Toward transgene-free genome editing in poplar plants using Agrobacterium-mediated delivery of a CRISPR/Cas9 cytidine base editor

Gilles Pilate, Florian Veillet, Orlane Touzet, Annabelle Déjardin

		EFOR Meeting 2021 - May 11th - **"Plants session"** Organized by: Anne-Cécile MEUNIER (CIRAD, Montpellier, France) and Eric GUIDERDONI (CIRAD, Montpellier, France)
	9.45 am - 10:00 am	**Welcome (15 minutes)**
	10.00 am - 10.30 am	Anouchka GUYON (UMR IJPB - INRAE Versailles, France) **- A blue-print for gene function analysis through Base Editing in the model plant *Physcomitrium (Physcomitrella) patens***
	10.30 am - 11.00 am	Jean Luc GALLOIS (GAFL - INRAE Avignon, France) **- Resistance to viruses by loss-of-susceptibility in tomato: from natural variation to edited genes**
	11.00 am - 11.30 am	Léo HERBERT (UMR AGAP - CIRAD Montpellier, France) **- Enhance Nitrogen Use Efficiency by precise editing of the rice transceptor NRT1.1b at near base**
	11.30 am - 12.00 am	Matthieu CHABANNES (UMR BGPI - CIRAD Montpellier, France) **- Edition of endogenous banana streak virus sequences (eBSV) in banana to lift the constraint linked to the presence of these sequences**
	12.00 am - 12.30 am	Mathieu ROUSSEAU-GUEUTIN & Maud FACON (UMR IGEPP - INRAE, Université de Rennes, France) **- Tuning a ménage à 4: how to deal with DUPLicated gene Expression**
	12.30 am - 2.00 pm	**LUNCH BREAK**
	2.00 pm - 2.30 pm	Gilles PILATE (UMR BioForA - INRAE - ONF Orléans, France) **- Toward transgene-free genome editing in poplar plants using Agrobacterium-mediated delivery of a CRISPR/Cas9 cytidine base editor**
	2.30 pm - 3.00 pm	Amélia GASTON (UMR Biologie du Fruit et Pathologie - INRAE, Villenave d'Ornon, France) **- Balance between sexual and asexual reproduction in strawberry: the added value of genome editing**
	3.00 pm - 3.30 pm	Florian VEILLET (UMR IGEPP - INRAE, Université de Rennes, France) **- Genome editing to confer disease resistance and tuber quality improvement in the cultivated potato**
	3.30 pm - 4.00 pm	Jean-Philippe MAUXION (UMR BFP - INARE Bordeaux, France) **- CRISPR/Cas9-genome editing in tomato**
	4.00 pm - 4.30 pm	**Break (30 minutes)**
	4.30 pm - 5.00 pm	Norbert BOLLIER (VIB-UGent Center for Plant System Biology, Gent, Belgium) **- Efficient simultaneous mutagenesis of multiple genes in specific plant tissues by multiplex CRISPR**
	5.00 pm - 5.30 pm	Jean-Paul CONCORDET (TACGENE, MNHN Paris, France) **- Stimulating HDR for precise gene editing**
	5.30 pm	**END OF THE DAY**

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We present here the first evidence of the precise targeting of point mutations in the genome of a forest tree species using a cytidine base editor (CBE). This was done using the classical Agrobacterium cocultivation method routinely used on our model hybrid poplar clone (INRA 717-1B4) for more than 30 years. Our ultimate goal is to produce transgene-free edited poplar plants. Indeed, in perennial species with long generation time, such as trees, it is virtually impossible to get rid of alien copies introduced into the plant genome during the cocultivation step. Therefore, using a strategy already shown to be successful in tomato and potato (Veillet et al., 2019), we targeted the endogenous poplar acetolactate synthase (ALS) gene by a CBE through Agrobacterium tumefaciens cocultivation. Using an optimized procedure, we were able to regenerate at high yield chlorsulfuron-resistant plants. Interestingly, a small number of these herbicide-resistant plants do not show evidence of T-DNA integration. Molecular analyses are under way to more accurately characterize these plants. Our most recent experiments aim to evaluate on this poplar model system the co-edition of ALS with another gene.

Veillet F, Perrot L, Chauvin L, Kermarrec M-P, Guyon-Debast A, Chauvin J-E, Nogué F, Mazier M. Transgene-Free Genome Editing in Tomato and Potato Plants Using Agrobacterium-Mediated Delivery of a CRISPR/Cas9 Cytidine Base Editor. Int. J. Mol. Sci. 2019, 20, 402. https://doi.org/10.3390/ijms20020402