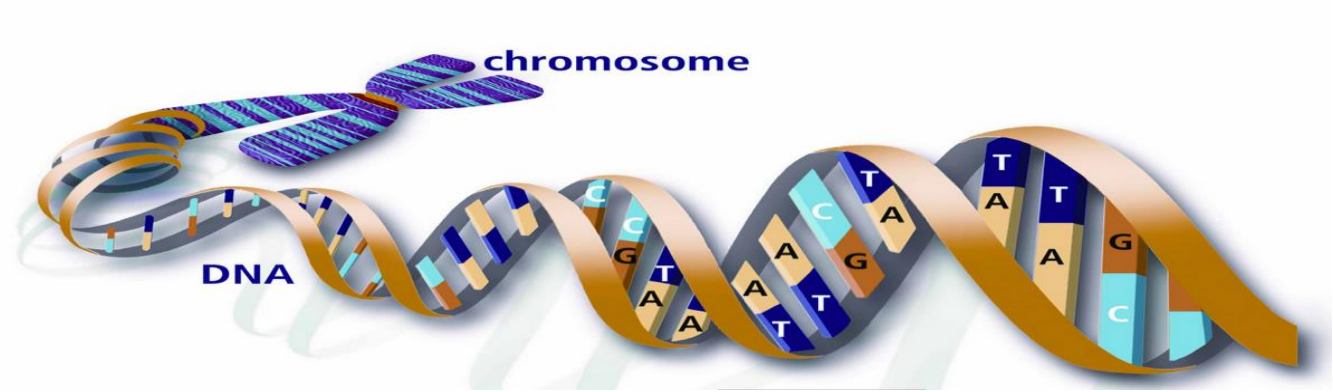
1872 PRIFYSGOL ABERYSTWYTH UNIVERSITY
IBERS
IBERS
Athrofa y Gwyddorau Biolegol, Amgylcheddol a Gwledig
Institute of Biological, Environmental and Rural Sciences
www.aber.ac.uk/ibers/

Genome editing; how it may change crop breeding with implications for agriculture and farm biodiversity.

Huw D Jones PhD FRSB

- Professor of Translational Genomics for Plant Breeding, IBERS, Aberystwyth University



VKM-EFSA Symposium 26-27 October 2017

Advancing environmental risk assessment by accounting for biodiversity and ecosystem services as protection goals.

- **Global agriculture must increase output while also minimising environmental impact**

- **Improved seed varieties is one way this can be done**

- **Genome editing is a collection of advanced molecular techniques for precise and targeted genomic changes that complement existing breeding systems**

- **The first applications will inevitably be through loss-of-function edits in well-characterised genes**

- **In the longer term, genome editing will underpin more fundamental changes and will ultimately merge with the tools and philosophies of synthetic biology**

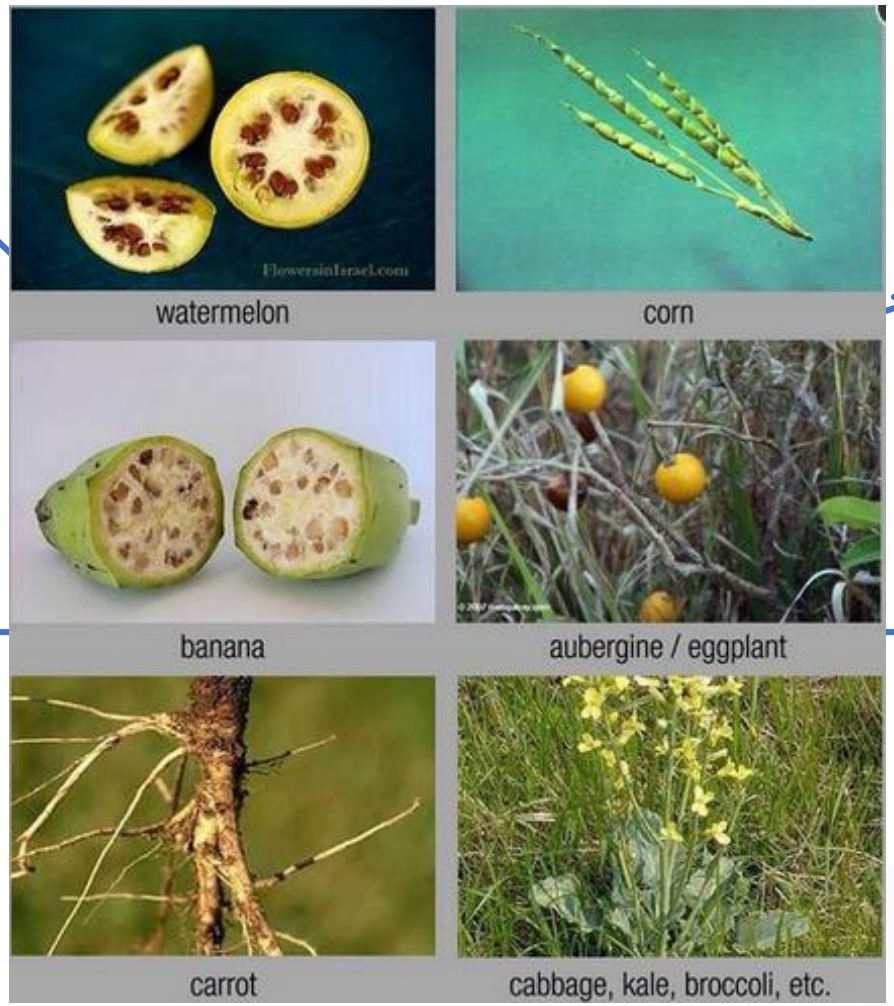
Genetics v. chemistry

Most farming interventions cost time and money, and have negative impacts on the environment.

The more ‘ADVANTAGEOUS GENETICS’ we can put into the seed

The FEWER INTERVENTIONS we need to make during cultivation which LOWERS COSTS and does LESS DAMAGE to the environment.

Wild relatives to Cultivated crops: the result of plant breeding



Classical plant breeding is a slow process: 10-15 years from selection of parents to varietal approval

Via ‘conventional‘ crossing

Breeding is based on ‘sex and selection’ but helped by a range of specialist/ laboratory techniques

Aneuploidy

Marker assisted selection

Mutation breeding

Genomic selection

Embryo rescue

Single-seed descent

Protoplast fusion

Synthetic polyploidy

Translocation breeding

Association Genetics

Grafting

F1 heterosis

Use of ‘Bridging’ spp.

Wide crossing

Inducible male sterility

Doubled Haploids

Diversity Arrays

Chromosome engineering

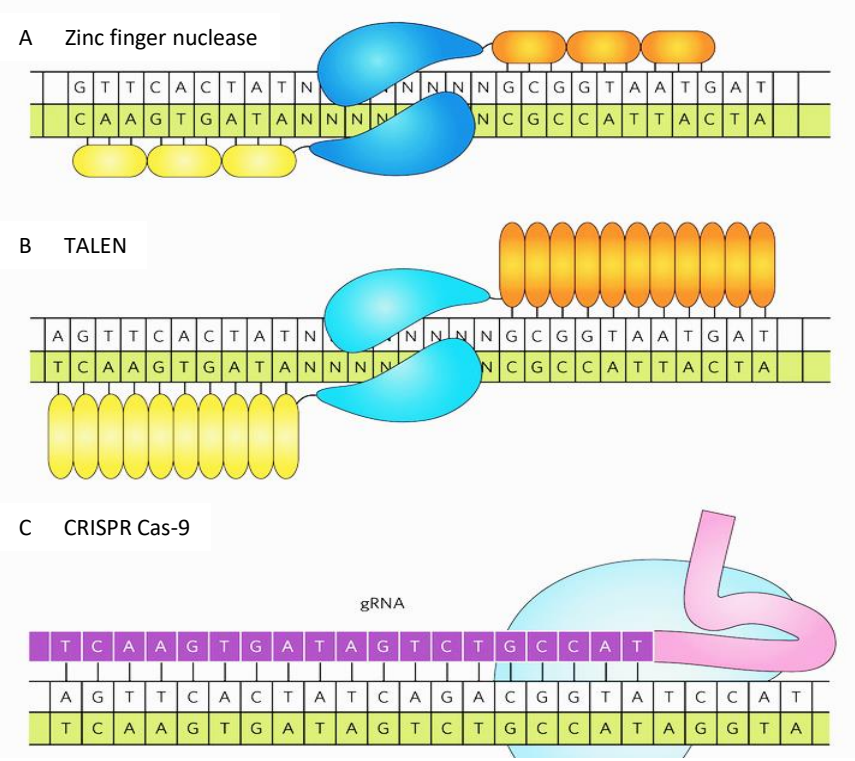
Pedigree breeding

Linkage analysis

Genome editing: Suite of technologies to make targeted changes to genomes

'A collection of advanced molecular biology techniques that have been developed over recent years that allow precise, targeted changes (adding, removing or replacing DNA) to an organism's DNA'. (BBSRC Genome editing working group 2017).

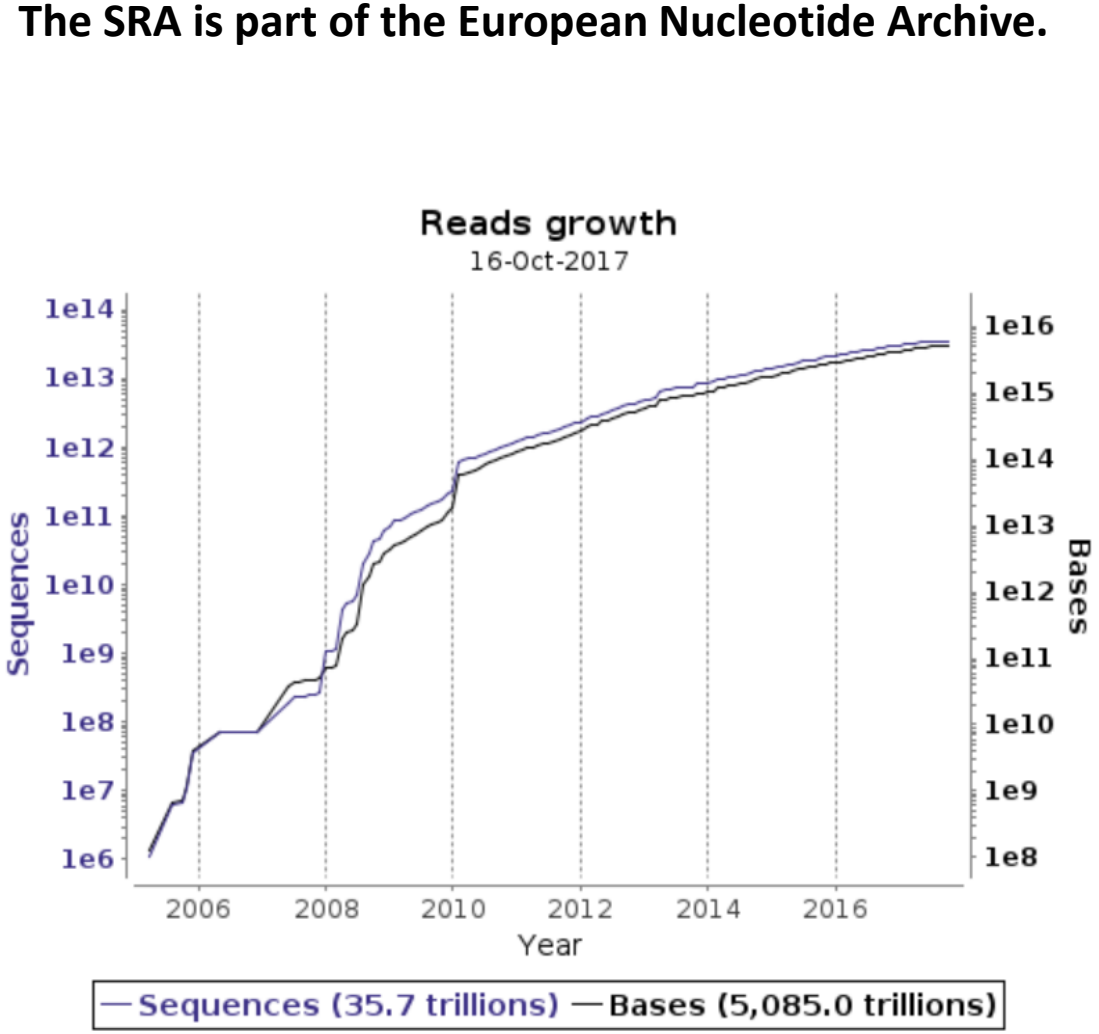
- **Meganucleases**
- **Zinc-Finger Nuclease (ZFN)**
- **Transcription activator-like effector nucleases (TALENs)**
- **Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR CAS-9)**
- **Oligonucleotide Directed Mutagenesis (ODM)**

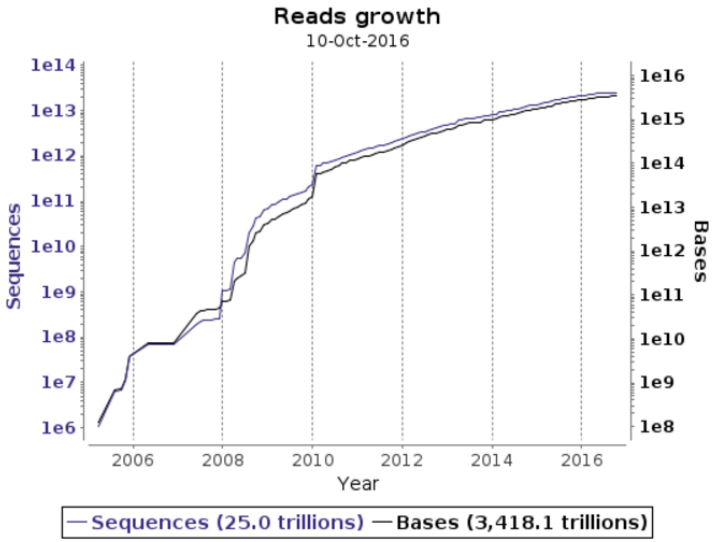


HD Jones (2015) Nature Plants 1: 14011

Nucleotide databases show explosive growth

The SRA is part of the European Nucleotide Archive.





Additional 10.7 trillion reads in 12 months (344,000/sec)

3K RGP *GigaScience* 2014, **3**:7
http://www.gigasciencejournal.com/content/3/1/7

DATA NOTE **Open Access**

The 3,000 rice genomes project

The 3,000 rice genomes project[1,2,3*†]

Abstract

Background: Rice, *Oryza sativa* L., is the staple food for half the world's population. By 2030, the production of rice must increase by at least 25% in order to keep up with global population growth and demand. Accelerated genetic gains in rice improvement are needed to mitigate the effects of climate change and loss of arable land, as well as to ensure a stable global food supply.

Findings: We resequenced a core collection of 3,000 rice accessions from 89 countries. All 3,000 genomes had an average sequencing depth of 14×, with average genome coverages and mapping rates of 94.0% and 92.5%, respectively. From our sequencing efforts, approximately 18.9 million single nucleotide polymorphisms (SNPs) in rice were discovered when aligned to the reference genome of the temperate *japonica* variety, Nipponbare. Phylogenetic analyses based on SNP data confirmed differentiation of the *O. sativa* gene pool into 5 varietal groups – *indica*, aus/boro, basmati/sadri, tropical *japonica* and temperate *japonica*.

Conclusions: Here, we report an international resequencing effort of 3,000 rice genomes. This data serves as a foundation for large-scale discovery of novel alleles for important rice phenotypes using various bioinformatics and/or genetic approaches. It also serves to understand the genomic diversity within *O. sativa* at a higher level of detail. With the release of the sequencing data, the project calls for the global rice community to take advantage of this data as a foundation for establishing a global, public rice genetic/genomic database and information platform for advancing rice breeding technology for future rice improvement.

Keywords: *Oryza sativa*, Genetic resources, Genome diversity, Sequence variants, Next generation sequencing

In vitro cell culture

Explant

Variety
Preculture
Size,

co-cultivation & regeneration protocols

pH
Acetosyringone
Surfactant:

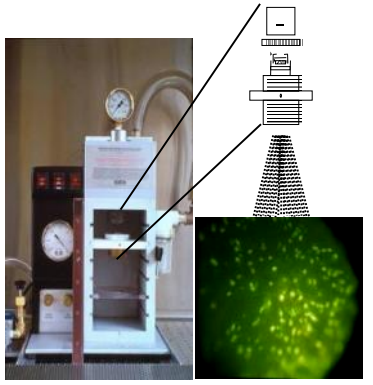
Basal media
Type
Hormones / Concentration

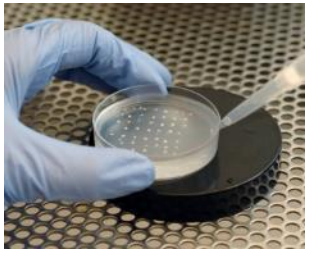
Dark / light
Temp / timing

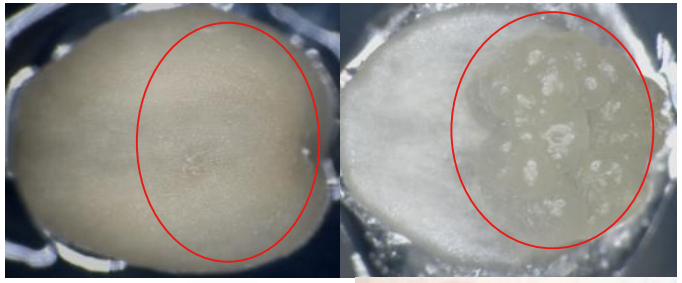
Amoah, Wu, Sparks & Jones (2001) *J Exp Bot* 52: 1135-1142
Wu, Sparks, Amoah & Jones (2003) *Plant Cell Rep* 21: 659-668
HD Jones, A Doherty and H Wu (2005). *Plant Methods* 1:5.
Wu, Doherty & Jones (2007) *Transgenic Res* 17(3): 425-436.
Freeman, Sparks, West, Shewry Jones (2011). *P. Biotech J* 9: 788–796
Zhang, Jones, Gao, Wang, Ma, Xia (2013). *BMC Genomics* 14:560

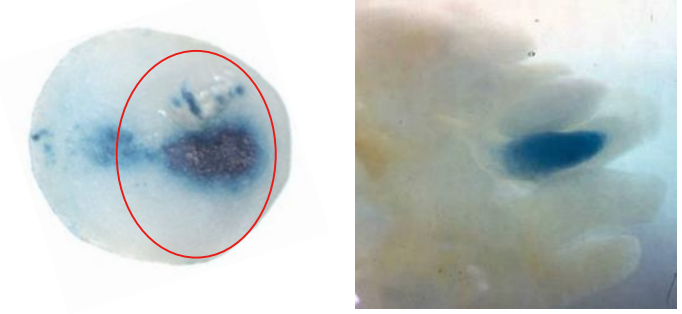
Gene Transformation

Optimised methods for DNA-delivery using Gene Gun or Agro strain & vectors

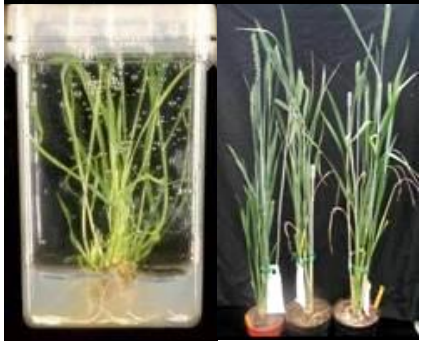


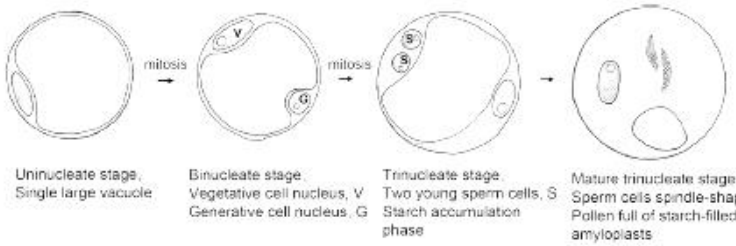


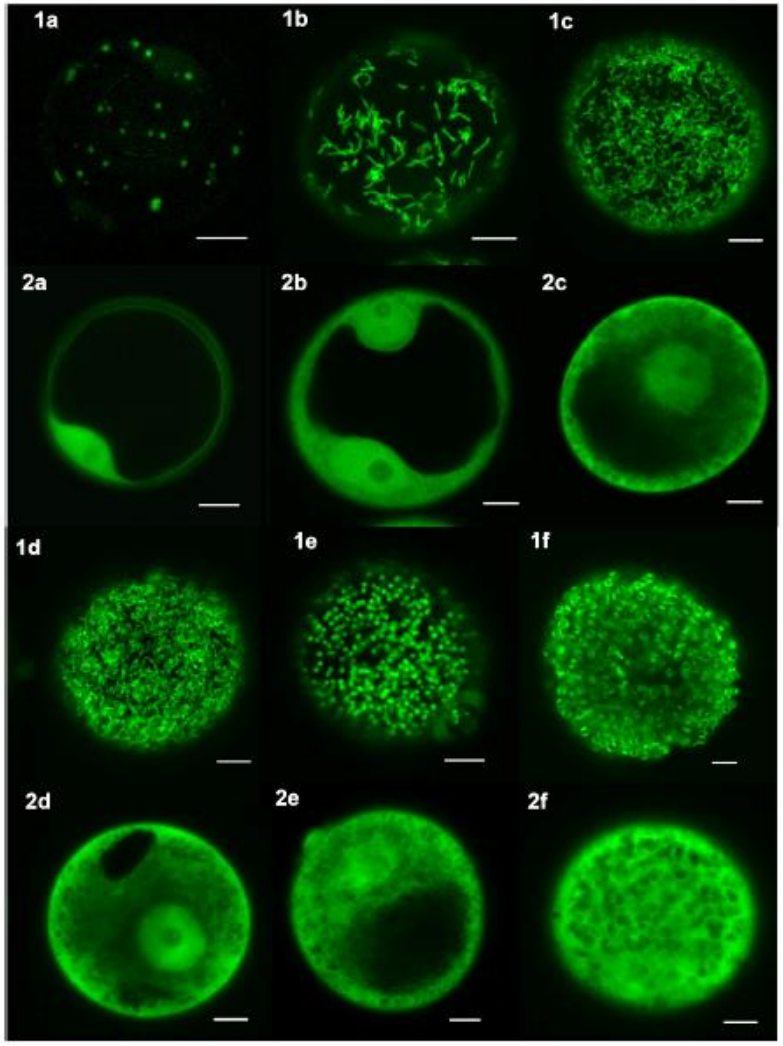




Tissue culture







Visualisation of plastid degradation in sperm cells of wheat pollen
LF. Primavesi H Wu EA. Mudd A Day & HD. Jones 2016 Protoplasma

Functional genomics

IBERS: NATIONAL PLANT PHENOMICS CENTRE

Site-directed nucleases (SDN) for gene editing

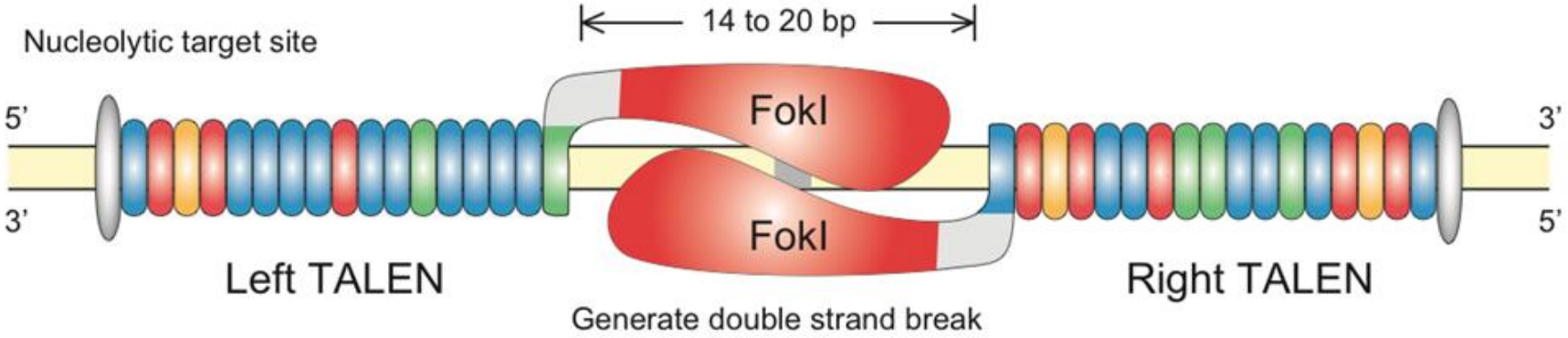


Fig 1. A pair of TALEN proteins each composed of a DNA-binding domain and a Fok1 nuclease designed to make a double stranded break in a pre-determined genetic locus. (From Open Resource for TAL effectors)

Table 1 Examples of genome editing in each of the major crop types following the United Nations Indicative Crop Classification [105] Part 1 of 2

CROP classification	Crop	Gene editor	Target gene	Heritable edits observed	Reference
1. Cereals	Wheat (durum)	CRISPR/Cas9	*GASR7*, *DEP1*, *NAC2*, *PIN1* and *LOX2*	Second generation	[64]
	Wheat (bread)	CRISPR/Cas9 and TALEN	*MLO*	Second generation	[62]
		CRISPR/Cas9	*GASR7/GW2-B1* and *D1*	Plants regenerated from embryos	[65]
	Maize	MN	*LG1*	Plants regenerated from embryos	[106]
		TALEN	*GL2*	First generation	[107]
		ZFNs	*IPK1*	Second generation	[108]
		CRISPR/Cas9 and TALEN	*PDS*, *IPK1A*, *IPK* and *MRP4*	Protoplasts and plants regenerated from embryos	[109]
		CRISPR/Cas9	*LIG1*, *Ms26/Ms45*, and *ALS1/ALS2*	Second generation	[110]
	Rice	ZFN	*GUS* insertion	Plants regenerated from embryos	[111]
		TALEN	*SWEET14*	Second generation	[112]
		CRISPR/Cas9	*SWEET11*	Second generation	[113]
	Sorghum	CRISPR/Cas9	*DsRED2*	Editing in embryos	[114]
	Barley	TALEN	*PAPhy_a*	Plants regenerated from embryos	[115]
		CRISPR/Cas9	*PM19*	Second generation	[116]
2. Vegetables and melons	Cucumber	CRISPR/Cas9	*eIF4e*	Third generation	
	Tomato	TALEN	*PRO*	First generation	[117]
		CRISPR/Cas9	*AGO7*	First generation (low fertility)	[118]
	Lettuce	CRISPR/Cas9	*BIN2*	Second generation	[119]
	Watermelon	CRISPR/Cas9	*PDS*	Edited generation only and protoplasts	[120]
3. Fruits and nuts	Grapefruit	CRISPR/Cas9	*LOB1*	Plants regenerated from cell suspension	[121]
	Oranges	CRISPR/Cas9	*PDS*	Transient expression in leaves	[122]
	Grapes	CRISPR/Cas9	*IdnDH*	Plants regenerated from callus	[123]

	Fig	ZFN	Transiently expressed *GUS*	Transient expression in leaves	[124]
	Apple	ZFN	Transiently expressed *GUS*	Transient expression in leaves	[124]
		CRISPR/Cas9	*PDS*	Edited generation only	[28]
4. Oilseed crops	Soybean	ZFN	*DCL*	Edited hairy roots	[125]
		CRISPR/Cas9 and TALEN	*PDS11/PDS18*	Edited generation (low fertility) and hairy roots	[126]
	Flax	ODM with CRISPR/Cas9 and TALEN	*EPSPS*	First generation	[127]
	Rapeseed	ZFN	*KASII* expression control	Second generation	[128]
		TALEN	*FRI*	Edited generation only	[129]
		CRISPR/Cas9	*BolC.GA4*	Second generation	[116]
5. Root/tuber crops	Potato	TALEN	*Vinv*	Plants regenerated from protoplasts	[130]
		TALEN	*ALS*	Editing in protoplasts	[131]
		CRISPR/Cas9	*GBSS*	Editing in protoplasts	[132]
6. Beverage and spice crops	None	–	–	–	–
7. Leguminous crops	None	–	–	–	–
8. Sugar crops	None	–	–	–	–
9. Other crops	Cotton	MN	*EPSPS* insertion	Second generation	[133]
		CRISPR/Cas9	*CLA1*	Edited generation (low fertility)	[134]
	Petunia	ZFN	*GUS* repair	Second generation	[135]

The first applications will be null edits in genes with known functions, because these are straightforward to generate and the results are predictable from the existence of null alleles in existing gene pools or other knockout or silencing approaches such as induced mutations or RNAi

SCIENCE

CRISPR-Modified Corn May Soon Be Ready For Market

It would be the first crop to go on sale that has been genetically altered with the enzyme

By **Alexandra Ossola** September 6, 2016

zqf503 via Pixabay

Could a field like this someday be full of CRISPR-modified crops?

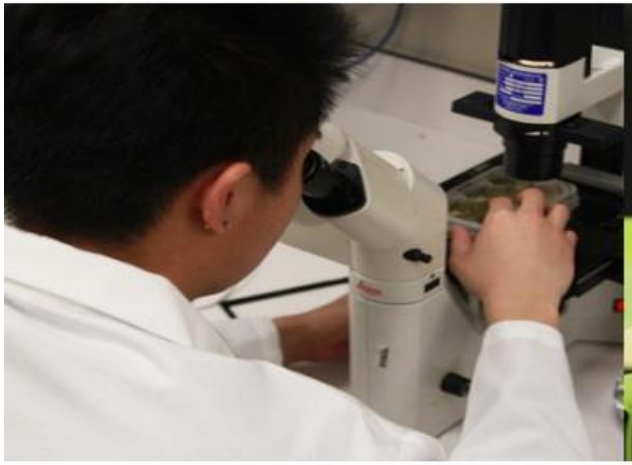
On April 18, 2016, DuPont Pioneer announced waxy corn hybrids as their first commercial agricultural product developed through the application of CRISPR-Cas.
On December 14, 2015, Pioneer requested confirmation from the USDA- APHIS's Biotechnology Regulatory Services (BRS) in regards to the regulatory status of waxy corn developed using CRISPR-Cas. The USDA state "APHIS has no reason to believe that this CRISPR-Cas waxy corn is a plant pest. Therefore, APHIS does not consider CRISPR-Cas waxy corn to be regulated pursuant to 7 CFR part 340."

SEARCH HOME CIBUS SCIENCE NUCELIS PRESS ROOM CAREERS

1872 PRIFYSGOL ABERYSTWYTH UNIVERSITY IBERS

Cibus

Harvesting Nature's Genetic Diversity



Gene Repair OligoNucleotide (GRON) technology

CIBUS PRESS RELEASE

MEDIA INQUIRIES
(858) 450-0008

FOR IMMEDIATE RELEASE

Cibus Global Announces Approval of First Commercial Product SU Canola in Canada

San Diego (March 18, 2014) Cibus Global, a cutting-edge precision gene editing firm, announced today that its first commercial product Sulfonylurea Tolerant Canola has received Plant Novel Trait approval by the Canadian Food Inspection Agency.

About Cibus Global

Cibus Global is a leading precision gene editing company with a unique, patented technology for naturally modifying cell functions. Its technology enables access to global multibillion-dollar markets in agriculture, specialty chemicals, and human health. It offers a disruptive alternative to transgenic (GM or GMO) approaches.

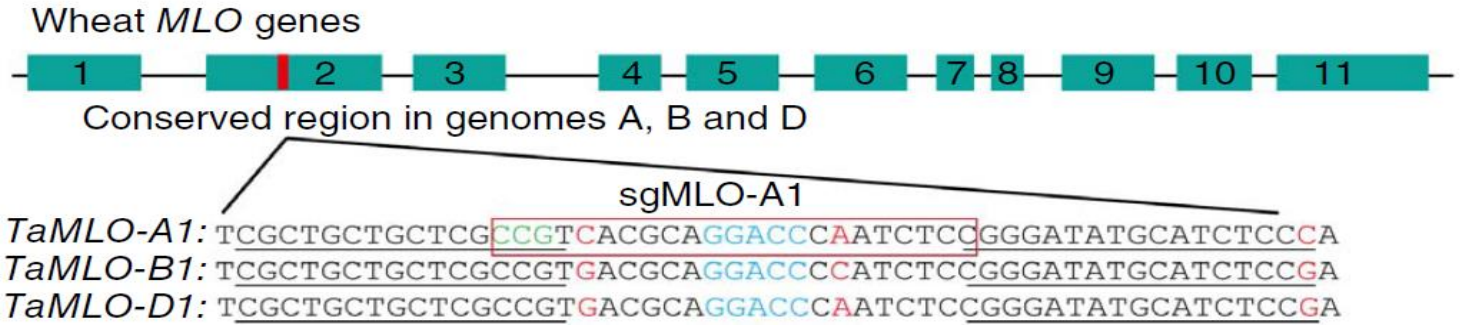
Our core purpose is to lead the transition to sustainable agricultural and industrial products and improved human health by safely harnessing Nature's own genetic diversity.

Table 2 Potential for genome editing to phenocopy existing, commercially valuable traits resulting from conventional biotechnology and classical mutagenesis Part 1 of 2

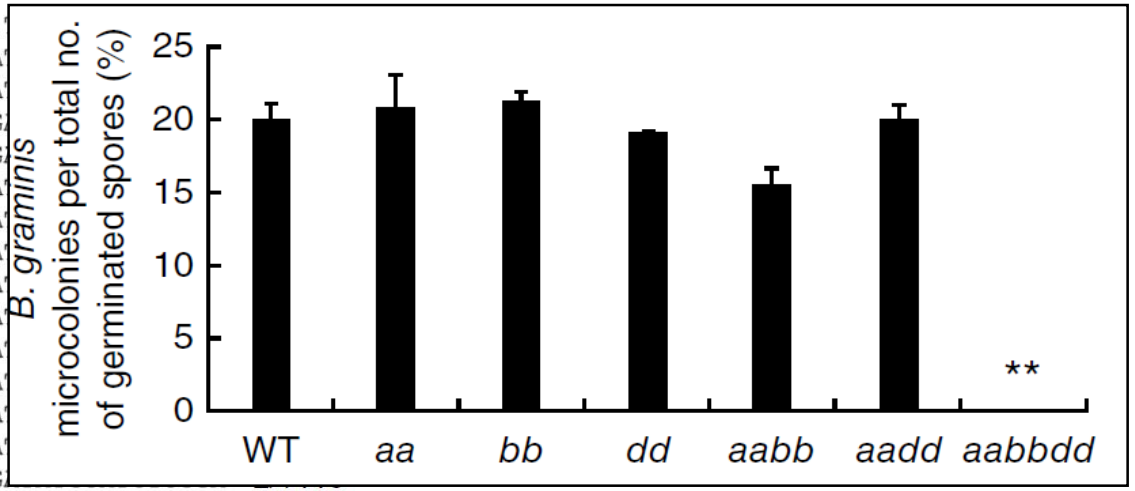
Target trait	Target sequence	Species	Classical/ non-genome-editing approach	Genome-editing proof-of-principle to phenocopy target trait
Reduced oxidative browning	*PPO*	Apple	Knockdown by RNAi [136]	PPO has been edited in mushroom [29]
		Potato	Knockdown by RNAi [137]	
Waxy starch	*Wx1*	Maize	Knockout found in natural/ induced mutants [138]	Waxy genes have been edited in maize [139]
		Potato	Knockout found in X-ray-induced mutagenesis [140]	
	Wx1-A1, *B1* and *D1*	Wheat	Mutants obtained using TILLING mutagenesis [141]	
High-amylose starch	*SBEs*	Barley	Knockdown by RNAi [73]	Two SBEs have been edited in rice [77]
		Maize	Knockdown by RNAi [69]	
		Wheat	Knockdown by RNAi [71]	
	SSII	Potato	Knockdown by RNAi [70]	
Low amylose	*SSSI*	Rice	Knockdown by RNAi [74]	
Improved cold storage	*Amy23*, *BAM1* and *BAM9*	Potato	Knockdown by RNAi [76]	Although these traits have not been obtained through editing, gene knockouts in potato have been obtained (Table 1)
	PhL and *SAR1*	Potato	Knockdown by RNAi [75]	
Fungal resistance	*EMR*	Barley	Knockout in EMS induced mutants [142]	Fungal resistance has been obtained in rice with a ERF922 knockout [143] and with a triple MLO knockout in wheat [62].
	MLO	Barley	Knockout obtained by chemically induced mutagenesis [144]	
	ERF922	Rice	Knockout isolated by breeding [145]	
	Gls1	Wheat	Knockdown by RNAi [146]	

Viral resistance (dsDNA)	Rep-binding motif	Tomato	Motif identification [147]	Resistance obtained occupying the binding site with ZFN [43].
Viral resistance (ssRNA)	*eIF4E*	Cucumber	Knockdown by RNAi [148]	Viral resistance has been obtained in cucumber using this edit [45].
		Apricot	Knockout found by QTL identification in natural mutants [149]	
	eIF4E/eIF4G	Tomato	Knockout in natural mutants [150]	
Herbicide resistance	*EPSPS*	Cotton	Transgenic expression [151]	The innate EPSPS has been modified in *Arabidopsis* [127]. AHAS and ALS have been edited in maize using ODM [152] and CRISPR/Cas9 [110]. Resistant forms of ALS have been introduced using HDR in rice [153,154]
		Maize	Transgenic expression [155]	
		Rice	Transgenic expression [156]	
		Soybean	Transgenic expression [157]	
	AHAS (*ALS*)	Canola	Amino acid substitution in natural mutants [158]	
		Rice	Amino acid substitution found in EMS mutants [159]	
Dwarf	*GA20ox2*	Rice	Knockout found in γ-ray-induced mutants [160]	Although these traits have not been obtained through editing, gene knockouts in rice and wheat have been obtained (Table 1).
	DELLA	Wheat	Amino acid substitution using TILLING mutants [94]	
Allergen reduction	Gliadin genes	Wheat	Knockdown by RNAi [161]	This has not been modified, but multiplex editing in wheat could replicate the outcome described [7]
Low phytate	*IPK1*	Rice	Knockdown by RNAi [162]	Replicated in maize using ZFN [108]
HOLL lines	*FAD2-1* and *FATB1*	Soybean	Knockdown by RNAi [163]	Replicated with TALEN [164]
Reduced acrylamide	*Vlnv*, *AS1* and *AS2*	Potato	Knockdown by RNAi [165]	Replicated with TALEN [130]

Mildew resistance by editing all mlo alleles in wheat




```
T0-1 A1:  TCGCTGCTGCTCGCCGTcacg.....
T0-2 A1:  TCGCTGCTGCTCGCCGTcacgcagga...aatctcCGGGA
T0-3 A1:  ..........................caatctcCGGGA
     B1:  TCGCTGCTGCTCGCCGTgacgcagga/ccccatctcCGGG
     D1:  TCGCTGCTGCTCGCCGTgacgcagga.........../GG
T0-4 D1:  TCGCTGCTGCTCGCCGTgacgcagg.....atctcCGGGA
T0-5 D1:  TCGCTGCTGCTCGCCGTgacgcag.....aatctcCGGGA
T0-6 A1:  TCGCTGCTGCTCGCCGTcacgca......aatctcCGGGA
          TCGCTGCTGCTCGCCGTcacgcagga...aatctcCGGGA
          TCGCTGCTGCTCGCCGTcacgcagga....atctcCGGGA
          TCGCTGCTGCTCGCCGTcacgcaggac..aatctcCGGGA
T0-7 D1:  TCGCTGCTGCTCGCCGTgacgcaggac..aatctcCGGGA
T0-8 A1:  TCGCTGCTGCTCGCCGTcacgcag.......tctcCGGGA
          TCGCTGCTGCTCGCCGTcacgcagg...caatctcCGGGA
     B1:  TCGCTGCTGCTCGCCGTgacgcagg../cccatctCCGGG
T0-9 A1:  TCGCTGCTGCTCGCCGTcacg.........tctcCGGGATATGCATCTCCCA  –10
     D1:  TCGCTGCTGCTCGCCGTgacgcaggac.....ctcCGGGATATGCATCTCCGA  –5
          TCGCTGCTGCTCGCCGTgacgcaggac....tctcCGGGATATGCATCTCCGA  –4
          TCGCTGCTGCTCGCCGTgacgcaggac..aatctcCGGGATATGCATCTCCGA  –2
T0-10 A1: TCGCTGCTGCTCGCCGTcacg..........ctcCGGGATATGCATCTCCCA  –11
T0-11 A1: TCGCTGCTGCTCGCCGTcacg.../gacccaatctcCGGGATATGCATCTCCCA  –3/+61
     D1:  TCGCTGCTGCTC.............................CATCTCCGA  –29
T0-12 A1: TCGCTGCCGCTCGCCGTcacgc........atctcCGGGATATGCATCTCCCA  –8
```



Mildew-resistance only when all 6 alleles were KO

Wang Y. *et al.* (2014) Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat. Biotech. **32** 947–951

Genome editing is not one thing

Type 0 – no change to DNA sequence

Deactivated CAS-9 / altered epigenetic marks / transcriptional control

Type 1 – Site-directed mutagen; double-stranded breaks

DNA repair via NHEJ

Type 2 – SDN plus repair template (also ODM)

Intentional 'small' edits to native DNA via HR

Type 3 – Insert new gene into 'safe harbour'

Targeted GMO via HR (but 'allele replacement' could be placed on other end of spectrum)

'Naturalness' / 'Interventionist' / 'Detectable'

Huw D Jones, (2016) Are plants engineered with CRISPR technology genetically modified organisms? The Biochemist June 2016

Next 25 years?

Longer term prospects for gene editing point to more fundamental changes for farming. Eg. could reduce 10,000 years of plant breeding into 5 or 10 years.

The only difference between undomesticated teosinte and current elite maize is the precise sequence of a few thousand alleles. Possible to recreate a new version of maize but with features that make it more harmonising with the environment

Global status of regulatory oversight of genome editing

- US FDA have ruled that at least 7 products generated using genome editing are NOT Regulated Products in the US (a low-phytate maize, a herbicide-tolerant (HT) canola, a mildew-resistant wheat; a non-browning mushroom, PPO knock-out potato, FAD3 knockout soybean and the first ‘CRISPR crop’, a waxy maize.

- Canada use product-based regulation so should accommodate editing

- Argentina and Brazil have given guidance that on a case-by-case basis Type I genome editing wound not be regulated as a GMO

- EC still not decided but if gene editing is classed as a GMO it will kill the technology in EU

In Conclusion

- Innovation in Plant breeding will pay significant part in ‘sustainable intensification’ and maybe also in extensive, organic cultivation if ‘rules’ set by the governing organisations change
- First applications of gene editing will be type 1 edits in well characterised genetic pathways
- Longer term potential is for more fundamental synthetic biology to create customised crops
- Not clear yet whether products of different gene editing approaches will be regulated under GMO legislation in EU
- If they are, the high cost of regulation and stigma of GMO labelling etc will kill the technology in EU