Engineering haploid inducer lines in chicory

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Context: *Cichorium intybus var. sativum*, also known as chicory, is an economically important crop that is mainly cultivated for the high inulin content in its roots, which has many applications in the food industry. Hybrid breeding, in which two homozygous lines are crossed to obtain high yield heterozygous offspring, can increase the root yield of chicory. To develop these homozygous chicory lines, we set out to create haploid inducer lines. Here, we describe a stepwise approach to find naturally occurring mutations that may lead to haploid induction or to create them through CRISPR.

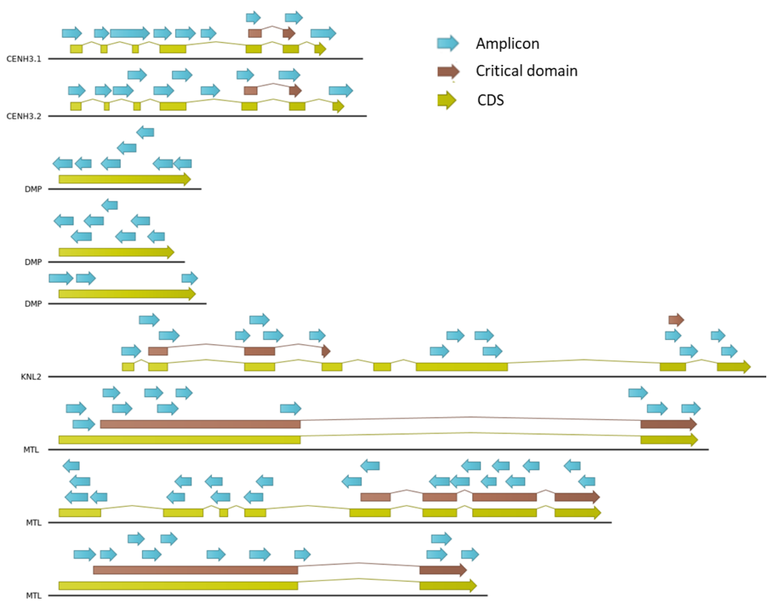
1 Primer design amplicon sequencing

AIMS: 1) Select candidate genes involved in haploid induction via genome elimination and 2) resequence via HiPlex amplicon sequencing.

9 paralogs selected of *CENH3*, *KNL2*, *MTL* and *DMP*

90 amplicons designed across these genes

Divided into 2 multiplex primer pools



2 Screen natural variation for rare defective alleles

AIMS: 1) Screen plants for carriers of naturally occurring knock-out (KO) mutations and 2) screen genes for regions of low sequence diversity in founding varieties of chicory breeding program.

35 chicory and 25 witloof varieties

30 plants per variety to cover genetic diversity

1554 plants screened in 163 pools

Predict mutated protein sequence

Identify defective KO alleles

Sequence 320 individual plants

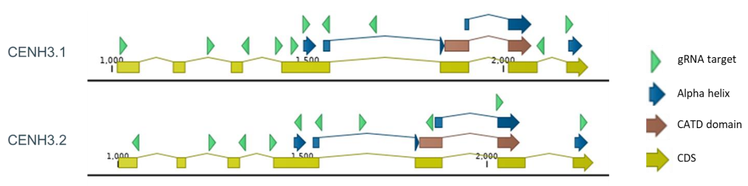
Predicted effect on protein function	# alleles	Genes
Strong (<10%*)	2	*KNL2, MTL ortholog*
Medium (>10% and <50%*)	4	*DMP orthologs, MTL orthologs*
Weak (>50% and <90%*)	1	*DMP ortholog*

*The percentage refers to protein sequence similarity between WT and mutant haplotype

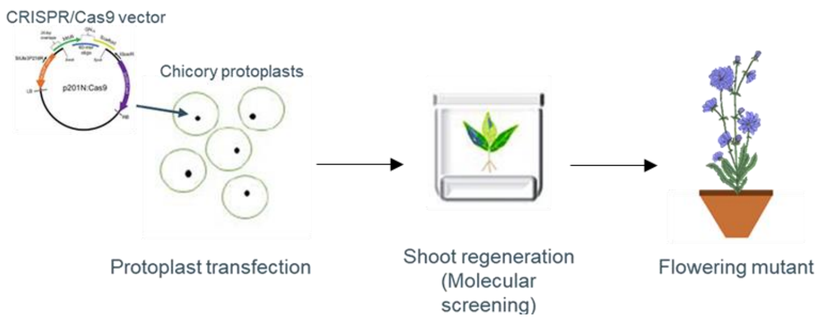
3 Create variation by CRISPR

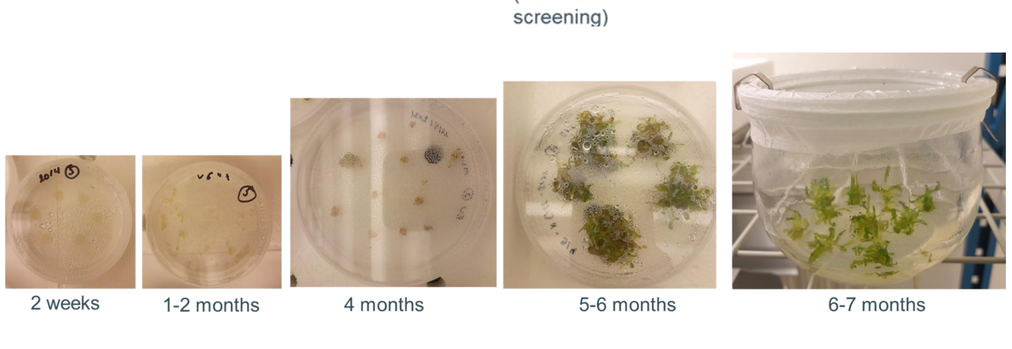
AIM: Create mutations in conserved regions of candidate genes to complement natural variation found in Step 2.

gRNA targeting conserved regions of genes



Protoplast transfection with CRISPR vectors and regeneration of callus and shoots





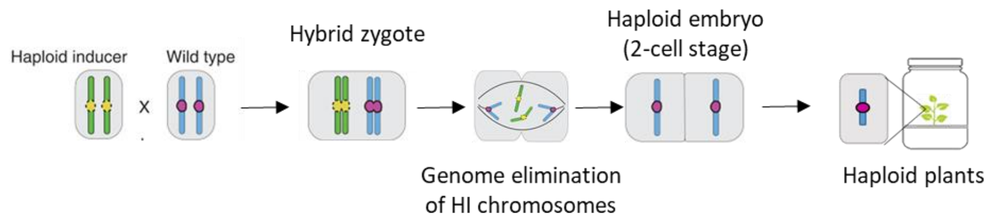
11 mutated alleles identified in *CENH3.2* in regenerating callus and shoot material

CENH3.1, *KNL2*, *MTL* and *DMP* CRISPR experiments ongoing

4 Check for haploid inducer phenotype

AIM: Screen plants with naturally occurring KO mutations (Step 2) and mutants created by CRISPR (Step 3) for a haploid inducer phenotype.

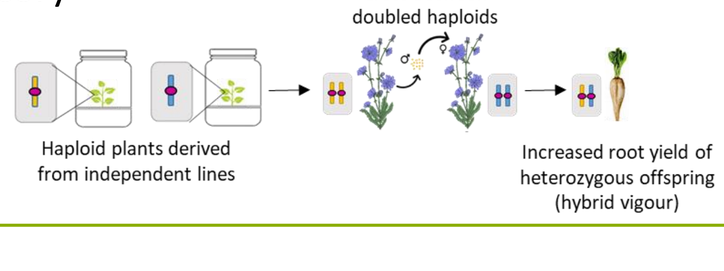
Crosses between putative haploid inducer and tester line



Ploidy assessment of offspring via flowcytometry, SSR analysis, HiPlex amplicon sequencing and cytogenetics

Conclusions and future perspectives:

- We identified naturally occurring KO mutations in candidate genes for haploid induction
- We created KO alleles via CRISPR in *CENH3*, which had no naturally occurring KO variants
- We used a complementary approach (natural and induced variation) to obtain a collection of potential haploid inducers
- Efficient haploid inducers can be used in hybrid breeding for chicory



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