Intelligent Breeding for consumer and processor traits

On April 6, 2011 Food Valley organised an Open Innovation Seminar at the KeyGene site in Wageningen where the food industry and the seed industry shared their experiences in how to benefit from the breakthrough research and innovations achieved in plant breeding and seed industry[3]. A number of introductions that described the context and the general innovation perspectives for the food and the seed industry was followed by four talks that focused on specific areas of innovation, ranging from a talk about general technologies such as molecular mutagenesis for precise gene editing and automated phenotyping by Mark van Haaren (KeyGene) to reverse engineering for taste, starting with consumer preferences by Peter de Kok (NIZO food research). Rich Ozminkowski (Heinz) focused on breeding for specific processor traits and Jeroen Rouppe van der Voort (ENZA seeds) presented three examples of breeding for extended shelf life.

Intelligent breeding: High resolution genome analysis combined with precise phenotyping

Mark van Haaren started to explain about KeyGene's work in Whole Genome Profiling and crop specific Databases, identification of candidate genes in several crops, induction of variation by molecular mutagenesis and Breeding by Design[4]. Yet, phenotyping is becoming a limiting factor, he stressed. Phenotyping is laborious and requires significant experience and a professional breeder's eye. The high complexity of agronomically important traits makes it even more difficult to perform objective and robust phenotyping in a high throughput manner. This is why KeyGene and LemnaTec, a German company specialised in image processing for high throughput screening, plant breeding and plant phenotyping decided to collaborate and develop KeyTrack, a system of fully automated phenotyping services.

The seminar was followed by the official opening of PhenoFab, one of the biggest fully automated plant phenotyping Research Facilities in Europe. This facility is based on the so-called 'moving field concept', automatic transportation of plants in the greenhouse on pre-defined or random patterns, thus homogenising growth conditions. With RFID technology each plant can be identified and tracked throughout its whole life-cycle in the greenhouse. This allows phenotyping at all growth stages of the plants, which makes it possible to see the development of a plant over time. Plants are imaged under highly defined conditions in specific chambers, resulting in images of the plants in visible light (3D). Other chambers employ near-infrared imaging and 3D fluorescence imaging. Near-infrared imaging and 3D fluorescence imaging can be employed, for example, to check the water status of leaves or root systems and chlorophyl and Green Fluorescence Protein (GFP) in transgenic plants. Moreover, the use of transparent pots enables measurement of the root system with algorithms based on the observed amount of pixels. Other high precision greenhouse technology can be used to mimic specific environmental (stress) conditions. Thus, thousands of data points are produced for a single plant about important factors and traits such as biomass and root development, abiotic and biotic stress, germination, fruit shape and fruit colour. Those data are stored in a fully

Photo by LemnaTec

[3] The purpose of each Food Valley Open Innovation seminar is to offer an opportunity to companies, active within the agri-food business, to share knowledge on how to innovate in a more sustainable, healthier and more profitable way. The seminars are held throughout the year and are organized in close cooperation with different parties (www.foodvalley.nl)

[4] Breeding by Design is a concept that aims to control all allelic variation for all genes of agronomic importance. This concept can be achieved through a combination of precise genetic mapping, high-resolution chromosome haplotyping and extensive phenotyping (Peleman, 2003).

integrated database. An informative video can be watched at http://www.lemnatec.com/about-us.
So in the end we have the DNA sequence of the genome, a genetic map and (sequenced) markers, information about the genes and their annotation, basic understanding of how a number of genes function in a gene regulatory network[5], and a lot of phenotypic data. The trick is to combine the data about phenotype, genetic maps, genes and gene regulatory networks and extract useful information for plant breeders. Does it usually take a couple of years to get from a phenotype to gene, Van Haaren thinks this can be done in three months now.

Breeding for extended shelf life: Finding the right balance

ENZA Seeds is a global player in about 20 vegetable crops. Tomato, cucumber, sweet pepper and lettuce are the major crops. Jeroen Rouppe van der Voort, manager biotechnology at ENZA Zaden, presented ENZA's approach to improvement of shelf-life of cucumber, green sweet pepper and lettuce.
Shelf-life is defined by a number of characteristics influenced by environmental factors, which makes it a complex trait. Shelf-life is a function of a range of properties such as taste, freshness, appearance, and crunchyness. Decolouration, moisture loss, microbial infection and loss of nutritional value are the major threats.
Cucumbers, for instance, can get soft and slimy patches that make them unsuitable for consumption. Cooling, which is generally used to extend the shelf life of fresh products, cannot be applied for cucumber fruits as these fruits are not suitable for storage at low temperatures. The shelf life of the fruits can be extended by wrapping them in a sealing foil or applying a wax-coating.


Browning of cut lettuce

By using a DNA marker ENZA Zaden managed to breed the cucumber *Borja*, a variety that is also available as organic seed from Vitalis Organic Seeds, which has an extended shelf-life and Cucumber Vein Yellowing Virus resistance. Another example is the *Sweetgreen* green pepper Usually, the colour of unripe green peppers turn into red, yellow or orange as they ripen with increasing sweetness and Vitamin C levels. It took Enza more than 10 years to develop a variety which stays deep dark green until ripening stage.

A third example that ENZA is still working on is the problem of browning on the cut surfaces of pre-cut lettuce, a convenience product of which the market share is still growing. Wound induced changes in phenolic metabolism[6] is thought to be involved in lettuce tissue browning. Increased activity of the enzyme phenylalanine ammonia lyase (PAL) has been correlated with a decrease in the subsequent shelf life and overall visual quality of minimally processed lettuce but PAL inhibitors that are very effective at inhibiting browning are not approved for food use. The mapping of Quantitative Trait Loci (QTLs) for shelf life in lettuce now opens opportunities for using DNA markers in breeding for extended shelf life.

Rouppe van der Voort empasized that in normal breeding it is very hard to find offspring with stable characteristics. Although molecular markers turn out to be very helpful, a lot still depends on the creativity of the breeder because breeding is balancing between characteristics such as taste and shelf-life that are often conflicting.

[5] The translation of a gene into a trait is usually not a straight-forward process. Genes interact with each other and with other substances in the cell. This is why the function of genes is studied in the context of regulatory networks that can include a large number of genes, environmental conditions and substances in the cell.

[6] Plant phenolics are the most widely distributed class of plant secondary metabolites and several thousand different compounds have been identified. They can be found in plants and have an antioxidant activity.

The need for diversity in tomato processing

“Most processors do not realize what plant breeding and picking the right variety can do”, Rich Ozminkowski told the audience. Ozminkowski is Heinz’s manager of Agricultural Research. He explained how Heinz decides on tomato varieties from a food processing perspective.

Selling 650 million bottles and 2 packets of tomato ketchup for every person on the planet per year, Heinz is one of the biggest tomato processors in the world. Annual sales are over 10 billion US dollars per year. Producing tomato ketchup since 1876, Heinz recognised the importance of the quality of its major ingredient soon after the turn of the century by starting its own agricultural research in 1916. Recognition of the impact variety has on quality and cost of the final product resulted in the start of tomato breeding in 1934. Nowadays, HeinzSeed based in Stockton, California, supplies seed of about 30% of the global processing tomato hybrids and has gained a 44% market share in California.

In selecting tomato varieties, the company focuses on four aspects:

1. Yield, which is what growers want;
2. Adaptability to environmental conditions to ensure that the crop is available, independent from weather conditions;
3. Factory yield, which requires high juice viscosity varieties;
4. Product quality to ensure consumer acceptance. Product quality is measured in terms of soluble solids, the consistency of juices and paste. Flavour is important too, but is not easy to breed for. Moreover, flavour is also a result of the recipe applied in processing.

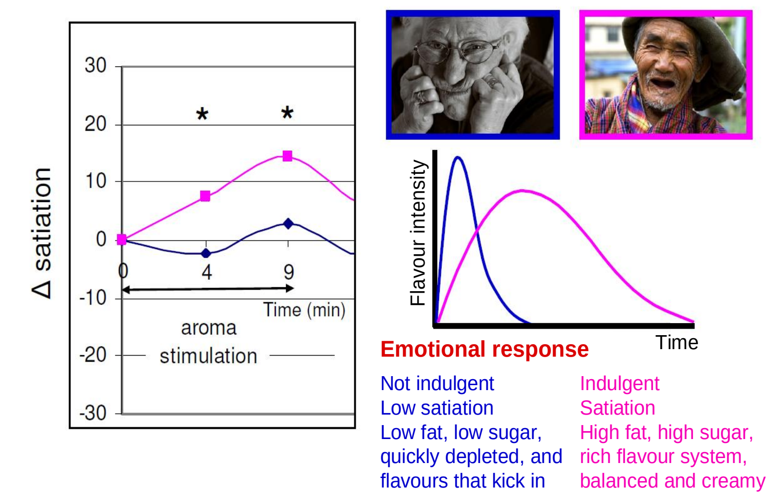
Ozminkowski stressed that plant variation is not only defined by the plant’s genetics, but also by environmental factors and the interaction between genetics and the environment; the “environment” includes the particular processing conditions. This makes breeding for the final product a real challenge. He also stressed that Heinz is using the natural genetic variability in tomatoes to improve their varieties. And, there is never a single winner: you have to account for environmental variation and you need diversity in your variety pack!

Seeding taste

“If we ask ourselves how to improve consumer appreciation of a tomato, we should look at a wide range of characteristics, such as taste, smell, mouthfeel, colour, shape, and even touch and sound”, Peter de Kok said. De Kok, principal scientist flavour, beverages and ingredients at NIZO Food Research, the Netherlands, further elaborated on flavour, which he called *‘the signature of the product’,* the key attribute. He proposed a system of virtual product optimisation based on current knowledge of consumer appreciation before starting breeding programs.

De Kok emphasized the complex of the relation between the presence of a certain flavour compound in the product and consumer appreciation. The flavour of a product is not only determined by the composition of the aroma and taste when the food is produced and prepared; and the release of the aroma and taste when the food is masticated and consumed. It is also determined by the way the brain amalgates all sensory information into complex hedonic liking and attributes such as “tastes good”, “creamy” or “fresh”.
Scientists from the Food Chemistry Group of Wageningen University demonstrated that viscosity affects flavour intensity perception, suggesting that the texture determines perception of flavour intensity rather than the in-nose flavour concentration (Weel, 2002). Moreover, we have to take into account that adding more of a key flavour compound does not automatically result in an increase of flavour sensation. Below a certain threshold concentration flavour compounds are usually not perceived by consumers at all. Increasing the flavour compound concentration beyond this threshold level usually results in an exponential (stronger than linear) increase of perceived flavour intensity. Then follows a phase where intensity increases linear with concentration and finally there is a logarithmic phase with intensity increasing much less than linear with concentration. Thus, adding more of a compound changes intensity depending on the concentration and the threshold. Twice as much aroma is not twice the aroma intensity!! Therefore a different recipe is required to maintain the balance in cocktails.

The consumer's (emotional) state of mind and the level of indulgence and satiation is also related to flavour intensity patterns over time. It has been demonstrated that a product with a short and high peak of flavour intensity is quickly depleted and associated with low fat and sugar, and flavours that kick in. A product exactly the same in calorie content with a more extended flavour release pattern, on the contrary, is perceived as a rich flavour system, balanced and creamy, high in fat and sugar (see illustration on the right).



Different flavour release patterns trigger different emotional response
(De Kok, NIZO Food Research, 2011)

Last but not least, apart from being the result of a physical process consumer preference is also a question of perception. Here we have to bear in mind that perception is hard to predict because consumers or assessors don't tell what they think, they don't think what they feel, and they don't feel like they behave.

Because of these complexities De Kok is convinced that a straightforward approach translating a key flavour component into a breeding target will lead to disappointing results. His proposal is a three step approach of virtual product optimisation by introduction of a verification step for the right flavours:

1. First establish what is required to improve your product in terms of customer or consumer preference.
2. With that knowledge at hand you can start investigating which metabolic routes (enzymes, genes) are required.
3. Only then start breeding programs.

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