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Genomics, Epigenetics & Synthetic Biology

Part II Plant Sciences Module L1

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Synthetic Biology and Plant Biotechnology

Lecture 1: Genetic modification in agriculture and the advent of Synthetic Biology.
Lecture 2: Genetic circuits and genome scale DNA engineering.
Lecture 3: Engineered logic and the control of gene expression.
Lecture 4: Self-organisation and reprogramming of multicellular systems.

The lecture images, handouts and references are available at:
http://www.haseloff-lab.org/education/index.html

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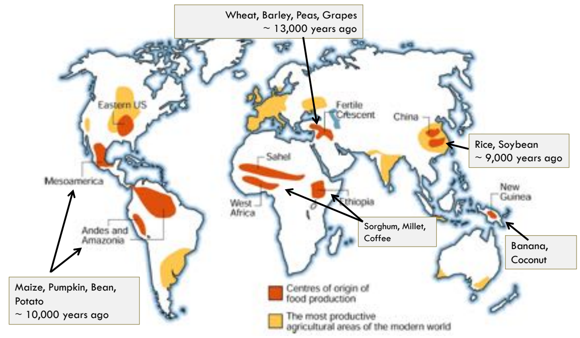
Origins of world crops



Nikolai Vavilov

Nicolai Vavilov was a Russian biologist who first popularised the idea of geographical centres of diversity for the origin of modern crop species. These centres corresponded to areas of botanical diversity that coincided with the establishment of early human societies and plant domestication.

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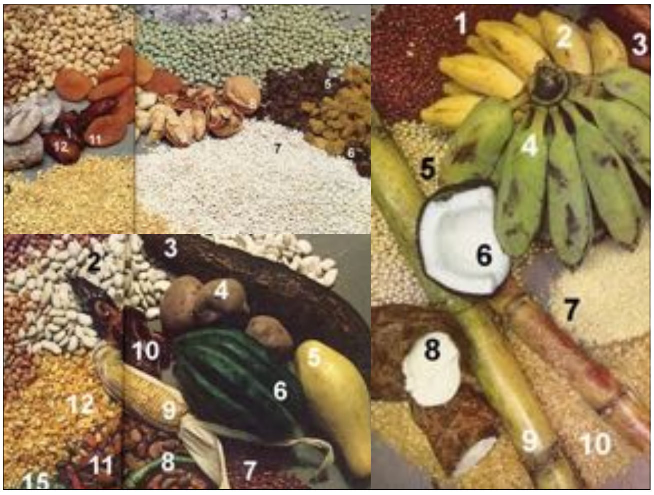

A pictorial representation of crops from the Middle East (top left) from the New World (bottom left) and from the Far East (right).

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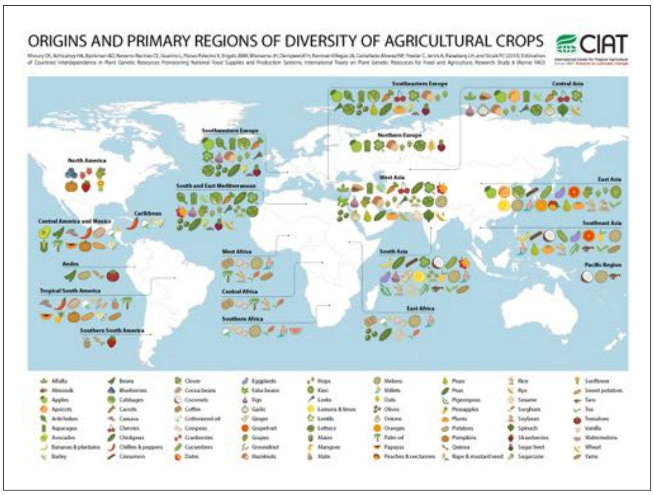
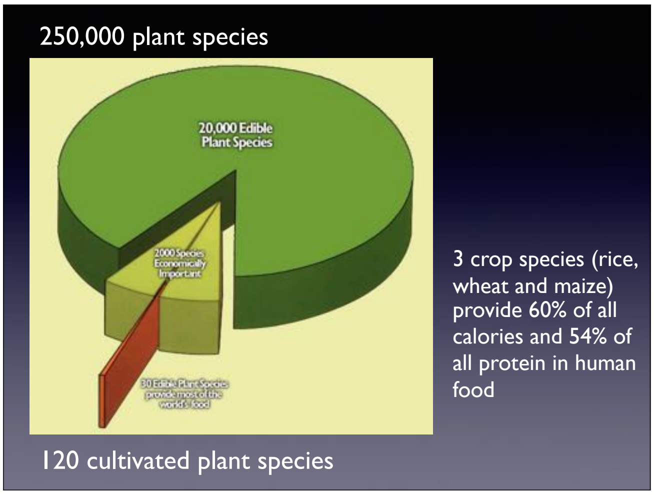


This diagram from the International Centre for tropical agriculture shows the global origin of a wide variety of agricultural crops. We tend to think of crop diversity as a cornucopia, but…



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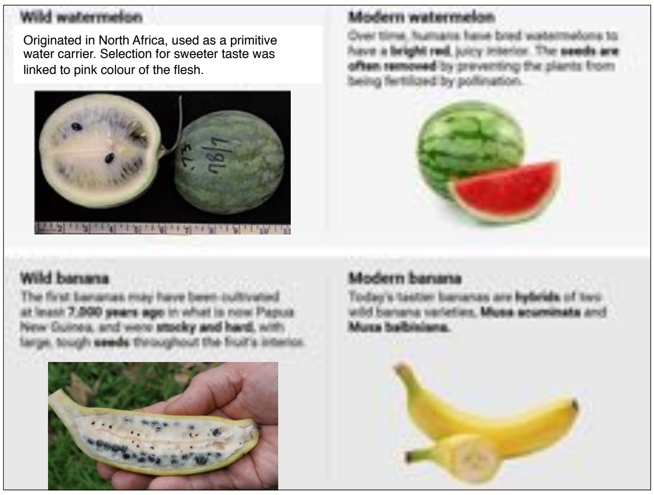
Crop plants sample a tiny fraction of total plant diversity. It estimated that there are around 400,000 plant species on Earth. Only around 20,000 of these have ever been used by humans as food, and only 2000 plant species have any economic importance as food crops. 30 species provide most of the world's food. Three species - rice, wheat and maize, provide 60% of calories and over half of the protein in human food. A vast reservoir of biological diversity remains untapped.



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Ancient species are provided raw material for domestication of crop plants. Domestication has occurred over millennia, and often accompanied by substantial changes in phenotype. For example, melons were thought to have been originally used in prehistoric times as natural water carriers in northern Africa. The wild melons have a high water content but are bitter. The selection for sweeter tasting melons unintentionally produced pink flesh, as the genetic loci for colour and sweetness are closely positioned. In addition, bananas were first domesticated in Papua New Guinea. These were diploid and contained seeds. Modern bananas are triploid, sterile and seedless…and genetically homogeneous.

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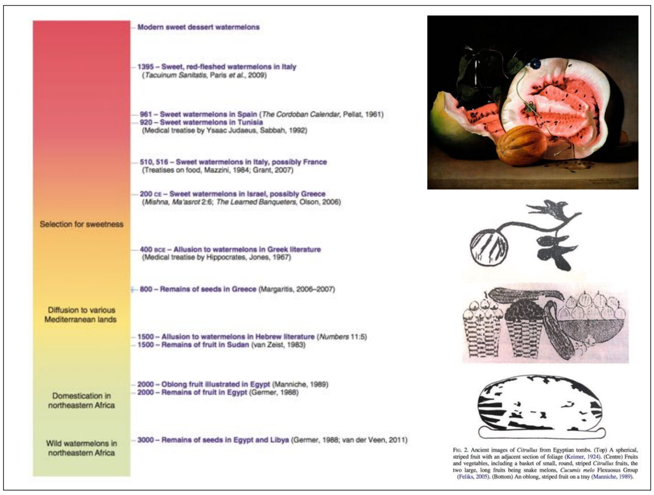
The ancestor of the modern watermelon is believed to have originated in northern Africa. These ancestral plants possessed fruit that were pale, heavily seeded and bitter. However they were useful as a means of transporting water.



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Images and the remains of watermelons have been found in 5000 year old Egyptian tombs. And there have been literary references to watermelons since that time. The first evidence of sweet watermelons occurs around 2000 years ago.

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Watermelon phenotypes: ranging from the ancestral form (lower left), through to modern varieties.

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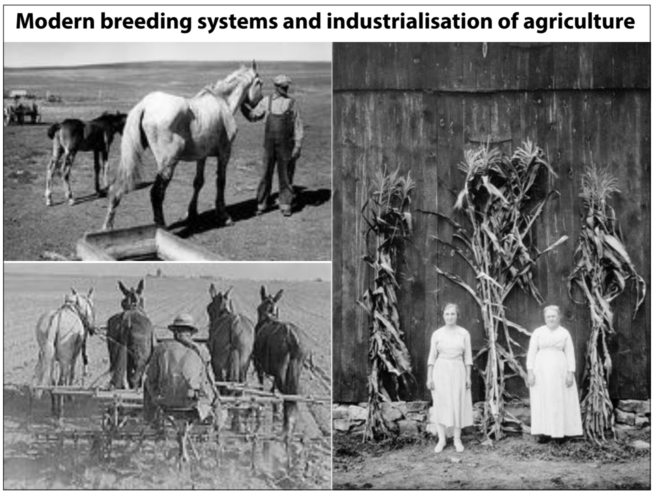
A wide variety of modern cultivars are shown. Modern breeding has produced an expanded variety of different characters including fruit colour, size and seed content.



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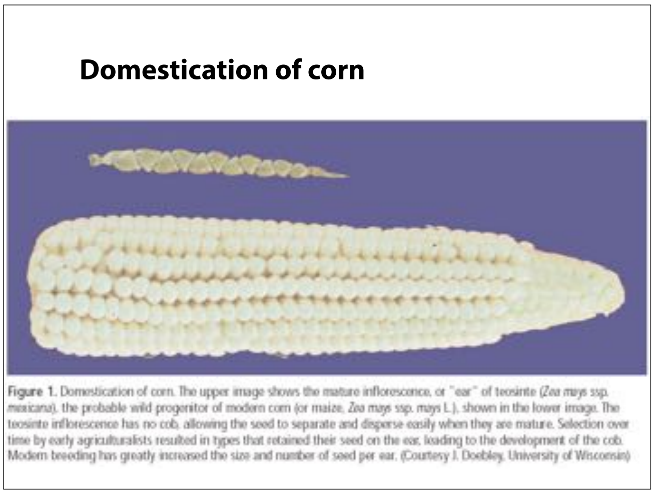
Europeans adopted maize as a crop and the 1800s saw large plantings across the Midwest of the United States. Before 1900 farmers in the Midwest were highly self-sufficient. They looked to the outside world for things like salt and nails, but external inputs into crops were minimal. Fertiliser inputs were limited to manure, pesticides were unknown and crops were true breeding and seed corn was obtained from previous year's crop. County fairs included competitions for the highest yielding corn plants.



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Early forms of maize strongly resemble teosinte, a plant endemic to Mesoamerica, and a subspecies of *Zea mays*. This likely progenitor has a strikingly distinct morphology, with smaller numbers of kernels arranged on a spike. It has been estimated that new varieties of maize been selected for over 9000 years. Modern varieties are characterised by a cob architecture with much larger numbers of kernels on each inflorescence.

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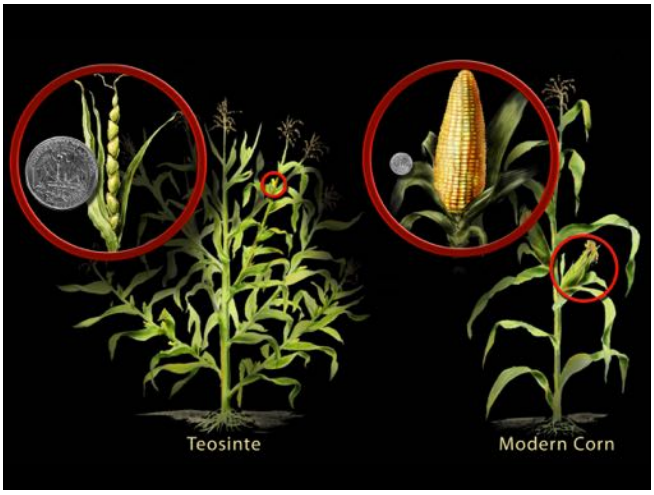

The overall habits of teosinte and modern maize plants are strikingly different. Teosinte plants are more highly branched with multiple male and female inflorescences. Graphical representations are shown with a coin added for scale. Modern maize plants are taller with a higher degree of apical dominance, and are better adapted for modern agricultural practices.

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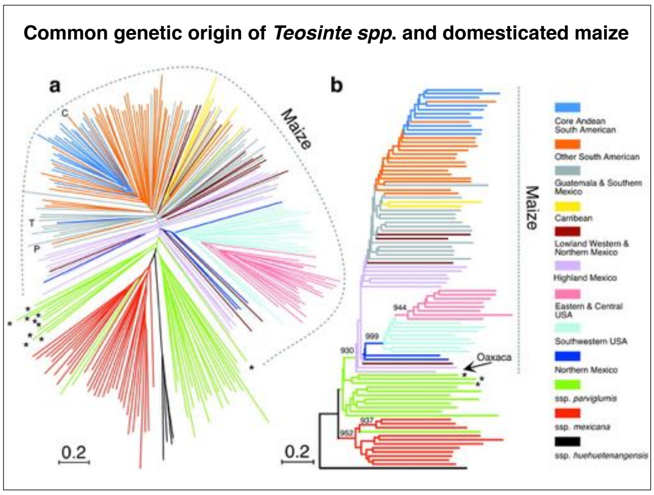

Genome sequencing of teosinte and domesticated maize demonstrates that they share a close common origin. Maize subspecies corresponding to teosinte are shown in green, red, and black.

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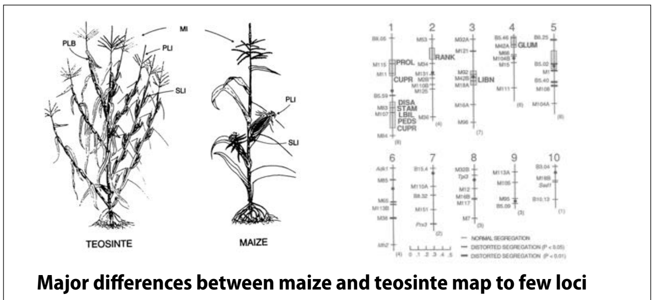

Table 1. List of principal traits distinguishing maize and teosinte

Trait	Description
CUPR (cupules per rank)	Number of cupules in a single rank
DISA (disarticulation score)	Tendency of ear to shatter (1–10 scale)
GLUM (glume score)	Hardness and angle of outer glume (1–10 scale)
LBIL (lateral branch internode)	Average length of internodes on the primary lateral branch
LIBN (branch number)	Number of branches in primary lateral inflorescence
PEDS (pedicellate spikelet score)	Percentage of cupules lacking the pedicellate spikelet
PROL (prolificacy)	Number of ears on the primary lateral branch
RANK (rank)	Number of rows of cupules
STAM (staminate score)	Percentage of male spikelets in primary lateral inflorescence

Doebley *et al.*, PNAS (USA) 87: 9888-9892 (1990)

Work from John Doebley's lab has mapped the genetic differences between teosinte and maize. genetic mapping studies have identified genes known to be involved in vegetative branching, morphology and floral architecture. Strikingly it was estimated that around 90% of the difference in form between teosinte and maize could be accounted for by less than ten genetic loci.

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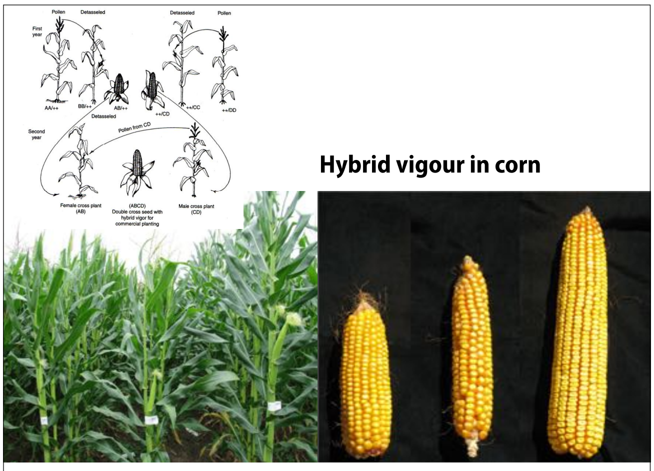

Inbred plant B73 (left), inbred plant Mo17 (middle), and hybrid plant B73 x Mo17 (right).

In the 1900s scientists like G.H. Shull observed that open pollinated inbred forms of maize became less productive over time. In contrast heterosis or out-crossing gave rise to highly productive progeny. (Maize plants have separate male and female flowers and detasseling of male flowers is a simple way of ensuring selective crossing). Through the 1920s, plant breeding stations were established to create parental inbred lines that could be used for different crosses and to create highly productive maize seed.

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Entrepreneurs like Roswell Garst helped transform US agriculture last century. He helped to establish sales of hybrid corn seed with the noted corn breeder Henry Wallace in 1930s in Iowa. Wallace established Pioneer Hi-Bred, and Garst established Garst seed. Farmers were previously highly self reliant - saving a portion of their crop for next year's seed, using manure for fertiliser, and using draft horses for ploughing and carting the hand-picked corn. Garst offered free bags of hybrid seed corn in return for half of the next seasons increased yield. When the new seed outperformed, he only accepted the cost of the seed corn - in return for a commitment for the following season. Farmers soon switched to purchasing seed corn for cash. Eventually this led to the conversion of farming from an occupation, to an industry. There was a loss of diversity, from 786 varieties in 1903 to 52 in 1983 - and increased application of synthetic fertilisers, pesticides and herbicides. Machinery was invented for handling of the more

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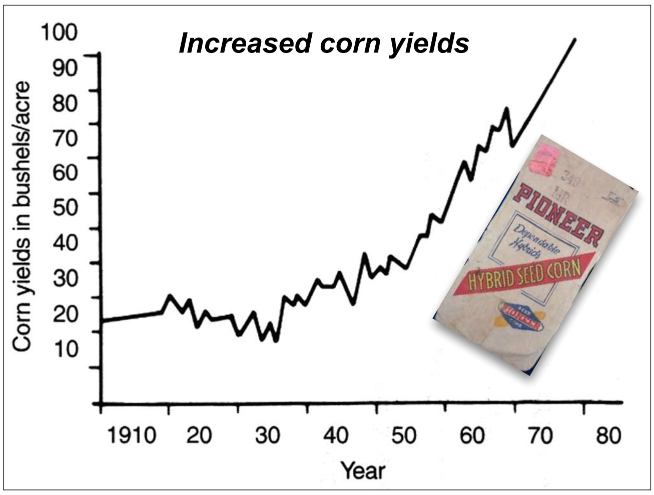

Hybrid maize seed saw rapid adoption in the US Midwest after its introduction in the 1930s. the overall percent plan planted with hybrid maize increased rapidly. In addition, new varieties of hybrid maize saw rapid increases in productivity over the coming decades. Photographs are shown of parental lines and hybrid progeny.

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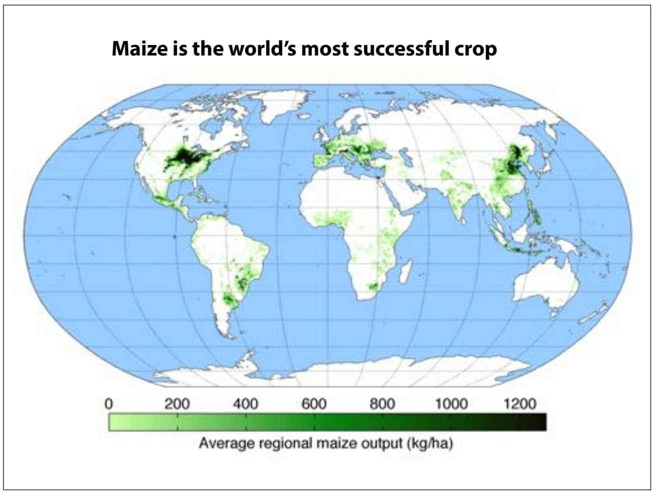

From its origin as a Mexican weed, worldwide production of maize is over 1 gigatonne per annum, more than wheat or rice. (http://www.fao.org/faostat/, and http://www.worldofcorn.com). The USA and China are the major producers of maize.

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Selective breeding of other crops has dramatically improved their yields also. The decades following 1960's saw the breeding of highly productive new varieties of wheat. Many of these varieties were dwarf, which provided agronomic benefits and allowed commitment of more resources to seed production during growth. In addition, improved response to inorganic fertilisers and introduction of disease resistance through cycles of out-crossing and back-crossing contributed to new elite varieties.

Norman Borlaug was a pioneer of these efforts. He is shown here with Sonora-64, one of the semi-dwarf, high-yield, disease-resistant varieties that was key to the Green Revolution, to a group of young international trainees, at what is now CIMMYT's CENEB station (Campo Experimental Norman E. Borlaug, or The Norman E. Borlaug Experiment Station), near Ciudad Obregón, Sonora, northern Mexico.

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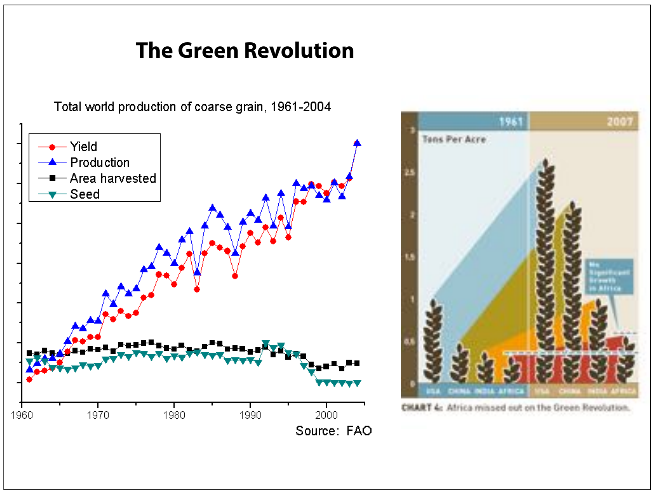
“The harvesters” by Pieter Bruegel the Elder (1565) - with a graphic representation of a partly harvested wheat field in northern Europe. Note that the height of these wheat crops reached shoulder height.
Modern wheat crops are much shorter, shown here with Norman Borlaug and colleagues at a trial field of Sonora-64.
The story of Borlaug career is inspiring, a short version can be found at https://en.wikipedia.org/wiki/Norman_Borlaug. He has been credited with saving a billion people from starvation, and his work has been extended to rice varieties.



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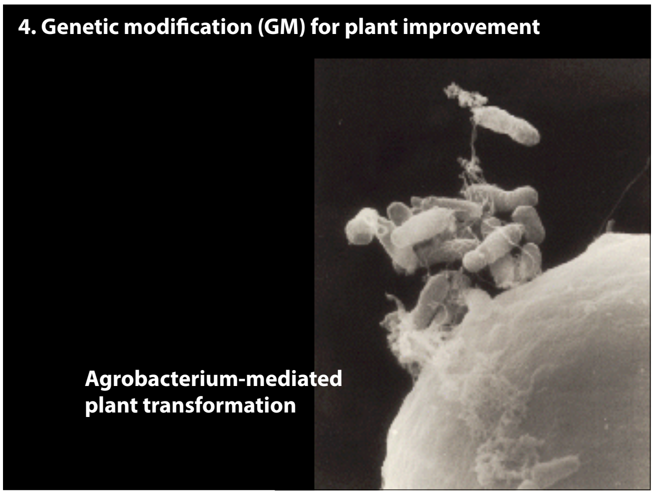
From the 1960s, the worldwide production of grain has increased dramatically in yield and total production despite relatively constant area of cultivation and planted seed. The bulk of these increases have been seen in the developed world, China and India. The benefits of increased production have not been so widely seen in Africa.



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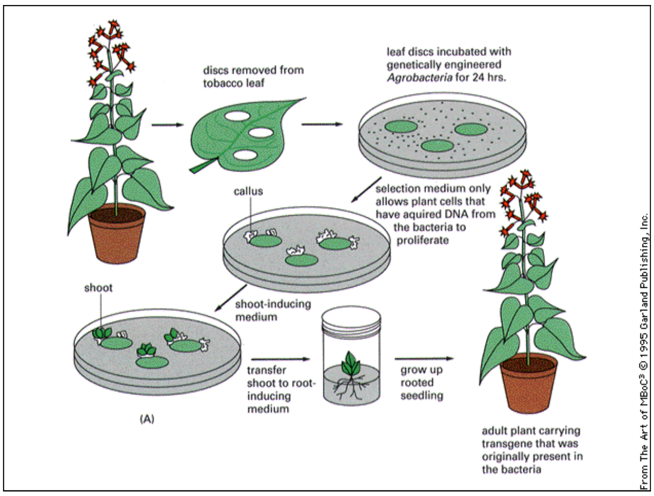
Agrobacterium tumefaciens is capable of binding to plant cells, forming a conjugation complex and transferring a specific and delimited segment of DNA. Here shown in an electron micrograph.



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In a normal infection, conjugation of a bacterium with a susceptible plant is followed by replicative transfer of a specific segment of DNA called the T-DNA (shown in red) - from a region of a Ti plasmid into a recipient plant cell. The transformed cells are then programmed to proliferate.
Plant transformation with a disarmed binary plasmid requires (i) co-cultivation of plant material with an engineered Agrobacterium strain, (ii) curing of the Agrobacterium by (microbial) antibiotic treatment, (iii) regeneration of plantlets from transformed cells under (plant specific) antibiotic selection. In this example, the engineered T-DNA contains kanamycin. (iv) Rescue of regenerated plants for grow and harvest transgenic seed. At this point transgenic plants can enter a breeding programme.

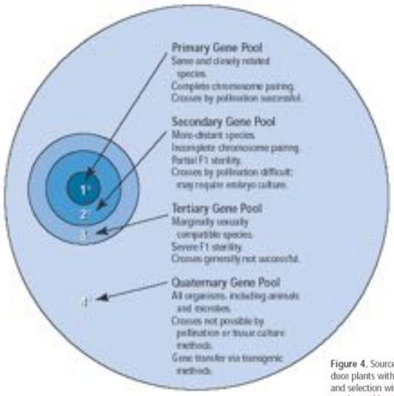

Figure 4. Sources of genetic variation for crop improvement. Breeders produce plants with improved combinations of genes by crossing (hybridization) and selection within the primary gene pool, which is comprised of a crop species and its closest related wild species. Tissue culture methods such as embryo culture are commonly used to enable genes from the secondary gene pool to be transferred into the cultivated species. Other methods such as somatic hybridization sometimes allow genes from the tertiary gene pool of more distantly related species to be transferred into crop plants. The immense gene resources of the quaternary gene pool (essentially all other organisms) can be used for crop improvement only via transgenic methods.

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Until the early 1980s, the genetic modification of crops required the introduction of new genes through sexual crossing and refinement of traits through breeding. Specialised breeding techniques can allow access to gene pools outside of the same species - but access is confined to closely related plants. The advent of techniques to create transgenic plants allows synthesis of effectively any engineered DNA construct and unconstrained modification of plant genomes. This breakthrough came in 1983 with the independent publication of the first Agrobacterium-mediated plant transformation papers from three groups. The most predominant transgenic traits are herbicide and pest resistance.

Weed-infested soybean plot (left) and
Roundup Ready® soybeans after Roundup treatment.

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An example of the use of herbicide resistant variety of soybean for weed control. In this case the plot has been sprayed with Roundup, a wide spectrum herbicide which is effective in reducing weed growth, however the transgenic herbicide resistant soybean plants are unaffected. The use of herbicides for weed control allows new approaches to no-till agriculture. However it has also led to the appearance of resistant weed strains.

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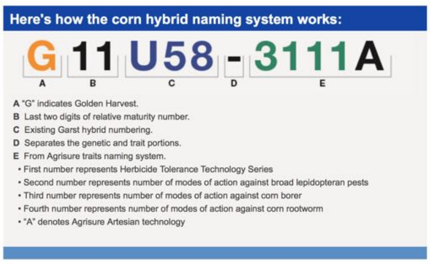
Bacillus thuringiensis strains contain variety of natural toxins that are highly selective and specific for different types of insects, including lepidopteran and beetle pests. Genes that encode different types of BT toxin have found wide use for protecting maize, cotton and soybean crops. Ingestion of the BT toxin by feeding insects results in disruption of ion channel function in the insect's gut. Above, corn kernels containing expressed BT toxin are protected from rootworm beetles.

Stacking of transgenic traits in hybrid corn



Syngenta

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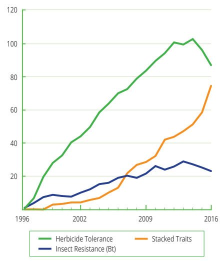
Single gene traits are commonly stacked. Here the code is shown for naming a Syngenta variety of corn. The name includes a reference to the hybrid maize line and transgenic traits.

Figure 12. Global Area of Biotech Crops, 1996 to 2016: by Trait (Million Hectares)



Source: ISAAA, 2016

Table 35. Global Area of Biotech Crops, 2015-2016: by Trait (Million Hectares)

Traits	2015	%	2016	%	+/–	%
Herbicide Tolerance	95.9	53	86.5	47	-9.3	-10
Stacked Traits	58.5	33	75.4	41	+16.9	+29
Insect Resistance	25.2	14	23.1	12	-2.1	-8
Virus Resistance/ Other	<1	<1	<1	<1	<1	<1
Total	**179.7**	**100**	**185.1**	**100**	**+5.4**	**+3.0**

Source: ISAAA, 2016

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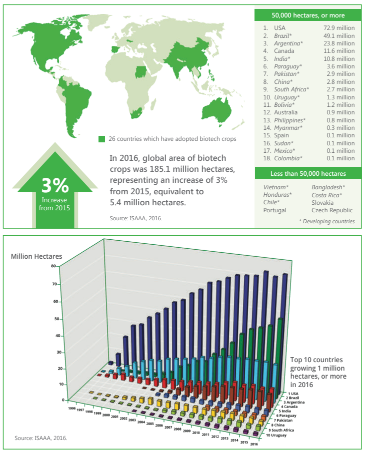
The first transgenic plants were created in the laboratory in the early 80s. By the mid-90s field trials of transgenic crops were underway. The first generation of traits included herbicide tolerance for weed control, and insect and virus pest resistance. In the subsequent 20 years there has been a rapid uptake in the use of these single gene traits in maize, cotton and soybean crops. We are seeing a sharp rise in the use of combined, or stacked, traits. In 2016, 185 million ha of transgenic crops were grown.

Figure 2. Global Area (Million Hectares) of Biotech Crops, 1996 to 2016, by Country, Mega-Countries, and for the Top Ten Countries



Source: ISAAA, 2016.

30

Countries in North and South America have seen the fastest and greatest increase in planting of biotech crops. They account for the overwhelming majority of GM producers globally. Outside of the Americas, there has been poor uptake of transgenic crops for food production. However, transgenic cotton is finding some adoption in Asia. Notably, there has not been wide adoption of transgenic crops in Europe or Africa to date.

Table 29. Biotech Crop Area in the European Union, 2006-2016

	Country	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
1	Spain	53,667	75,148	79,269	76,057	76,575	97,326	116,307	136,962	131,538	107,749	129,081
2	Portugal	1,250	4,263	4,851	5,094	4,868	7,724	9,278	8,171	8,542	8,017	7,069
3	Czechia	1,290	5,000	8,380	6,480	4,680	5,091	3,080	2,560	1,754	997	75
4	Romania	--	350	7,146	3,244	822	588	217	220	771	3	--
5	Slovakia	30	900	1,900	875	1,248	761	189	100	411	104	138
6	Germany	950	2,685	3,173	--	--	--	--	--	--	----	---
7	Poland	100	327	3,000	3,000	3,000	3,000	N/A	--	--	----	---
	Total	**57,287**	**88,673**	**107,719**	**94,750**	**91,193**	**114,490**	**129,071**	**148,013**	**143,016**	**116,870**	**136,363**

Source: ISAAA, 2016

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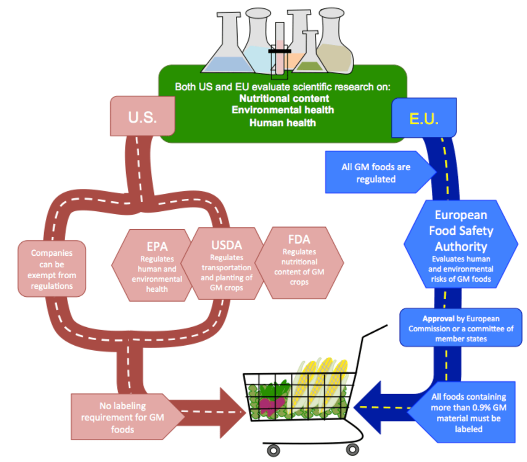
Latest figures for the adoption of transgenic crops in Europe. There are relatively small areas of transgenic crops grown in Spain and Portugal - corresponding to transgenic maize. And very little grown elsewhere.



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The US and Europe have adopted very different regulatory systems for GM foods. Food companies submit the same types of scientific data to U.S. and EU regulatory bodies for approval. Three separate agencies in the U.S. evaluate the potential risks of GM foods, while a centralised approval process is established in the EU. Approval and labeling requirements are stricter in the EU. (http://sitn.hms.harvard.edu/category/flash/special-edition-on-gmos/)

Different approaches to GMO regulation:

Precautionary Principle (Europe)- GM crops are potentially dangerous and pose new risks and thus their use should be avoided until they are proven safe.

Substantial Equivalence Principle (USA) - GMOs are no different from conventional crops, if the products so derived are "substantially equivalent" in composition, nutritive value or safety after thorough comparative testing.

Table 40. Reduction in Pesticides and Environmental Impact Quotient*

	1996-2014	1996-2015	2014 alone	2015 alone
Reduction in pesticides (Million kgs active ingredient, a.i.)	583.5	619	40.4	37.4
Pesticides savings (%)	8.2%	8.1%	6.4%	6.1
Reduction in (EIQ)**	18.5%	19%	17.6%	18.5

** Environmental Impact Quotient (EIQ) = a composite measure based on the various factors contributing to the environmental impact of an individual active ingredient.
* Brookes and Barfoot, 2017, Forthcoming.

Table 41. Savings on CO2 Emissions Equated with Number of Cars off the Road*

	2014 alone	2015 alone
Savings in CO2 emissions due to reduced use of fossil-based fuels (Billion kgs)		
a. Due to reduced insecticide and herbicide sprays	2.20	2.80
b. Due to reduced ploughing	24.8	23.9
Total CO2 emissions	27.0	26.7
Reduction in number of cars off the road (Million)		
a. Due to reduced insecticide and herbicide sprays	0.97	1.25
b. Due to reduced ploughing	11	~11 (10.6)
Total cars off the road	12	~12 (11.9)

* Brookes and Barfoot, 2017, Forthcoming

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The use of GM crops has resulted in reduced use of chemical pesticides, and reduced ploughing for weed control. These are estimated to have beneficial impacts on the environment and CO_2 emissions.

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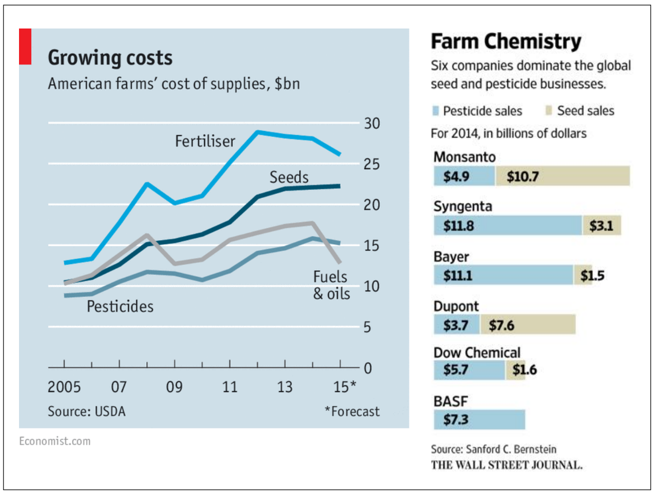
The introduction of unlabelled GM corn and soybean products from the US during the 90s caused a consumer backlash in Europe. This is partly due to the lack of choice and benefit for the consumer and perceived risks associated with the new technology - in the wake of the BSE crisis. Further there has been strong distrust of the large agrochemical companies who are exploiting the new technology.



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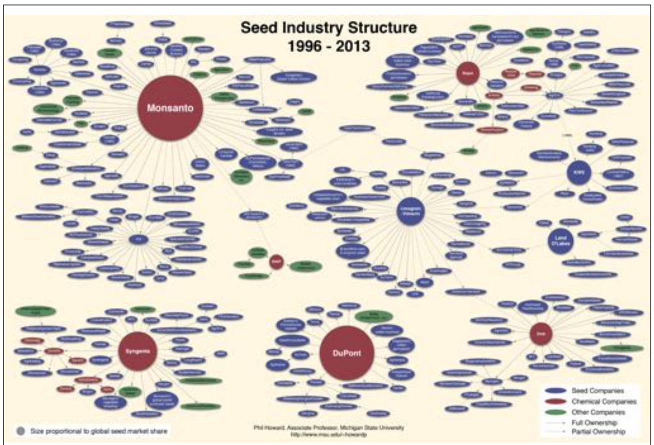
The intensive nature of modern agriculture has led to increasing costs and complexity for farmers. Increasing yields come at the expense of increased fertiliser, pesticide, fuel and seed costs. The industry seen ever increasing levels of integration, so that a few companies are the major players in global agriculture.



Revision to Howard, P.H. **Visualizing Consolidation in the Global Seed Industry: 1996–2008**. Sustainability 2009, 1, 1266-1287

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Diagrammatic representation of the global seed industry. A few major agrochemical companies (shown in red) own, or have an interest in, clusters of the many seed companies. These agricultural combines are characterised by increasing vertical integration and consolidation.

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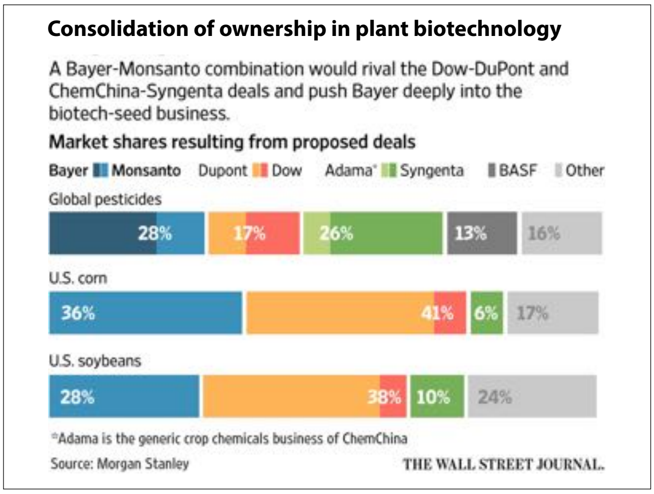

Six major agrochemical companies are undergoing further mergers, and we may see three new companies owning 60% to 80% of key agricultural activities worldwide.

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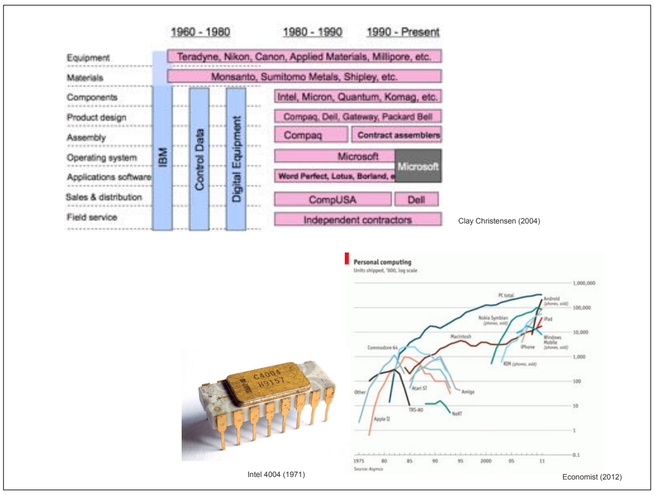

This level of consolidation has been seen in other industries. For example, the minicomputer industry was dominated by three companies (IBM, Control Data and DEC) through the 1960s. However the invention of the microprocessor in the early 70s, and the emergence of low-cost microcomputers cause disruption and saw the decline of these companies, and the emergence of a whole new range of businesses. The microcomputer industry was itself disrupted by the emergence of smart phones and apps. GM agribusiness is based on the use of 1980s technologies. Could this be due for disruption?

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Disruptive technologies:

Genome editing

Synthetic Biology - Engineering

The last few years have seen the emergence of both new technologies for direct genome editing, and for new engineering approaches that promise both highly efficient modular construction of DNA systems and systems for rational design. These have the potential to disrupt existing products and ways of working.

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Introduction/revision for CRISPR-Cas9 gene editing

There has been an explosion of new gene editing techniques and their application for biomedical and agricultural uses. Accessible articles and reviews that describe the new wave of editing techniques can be found with other lecture materials at http://www.haseloff-lab.org/education

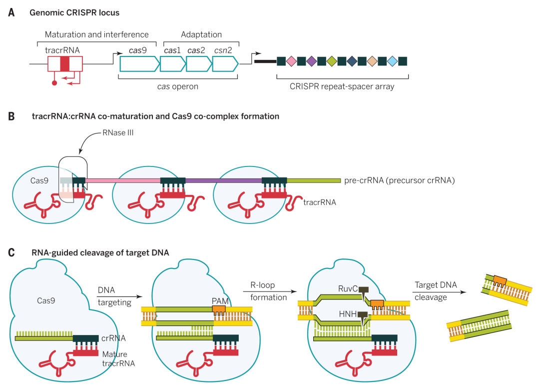

Fig. 2. Biology of the type II-A CRISPR-Cas system. The type II-A system from *S. pyogenes* is shown as an example. (**A**) The *cas* gene operon with tracrRNA and the CRISPR array. (**B**) The natural pathway of antiviral defense involves association of Cas9 with the antirepeat-repeat RNA (tracrRNA: crRNA) duplexes, RNA co-processing by ribonuclease III, further trimming, R-loop formation, and target DNA cleavage. (**C**) Details of the natural DNA cleavage with the duplex tracrRNA:crRNA.

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The CRISPR class of gene editing tools are derived from natural systems for bacterial immunity. Bacteria contain mechanisms for converting foreign DNA to embedded interspersed segments of sequence of defined length - the CRISPR arrays. These act as a reservoir of elements that can be used to attack incoming homologous sequences - such as phage DNAs. CRISPR sequences are transcribed, paired with the tracrRNA and bound to the Cas9 protein to produce a targeted, RNA-programmed nuclease.

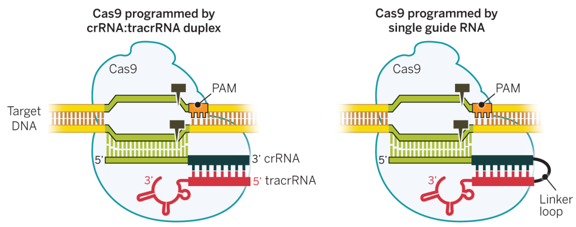

Fig. 3. Evolution and structure of Cas9. The structure of *S. pyogenes* Cas9 in the unliganded and RNA-DNA–bound forms [from (77, 81)].

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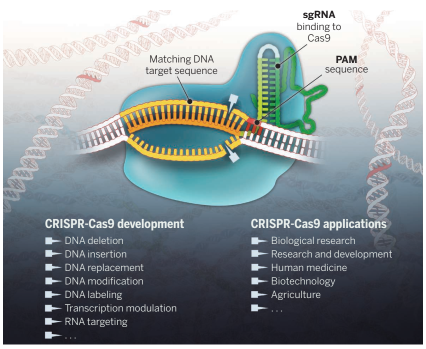
The tracrRNA and crRNA components of the nuclease can be fused to create a single guide sequence that, in combination with Cas9, will produce a nuclease that can be targeted to any DNA sequence adjacent to a 3 nucleotide PAM sequence.



The Cas9 enzyme (blue) generates breaks in double-stranded DNA by using its two catalytic centers (blades) to cleave each strand of a DNA target site (gold) next to a PAM sequence (red) and matching the 20-nucleotide sequence (orange) of the single guide RNA (sgRNA). The sgRNA includes a dual-RNA sequence derived from CRISPR RNA (light green) and a separate transcript (tracrRNA, dark green) that binds and stabilizes the Cas9 protein. Cas9-sgRNA–mediated DNA cleavage produces a blunt double-stranded break that triggers repair enzymes to disrupt or replace DNA sequences at or near the cleavage site. Catalytically inactive forms of Cas9 can also be used for programmable regulation of transcription and visualization of genomic loci.

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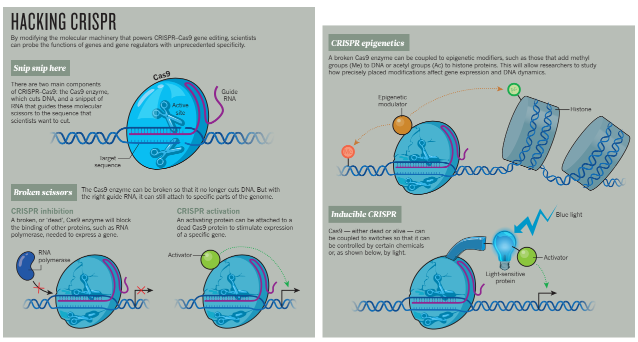
The CRISPR-Cas9 system can be used to create a programmable DNA binding complex. This will normally create a double-strand break at the target site. This has ben used widely for targeted mutagenesis, via error-prone repair of dsDNA breaks in vivo, and to promote DNA replacement through homologous repair. In addition, the CRISPR-Cas9 complex has been engineering to have a wide range of other activities…



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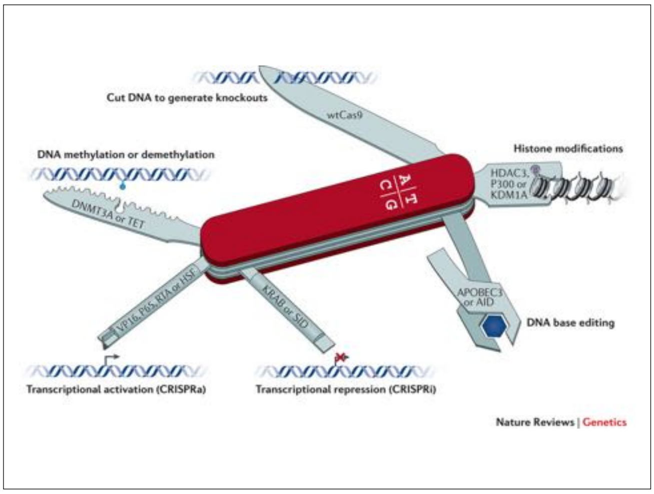
Use of the CRISPR-Cas9 complex to catalyse dsDNA breaks, site-specific delivery of inhibitor or activators of transcription, recruitment of chromatin modifiers, and as an inducible (and targeted) regulator of gene expression.



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The RNA-programmable DNA binding element is finding many applications as a tool for genome manipulation *in vivo*.

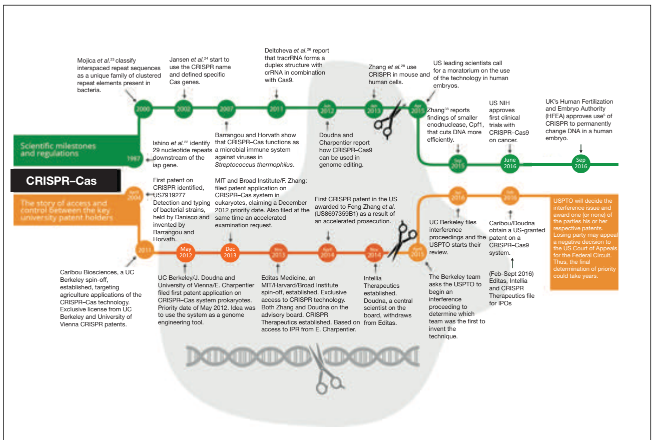

Figure 1 CRISPR–Cas scientific and regulatory milestones (upper strand) as well as milestones in patenting activity (lower strand). IPR, intellectual property rights.

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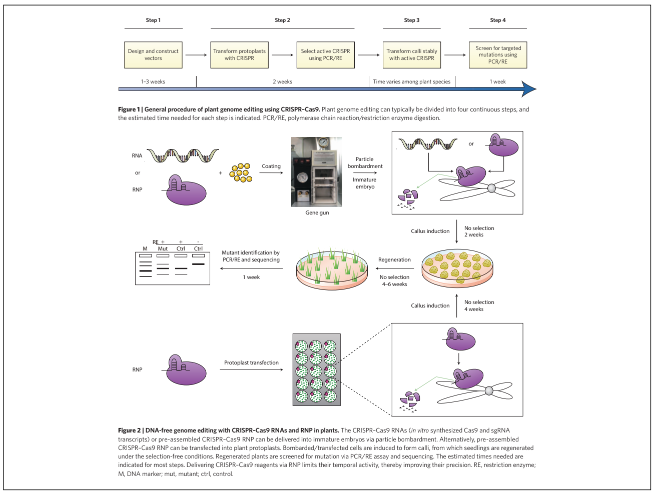
History of CRISPR-Cas9 manipulation and commercial exploitation.



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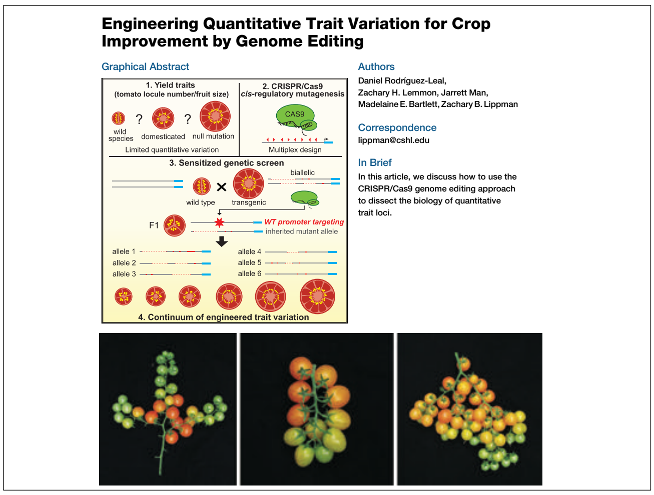
DNA-free manipulation of crop plants. Delivery of CRISPR-Cas9 ribonucleoprotein into plant cells by protoplast transformation or biolistic delivery allows precise manipulation of plant genomes without the introduction of plant pathogen sequences (e.g. Agrobacterium), or other foreign DNA. This allows the production of modified plants with engineered genomes - which would be indistinguishable from, say, mutant plants produced by random mutagenesis.



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In a recently published experiment, Lippman and colleagues targeted regulatory elements in the tomato genome, using CRISPR-Cas9 delivery. They could generate variant traits in a targeted way, and produce plant lines with traits that could be introduced directly into a breeding programme. This is demonstration of an alternative to conventional plant transformation, and introduction of foreign activities - that has be potential to be regulated differently from existing GM crop systems.

Lecture 1

1. **Origins of modern crops**
2. **Selection and breeding of new crop varieties**
3. **Industrialisation of agriculture**
4. **Genetic modification (GM) for plant improvement**
5. **Genome editing**

Lecture 2: Synthetic Biology and DNA engineering.

Lecture 3: Engineered logic and the control of gene expression.

Lecture 3: Self-organisation and reprogramming of multicellular systems.

Additional resources: **http://www.haseloff-lab.org** (Education)

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The next lecture will introduce a second disruptive technology - synthetic biology.