USE OF THE PRE-ASSEMBLED RIBONUCLEOPROTEIN (RNP) COMPLEX FOR GENE EDITING OF WHEAT AND POPLAR

ROTHAMSTED RESEARCH

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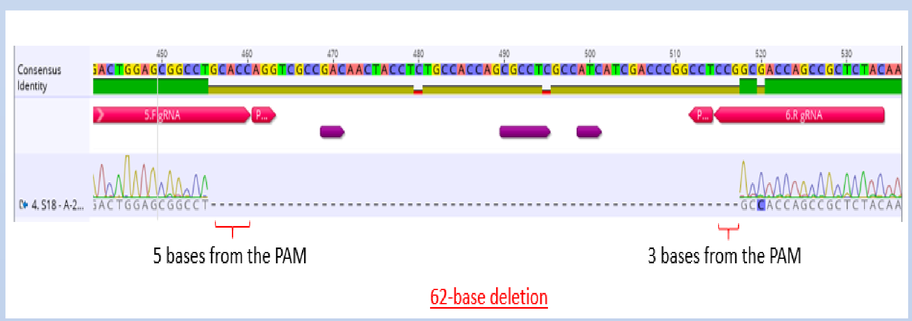
- Gene editing is much more targeted than conventional genetic modification, making more subtle changes to genes already present within the plant.
- Integration of Cas9 and guide RNA sequences into the genome can be disadvantageous causing offsite cleavage and/or continued cleavage of target sites in subsequent generations.
- Use of purified protein in the form of a pre-assembled RNP complex could overcome this issue and would also avoid reliance on the cell's expression machinery.
- The use of purified proteins also has potential to avoid regulatory issues if it is concluded that resultant plants need not be classified as GMOs.

GENE EDITING USING CONVENTIONAL METHODS

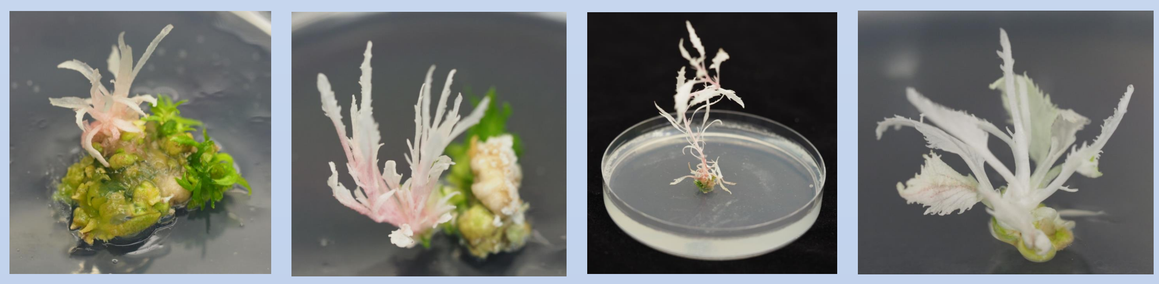
Successful gene editing has been achieved in both wheat and poplar using standard transformation methods which involve integration of transforming constructs +/- a selectable marker gene.

For wheat, the *ASN2* gene has been targeted in a project to reduce asparagine content (Sarah Raffan).



Sequence data showing ASN2 gene editing

In the case of poplar, the phytoene desaturase (*PDS*) gene was chosen since it gives an obvious bleached phenotype that is easily monitored (Sergio Cerezo-Medina).



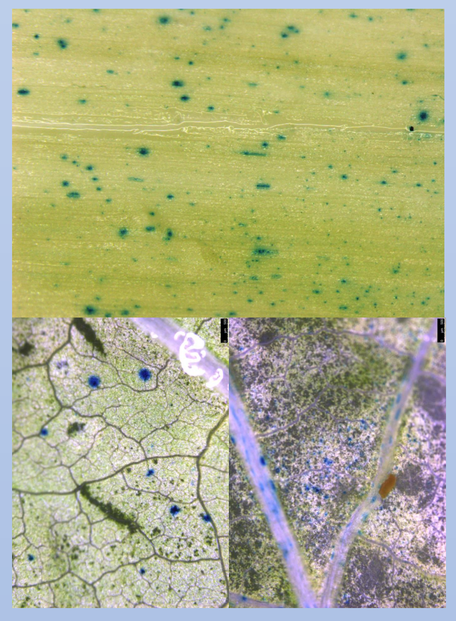
Albino poplar explants demonstrating editing of PDS gene

PRELIMINARY WORK TOWARDS USE OF RNP COMPLEX

Prior to transforming with the RNP complex it was important to establish that protein could be successfully delivered to cells and that a functional RNP complex could be formed.

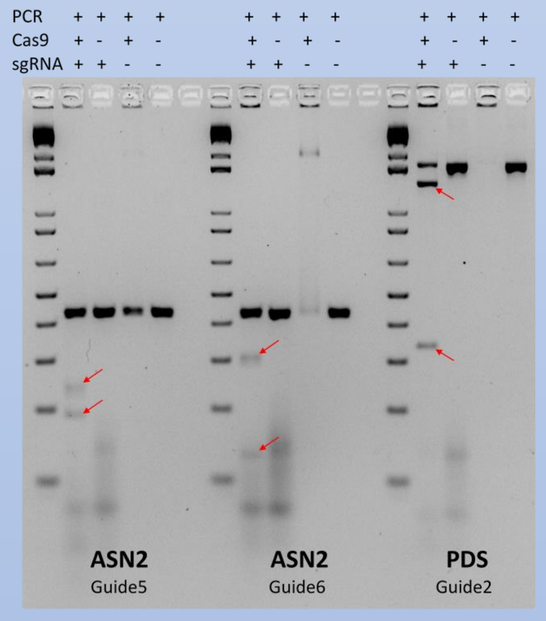
Wheat

Poplar



Bombardment of purified GUS protein

Initial experiments using purified GUS protein have demonstrated that it is possible to deliver protein to cells using biolistics.



In vitro cleavage of PCR product by RNP complex

Formation of an active ribonucleoprotein (RNP) complex has been achieved *in vitro* using purified Cas9 enzyme and synthetic single guide RNAs (Synthego) to *ASN2* and *PDS*.

WORK IN PROGRESS

Both wheat and poplar are now being targeted with a pre-assembled RNP complex which is being delivered via gold particles using the BioRad PDS-1000/He particle gun. In order to be DNA-free, no selectable markers are being used. Edited poplar explants should be distinguishable by their bleached phenotype but screening of the wheat regenerants is likely to be more challenging, especially if efficiencies of editing with the RNP complex are not anticipated to be very high. However, demonstration of even a few edited lines will show it is a technique worth pursuing and optimizing.

BBSRC bioscience for the future

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