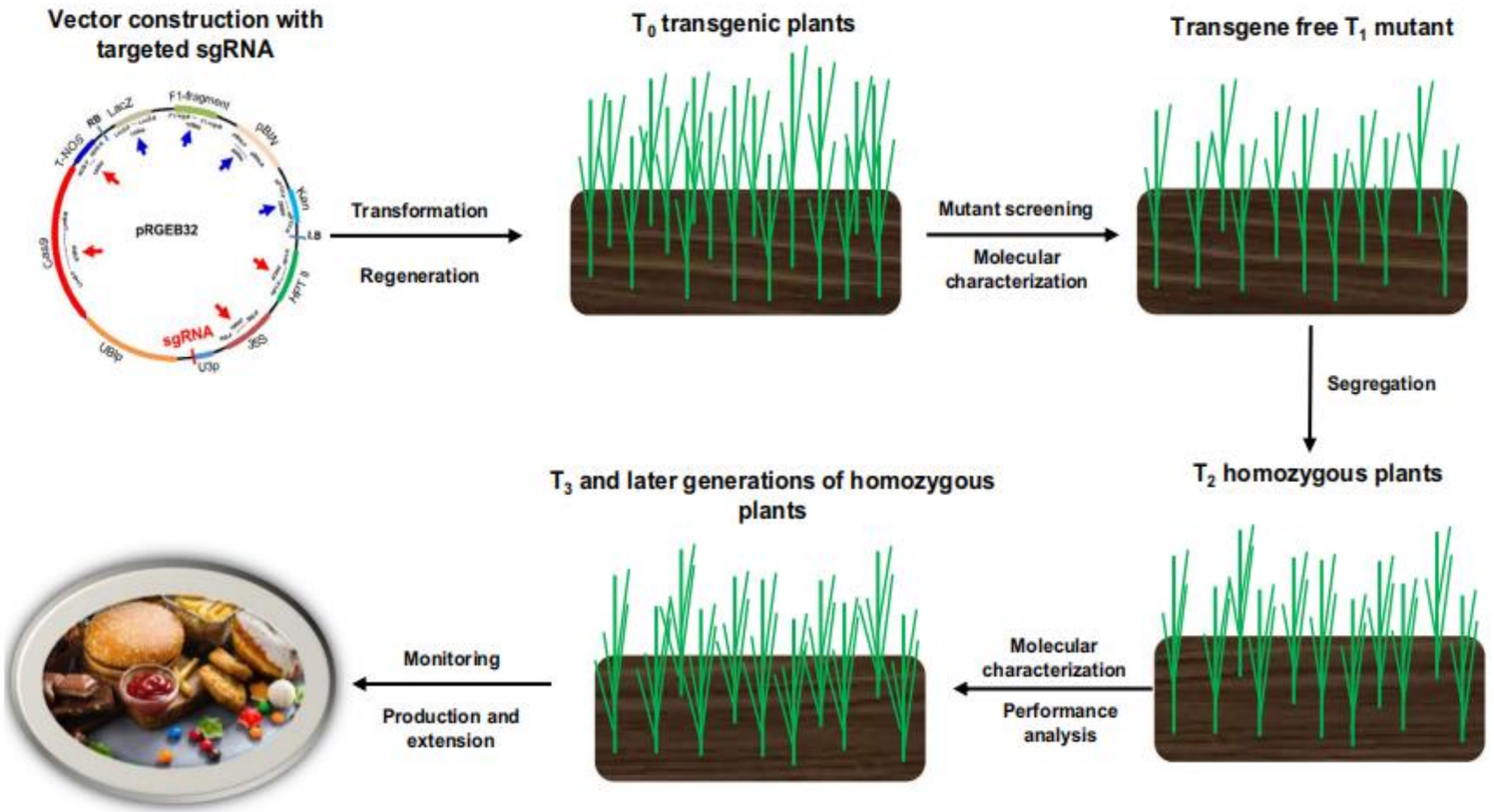


Fig. 2 Molecular characterization of genome-edited crops along the process from the lab to the field

CRISPR/Cas – Aplicações no melhoramento

1. Selection of target genomic site
- 20 bp sequence just upstream of the PAM (NGG) (CRISPR-Cas9)
- 20-24 bp sequence just downstream of the PAM (TTTV) in CRISPR-Cas12a /Cpf1
- Use online tools to minimize off-targeting

2. Designing of sgRNA (Cas9 system)
- sgRNA is expressed using a small RNA promoter such as U6p or U3p
- First nucleotide in the guide sequence is a "G" if U6p is used or an "A" if U3p is used
- Guide sequence should match the target except for the first nucleotide (5′ G or A)

3. Construction of expression vector
- Assemble DNA sequences producing sgRNA and Cas9 nuclease (Cas9 vector)
- Assemble DNA sequences producing crRNA and Cas12a nuclease (Cas12a vector)

4. Stable transformation of plants
- Agrobacterium transformation
- Callus bombardment
- Protoplast transformation

5. Screening of putative transformed plants
- Selection of transformed plants via selectable markers
- Analyzing gel bands to confirm inserted sequence

6. Genotyping (genome editing events) of T_0 lines
- Different screening methods such as PCR/RE, SSCP, T7E1 and ACT-PCR etc. have been used to detect CRISPR-Cas induced mutants

7. Analysis of gene function in T_1 generation
- To observe and analyze the phenotypic consequences induced by CRISPR-Cas genome editing
- To study molecular functions of the edited genes in plants

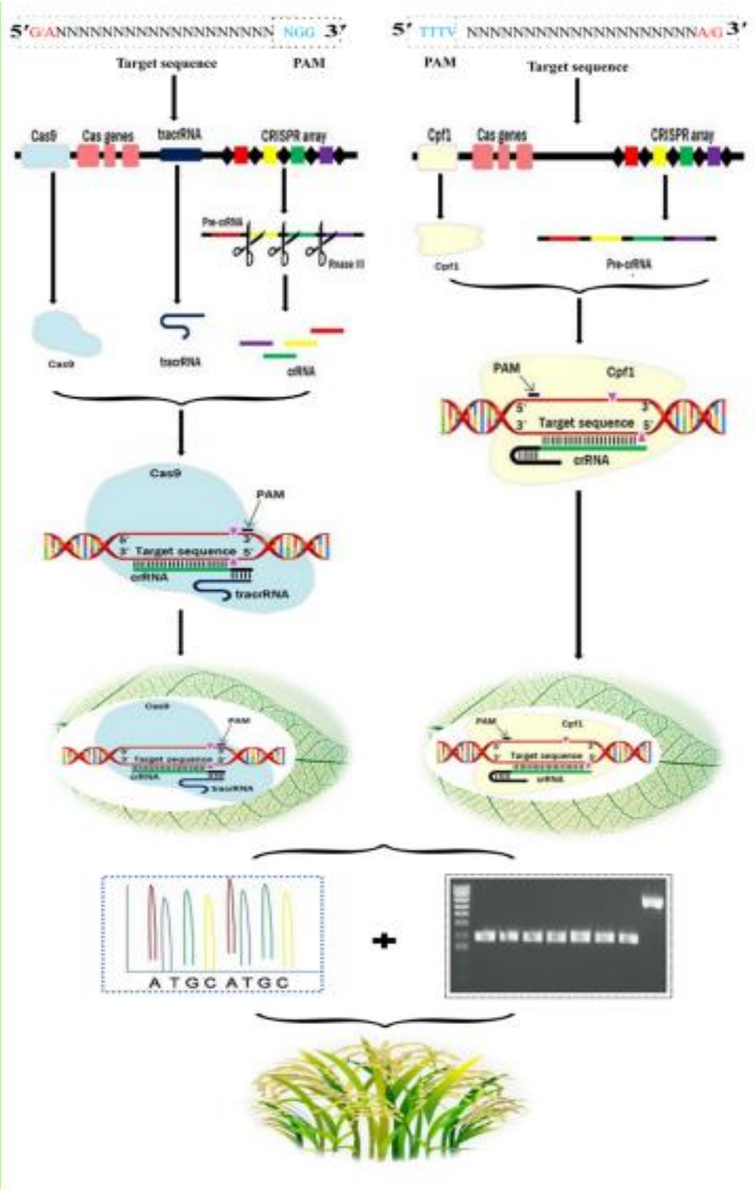


Fig. 2 Basic strategy of plant genome editing with Cas9 or Cas12/sgRNA system. Modified after Świat et al. [94]

CRISPR/Cas – Aplicações no melhoramento

Table 1 Genes identified in rice cultivars which lead to salinity stress tolerance

S. No	Gene name	Specific function	References
1	*Oshkt1;1* and *Oshkt2:1*	Play an important role in reducing Na+ accumulation in shoots to cope with salt stress	[36]
2	*OsSHMT3*	Play a vital role to confer tolerance toward salt stress	[46]
3	*SIDP361*	Encodes a *DUF1644* family protein to induce salt tolerance	[47]
4	*OrbHLH001*	Overexpression of *OrbHLH001* gene enhances tolerance to salt stress in shoots and roots of rice	[48]
5	*OsP5CS1*	Its expression is inducing under endogenous supply of ABA and salt stress	[49]
6	*OsSAPK2*	*SAPK2* rapidly activated in response to salt stress in rice plants	[50]
7	*OsP5CR*	Higher level of *OsP5CR* transcripts accumulation were observed in rice cultivars during salt stress	[49]
8	*OsCIPK15*	Overexpression of *OsCIPK15* shows enhances salt tolerance in rice	[51]
9	*OsMYB3R-2*	Rice plants expressing *OsMYB3R-2* showed increased salt tolerance	[52]
10	*OsHAK5*	Overexpression of this gene confer salt tolerance in rice shoots	[53]
11	*ZFP179*	Overexpression of *ZFP179* gene increased salt tolerance and is useful to develop transgenic crops with enhanced salt tolerance	[54]
12	*SAPK4*	Induced expression of *SAPK4* result in improved germination, growth and development under salt stress both in seedlings and mature plants	[55]
13	*AKT1*	Over expression of *AKT1* enhances $K^+$and Na^+ uptake by root tissues	[56]
14	*OsHAK21*	The potassium transporter *OsHAK21* functions in the maintenance of ion homeostasis and tolerance to salt stress	[57]
15	*OsPP1a*	Overexpression of *OsPP1a* enhancing tolerance to high salt treatment in rice	[58]
16	*OsNHX1*	*OsNHX1* encoding a Na+ /H+ exchanger and involve to increase salt resistance in rice by decreasing osmotic potential under NaCl stress	[59]
17	*PtCYP714A3*	PtCYP714A3 regulates homeostasis of Na+/K+in transgenic rice plants to support their survival under salinity stress	[60]
18	*OsSUV3*	In transgenic rice *OsSUV3* helicase improves photosynthesis to comate salinity stress	[61]
19	*OsRMC*	*OsRMC* encodes a receptor like kinase which negatively regulate salt stress response in rice	[62]
20	*OsCPK4*	Under salt stress condition *OsCPK4* gene expression rate is enhancing to increase water holding capacity of plant cells, prevent lipid peroxidation and control electrolytes leakage from the membranes	[63]
21	*P5CS*	A strong correlation between *P5CS* expression and the accumulation of proline has been shown in rice	[64]
22	*codA*	Promotes synthesis of glycine betaine to protect damage of membrane, enzymatic activity, photosynthesis and helps to regulate ROS detoxification	[65]
23	*OsTPS1*	Overexpression of *OsTPS1* improve the tolerance of rice seedling to high salinity by increasing the amount of trehalose and proline	[66]
24	*PINO1*	Overexpression of this gene allows growth of the transgenic plants in salty environment by increasing inositol production	[67]
25	*OsTIP1;1*	Expression of *OsTIP1;1* increases during high salt stress	[68]
26	*HvPIP2;1*	Under salt stress transcription of *HvPIP2;1* is increases	[69]
27	*OsDREB1A*	Transcript levels of *OsDREB1A* increases rapidly when rice plants are exposed to salt tolerance	[70]
28	*OsDREB2A* and *OsDREB1D*	Expression of *OsDREB2A and OsDREB1D* transcription factors confer enhancing dehydration and salt stress tolerance in rice	[71]
29	*OsDREB1F*	Transgenic plants harboring *OsDREB1F* gene led to enhanced tolerance to salt	[72]
30	*OsCDPK7*	*OsCDPK7* encoding a Ca2+dependent protein kinases and its overexpression enhance salt stress tolerance in transgenic rice	[73]
31	*GlyII*	Studies indicated that glyII enzyme may have an important and independent role in conferring salt stress tolerance	[74]
32	*GS2*	Expression of *GS2* confer resistant to salt in rice plants by enhancement photorespiration	[75]
33	*OsKAT1*	*OsKAT1* with cooperation of K1 channels maintain cytosolic cation homeostasis during salt stress and thus protect leakage of Na^+ from cells	[76]
34	*OsMAPK5*	*OsMAPK5* encode different spliced transcripts which eventually positively regulate salt resistance in plants	[77]

Table 1 (continued)

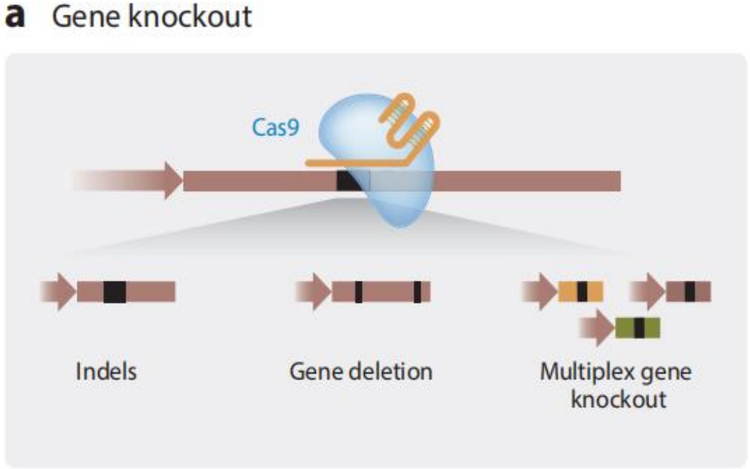
S. No	Gene name	Specific function	References
35	*Em*	Treatment with saturated levels of NaCl and ABA results in a double level of *Em* transcripts in rice cells	[78]
36	*rMip1*and *rTip1*	These two genes have role in cell transportation and their expression is increases in rice seedlings under high salt and drought stress	[79]
37	*sodA1*, *sodCc1*and *sodCc2*	The expression of *sodA1* and *sodCc2* is strongly increases with treatment of high salt and ABA concentration. and transcription of *sodCc1* gene is increases during salt stress	[80]
38	*NAC*	Expression of *NAC* (*NAM*, *ATAF* and *CUC*) gene family can be induced significantly in guard cells of rice under salt stress	[81]
39	*NADP-ME*	*NADP*-malic enzyme plays an important role in plant defense and its expression is induced by salt stress	[82]
40	*ZFP252*	*ZFP252* plays an important role in rice response to salt and drought stresses and is useful in engineering crop plants with enhanced tolerance to salt and drought stresses	[83]
41	*OsMSR2*	Expression of *OsMSR2* gene conferred enhanced tolerance to high salt in Arabidopsis	[84]
42	*OsGAPC3*	This gene can alleviate salt toxicity in transgenic plants through the regulation of H_2O_2 level	[85]
43	*NBRISN13*	Studies imply that expression of *NBRISN13* (*SN13*) gene confers salt tolerance in rice by modulating differential transcription in a set of different 14 genes	[86]
44	*ONAC106*	Transcripts of *ONAC106* delays leaf yellowing and enhance salt stress in rice plants	[87]
45	*OsBIERF3*	Studies suggest that transcripts of *OsBIERF3*gene plays an important role in disease resistance response and salt tolerance	[88]
46	*Rab16A*	Overexpression of *Rab16A* gene in transgenic plants reduced chlorophyll damages, decease Na^+ accumulation and generate salt tolerance	[89]
47	*CYP94*	Elevated level of *CYP94* genes (*CYP94C1* and *CYP94C2b*) transcripts confer salt tolerance in rice cells	[90]
48	*OsCPK21*	The transcript of *OsCPK21* is a calcium-dependent protein kinase that confers salt tolerance in rice	[91]
49	*OsCYP2*	Transgenic rice seedlings with high level of *OsCYP2* showed better tolerance to salt than wild-type seedlings	[92]
50	*OSRIP18*	Overexpression of *OsRIP18* gene in transgenic rice induced tolerance against drought and salt stresses	[93]
51	*OsCam1-1*	Overexpression of *OsCam1-1* gene plays an important role in ABA biosynthesis which lead to salt tolerance in rice	[95]

CRISPR/Cas – Aplicações no melhoramento

Article

CRISPR/Cas9 Mediated Knockout of the *OsbHLH024* Transcription Factor Improves Salt Stress Resistance in Rice (*Oryza sativa* L.)

Mohammad Shah Alam [1], Jiarui Kong [1], Ruofu Tao [1], Temoor Ahmed [2], Md. Alamin [1], Saqer S. Alotaibi [3], Nader R. Abdelsalam [4] and Jian-Hong Xu [1,5,*]



ROS decorrentes do estresse salino — danos oxidativos, peroxidação de lipídeos, danos na membrana e morte celular.

Limpeza dos ROS (antioxidantes) e a homeostase iônica — aumentar a tolerância do arroz.

Vários genes estão envolvidos na regulação do estresse salino.

Os genes bHLH subfamília U ainda não foram explorados. O gene OsbHLH024 foi selecionado para o estudo.

Foram investigados a **função do gene** e a **perda da função no mutante**.

CRISPR/Cas – Aplicações no melhoramento

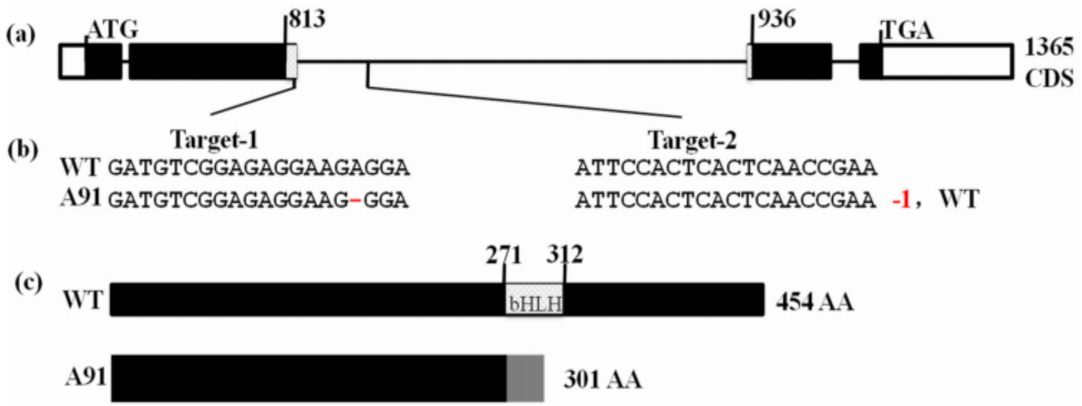


Figure 2. CRISPR/Cas9-induced mutation in the *OsbHLH024* (*LOC_Os01g39330*) gene. (**a**) Schematic diagram of gene structure and two CRISPR/Cas9 target locations, with UTRs, exons, and introns and bHLH domain shown by blank rectangles, black rectangles, black lines, and dotted rectangles, respectively. The 20-nt target sequences are shown at the bottom of the gene structure; (**b**) DNA sequencing and alignments with WT identify the editing genotypes; the deletion is indicated by the red dash; (**c**) the expected protein structure of WT and A91. The dotted and grey showed the bHLH domain in WT and frameshift or premature stop in A91.

OsbHLH024 editado (**A91**).

Dois locais foram usados como alvo.

Introdução por ***Agrobacterium*** em cultura de tecidos da cultivar WT.

O mutante homozigoto A91 com uma adenina deletada no alvo 1 (***stop codon prematuro***).

CRISPR/Cas – Aplicações no melhoramento

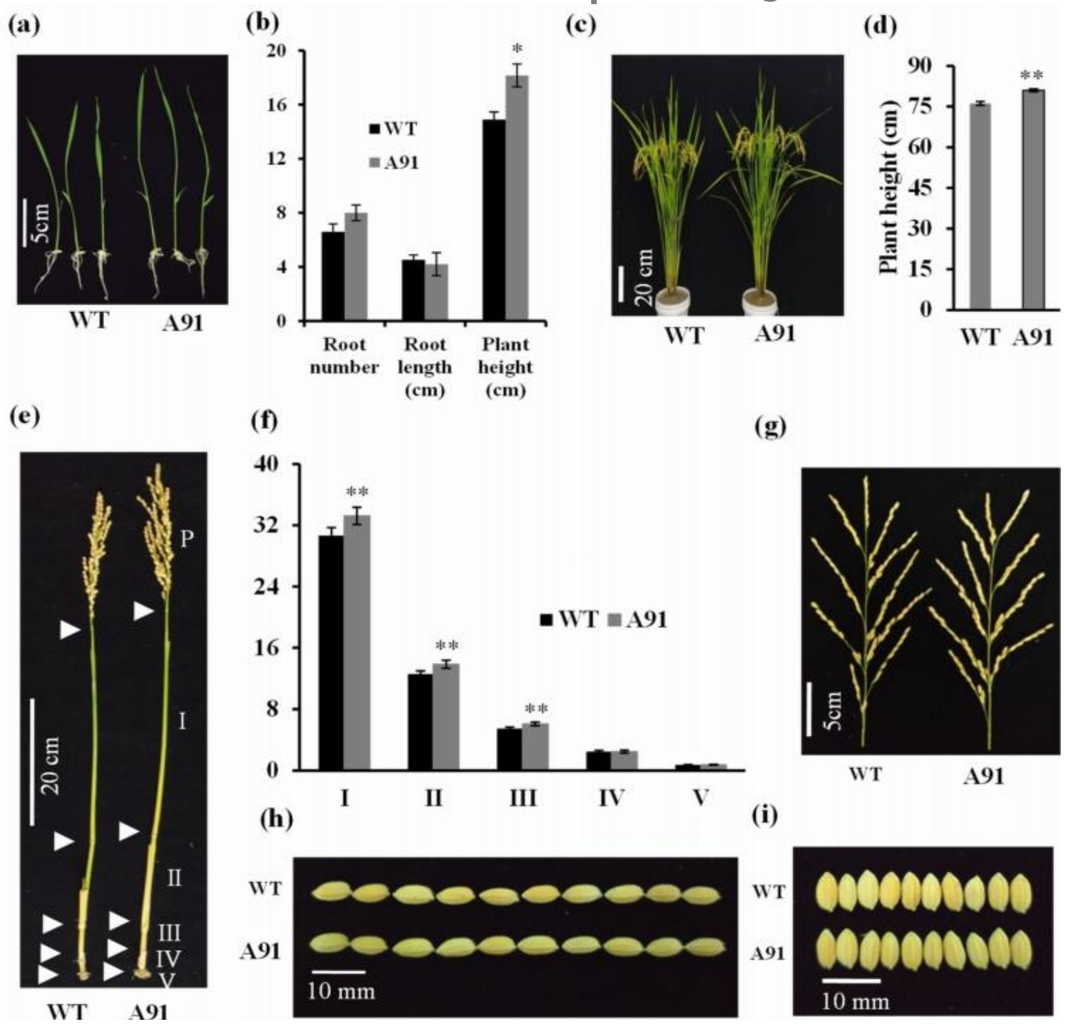


Figure 3. The phenotypic comparison of the A91 and WT. (**a**) The height of seedlings at one-week-old growing in $\frac{1}{2}$ MS media; (**b**) the comparison of the root number, the root length and the shoot length in WT and A91 (n = 9 biological replicates); (**c**) the morphology of plants at the reproductive stage; (**d**) the measurement of plant height (n = 30 biological replicates); (**e**) the phenotype of tiller; (**f**) the measurement of internode length (n = 30 biological replicates); (**g**) the structure of panicle; (**h**) 10 seeds length; (**i**) 10 seeds width, values are expressed as means ± SE; * and ** denote significant *t*-test results at $p < 0.05$ and $p < 0.01$, respectively.

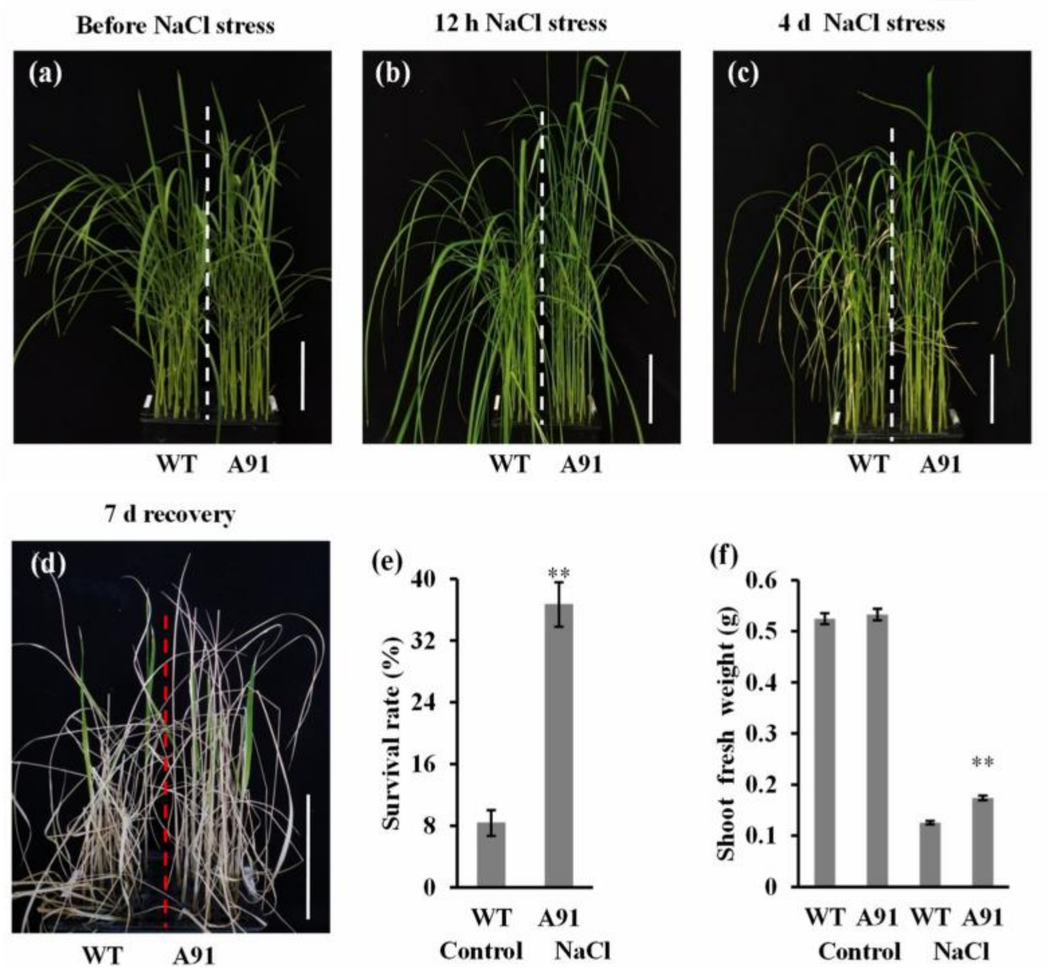


Figure 4. The growth characteristics of the A91 and WT exposed to salt stress treatments (0 and 150 mM NaCl). (**a**) The growth of 21-day-old seedlings before salt stress; (**b**) the growth of 21-day-old seedlings after 12 h stress; (**c**) the growth of 21-day-old seedlings after 4 days of stress; (**d**) 7 days recovery after 7 days of salt stress; (**e**) the survival rate after 7 days of recovery; (**f**) the fresh shoot weight after 7 days of stress; (**g**) total chlorophyll content; (**h**) SPAD value; (**i**) fluorescence; n = 3 biological replicates (**e**,**f**, each replicate represented 20 seedlings); 3 biological replicates (**g**), 9 biological replicates (**h**,**i**); data represented as means ± SE; ** indicates significant *t*-test results at $p < 0.01$; scale bar 10 cm.

CRISPR/Cas – Aplicações no melhoramento

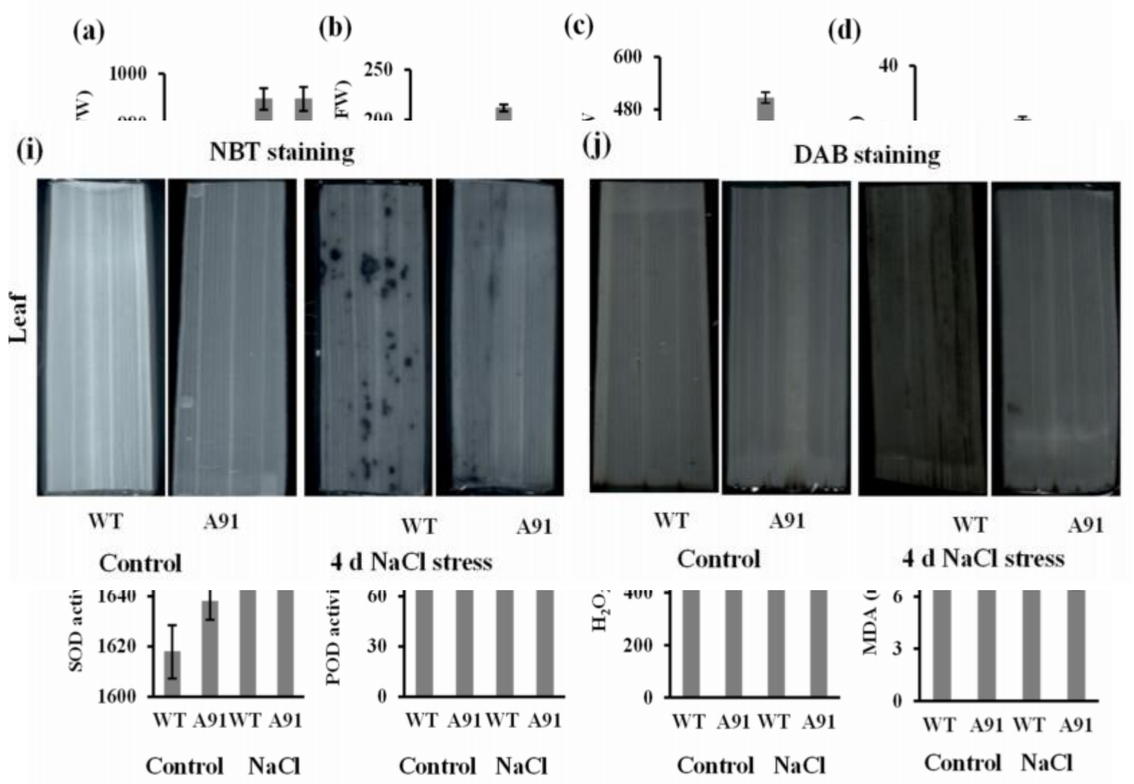


Figure 5. The oxidative stress in the shoot and root of WT and A91 seedlings under salt stress. (**a–d**) activities of SOD, POD, H_2O_2, and MDA in shoot; (**e–h**) activities of SOD, POD, H_2O_2, and MDA in the root; (**i**) NBT staining (indicates $O_2^{\bullet-}$); (**j**) DAB staining (indicates H_2O_2); n = 3 biological replicates, data represent means ± SE; * and ** indicate significant *t*-test results at $p < 0.05$ and $p < 0.01$, respectively.

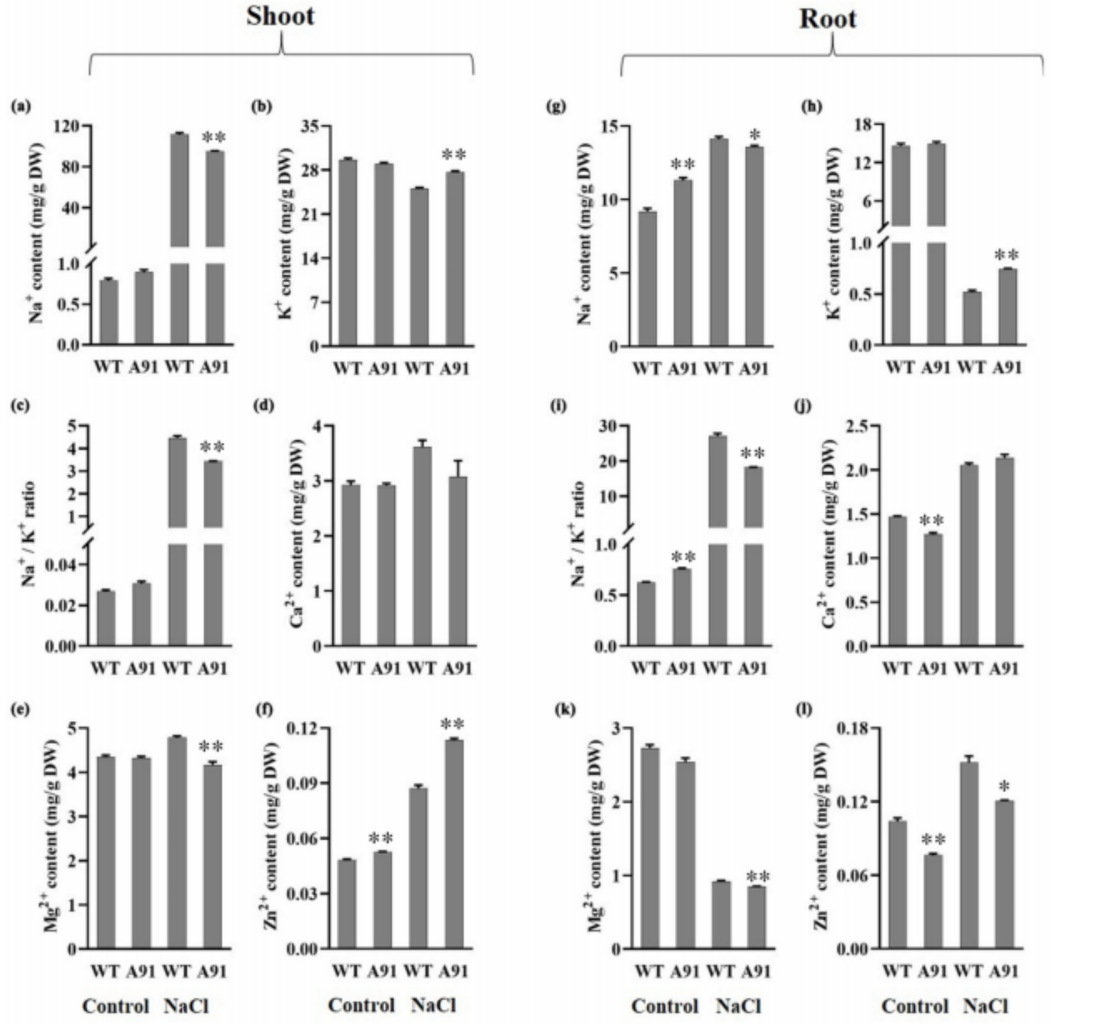


Figure 6. Measurement of nutrient elements in the shoots and roots of WT and A91 seedlings exposed to salt stress for 7 days. (**a–f**) Na^+, K^+, Na^+/K^+, Ca^{2+}, Mg^{2+}, and Zn^{2+} in shoots; (**g–l**) Na^+, K^+, Na^+/K^+, Ca^{2+}, Mg^{2+}, and Zn^{2+} in roots; n = 3 biological replicates; data represent means ± SE; * and ** indicate significant *t*-test results at $p < 0.05$ and $p < 0.01$, respectively.

CRISPR/Cas – Aplicações no melhoramento

Esses resultados sugerem que esse gene confere resistência ao estresse salino por melhorar as condições fisiológicas das folhas, retardando o aparecimento dos sintomas do estresse e promovendo uma melhor sobrevivência.

O mutante para esse gene exibiu uma **maior tolerância ao estresse** salino, menor acumulo de ROS, Na^+, oxidação de lipídeos e mais K^+ com um nível nutricional balanceado.

CRISPR/Cas – Aplicações no melhoramento

Generation of semi-dwarf rice (*Oryza sativa* L.) lines by CRISPR/Cas9-directed mutagenesis of *OsGA20ox2* and proteomic analysis of unveiled changes caused by mutations

Yue Han[1] · Kaichong Teng[1] · Gul Nawaz[1] · Xuan Feng[1] · Babar Usman[1] · Xin Wang[1] · Liang Luo[1] · Neng Zhao[1] · Yaoguang Liu[2] · Rongbai Li[1]

Fig. 1 Schematic diagram of sgRNA target sites in *OsGA20ox2*. Exons are indicated as black boxes. T1 and T2 represent Target 1 and Target 2, respectively

Fig. 3 Detection of positive mutant lines and analysis of mutations. **a** T_0 positive mutant lines; *M* DNA marker, *WT* wild type, 1–19 T_0 transgenic lines; **b** nucleotide sequence alignment and mutation frequency for both target regions. Deletions and insertions are indicated by red dashes and letters, respectively, while yellow highlighted is PAM (protospacer adjacent motif). The numbers on the right side show the sizes of the indels, with "–" and "+" indicating deletion and insertion of the nucleotides

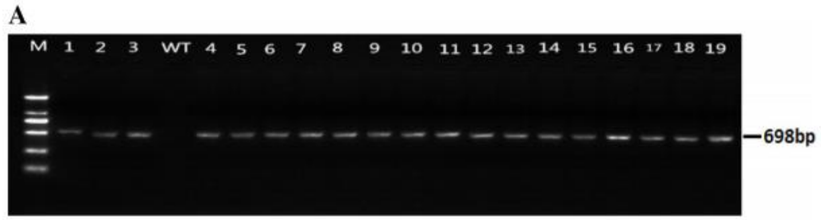


Table 1 Mutation rates of both targets in T_0 genration

Mutation type						
Targets	Bi-allelic	Homozygous	Heterozygous	Chimeric	WT	Total
SD1T1						
NOP	0	17	3	0	4	24
MR	0	70	12	0		83
SD1T2						
NOP	7	6	5	0	6	24
MR	29	25	20	0		74
					Mean MR	73.5

NOP number of plants, *MR* mutation rate (%), *WT* wild type

CRISPR/Cas – Aplicações no melhoramento

Table 3 Plant height and endogenous GA levels in wild-type and T-DNA-free T_1 mutant plants

Genotypes	PH (cm)	GA (μg/kg Fw)
WT	145.42 ± 3.93	1.82 ± 0.2
G7-1-3	120.32 ± 2.55**	1.22 ± 0.13**
G7-1-5	122.65 ± 4.11**	1.25 ± 0.12**
G7-1-8	119.63 ± 2.52**	1.24 ± 0.42**
G7-3-1	116.74 ± 3.18**	1.22 ± 0.31**
G7-3-6	119.62 ± 1.92**	1.23 ± 0.12**
G7-3-9	118.25 ± 2.63**	1.21 ± 0.24**
G7-6-3	115.35 ± 3.25**	1.19 ± 0.13**
G7-6-4	113.15 ± 3.79**	1.15 ± 0.13**
G7-6-7	114.26 ± 2.95**	1.18 ± 0.08**
G7-12-2	118.63 ± 3.42**	1.20 ± 0.16**
G7-12-7	119.53 ± 4.14**	1.21 ± 0.11**
G7-12-9	121.21 ± 2.43**	1.22 ± 0.09**
Mean	111.04	1.26
	$R = 0.97$, p value = 0.00001	

Data are the mean of three replicates

WT wild type, *PH* plant height, *GA* gibberellins, *R* Pearson correlation co-efficient

**Significant difference ($p < 0.01$)

Table 4 Agronomic traits of the high stalk and semi-dwarf mutants and their wild type

Genotypes	PN	PL (cm)	FLL (cm)	FLW (cm)	GNPP	SSR (%)	GW (g)	YPP (g)	GL (mm)	GWD (mm)
WT	5.9 ± 0.8	28.6 ± 2.5	47.0 ± 4.6	1.6 ± 0.1	135 ± 12	88.4 ± 8.9	24.9 ± 5.3	15.7 ± 2.6	2.92 ± 0.1	2.43 ± 0.2
G7-1-3	6.0 ± 0.4^{ns}	25.4 ± 1.1^{ns}	$39.6 \pm 2.1^{*}$	1.7 ± 0.4^{ns}	138 ± 10^{ns}	89.1 ± 7.2^{ns}	25.0 ± 3.8^{ns}	16.3 ± 2.3^{ns}	2.96 ± 0.1^{ns}	2.40 ± 0.6^{ns}
G7-1-5	5.8 ± 0.2^{ns}	24.2 ± 2.7^{ns}	$34.4 \pm 3.2^{*}$	1.6 ± 0.2^{ns}	136 ± 11^{ns}	88.8 ± 8.3^{ns}	25.7 ± 6.1^{ns}	16.2 ± 2.2^{ns}	2.95 ± 0.4^{ns}	2.41 ± 0.4^{ns}
G7-1-8	5.6 ± 0.1^{ns}	26.0 ± 3.5^{ns}	$36.1 \pm 2.9^{*}$	1.6 ± 0.1^{ns}	132 ± 9^{ns}	88.0 ± 5.6^{ns}	25.2 ± 4.2^{ns}	15.9 ± 1.4^{ns}	2.91 ± 0.4^{ns}	2.45 ± 0.4^{ns}
G7-3-1	6.0 ± 0.4^{ns}	23.5 ± 2.6^{ns}	$35.7 \pm 2.4^{*}$	1.7 ± 0.3^{ns}	130 ± 13^{ns}	87.5 ± 4.5^{ns}	25.8 ± 3.5^{ns}	16.1 ± 1.1^{ns}	2.92 ± 0.2^{ns}	2.45 ± 0.7^{ns}
G7-3-6	6.3 ± 0.7^{ns}	25.3 ± 2.7^{ns}	$34.4 \pm 3.2^{*}$	1.7 ± 0.2^{ns}	126 ± 14^{ns}	88.2 ± 4.2^{ns}	26.4 ± 4.9^{ns}	15.9 ± 2.4^{ns}	2.93 ± 0.2^{ns}	2.45 ± 0.6^{ns}
G7-3-9	6.4 ± 0.3^{ns}	25.0 ± 2.9^{ns}	$35.5 \pm 3.6^{*}$	1.8 ± 0.5^{ns}	129 ± 13^{ns}	88.1 ± 3.6^{ns}	24.9 ± 2.7^{ns}	16.2 ± 3.2^{ns}	2.96 ± 0.3^{ns}	2.42 ± 0.3^{ns}
G7-6-3	6.6 ± 0.5^{ns}	23.8 ± 3.1^{ns}	$37.3 \pm 1.8^{*}$	1.6 ± 0.4^{ns}	131 ± 16^{ns}	87.9 ± 4.2^{ns}	26.1 ± 5.1^{ns}	16.7 ± 2.7^{ns}	2.91 ± 0.3^{ns}	2.44 ± 0.2^{ns}
G7-6-4	6.5 ± 0.6^{ns}	24.4 ± 2.5^{ns}	$37.9 \pm 3.9^{*}$	1.6 ± 0.3^{ns}	134 ± 13^{ns}	88.8 ± 3.5^{ns}	26.3 ± 1.6^{ns}	16.6 ± 2.3^{ns}	2.93 ± 0.1^{ns}	2.43 ± 0.4^{ns}
G7-6-7	6.6 ± 0.1^{ns}	25.8 ± 1.2^{ns}	$35.5 \pm 4.1^{*}$	1.7 ± 0.6^{ns}	130 ± 12^{ns}	87.3 ± 5.9^{ns}	26.9 ± 3.2^{ns}	16.7 ± 2.5^{ns}	2.94 ± 0.5^{ns}	2.43 ± 0.1^{ns}
G7-12-2	6.4 ± 0.4^{ns}	26.6 ± 1.3^{ns}	$38.2 \pm 2.1^{*}$	1.8 ± 0.4^{ns}	129 ± 14^{ns}	87.6 ± 3.4^{ns}	25.5 ± 2.2^{ns}	16.4 ± 1.5^{ns}	2.93 ± 0.1^{ns}	2.44 ± 0.6^{ns}
G7-12-7	5.8 ± 0.2^{ns}	25.0 ± 1.6^{ns}	$38.9 \pm 3.1^{*}$	1.7 ± 0.3^{ns}	126 ± 16^{ns}	88.7 ± 2.5^{ns}	25.2 ± 2.9^{ns}	16.5 ± 2.4^{ns}	2.91 ± 0.4^{ns}	2.47 ± 0.4^{ns}
G7-12-9	6.0 ± 0.3^{ns}	24.5 ± 1.7^{ns}	$37.6 \pm 2.9^{*}$	1.9 ± 0.2^{ns}	127 ± 15^{ns}	87.6 ± 3.1^{ns}	25.9 ± 2.7^{ns}	16.3 ± 0.3^{ns}	2.72 ± 0.6^{ns}	2.46 ± 0.3^{ns}

Data are the mean of five replicates

WT wild type, *PN* panicle numbers, *PL* panicle length, *FLL* flag leaf length, *FLW* flag leaf width, *GNPP* grain number per panicle, *SSR* seed setting rate, *GW* 1000-grain weight, *YPP* yield per plant, *GL* grain length, *GWD* grain width

*Significant difference and ^{ns}represents no significant difference ($p < 0.01$)

CRISPR/Cas – Aplicações no melhoramento

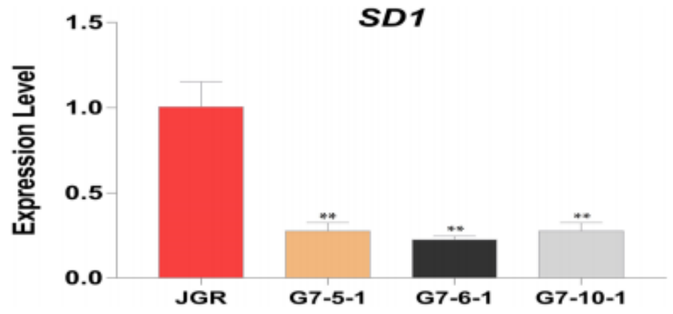


Fig. 5 *SD1* expression level of wild type (JGR) and T_1 mutant plants by RT-qPCR analysis. Data are the mean ± SD of three independent PCR values

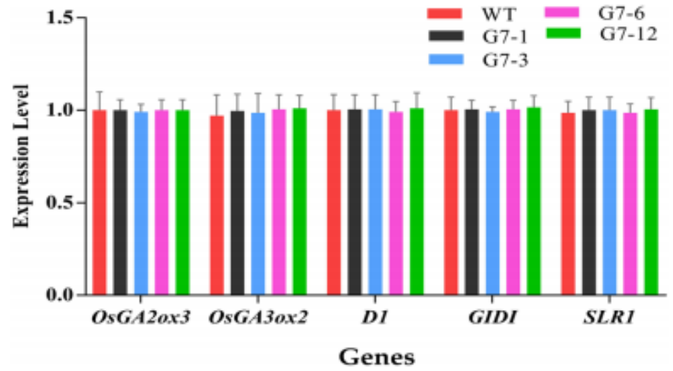


Fig. 6 Expression analysis of GA biosynthesis genes (OsGA2ox3 and GA3ox2) and signaling genes (*D1*, *GIDI* and *SLR1*) in panicle tissues of wild type and mutant lines. The data are the mean ± SD of three independent values

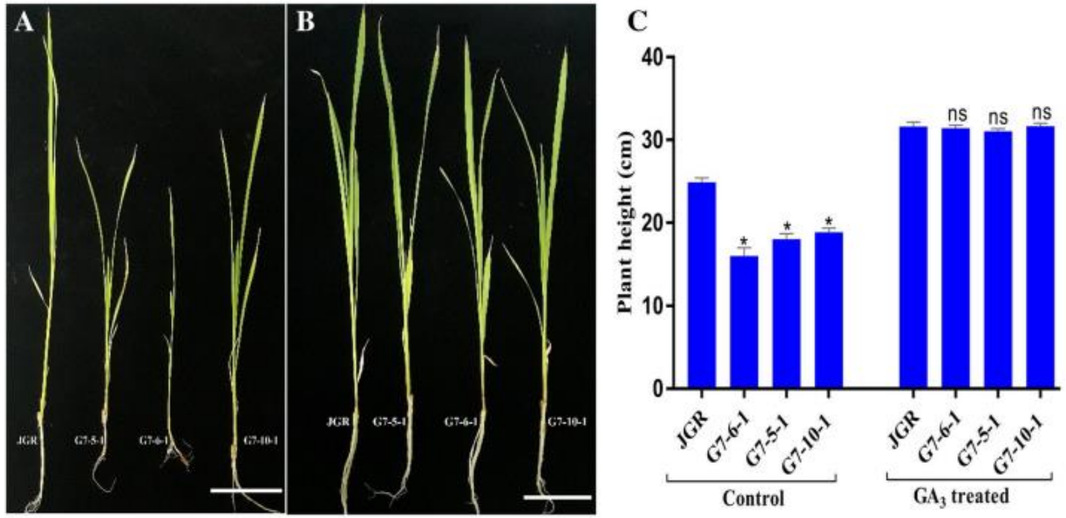


Fig. 7 Seedling phenotype of wild JGR and mutant plants under controlled conditions and GA_3 treatment **a** plants under controlled conditions, **b** plants treated with 10 μM GA_3, **c** plant height of control and GA_3-treated seedling

CRISPR/Cas – Aplicações no melhoramento

A mutação realizada com a ferramenta CRISPR no alelo SD1 desenvolveu com **sucesso mutantes semi-anãs** e com aumento ligeiro no rendimento de grãos.

A perda de função nesse alelo sugere que a produção de GA ativo para elongação foi afetada.

Os dados produzidos promovem um exemplo de genes editados com potencial para comercialização.

CRISPR/Cas – Aplicações no melhoramento

Plant Biotechnology Journal
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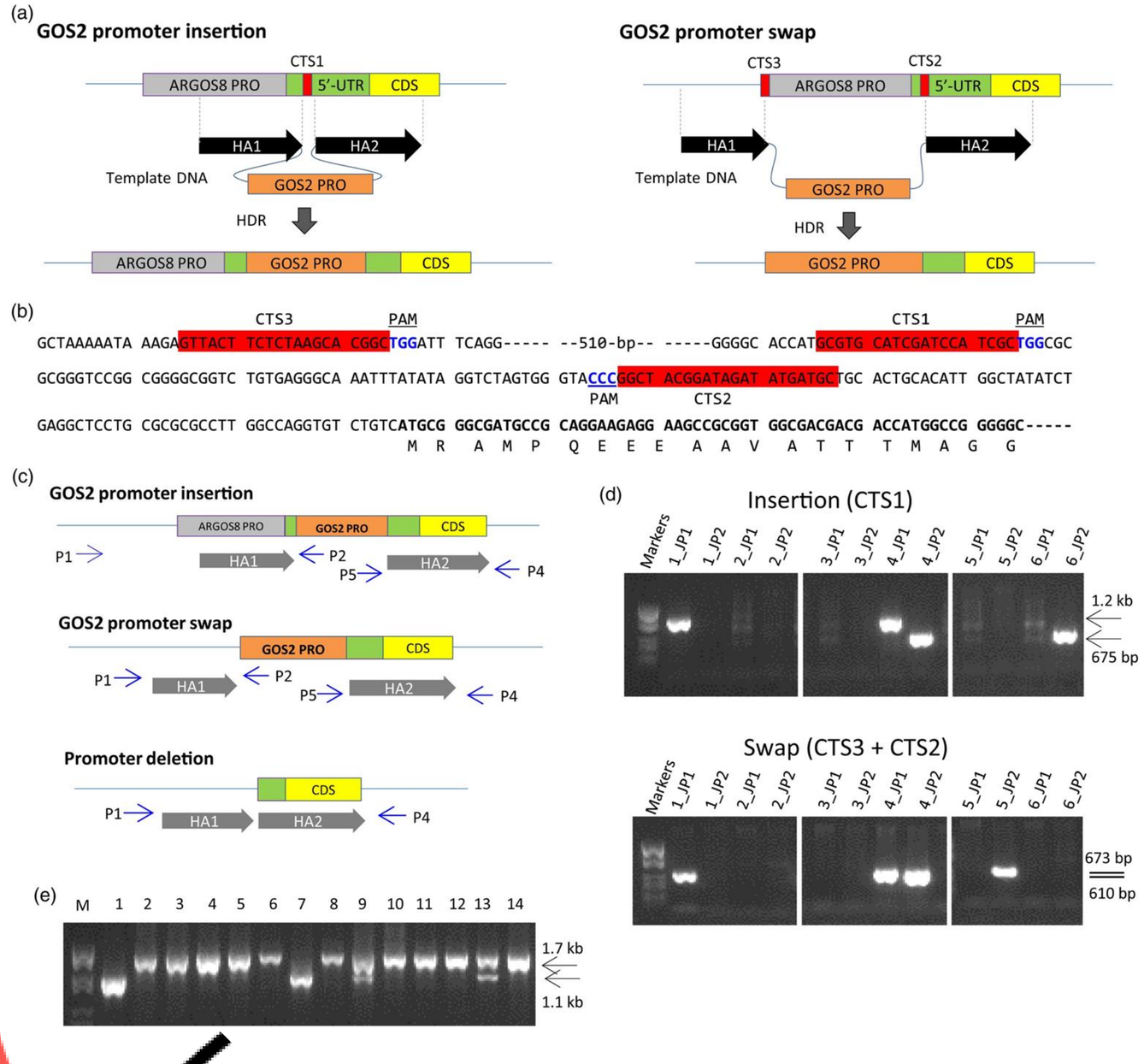
ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions

Jinrui Shi, Huirong Gao, Hongyu Wang, H. Renee Lafitte, Rayeann L. Archibald, Meizhu Yang, Salim M. Hakimi, Hua Mo, Jeffrey E. Habben

Objetivo: **Novas variantes do ARGOS8**, através da substituição do promotor.

Em duas linhagens : **B73** (pública) e **PH184C** (linhagem comercial da DuPont).

CRISPR/Cas – Aplicações no melhoramento



GOS2 Pro = promotor homólogo ao GOS2 do arroz

CRISPR/Cas – Aplicações no melhoramento

X

T0

PH184C

⊗

F2

F1

X

PH184C

BC1

CRISPR/Cas – Aplicações no melhoramento

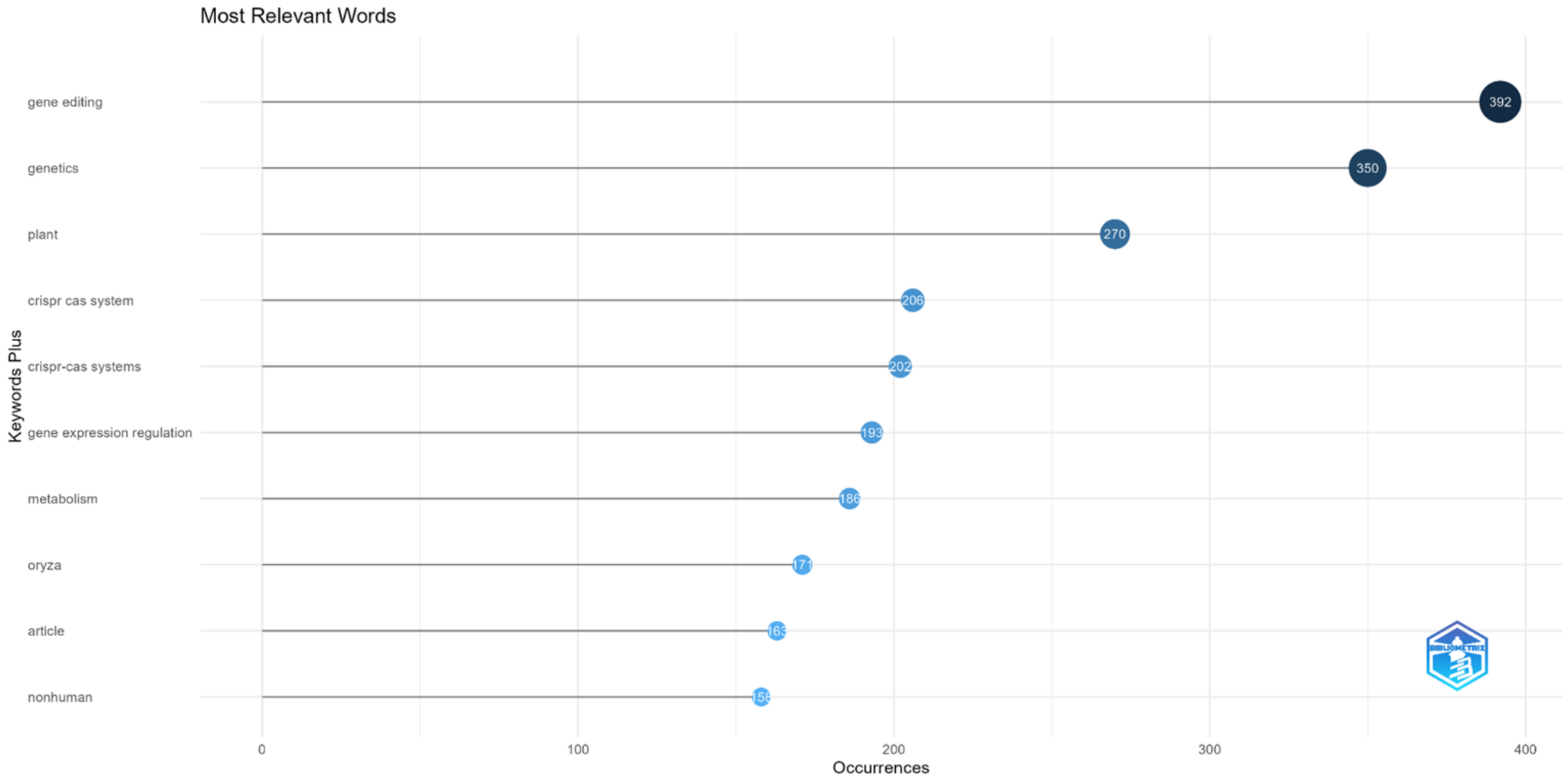
Table 1. Grain yield of *ARGOS8* genome-edited variants and wild type under flowering stress, grain-filling stress and optimal (well-watered) conditions

	Flowering Stress	Grain-filling Stress	Optimal
	ton ha^{-1} (bushel $acre^{-1}$)		
ARGOS8-v1	8.67 (138.0)[a]	7.47 (119.0)	13.13 (209.0)
ARGOS8-v2	8.67 (138.0)[a]	7.54 (120.0)	13.19 (210.0)
WT	8.34 (132.8)	7.72 (122.9)	13.01 (207.1)

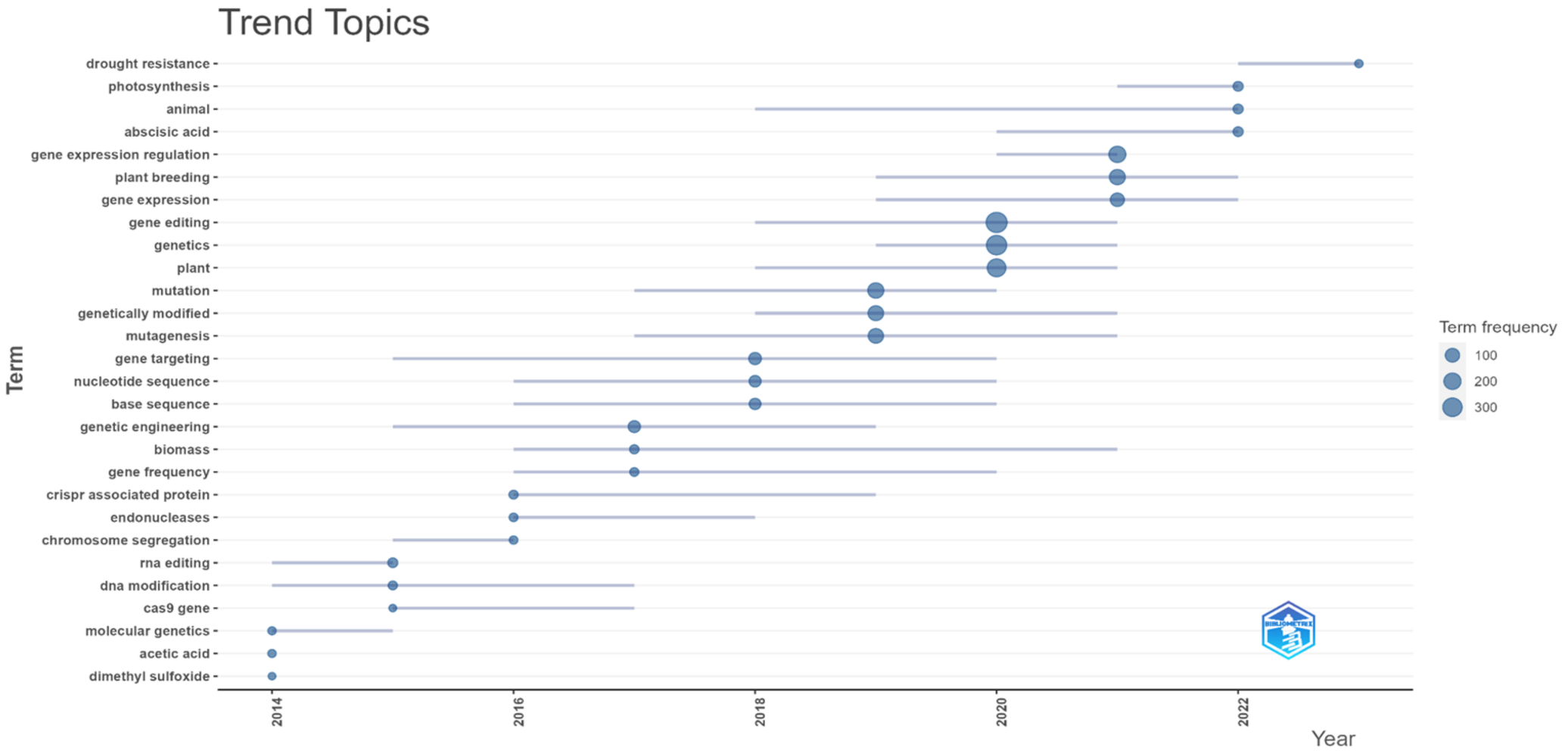
O gene ARGOS8 é um regulador da resposta à etileno.

Superexpressão = menor sensibilidade ao etileno, maior crescimento das plantas.

Perspectivas futuras



Perspectivas futuras



Perspectivas futuras

Ser amplamente utilizado para caracteres poligênicos com a possibilidade de modificar mais de um gene ou várias partes de um gene de uma mesma vez, por exemplo através da multiplexagem de gRNAs (Ali et al., 2022).

Exploração do potencial comercial dos estudos feitos atualmente (Wang et al, 2022).

Perspectivas futuras

Precisamos nos concentrar em mecanismos epigenéticos estáveis e hereditários ao longo das gerações.

Epigenética

Manipulação mais precisa e eficiente do genoma de plantas.

Evolução

Entender como as populações modificadas irão operar na natureza.

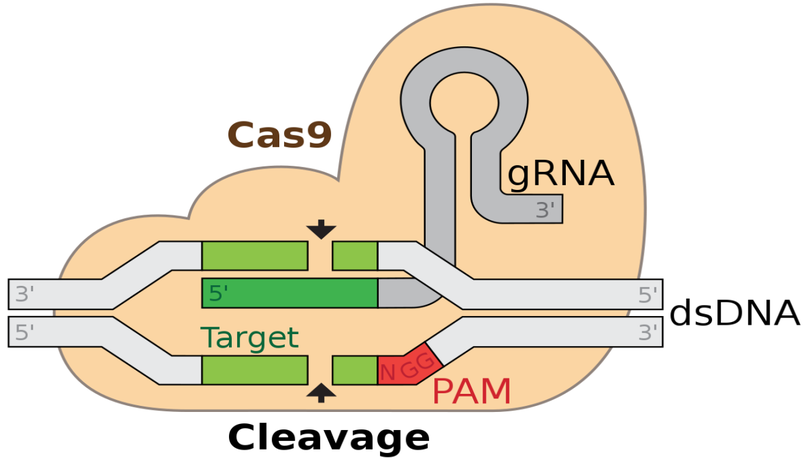
Conservação

Habilidade de múltiplos alvos/locos e minimizar os *off-target.*

Melhoramento

Dúvidas?
Considerações?

Ficamos à disposição!



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CRISPR/Cas – Navegando pelo seu universo de aplicações