Strong seed pods

Canola seed pods contain a valuable freight: the black seeds inside are pressed to extract an oil that is in global demand. However, if the seed pods open prematurely in the field, many of the seeds end up on the ground, resulting in lost yield. Researchers at Bayer CropScience have now developed a variety of canola with especially robust seed pods which are increasing yields for farmers in Canada.



Precious pearls: to make sure that as many of the black, oily canola seeds as possible can be harvested, Bayer researchers are developing robust seed pods.



All for the yellow flowers: Dr. Bart Lambert (photo, right) and his team are developing canola varieties with stronger seed pods. The plant experts use state-of-the-art biotechnology to selectively adjust the stability of the canola pods, thereby preventing the seeds from spoiling before the harvest.

For a plant, getting started in life is no easy task: in order for seeds to become productive plants, the delicate seedlings must first break through a hard shell. Mother Nature makes the joints in seeds especially thin for precisely this reason. They split open more easily, making it possible for the sprouts inside to germinate. Canola pods have exactly this type of "pre-set breaking point" as well. The pods are composed of two halves, also known as valves, firmly held together by a specialized tissue. When the pod is ripe, the tissue disintegrates - the pod splits open, releasing the black seeds inside. It's a natural process, but one that can drive canola farmers to despair. "If the ripe plants are buffeted by strong winds, for instance, the pods break open in the field and yield is lost. The seeds fall to the ground and can then no longer be used," explains Dr. Bart Lambert, who was previously responsible for global canola trait research at Bayer CropScience. "In 2012, for example, heavy summer storms ruined a large part of the harvest for farmers in Canada," continues Lambert. Canada is one of the world's largest growing regions for spring swede rape (known as canola in Canada). Canola, which is sown in May and harvested in August, is especially common in Canada and is the country's most valuable crop.

At present, canola growers manage seed loss by swathing – cutting the plants down in windrows three weeks before they become completely ripe. The canola plants then ripen on the ground.

Improving shattering resistance of canola pods to protect yields

This less-than-ideal practice is a necessity, even though it is associated with disadvantages for farmers. It requires an extra round of field work prior to harvest and, in addition, swathing the plants cuts off their supply of nutrients so the seed pods do not fully ripen and the seeds contain less oil as a result. Yet it is precisely this oil that is in such demand. Canola oil is popular worldwide, in particular as a cooking oil, because of the high proportion of healthy, unsaturated fatty acids it contains. Canola oil also plays a role in very different areas, such as the production of biofuels. It likewise serves as an important raw material in the making of

varnishes, paints and lubricants. Canada alone exported 7.1 million tons of canola seed worldwide in 2013.

Lambert's team has now developed a new solution that protects the plant's valuable oil-rich seeds: a type of canola with a more robust seed pod. In order to accomplish this, the biotechnology specialists specifically modified the characteristics of the pod's pre-set breaking point, which botanists refer to as the dehiscence zone. It was no easy task: "The formation of this brittle tissue is controlled by several different genes," explains Benjamin Laga, who led the pod

> **7.1** million tons Canada exported 7.1 million tons of canola seed in 2013.



Pod check: Kevin and Herbert Serfas (photo left, right to left) assess the canola plants at Ironsprings Farm in Canada. The ripe, black seeds contain large amounts of canola oil (photo right).



Strategy for shatter-resistant pods

The stability of the canola pods can be adjusted using reverse genetics. Researchers generate chemical changes (mutations) in the genotype. The candidates with an IND mutation are backcrossed with the original plant. The canola plants that result from this cross have stronger seed pods. The seeds stay in the pod and do not fall out when buffeted by the wind.







Delicate seedlings: the characteristics of the young canola plants (photo above) are comprehensively examined in laboratory tests and field trials. This is the first point at which scientists can tell whether the plants carry all of the desired characteristics. Bart Lambert and Benjamin Laga (photo right, left to right) monitor the canola plants' growth.

shatter reduction program. The team of researchers focused on one of them: the INDEHISCENT gene, or IND for short. "When this gene stops working," says Laga, "a pre-set breaking point does not form. The valves of the seed pod grow firmly together."

Using reverse genetics and backcrossing to create shatterproof seed pods

Nature didn't just hand this success to the scientists on a silver platter, however. It took more than ten years of research in laboratories, greenhouses and fields. For example, the scientists blocked the IND gene with ribonucleic acid interference, a natural mechanism that shuts down targeted genes in biological cells. This method is also used by plant and animal cells to protect themselves against threats such as dangerous viruses. Ribonucleic acid - known as RNA for short - serves as a mediator between genes and protein, translating the information from the genotype into proteins. In order to switch off the IND gene, Lambert and his team first developed a tailor-made sequence that fits precisely onto the RNA of the unwanted IND gene - like a Velcro fastener - and completely interrupts the chain of information. This approach worked well - a little too well, in fact. "The seed pods were so strong that we were almost unable to open them," says Lambert. The

seed pods would not break open in the wind on the field – but they would also resist opening by farmers using conventional harvesting methods.

From technical concept to a commercial trait

After several modification attempts, however, the scientists were able to custom tailor the strength of the seed pods. The key to their success: reverse genetics. It begins directly in the DNA – the genotype of the canola plant. The researchers first used a chemical technique to make small random changes, known as mutations, across the entire DNA. From among the thousands of randomly changed plant genomes, they then selected those with a mutation in the IND gene.

This change is not visible in the seeds, however. The biotechnology specialists therefore had to analyze the genotype by amplifying and sequencing the IND section of the various genes. When this was compared with the original gene sequence, researchers could tell which canola genes had promising IND gene mutations. Using this method, the specialists at Bayer were able to reduce the huge pool of possibilities to some 20 candidates.

Unwanted mutations also occurred outside of the IND gene, however. Researchers therefore had to eliminate these by repeatedly backcrossing the new plants with the original unmodified one. The resulting seeds could then leave the laboratories and greenhouses. They were planted in Belgium and Canada as part of the field trials phase. It was an exciting moment for the scientists. The field trials took three years. In each season, the researchers had to wait patiently until the plants were ripe.

Oily delight

Canola oil is widely used on salads, for frying and in margarine. Just a few decades ago, however, this vegetable oil played only a minor role in the food industry. The reason: it contained bitter substances such as erucic acid. In 1974, Canadian plant researchers succeeded in breeding an oilseed rape plant with significantly fewer bitter constituents and thus made canola oil palatable.

The seed pods on some plants were very difficult to open. Others were more promising and were put into the next phase of cultivation. In this way, the scientists were able to continuously reduce the selection – until one plant had the characteristics they were looking for. "Its seed pods were not too robust, but not too easy to open either," says Laga.

But the scientists wanted more than just shatter-resistant pods, however, so they transferred and tested the final mu-



Golden prospects: at the Monheim research site, Bayer CropScience scientists investigate how the new canola varieties grow in the greenhouse and in field tests (photo left), and take tissue samples for detailed analysis in the laboratory (photo right).

tant IND gene in hybrid canola. Hybrids are generally more productive and more resistant to stress than open-pollinated varieties – an effect experts refer to as heterosis. The result: hybrid canola with lots of large, good-quality seeds that remain safely in their seed pods until harvest.

A first hybrid is now available to farmers in Canada under the name InVigor™ L140P. This is a reason to celebrate for researchers and for farmers as well: the variety carries the first yield trait created with this method to be commercialized in the history of Bayer CropScience. Canadian farmers can now be more relaxed during harvesting time as they do not have to fear significant seed loss when the weather gets worse and the wind grows stronger. They also have the option to harvest their canola fields at just the right time without having to swath them first. This gives the plants enough time to ripen. "They form better-quality seeds and fuller seed pods," explains Lambert, "which in turn yields larger harvests." The scientists at Bayer are not resting on their laurels, however. "Canola is a relatively young crop plant. So there is still a lot of potential for improvement here," says Lambert. Canola has only been commercially grown since the 1970s – and has already enjoyed a meteoric career.

Resistance to fungal infections: no end to the ideas for new canola varieties

The crop is now the most important oil seed after soy. "We want to gradually continue improving the canola plant," says Lambert. The mutant IND gene for strong seed pods is now being incorporated into other canola hybrids for the European market. Bayer CropScience Trait Research teams are also working on further increasing yields by optimizing the supply of nutrients to the pods. These plant characteristics can then be combined by conventional breeding with resistances to dangerous fungal infections, for instance. "This enables regionally tailored varieties that farmers can use to respond to the special challenges in their cultivation area in a very individual way," says Lambert.

The most important canola-growing nations

The world map shows the five countries that produced the largest quantities of canola (in millions of tons) in 2012. The biggest producer was Canada, with 15.4 million tons.



<u>www.research.bayer.com/canola</u> Further information on this topic