Boosting Innovations for better Crops

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Our World

Rocked by change: Wow! What is happening?

Major developments:

- China, India as emerging economies are growing, political turnarounds in USA & Turkey, Brexit, EU under pressure
- Growing and aging world population
- Growing economies
- **Need** for more, healthy and better food
- **Need** for sustainable agricultural production
- **Need** to adapt to climate change

**Significant Crop Improvements are part of the solution**
Our Agro Food world
Rocked by change: Wow! What is happening?

Global Agro Food developments:
- Large consolidations (resulting in the Big Five: “MonBayer”, “SynChina”, “DowPio”, KWS and Limagrain)
- Countries on the move (e.g. China & India)
- Agro Food Patents: on Genes, Traits & Plants, relation to Breeders right’s
- Regulations on Novel Breeding Technologies and Biodiversity / Germplasm availability
- Technologies: HT DNA sequencing, Big Data, HT Trait Discovery + crossovers with other fields (e.g. ICT, Nanotechnology, BioMedical field, Robotics, Defense Industry, Creative Industry)

KeyGene addresses these challenges by collaborating with industry leaders in partnerships to generate breakthrough innovations.

KeyGene develops:
new Crops & Traits & Technologies for the Future
Our Crops for the Future
Genomics enables innovations in all crops

- **Accelerate** improvement of current cash crops (e.g. corn, soybean, tomato, lettuce, onion)
- **Start** improvement of Industrial crops (e.g. oil palm, banana)
- **Increase** improvement of orphan crops (e.g. cassava, millet, plantain)
- **Develop** novel protein crops (e.g. pulses, quinoa)
- **Domesticate** crops (e.g. dandelion for rubber, stevia for sweetener)

*My view: because of cost reductions in genomics we can now molecularly improve crops that we could not touch earlier*
Our Traits for the Future
Interesting “old” and new traits

• Yield & Yield Stability *(feed the world)*
• Abiotic Stress Tolerance *(adapt our crops to climate change)*
• Biotic Stress Resistances *(sustainability, reduce the use of agro chemicals)*
• Quality, Taste *(more middle class consumers)*
• Long shelf life *(stop reducing waste!)*
• Health *(longer, better life for all of us)*

*My view: besides some new traits, most “old” traits are still of high interest*
Our Technologies for the Future for Exponential Ag Biotech Innovations

• **Farming:** variety development via crossing and selection supported by DNA technologies & Genomics. Agronomy: with fertilizers and sometimes chemicals.
  
  *My view: good quality products for acceptable price*

• **Industrial Farming:** development via GM: corn, soybean, canola, cotton, sugarbeet. Multinationals. Agronomy: large monocultures.
  
  *My view: large scale, innovative, cost effective for farmer, risk perception often high*

• **New Farming:** city farming, vertical farming, NEW. Agronomy: production in multiple layers in factories in the city or at home. Leafy vegetables first.
  
  *My view: because of food safety, sustainability, local production, too technical?*
KeyGene is the go-to AgBiotech company for higher crop yield & quality. With our intellectual capital, solution driven approach and collaborative spirit, we work for the future of global agriculture with partners in the AgriFood sector.
For the improvement of vegetable crops KeyGene has strong & strategic partners:
- Leading seed companies
- Family owned / cooperative
- Growing strongly
- Innovation is key

Limagrain Vegetables Seeds

For the improvement of other crops KeyGene has strong & strategic partners:
- in ornamentals
- in field crops (e.g. rice, (tropical) corn, cotton)
- in new crops (e.g. stevia, dandelion)
- in polyploid crops (e.g. wheat, potato)
- in orphan crops (e.g. banana, eucalyptus, cassava)
KeyGene

Our R&D and Innovation strategy

Fundamental research  Developing technologies & traits  Applying molecular breeding of crops  Breeding  Seed products  Market

Academia  KeyGene  Partners breeding industry

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KeyGene
Our Innovative Technology platforms

**CropPedia**
The best platform for gene / trait big data analysis

**KeySeeQ®**
Lead gene discovery for trait & crop improvement

**Gene Editing & KeyBase®**
Better traits in your germplasm

**KeyPoint® Mutation Breeding**
Better Traits in your germplasm

**Sequence Based Genotyping**
in all your crops

**KeyGene®SNPSelect**
for all your traits

**Breeding Strategies**
Fast stacking of traits in your germplasm

**Digital Phenotyping**
Measuring better traits in your germplasm

**Virtual Reality Breeding**
Interactive platform to analyze and visualize trait and crop performance

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Our Innovative Trait platforms

- Drought resistance
- Abiotic Stresses
- Biotic Stresses
- Crop Reproduction
- Clonal seeds without fertilization (Apomixis)
  Sterility
  Double Haploid induction

- Fungi
- Bacteria
- Virusses
- Sucking Insects

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DNA: source of genetic information

DNA: 63 yr

DNA has 4 building blocks (GATC)

Not digital but quatro-code

In all plant cells
DNA variation in our crops
DNA from each crop plants differs = genetic variation!

Tomato DNA consists of 1.000.000.000 code letters (human: 3 x more)
In DNA of 2 different commercial tomato plants: ~ 500.000 differences
With a third plant: ~ 800.000 differences
In 100 different commercial tomato varieties: ~ 10 million differences
Between commercial and wild tomato species: ~ 100 million differences

Which DNA differences are important?
How can we increase the amount of useful differences?
Which differences can we use to develop better crops in a non GM way?
### KeyGene & Genetic Variation

**Use of genetic variation for crop improvement**

#### Existing variation

- **I. Advanced Molecular Breeding**
  - slow
  - few mutations searching track record

- **II. Mol Mutagenesis**
  - KeyPoint®MB
  - CRiSPR Cas9
  - many mutations targeted

#### New variation

- **GM not by KeyGene**

#### Genetic modification

- Too expensive: 20 – 100 million €
- Technical issues
- 20 yrs experience: safe
- Societal aspects (EU / Japan)
I

Advanced Molecular Breeding
KeyGene
Start of modern Breeding: Genome Sequences

**Perfect Reference Genome Sequences**

1000s of genomes resequenced

Info in proprietary interactive databases: CropPedia

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**Food**

- Tomato
- Potato
- Cucumber
- Melon
- Pepper
- Onion
- Watermelon
- *Brassica rapa*
- *Brassica oleracea*
- Lettuce
- Chickpea
- Rice
- Stevia
- Sugar beet
- Wheat
- peach
- Apple
- Papaya
- Orange
- Strawberry
- Banana
- Bean
- Pigeon pea
- Cassava

**Feed**

- Soybean
- Medicago
- (Trop) Corn
- Sorghum
- Flax
- Cotton
- Dandelion
- Mose bamboo
- Oilseed rape
- Poplar
- Hevea
- Jatropha
- Castor bean
- Sunflower
- Chrysanthemum
- Petunia
- Orchid
- Cacao
- Barley
- Grape
- Tobacco
- Oilpalm

**Fiber**

- Food
- Feed
- Fiber
- Fuel
- Flowers
- Fun

GREEN: by KeyGene

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DNA markers & Traits

Variation in genes relates to commercial traits

True orange in pepper

1 basepair difference in 2,700,000,000 base pairs

Value for breeding partner: fast development of orange pepper varieties
II
Molecular Mutagenesis Approaches and examples
I. Selection of Lead Genes by KeySeeQ® using CropPedia

II. Gene Editing of selected genes

a. via CRISPR/Cas9/TALENs, etc.
   - fast and robust technology
   - issues: FTO and regulation in EU & others

b. via KeyPoint® Mutation Breeding: KeyGene’s highly successful mutagenesis system using chemicals & Next Generation Sequencing
   - large track record
   - high throughput & broad mutation spectrum
   - allelic series provide new opportunities
   - strong proprietary position KeyGene
   - mutants obtained: non GM + patentable
   - direct input for breeding programs
**KeyPoint® Mutation Breeding**

*Gene targeted random mutagenesis*

- **Chemical Mutagens-treated seed**
  - Young M1/M2 plants
  - 15,000 seeds

- **Targeted sequence based detection of novel variants**
  - Verification of novel variant plants
  - 10,000 plants

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Immortal Bulked-M2

Mutant Rice Population

- Rice Japonica type cultivar ‘Volano’ (risotto)
- Mutagenized plants in field, Italy
  - Sampling ~8000 plants
  - KeyGene & University Milano
- Seed harvesting per plant
- DNA isolated per plant
KeyPoint® Mutation Breeding

Track record

KeyGene's successful crop track record

- Tomato: 0.002 variants/Kb/plant
- Cucumber: 0.015 variants/Kb/plant
- Melon: 0.007 variants/Kb/plant
- Sweet pepper: 0.016 variants/Kb/plant
- Hot pepper: 0.012 variants/Kb/plant
- Dandelion: 0.002 variants/Kb/plant
- Sunflower: 0.008 variants/Kb/plant
- Soybean: 0.012 variants/Kb/plant
- Spring Wheat: 0.007 variants/Kb/plant
- Corn: 0.015 variants/Kb/plant
- Canola: 0.038 variants/Kb/plant
- Tobacco: 0.024 variants/Kb/plant
- Sorghum: 0.025 variants/Kb/plant
- Rice: 0.004 variants/Kb/plant
- Winter Wheat: in progress
- Barley: 0.004 variants/Kb/plant
- Cassava: 0.006 variants/Kb/plant

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Crop & Trait Improvement Teasers: everything comes together: partners, bio-informatics, (bio) technologies, traits, patents, commercial value
Trait 1: Insect Resistance by Molecular Breeding

Molecular Breeding has enabled development of Aphid resistant Lettuce varieties, strongly reducing the need for the use of chemicals.

Market leading position RijkZwaan
Trait 2: Nematode Resistance via KeyPoint® Mutation Breeding Molecular Mutagenesis

Genomics based molecular mutagenesis will yield durable nematode resistance in tomato and will deliver sustained value for the breeder.
Trait 3: Development of Hybrid Rye; PollenPlus

Hybrid rye development was impacted by linkage drag between ergot resistance and seed set. Molecular breeding solved the issue.

Resulting in toxin free rye with high flour and feed quality. And an important commercial success for KWS.
Trait 4: potential yield improvement in rice

Trials Italy, Po Delta 2016

Rice is a main staple food all around the world including Europe where we have “Risotto” rice production in the Po delta.

KeyPoint®Mutation Breeding resulted in rice with a single base pair mutation in a gene that has high potential for yield improvement and has erect panicles.
Trait 5: Breakthrough Double Haploid induction in rice

Double Haploids enable a substantial speed increase of breeding programs

KeyPoint® Mutation Breeding of candidate genes enables the generation of pre-breeding material to induce Double Haploids in commercial rice germplasm. Patent applications published 02/04/2017
 Trait 6: Breakthrough in sustainably produced Natural Rubber

Natural rubber remains essential in today’s economy. The production is however exposed to threats, including price/supply volatility, diseases and environmental concern.

Early 2016, the application of state of the art molecular technologies has lead to the world first interspecies hybrid dandelion variety. This novel agricultural crop enables annual rubber production in temperate climates.
An example of an innovation for better crops for the future

1. We know much about the DNA of our crops
2. Phenotyping has become a bottleneck now

Therefore: investments in
- Digital & Robotized Phenotyping
- Big data handling & analysis
- Visualization of analysis: Virtual Reality Breeding
Digital Phenotyping - PhenoFab®

Robust, automated digital phenotyping

The traditional way of reporting:
• Paper text
• Photo’s
• Graphics

An experiment with 900 plants and 9 photo’s per plant per day, will generate 396,900 digital images in seven weeks!
BIG Data!
Strong need to interactively visualize such data: VR Greenhouse
Virtual Reality Breeding
A new proprietary way towards collaborative and VR Breeding

With respect to visualization of big data and reporting, KeyGene researchers have developed the Virtual Reality Breeding tool. It was successfully introduced and demonstrated in January 2017 at the Plant and Animal Genome conference in San Diego. At the Crop Innovation & Business Conference in Amsterdam, held April 2 – 4, 2017 the Virtual Reality Breeding tool was explained by KeyGene’s CEO Arjen van Tunen. Conference attendees were able to step into the virtual breeding world by a demo in KeyGene’s booth.

For a preview of the virtual reality breeding tool:

https://www.youtube.com/watch?v=ou5_Q1mMLZs&feature=youtu.be
With all of this we, together, will improve our crops for the Future!

THANK YOU