New Breeding Nethods

More than just a "test-tube scenario"

Climate change poses a major challenge to all of us, with increasing temperatures, more frequent droughts, flooding and other extreme weather phenomena impacting agriculture all around the world. Concurrently, our eating habits and modern lifestyle are affecting the environment and our own health. In many areas, access to affordable and nutritious food remains a major issue.

To tackle these challenges, we need plant breeding innovations that build on scientific data and evidence. Plant breeders play a key role in addressing the effects of climate change by fostering sustainable agriculture in the first step of the agri-food chain. Advanced molecular breeding methods, used in combination with traditional plant breeding, offer additional, more efficient possibilities to systematically develop desired plant characteristics.

In this overview, we highlight some examples of the application of new breeding methods and their intended or proven benefits. These examples demonstrate that new breeding methods can be used to transfer traits that can ultimately aid society and the environment by, for example, increasing yields and reducing the need for inputs (producing "more with less"), supplying climate mitigation and adaptation solutions, and providing various benefits to human health.

Products on the market



Source: https://sanatech-seed. com/en/20201211-1-2/

Method applied Targeted mutagenesis (SDN-1) – CRISPR/Cas

Benefits: Nutritional values

GABA (Gamma-aminobutyric acid) is normally present in high quantities in raw tomatoes, but reduces as the tomato matures. It provides several benefits to human health, such as lowering blood pressure. The "GABA tomato", developed using CRISPR/Cas9 genome editing, maintains its naturally high GABA content through the ripening stage. Mature tomatoes have five to six times the GABA content of conventional tomatoes.



Source: https://calyxt.com/firstcommercial-sale-of-calyxt-high-oleic-soybeanoil-on-the-u-s-market/

Method applied Targeted mutagenesis (SDN-1) – TALEN

Benefits: Nutritional values

Oleic acid is a monounsaturated omega-9 fatty acid that has many health benefits for humans, such as reducing coronary heart disease. Soybean oil that has been developed through TALEN genome editing contains approximately 80% oleic acid. It has 20% less saturated fatty acids compared with conventional soybean oil and no trans fats, which are harmful to human health. Furthermore, it has a better shelf life compared with traditional soybean oil.

Research projects



Method applied Targeted mutagenesis (SDN-1) – CRISPR/Cas

Benefits: Improved quality – food safety

Wheat is one of the top staple food crops, broadly consumed across the world. During the baking, toasting and high-temperature processing of wheat-based foods, acrylamide, a carcinogenic substance, is formed. An effective means of reducing acrylamide in food products is lowering the content of its precursor, free asparagine, in the wheat plant. Using CRISPR/Cas9 genome editing, researchers were able to affect the gene responsible for asparagine synthetase in wheat grain, thereby reducing the concentration of free asparagine in the grain.



Method applied Targeted mutagenesis (SDN-1) – CRISPR/Cas

Benefits: Reduced use of fertilizers

Nitrogen fertilization is an important environmental concern, especially where crops are unable to absorb the fertilizers spread on the field, and instead nutrients leach into the environment. By targeting genes involved in nitrogen uptake, researchers developed winter wheat that is better able to utilize soil nitrogen. The genome-edited wheat showed better tolerance to low nitrogen levels in the soil and produced higher grain yields.



Source: https://www.nature.com/ articles/s41587-020-0444-0

Method applied Targeted mutagenesis (SDN-1) – CRISPR/Cas

Benefits: Improved field performance



Source: https://link.springer.com/ article/10.1007/s11816-<u>021-00732-y</u>

Method applied Targeted mutagenesis (SDN-1) - CRISPR/Cas

Benefits: Nutritional value

Corn known as "waxy corn" has seed starch consisting solely of amylopectin. This causes it to have a special consistency that makes it suitable for many industrial uses. Waxy corn varieties can be developed significantly faster with genome editing than with conventional breeding methods. Moreover, CRISPR-produced waxy corn hybrids have been found to be higher-yielding than those hybrids produced with conventional trait introgression through backcrossing and marker-assisted selection.



Source: https://www.nature.com/ articles/s41598-019-<u>55757-9</u>

Method applied Targeted mutagenesis (SDN-1) – CRISPR/Cas

Benefits: Improved resistance to insects and pathogens – tolerance to abiotic stressors

Genetic erosion refers to the loss of genetic diversity, and thus the loss of agronomic traits present in wild relatives or landraces (such as resistance to pests or diseases). Genetic erosion poses a particular threat to rice production in China, especially as climate change increases problems caused by crop pests and pathogens. Using CRISPR/Cas9, researchers were able to successfully edit a height gene to establish the semidwarf phenotype in landraces. The edited lines showed resistance to lodging, better tolerance of low-nutrient conditions and higher resistance to pathogens and insects compared with the modern varieties cultivated in China.



Method applied Targeted mutagenesis (SDN-1) – CRISPR/Cas

Benefits: Modified plant structure and flowering time to suit urban farming

Urban farming is gaining popularity due to its many benefits to the environment and society. Urban farming, however, requires crop varieties that are different in many ways from those used in conventional farming: Their size needs to be compact, and they need to yield at suitable times. Using genome-editing approaches to affect genes responsible for the tomato plant's architecture and flowering time, researchers were able to create compact, early-flowering plants better suited to urban farming than conventional varieties.



Source: https://bmcplantbiol. biomedcentral.com/arti-<u>cles/10.1186/s12870-019-</u> <u>1939-z</u>

Method applied Targeted mutagenesis (SDN-1) – CRISPR/Cas

Benefits: Climate adaptation – Tolerance to heat stress

With climate change causing temperatures to rise in many of the world's important agricultural areas, it is becoming critical to

Broccoli is an important vegetable crop due to its good nutritional value. Glucoraphanin is a major glucosinolate substance present in broccoli. Glucoraphanin converts into sulforaphane, which benefits human health by preventing a variety of chronic diseases. Researchers used CRISPR/Cas to develop broccoli with increased glucoraphanin content.



Source: https://link.springer.com/ article/10.1007/s11103-019-00952-5#Sec9

Method applied Targeted mutagenesis (SDN-1) - CRISPR/Cas

Benefits: Climate adaptation – increased yield under drought and reduced nitrogen availability

By editing genes involved in cytokinin-activation, researchers were able to develop rice varieties that produced higher yields through increased grain quantity and weight. Results from field tests performed under disparate conditions implied the edited varieties produced more even under drought or low-nitrogen conditions.



Source: https://link.springer.com/ article/10.1007/s00299-020-02622-z#Abs1

Method applied Targeted mutagenesis (SDN-1) – CRISPR/Cas

Benefits: Climate adaptation – Tolerance to salt stress

Soil salinity is a serious threat to agriculture, especially in many dry and irrigated areas. Generally, high soil salinity prevents plants' ability to uptake water and nutrients. Researchers have targeted negative stress-response regulators to develop tomato plants that are tolerant to high salinity levels at the germination and vegetative stages under experimental conditions.



Source: https://onlinelibrary.wiley.com/doi/full/10.1111/ <u>pbi.13673</u>

Method applied Targeted mutagenesis (SDN-1) - CRISPR/Cas9

Benefits: Increased yield

The number of seeds per pod is one of the main determinants of soybean yield. Using CRISPR/Cas9, researchers were able to in-

manage heat stress, which can limit plant growth and productivity. By using CRISPR/Cas9, researchers knocked out one of the genes related to a heat-stress response. The edited tomato plants showed improved tolerance to heat stress compared with wildtype tomato plants.



Source: https://www.biorxiv.org/ content/biorxiv/early/ 2022/06/05/2022.06.03. 494728.full.pdf

Method applied Targeted mutagenesis (SDN-1) – CRISPR/Cas

Benefits: Climate mitigation – diversified crop cycle

Pennycress is used as a 'climate-smart' cover crop to protect soil and control carbon loss outside of cropping seasons. With genome editing, pennycress was developed into an "off-season" crop with multiple uses that allow for sustainable optimization of agricultural systems. Containing approximately 30 % oil and a protein composition like canola, CoverCress[™] has the ideal composition to be used as e.g. a low-input feed for various animals, and as a low-carbon-intensity feedstock for the production of renewable fuels. Various further potential uses are envisioned.

troduce an allele to a low-altitude soybean variety and increase its yield by approximately 8 % to 10 %. The allele is present in many high-yielding soybean varieties and can now be used to breed superior varieties for tropical and subtropical regions, as intercross breeding between varieties from different latitudes is difficult.



Source: https://link.springer. com/article/10.1134/ <u>S1607672919010241</u>

Method applied Targeted mutagenesis (SDN-1) - CRISPR/Cas

Benefits: Improved resistance to pathogens – abiotic stress tolerance

Potato virus Y (PVY) is one of the most economically destructive potato diseases. When researchers edited an allele encoding a domain of coilin, a structural protein involved in RNA metabolism and other cellular functions in potatoes, the edited potatoes showed increased resistance to PVY and improved tolerance to salinity and osmotic stress.



Source: https://onlinelibrary.wiley.com/doi/full/10.1111/ <u>pbi.13228</u>

Method applied Targeted mutagenesis (SDN-1) – CRISPR/Cas

Benefits: Increased yield

In oilseed rape, plant height and branching directly correlate with yield. It has been challenging to develop plants with a betteryielding plant architecture using traditional breeding methods. With CRISPR/Cas, researchers were able to directly edit genes that regulate plant height and axillary bud outgrowth. This resulted in semidwarf plants with increased branching that provide desirable germplasm for further breeding of high-yielding oilseed rape.



Source: https://cen.acs.org/food/ agriculture/Cibus-advances-gene-edited-crops/98/i40

Method applied Targeted mutagenesis – ODM

Benefits: Reduced pod shattering

In canola and oilseed rape, yield losses occur when seeds release before harvest (pod shattering). Since extreme weather conditions often increase pod shattering, reduced pod shattering is an important productivity trait. The pod shatter reduction (PSR) trait, developed through ODM genome editing of canola and oilseed rape, increases yield potentials by preventing preharvest losses and addresses sustainability in farming by improving harvest management options.

