Slide 1

Genetic modification – applications

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Examples of applications and their sustainability

- Sustainability, three themes:
  - "People": living and working conditions for people
  - "Planet": decreasing environmental impact
  - "Profit": commercial viability and revenues for growers, processors and consumers

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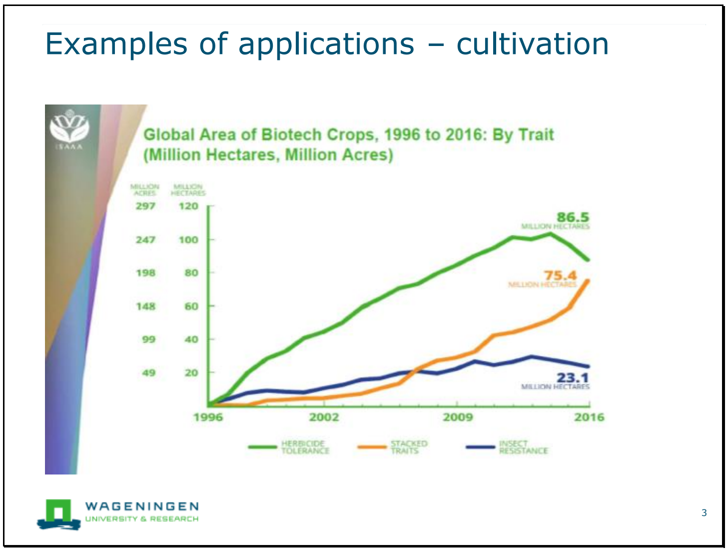

Main traits HT & IR (Bt). Herbicide tolerance at 47% and Stacked traits occupied 41% of the global acreage. Decline in HT & IR related to increase in stacked (combined HT & IR).

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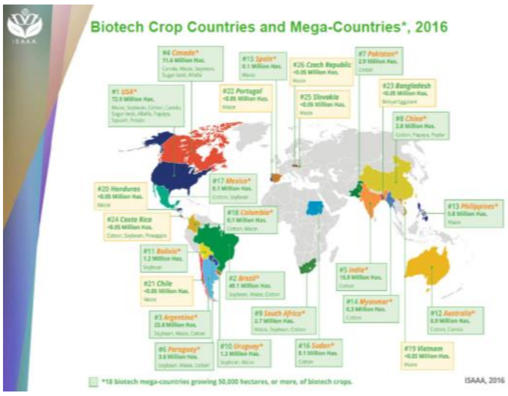
Examples of applications – cultivation



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Herbicide tolerance (HT)

- Most cultivated GM since 1996, particularly in US and South America: tolerance for glyphosate (Roundup)
- Based on a gene from a bacterium that developed resistance against Roundup
- The transgene expresses a resistant version of an enzyme responsible for production of essential amino acids, EPSPS, normally sensitive to Roundup: Roundup Ready (RR) crop
- RR in oilseed rape (canola), soybean, maize and cotton

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Gene encoding EPSPS with lower binding affinity for glyphosate from *Agrobacterium tumefaciens* CP4 (not the one used in plant transformation) found at a site polluted by glyphosate. EPSPS = 5-enolpyruvylshikimate-3-phosphate synthase, enzyme involved in shikimate pathway synthesising aromatic amino acids in plants, fungi and bacteria; it is inhibited by glyphosate.

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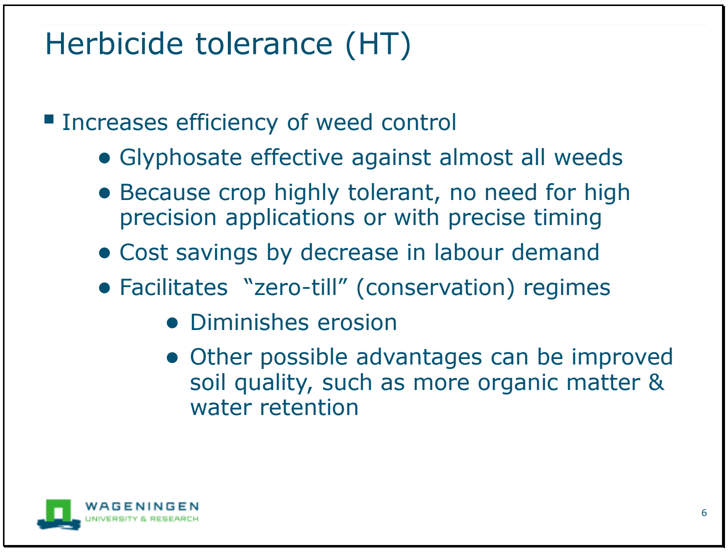

HT logical advantage for “zero-till”: sowing can be easily performed after only a herbicide treatment. Zero-till not completely dependent on HT, also implemented without HT. Organic matter sequestration also dependent on other factors, such as treatments at other times in rotation. Also disadvantages to zero till, such as increased disease pressure.

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Herbicide tolerance (HT)

- Glyphosate shows a lower toxicity compared to many other herbicides
  - Controversial: much attention from NGOs and discussions about scientific studies
  - By IARC (WHO) characterized as potentially carcinogenic, but according to Echa (European Chemicals Agency) not classifiable as carcinogenic
  - By European agency EFSA recently characterized as safe to use in an agricultural context

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IARC = International Agency for Research on Cancer (WHO = World Health Organization). EFSA = European Food Safety Authority.

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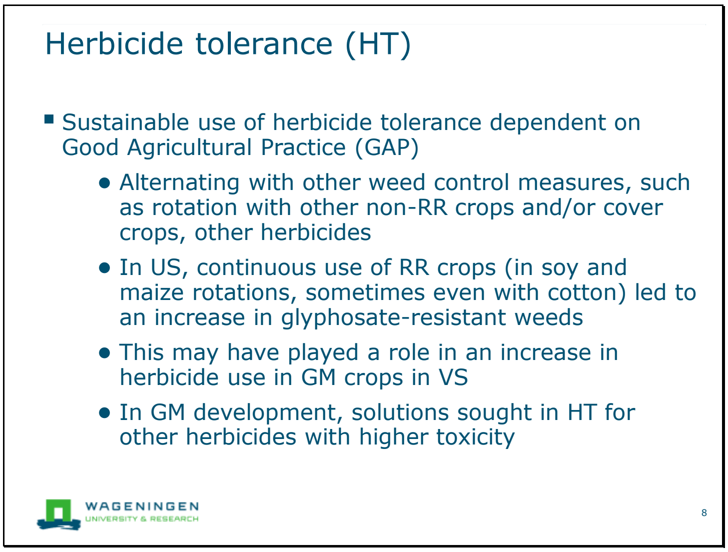

Increase in herbicide use complex issue: some studies point to a relationship between herbicide-resistant weeds and an increase in herbicide use, at least the expected decrease shortly after GM introduction soon disappeared. This is not always clear and moreover, toxicity level of glyphosate also important: being lower than other commonly used herbicides, impact may still be lower even when use in terms of amounts (kg) is higher. There are far less studies well addressing herbicide impacts. In addition, at the same time, herbicide usage has also increased in conventional crops in US. There are also weeds resistant to other herbicides than glyphosate, presently used in GM.

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Insect resistance (IR): Bt

- Cultivated since 1996: resistance against European corn borer (caterpillar of moth *Ostrinia nubilalis*), later on also against Western corn rootworm (caterpillar of beetle *Diabrotica virgifera*)
- Based on genes from bacterium *Bacillus thuringiensis*
- Transgenes express proteins (Cry) toxic to specific insect groups, i.e. different types of Cry against moths and beetles, respectively
    - Bt was already used in sprays
- Bt in maize, cotton and recently also in soybean, and even in a vegetable, eggplant (brinjal)

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Rootworm occasionally observed in NL through traps (hitchhiked with transports, e.g. airplane), then immediate eradication is obligatory, including destroying maize in the surroundings. Corn borer gradually increasing in NL, particularly in Province of Limburg, probably by climate warming. Bt may even be used as sprays in organic cultivation (as last resort). Spraying against corn borer difficult by its presence within the plant. Alternative for Bt is seed coating with insecticide (neonicotinoids). Neonicotinoids become more and more prohibited because of effects on non-target insects in nature, in particular, pollinators, such as bees. There has been an example in Germany in 2008 of honey bees impacted by an unfortunate combination of events in which insecticide from a seed coating had become dispersed. The seed coating with relatively high neonic levels against rootworm also showed relatively high abrasion. This neonic-containing dust was blown away at sowing using pneumatic machinery at a time when sowing was delayed by poor weather conditions resulting in co-incidence with flowering oilseed rape and fruit trees in neighbouring fields attracting bees, combined with dry and windy weather blowing in the contaminated dust from the maize fields (Forster 2009).

Bt in eggplant (brinjal in India) against fruit and shoot borer, developed in India, yet presently strong opposition against GM in India. Therefore as of yet only extensive Bt cotton cultivation in India (already introduced early 2000s). Bt brinjal cultivation only started in Bangladesh.

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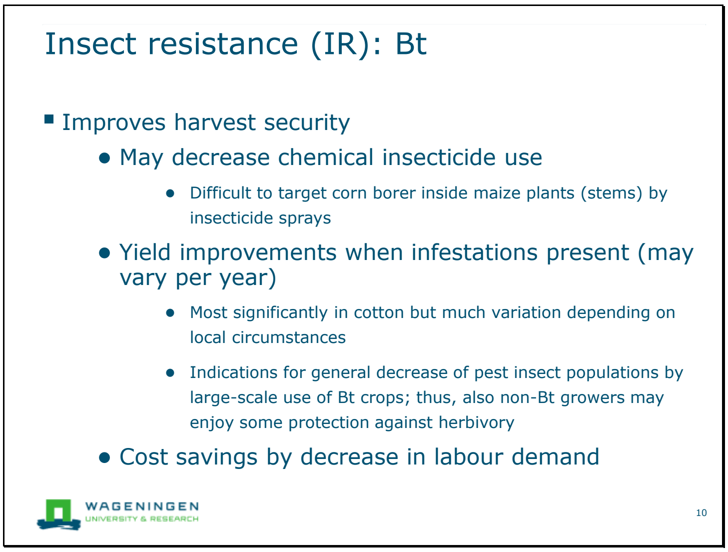

Many studies on Bt cotton in China and India, generally indicating better yields and labour safety due to reduced insecticide use. However, also lots of variation between farmers: cotton is risky cultivation for small farmer, potentially high revenues but also high investments with risks of debt when harvest fails, harvest not only dependent on Bt but also availability of water and fertilizer, reliable cultivar seed lots and accompanying cultivation knowledge etc, which were not always well supported, particularly at the beginning of Bt cotton cultivation in India. Profitability of Bt depends on risk of infestation weighed against higher seed costs and the importance attached to harvest security provided by Bt.

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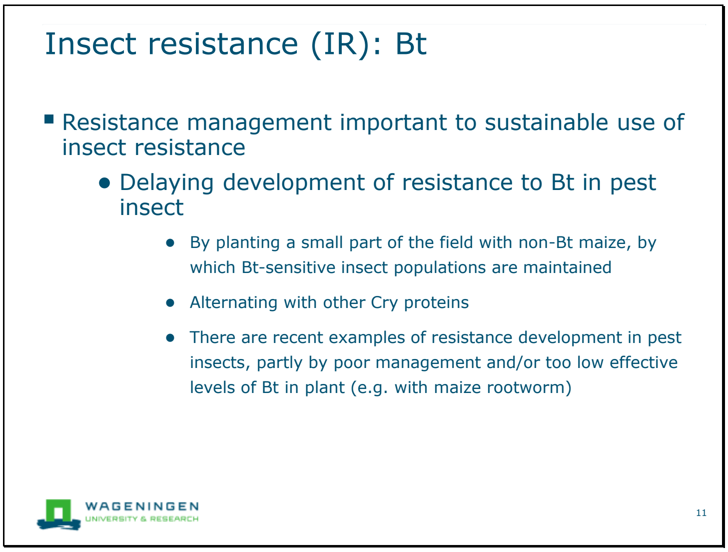

Bt resistance management by so-called “high dose – refuge” strategy: production of sufficient amounts of strongly impacting Bt in the plant so that also insects heterozygous for Bt resistance are killed, at the same time maintaining influx of alleles for sensitivity to Bt in the refuge consisting of non-Bt plants. With low insecticide use consequent to Bt cultivation, additional risk of the rise of secondary pests, i.e. insects sensitive to insecticides but insensitive to Bt (e.g. aphids or other sucking insects), thus need for monitoring the crop for this.

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Virus resistance: papaya

- Cultivated since 1998 on Hawaii (China since 2007): resistance against papaya ringspot virus (PRSV)
- Based on genes from virus itself (coat protein in US, replicase in China)
- Transgenes express RNAs from virus leading to gene silencing of the virus hampering its multiplication in the plant
- Virus resistance also developed in e.g. potato, common bean, squash, tomato, pepper, plum, cassava

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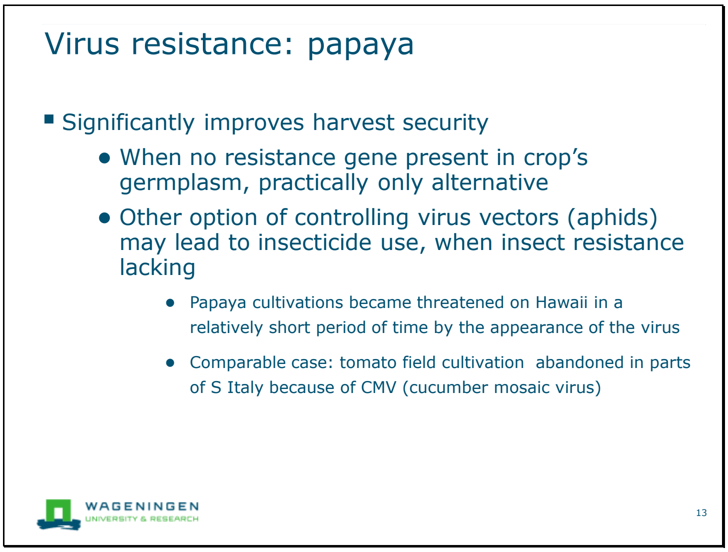

Papaya cultivation had already been given up on another isle of the Hawaii group (Gonsalves 2004; VIB 2014).

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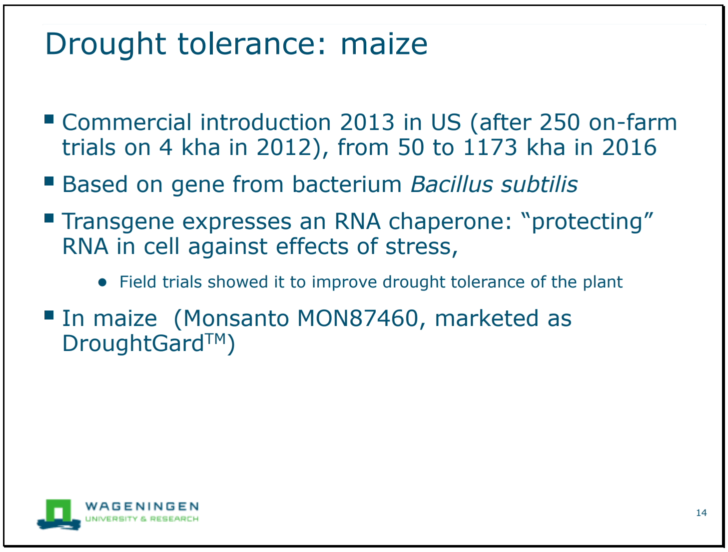

Bacillus gene for cold-shock protein B (cspB), identified as RNA chaperone (“protecting” RNA in the cell under stress conditions) (Castiglioni et al. 2008).

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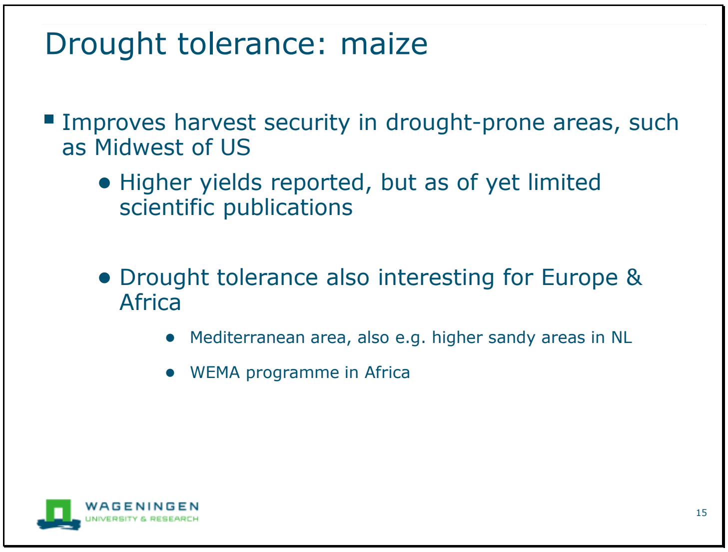

Increased yield under drought in field trials reported by Monsanto (~6%, Nemali et al. 2015). Drought tolerance complex trait (usually combination of genes involved), single genes may be rarely successful, particularly when often accompanied by negative pleiotropic effects under normal growth conditions. Sometimes, such negative effects can be reduced by using a stress-inducible plant promoter, such as rd29A, leading to the transgene only being expressed under stress conditions. In addition, drought stress can be confounded with heat stress that can also significantly affect yields, particularly during flowering. Thus, also drought-tolerant conventional hybrids developed with DNA markers (MAB): AQUAmax (Pioneer) yield improvements under drought average 5-6.5% based on large on-farm dataset (Gaffney et al. 2015). In addition, there is Syngenta Artesian. Adee et al. 2016 showed in field trials for all three of them together yield improvements by 5-7% compared to non-tolerant hybrids under high evapotranspiration conditions, with no yield penalty under well watered conditions. WEMA = Water Efficient Maize for Africa.

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Drought tolerance: maize

- Sustainability of drought tolerance
  - Could improve water use efficiency in arable crop cultivation
  - Potential of shift of crop cultivation to marginal areas
    - This could impact nature

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USDA in its environmental assessment deemed it unlikely for the GM drought-tolerant maize to significantly extend cultivation to other areas as the changes in yield are not outside of the complete crop range. Also, significant changes in market demand are important for such developments.

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Hybrid cultivars production: male sterility

- Used in US & Canada
- Based on genes from bacterium *Bacillus amyloliquefaciens*
  - A transgene that specifically expresses barnase in the tapetum of the anther, barnase degrades RNA thus disrupting pollen production in maternal lines;
  - Another transgene in the paternal line encodes barstar that inhibits barnase to restore fertility of the hybrid
- In oilseed rape (Bayer MS8 x RF3, marketed as InVigor^{TM})

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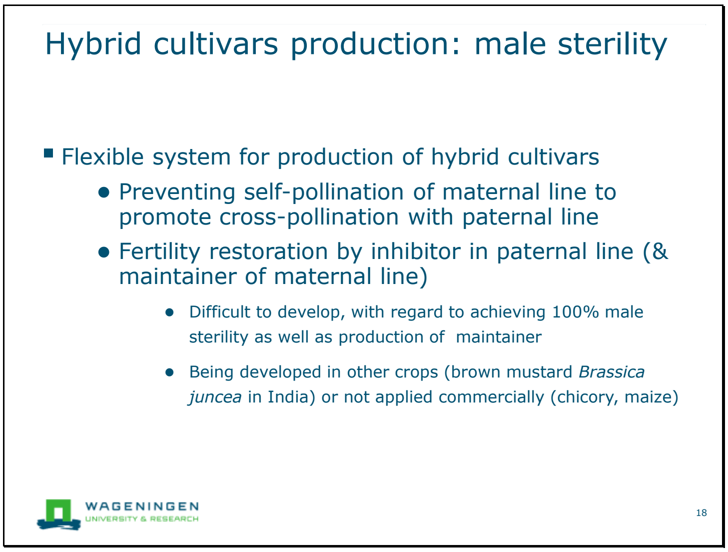

Also see Hybrid cultivars ppt.
Authorisation of *B. juncea* in India delayed because of an ongoing societal debate.

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Examples of applications – pipeline

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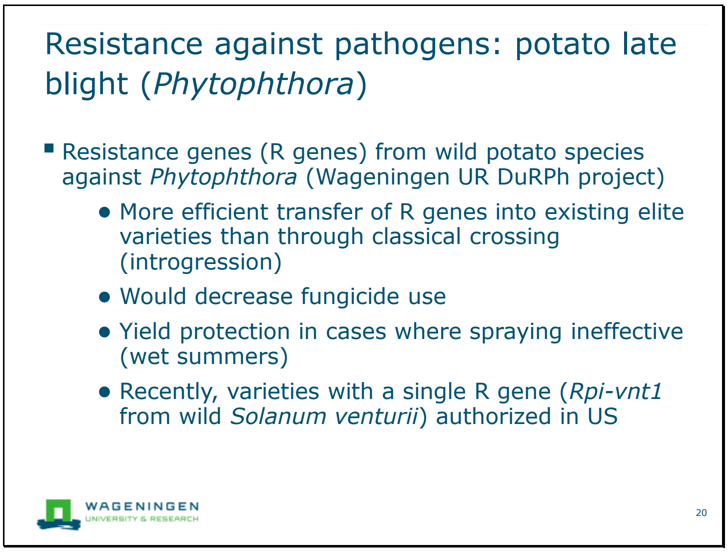

Fungicide use could diminish by 75% (Haverkort et al. 2016). Resistant varieties authorized in US combination with Innate product quality traits (Simplot).

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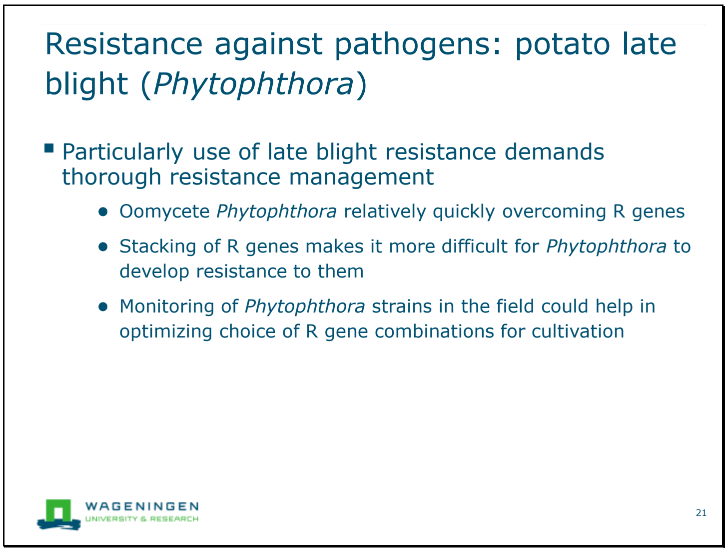

Production of varieties with several combinations of R genes that could be alternated to avoid *Phytophthora* resistance development. When necessary, R gene overcoming by *Phytophthora* can be delayed by additional spraying with fungicide. In that way, AVEBE is managing resistance development with its conventional starch variety Avito, containing a single resistance from a wild species. This is naturally not allowed in organic cultivation.

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Demonstration field trial with Desiree containing varying numbers of R genes

From front to back: Desiree, Desiree with 1 R gene, 2 R genes, 3 R genes. Left: support of resistance by fungicide spray; right: no spray.

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Product quality: lignin content

- Low level of lignin in cell walls

- Based on silencing of plant genes involved in lignin production (e.g. CCR, CCOMT)
  - Using transgenic versions producing RNAs interfering with gene expression

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Gene silencing using RNA interference special type of intragenesis (see NPBTs)

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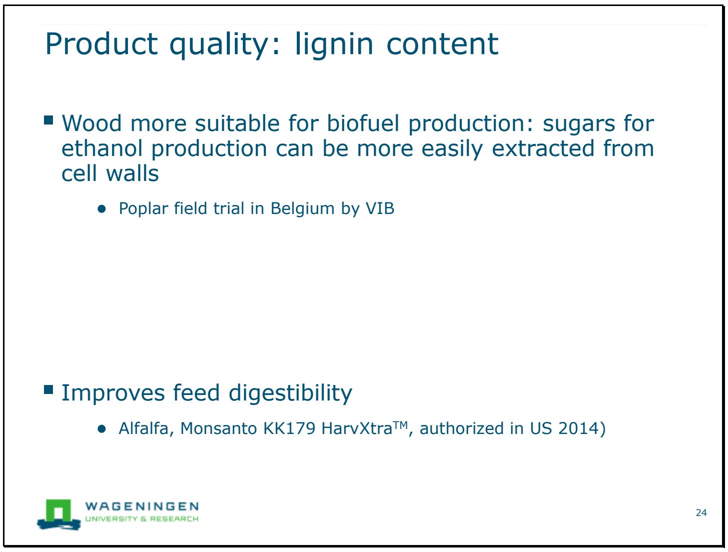

Lignin crosslinks cell walls and is poorly degradable, only by specialized fungi or by harsh chemical processes. VIB = Vlaams Instituut voor Biotechnologie (life sciences research institute in Flanders, Belgium).

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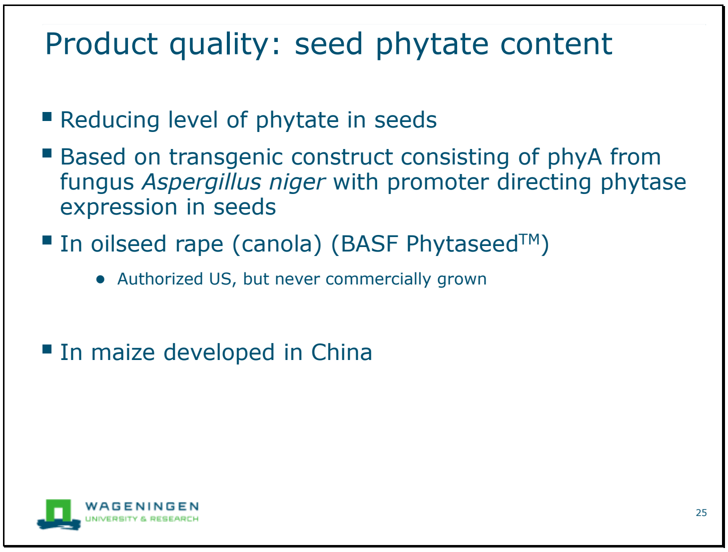

Product quality: seed phytate content
Reducing level of phytate in seeds
Based on transgenic construct consisting of phyA from fungus Aspergillus niger with promoter directing phytase expression in seeds
In oilseed rape (canola) (BASF Phytaseed™)
Authorized US, but never commercially grown
In maize developed in China
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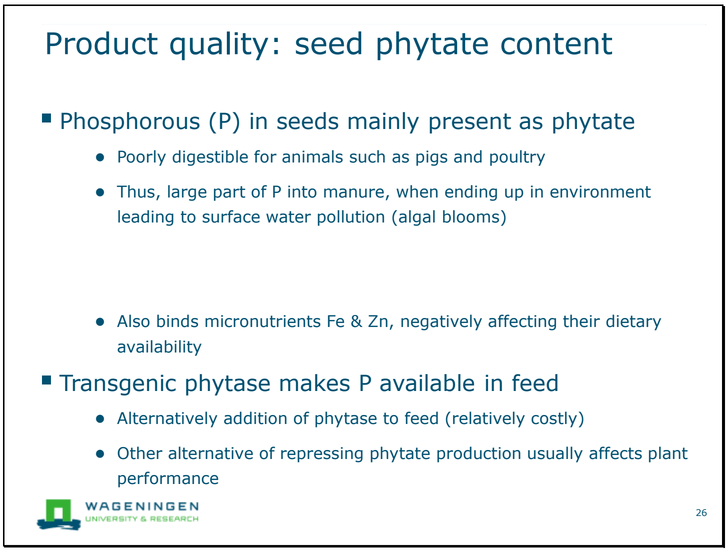

Added phytase needs to be produced by microbes, which is rather costly. Plant performance: e.g. seedling development dependent on P from phytate.

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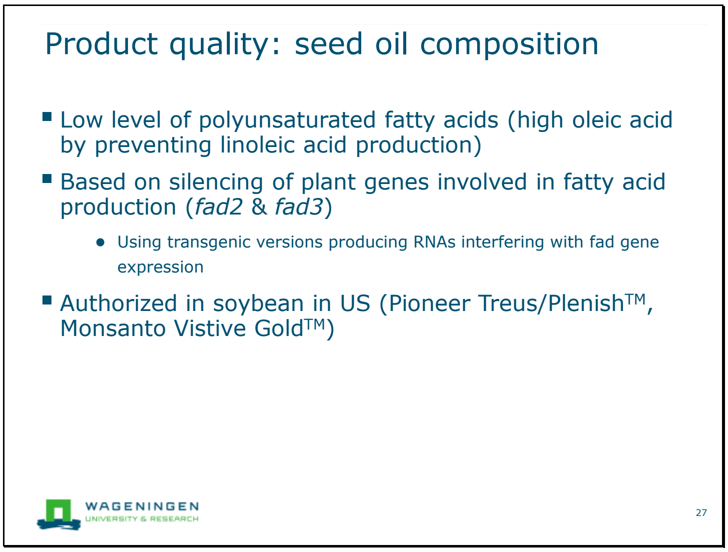

Gene silencing using RNA interference special type of intragenesis (see NPBTs)

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Product quality: seed oil composition

- Improves oil shelf life (sensitivity to oxidation making oil rancid)
  - Alternatively, a chemical process (hydrogenation) is used in which trans fatty acids are produced purportedly having negative health effects
- Oil also more attractive for industrial products and/or biofuel

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Trans fats associated with coronary heart disease

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Product quality: seed oil composition

- High level of omega-3 fatty acids
- Based on two genes involved in fatty acid production
  - delta 6 desaturase from plant (*Primula juliae*)
  - delta 15 desaturase from fungus (*Neurospora crassa*)
- In soybean (Monsanto MON87769)
- Omega-3 fatty acids regarded as health-promoting (marine oils)

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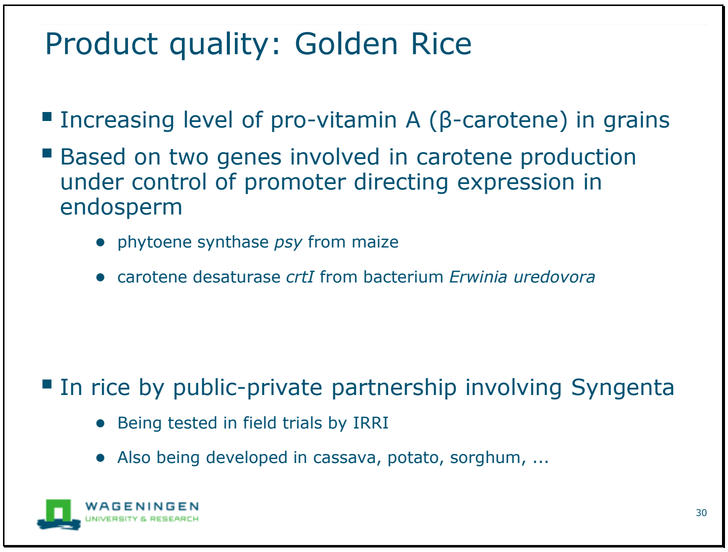

First generation golden rice with psy from daffodil still too low in vitamin A, significantly improved by using psy from maize (Paine et al. 2005). The partnership involves free availability of IPR (patents) on the production process of the GM crop. The slow progress in introducing golden rice is related to the GM debate & regulatory issues, but presently also to the event being not yet available in optimal varieties for rice growers (also partly related to regulatory costs making it cheaper to introgress a single event into other varieties than to directly introduce the construct in several varieties). IRRI = International Rice Research Institute (Philippines).

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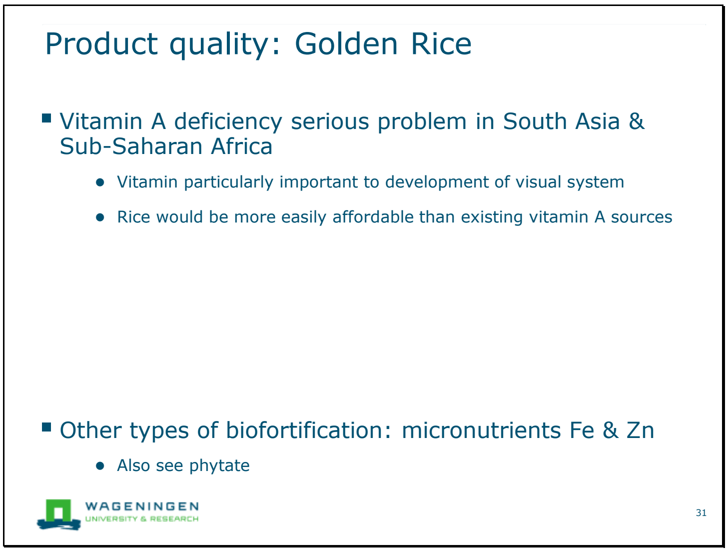

Although optimally improvement would come from enriching diets with existing vitamin A sources (vegetables), this appears to be no realistic option in serious low-income situations with regard to costs. Economists have estimated costs of not introducing Golden rice using a concept of “disability-adjusted life years” (DALY) to be fairly high (e.g. Wesseler & Zilberman 2017).