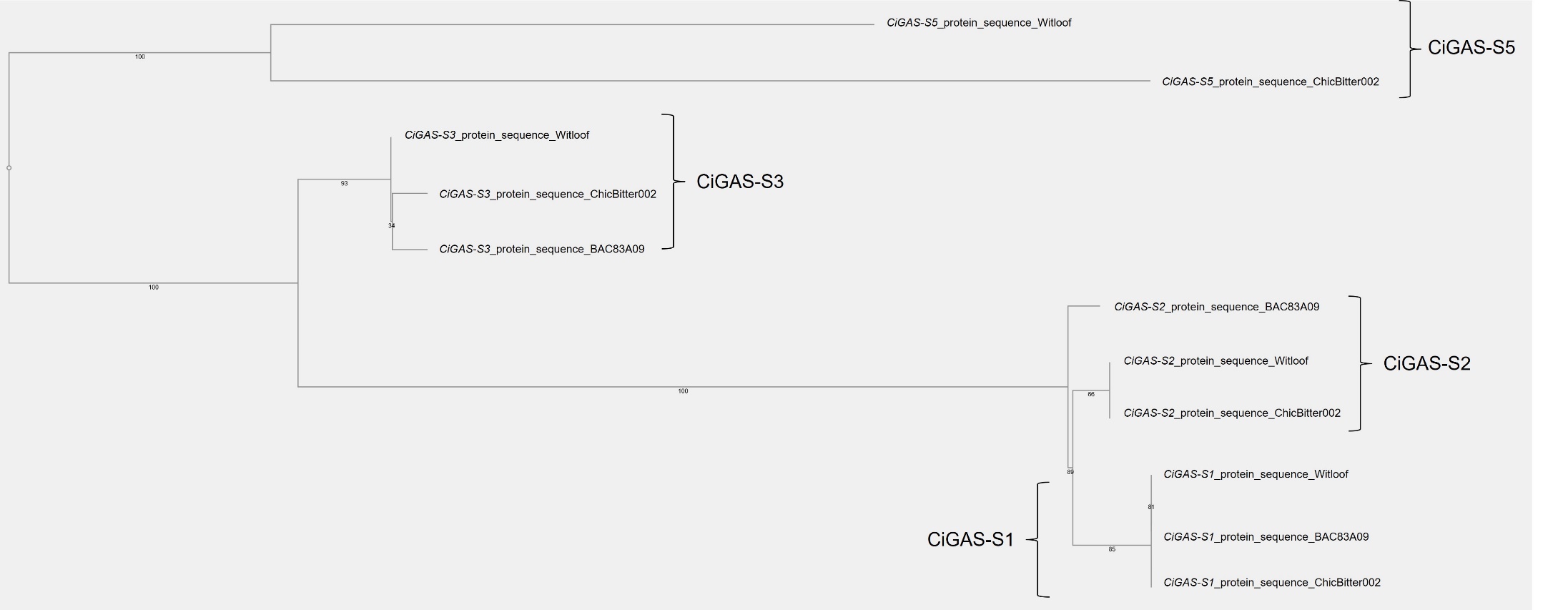
Supplementary Table 1. List of oligonucleotides.

|  |  |  |
| --- | --- | --- |
| Name | Sens | 5’-3’ sequence |
| CiGAS\_T1 | Forward | **ATCG**GTGTAGTAAACACTCTCGA |
| Reverse | **AAAC**TCGAGAGTGTTTACTACAC |
| CiGAS\_T2 | Forward | **ATCG**AACCATTGGCATCCCCTGA |
| Reverse | **AAAC**TCAGGGGATGCCAATGGTT |
| CiGAO\_T1 | Forward | **ATCG**TTGTTGGATTTGGGACGAG |
| Reverse | **AAAC**CTCGTCCCAAATCCAACAA |
| CiGAO\_T2 | Forward | **ATCG**GAGTCGCGATGCCTCTGGA |
| Reverse | **AAAC**TCCAGAGGCATCGCGACTC |
| GG1-F | Forward | ATATATGGTCTCACTCGAAAGAACCAACCTGTTTTCATAGC |
| GG1-R | Reverse | ATTATTGGTCTCAACCTAAAAAAAGCACCGACTCGGTG |
| GG2-F | Forward | ATATATGGTCTCAAGGTAAAGAACCAACCTGTTTTCATAGC |
| GG2-R | Reverse | ATTATTGGTCTCAACCGAAAAAAAGCACCGACTCGGTG |
| RolB | Forward | TGGATCCCAAATTGCTATTCCTTCCACGA |
| Reverse | TTAGGCTTCTTTCTTCAGGTTTACTGCAGC |
| C9-F | Forward | AAGCACGTTGCTCAGATCCT |
| C9-R | Reverse | CCGTTCGTCTCGATAAGAGG |
| S1 | Forward | AGAATCTATTAATCGGCTTT |
| Reverse | [HEX] TAAGGATCATCGCTTAATTA |
| S2 | Forward | [HEX] CCCAGAATCTATTAATTGGT |
| Reverse | AAGTTAGGTTATTTCCGTTG |
| S3 | Forward | [6-FAM] AATGATTACTCCGGTTTAGT |
| Reverse | TTCCGATTTCTTAATACTGA |
| S5 | Forward | [6-FAM] GTTCTCGTGCATTATACTTG |
| Reverse | ACCTTCCCCTATGTATGTAT |
| GAO | Forward | [HEX] TCACTACTTCCATTGCTCT |
| Reverse | ACTCTCTTTGATCCTTGATT |

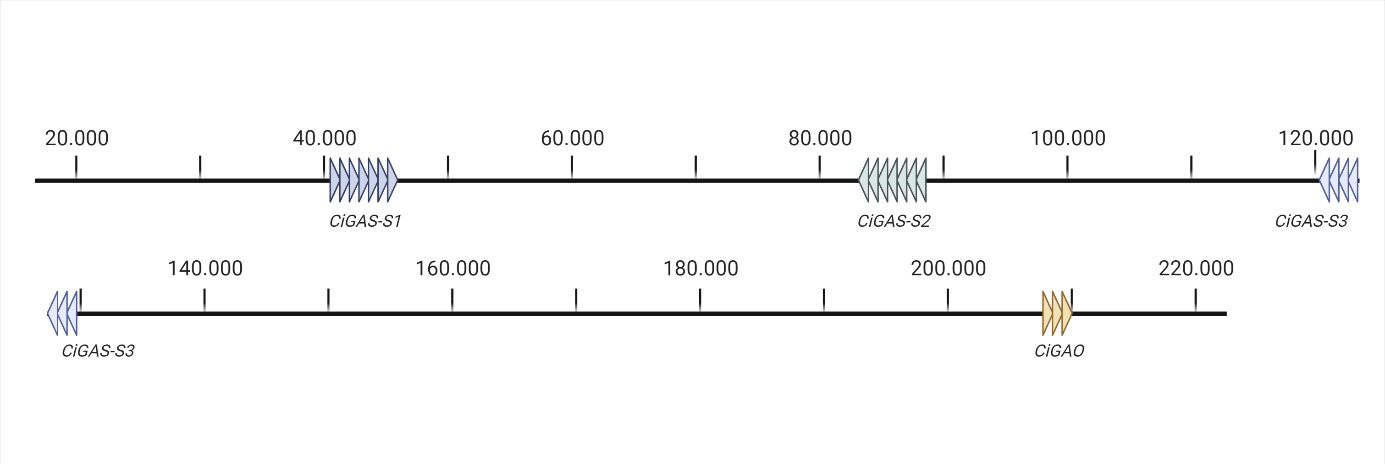
Binding sites used to insert sgRNA into the intermediate plasmid are in bold. [6-FAM] and [HEX] are fluorescent dyes used to labelled primers at the 5’ extremity.



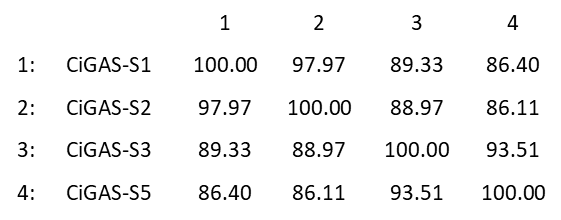
Supplementary Fig. 1. Phylogenetic tree of *CiGAS-short* genes obtained by MAFFT protein alignment. The alignment was performed between the four expressed copies of *CiGAS-short* genes identified in the witloof chicory genome (protein\_sequence\_Witloof), in the ChicBitter002 chicory genome (protein\_sequence\_ChicBitter002) and in the BAC clones published by Bogandovic *et* *al.* [16] (protein\_sequence\_BAC83A09).

Une image contenant table

Description générée automatiquementSupplementary Fig. 2. Jbrowse visualization of the 3 exons-structured *CiGAO* gene. The predicted (g24057.t1= *CiGAO* gene) is aligned against the ChicBitter002 reference sequence in the first track. The second track is the predicted witloof gene sequence from the Cargese program. The 3 following tracks are putative orthologues from related Asteraceae species. The 2 last tracks are transcripts sequences issued from RNA-seq data (Qualichic, Cargese programs) thus confirming the expression of the gene. Sequence IDs are indicated on the top of each track. Annotation information is given on the right of the tracks.



**Supplementary Fig. 3. Schematic representation of the genomic organization of the contig\_403\_pilon.** Genes *CiGAS-S1, CiGAS-S2, CiGAS-S3* and *CiGAO* are colocalized in the chicory genome. Each exon is represented by an arrow. The orientation of the arrows indicates the direction of transcription.



**Supplementary Fig. 4.** **Percentage of identity matrices found in the sequence comparisons between coding regions of *CiGAS-short.***

Supplementary Table 2. Relationship between total STL content and bitterness score.

A

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Samples** | **Total STL content (peak area (mUA))** | **Bitterness score** |  | **Samples** | **Total STL content (peak area (mUA))** | **Bitterness score** |
| **R01** | 67.77 | 3.65 |  | **Ctrl\_HR** | 208.20 | *10.10* |
| **R02** | 61.90 | 3.64 | B | **HR18** | 82.88 | *4.26* |
| **R03** | 93.95 | 4.19 |  | **HR16** | 202.04 | *9.81* |
| **R04** | 51.40 | 2.21 |  | **HR3** | 136.4 | *6.75* |
| **R05** | 67.28 | 4.4 |  | **HR9** | 300.73 | *14.41* |
| **R06** | 84.16 | 6.13 |  | **HR2** | 196.83 | *9.57* |
| **R07** | 72.39 | 3.61 |  | **HR12** | 19.31 | *1.29* |
| **R08** | 75.95 | 3.6 |  | **HR12\*** | 23.24 | *1.48* |
| **R09** | 131.23 | 7.83 |  | **Ctrl** | 100.08 | *5.06* |
| **R10** | 65.20 | 3.32 |  |  |  |  |
| **R11** | 68.57 | 4.47 |  |  |  |  |
| **R12** | 108.28 | 3.83 |  |  |  |  |
| **R13** | 86.86 | 2.49 |  |  |  |  |

Panel A) represents data for the 10 field-grown chicories analyzed by sensory analysis. Panel B) represents data for the controls and the CRISPR/Cas9 hairy root mutants. Data in italic represent theoretical bitterness score calculated with the equation Y = 0.06366\*X-0.6515 obtained by linear regression analysis. Total STL content (X) of each CRISPR/Cas9 HR lines samples was estimated by HPLC and sum of the STL peaks.

**Supplementary Table 3. Correlation between individual STL and bitter score.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **STL** | **R²** | **Pearson’s r** | ***p-value*** | **Significant?** |
| **DHLc-gly** | 0.01648 | 0.1284 | 0.6759 | No |
| **DHLc-ox** | 0.1633 | 0.4041 | 0.1709 | No |
| **DHLc** | 0.1107 | 0.3327 | 0.2666 | No |
| **Lc-ox** | 0.03821 | 0.1955 | 0.5222 | No |
| **Lc** | 0.3121 | 0.5587 | 0.0472 | Yes |
| **dLc-gly** | 0.8506 | 0.9223 | <0.0001 | Yes |
| **dLc-ox** | 0.1665 | 0.4080 | 0.1663 | No |
| **dLc** | 0.5723 | 0.7565 | 0.0028 | Yes |
| **DHdLc-gly** | 0.448 | 0.669 | 0.0122 | Yes |
| **DHdLc-ox** | 0.4171 | 0.6458 | 0.0171 | Yes |
| **DHdLc** | 0.5728 | 0.7568 | 0.0027 | Yes |
| **DHLp-ox** | 0.1603 | 0.4004 | 0.1851 | No |
| **DHLp** | 0.6668 | 0.8166 | 0.0007 | Yes |
| **Lp-ox** | 0.3745 | 0.6120 | 0.0262 | Yes |
| **Lp** | 0.5779 | 0.7602 | 0.0026 | Yes |

Linear regression and Pearson’s correlation test was performed between the bitter score and individual STL of the thirteen field-grown chicories used for sensorial analysis. DHLc-gly: 11(S),13-dihydrolactucin-15-glycoside; DHLc-ox: 11(S),13-dihydrolactucin-15-oxalate; DHLc: 11(S),13-dihydrolactucin; Lc-ox: Lactucin-15-oxalate; Lc: Lactucin ; dLc-gly: 8-deoxylactucin-15-glycoside; dLc-ox: 8-deoxylactucin-15-oxalate; dLc: 8-deoxylactucin; DHdLc-gly: 11(S),13-dihydro-8-deoxylactucin-glycoside; DHdLc-ox: 11(S),13-dihydro-8-deoxylactucin-15-oxalate; DHdLc: 11(S),13-dihydro-8-deoxylactucin; DHLp-ox: 11(S),13-dihydrolactucopicrin-15-oxalate; DHLp: 11(S),13-dihydrolactucopicrin; Lp-ox: Lactucopicrin-15-oxalate; Lp: Lactucopicrin

Une image contenant table

Description générée automatiquement

Supplementary Fig. 5. Protein sequence of the gene *CiGAS-S1* of mutant HR lines. Sequences of WT *CiGAS-S1* were aligned against the two mutated alleles of HR12 and HR12\* lines. For both HR12 and HR12\*, allele 1 have an insertion of a T and allele 2 have a deletion of 58 nucleotides in their exon 3. Both resulting in a change in the coding frame and appearance of stop codons. Stop codons were highlighted in pink.

Une image contenant table

Description générée automatiquement

Supplementary Fig. 6. Protein sequence of the gene *CiGAS-S2* of mutant HR lines. Sequences of WT *CiGAS-S2* were aligned against the two mutated alleles of HR12 and HR12\* line. For both HR12 and HR12\*, allele 1 and allele 2 have an insertion of an A in their exon 3. Both resulting in a change in the coding frame and appearance of stop codons. Stop codons were highlighted in ink.

Une image contenant table

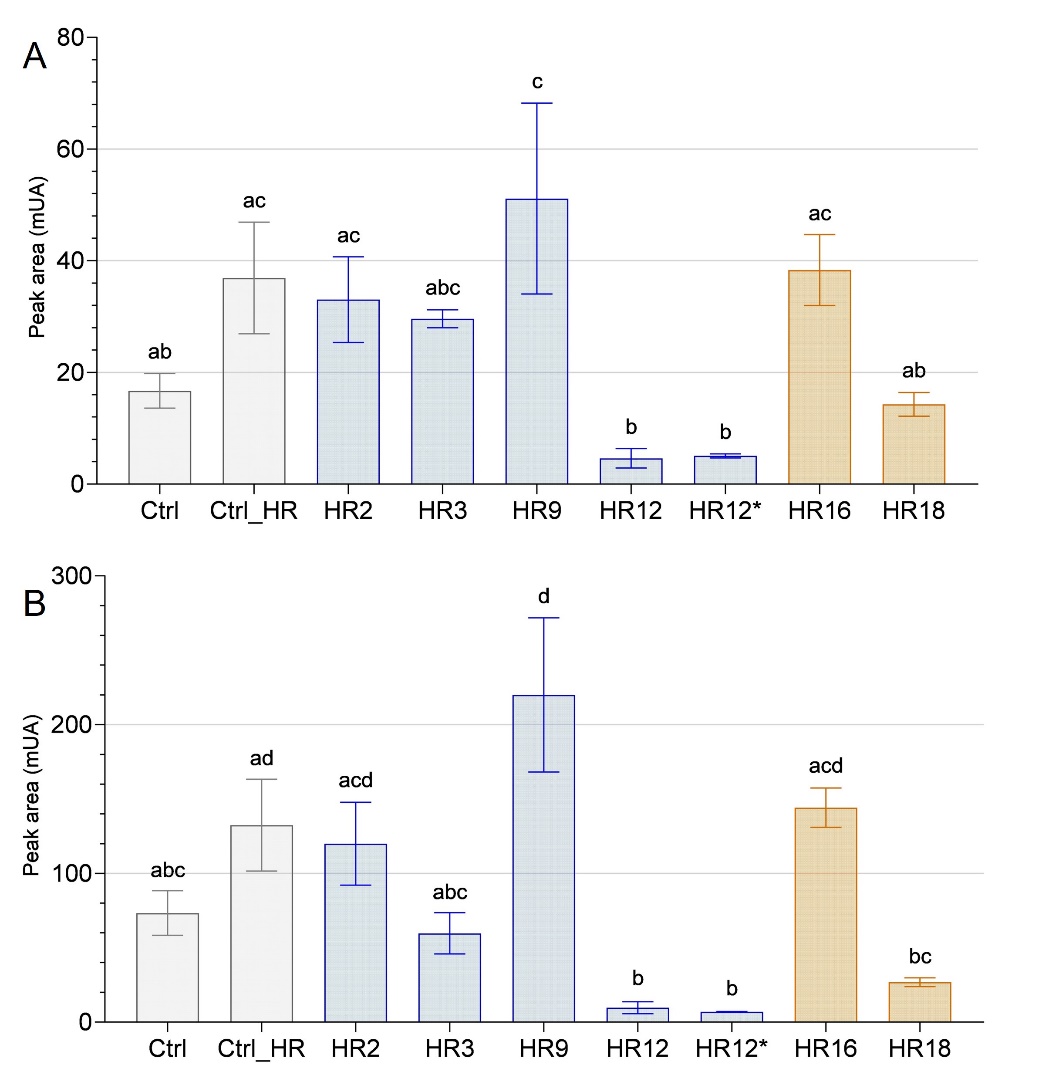
Description générée automatiquement

Supplementary Fig. 7. Protein sequence of the gene *CiGAS-S5* of mutant HR12\* line. Sequences of WT *CiGAS-S5* were aligned against the two mutated alleles of HR12\* line. Allele 1 (HR12\*.1) and allele 2 (HR12\*.2) have an insertion of an A in their exon 3. Both resulting in a change in the coding frame and appearance of stop codons. Stop codons were highlighted in pink.

Une image contenant table

Description générée automatiquement

Supplementary Fig. 8. Protein sequence of the gene *CiGAO* of mutant HR18 line.Sequences of WT *CiGAO* were aligned against the two mutated alleles of HR18 line. Allele 1 (HR18.1) has an insertion of an A and allele 2 (HR18.2) has a deletion of 8 nucleotide in their exon 1. Both resulting in a change in the coding frame and in appearance of stop codons. Stop codons were highlighted in pink.



Supplementary Fig. 9. Sesquiterpene lactone content of roots of edited chicory regenerated from HR lines. The peak areas (mUA) of STLs in the roots of 7 genome edited chicory regenerated from HR lines were analyzed by HPLC and compared to WT chicory lines (Ctrl and Ctrl\_HR). Panel A) is the total content of free forms of STLs and panel B) is the total content of oxalated forms of STLs. Gray bars correspond to control chicory lines, blue bars to *CiGAS-short* edited lines and orange bars to *CiGAO* mutants. The histograms show the modulation of the total content of free forms of STLs (A) or oxalated form of STLs (B) as a function of mutation on chicory lines. Statistical analysis was performed using non-parametric one-way ANOVA and Dunn’s post-hoc test (*p<0.005*).