



Novel Crop Breeding in Legumes

I am part-way through a PhD looking at developing tools to control trait inheritance in legumes. Based at the University of Cambridge, <u>Department of Plant Sciences</u>, I am part of the <u>Crop Breeding Technologies</u> <u>Group</u> at the <u>Crop Science Centre</u> which is led by <u>Natasha Yelina</u>. In September, Elena and Natasha joined an AFCP webinar to explain Elena's work so far.

My PhD Studentship is jointly funded by the <u>University of Cambridge</u> and four AgriFood Charities Partnership (AFCP) members: <u>Chadacre Agricultural Trust</u>; <u>Felix Thornley Cobbold Agricultural Trust</u>; <u>Perry</u> <u>Foundation</u>; and <u>The Morley Agricultural Foundation</u>.

Crop Breeding Technologies: Advances and Challenges

The current pace of conventional breeding is insufficient to feed the growing global population in the face of climate change. Therefore innovation is needed to increase food production sustainably.

Meiosis, a specialised cell division that produces eggs and pollen, is central for reproduction and breeding. During meiosis, parental chromosomes come together and physically exchange parts in a process called crossover. Naturally occurring crossovers reshuffle parental traits and create novel trait combinations that can be selected by breeders to produce new improved crop varieties. However, despite their key importance for breeding, <u>crossovers are limiting</u> – they are rare and uneven, resulting in lengthy breeding programmes.



<u>Elena Bidash</u>

The Crop Breeding Technologies Group aims to develop novel approaches that would overcome crossover limitations in crops, enhance trait reassortment and expedite crop improvement.



JI2822 - one of the pea varieties being used for VIGS experiments. Credit: Elena Bidash

Peas and Soybean

Pea is a second most important grain legume in the UK. Like many staple crops, pea genetic improvement is limited by its narrow genetic base. This can be overcome by introgression [the gradual movement of genes from one species into the gene pool of another] of valuable agronomic traits from pea wild relatives via conventional breeding.

Soybean is mainly grown in North and South America and imported into the UK. Deforestation associated with agricultural soybean production and the need to transport soybean globally represent sustainability issues. One solution would be to develop soybean varieties adapted to the UK climate, for example, via conventional breeding where traits that control soybean early flowering and maturity will be introgressed into commercial cultivars. My PhD study aims to enhance trait reassortment to expedite pea improvement and soybean adaptation for the UK climate via conventional breeding.

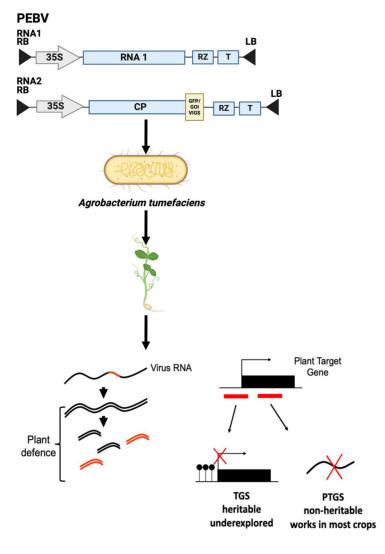
Virus-Induced Gene Silencing and Gene Editing Techniques

Plant gene expression can be controlled in several ways. For example, via <u>Virus-</u><u>Induced Gene Silencing</u> (VIGS), which uses modified plant viruses and a natural plant anti-viral defence mechanism to downregulate plant gene expression. VIGS is a transient way to downregulate plant genes, it only works while the virus is present in the plant and does not induce heritable changes to the plant genome.

Another way to control gene expression is CRISPR/Cas9 gene editing that requires genetic transformation to introduce heritable changes to the plant DNA, for example, mutations in plant genes. Both techniques can be used to switch off plant genes, for example, anti-crossover factors that naturally limit the frequencies of plant trait reassortment.

Increasing Crossovers

My first project aims to enhance trait reassortment in peas by reducing the levels of anti-crossover factors via VIGS. VIGS is routinely used to downregulate somatic genes and has been used to downregulate meiotic genes in a model plant. The novelty of this project is in downregulating meiotic genes, i.e. anticrossover factors, via VIGS in a crop.



Schematic representation/summary of VIGS method using pea. Credit: Elena Bidash

To achieve this, I am first targeting genes which reduced expression leads to visible changes in peas – a somatic gene the loss of which results in white patches on leaves (photobleaching) and a meiotic gene the loss of which results in infertility. The next step will be to reduce the levels of an anti-crossover factor, followed by measurements of trait reassortment frequencies with the expectation to observe increased crossovers.

The advantage of VIGS is that its effects are reversible, VIGS is only effective while the virus persists in the infected plant. Therefore, enhanced crossover frequencies achieved via VIGS can be limited to prebreeding where enhanced trait reassortment is beneficial and removed at later breeding stages when desired traits combinations have been obtained and enhanced trait reassortment can have a negative effect of breaking them apart. The downside is that VIGS does not always lead to a complete loss-of-function. Elena's task is optimise VIGS to achieve maximum possible downregulation of anti-crossover factors leading to a breeding technology to enhance trait reassortment.

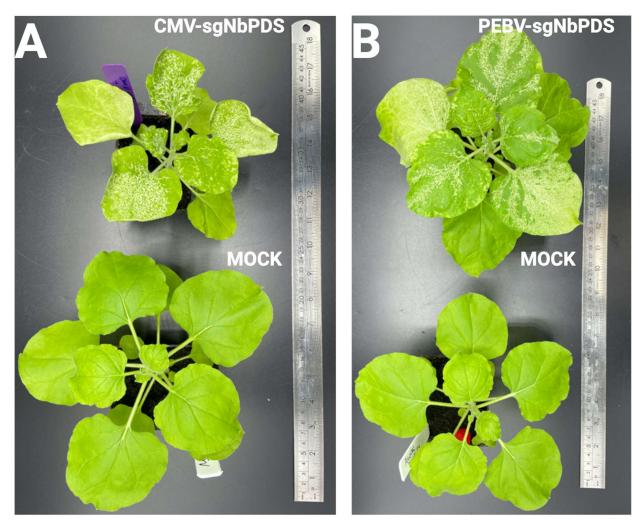
Developing VIGE in Soybean and Peas

My second project aims to reduce the requirement for stable genetic transformation in CRISPR/Cas9 gene editing. In conventional CRISPR/Cas9 gene editing, genetic transformation is required to edit each new gene or a group of several genes. Stable genetic transformation is lengthy and costly limiting throughput of CRISPR/Cas9 gene editing.

<u>Virus-Induced Gene Editing</u> is a recent advance demonstrated in a model plant, *Nicotiana benthamiana*, a relative of tobacco. VIGE allows the editing of any gene of interest using just one Cas9-overexpressing transgenic line because in VIGE, guide RNAs, molecules that 'instruct' the Cas9 enzyme which gene to mutate, are delivered into a Cas9-overexpressing transgenic line via a plant virus.

Originally VIGE has been established using a virus that only infects tobacco and its relatives, e.g. tomatoes. I have engineered two other viruses, *Cucumber mosaic virus* (*CMV*) that has the widest host range including soybean, and *Pea Early Browning Virus* (*PEBV*) that infects peas, to deliver guide RNAs. I have successfully showed that both *CMV* and *PEBV* can induce heritable edits in *Nicotiana benthamiana*.

The next step is to establish VIGE in soybean and, once genetic transformation and Cas9-overexpressing pea lines become available in the research community, translate the technology into peas.



CMV (left) and PEBV (right) can induce somatic edits in Nicotiana benthamiana. Credit: Elena Bidash

Plants and Seeds for the Market

A core discussion for the AFCP webinar focused on the practical implications for UK farming of this work. Whilst no specific timeline can be given at this stage, we are hopeful. Convention breeding can take between 10 and 15 years to deliver changes to seed varieties for farmers. These technologies will reduce this timescale.

At the moment, the technologies are still in a discovery stage. Work is needed to improve the technologies as well as to establish which traits need to be changed to support future food production and food security.

We look forward to catching up later to see how this develops.