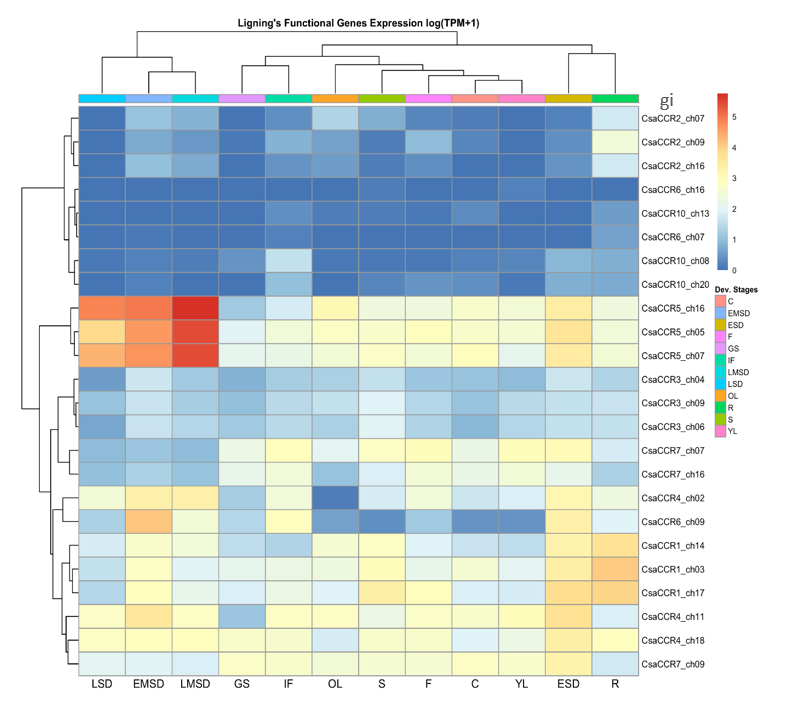
**Supplementary data 1-a**

CsCCR4 gene expression during normal development stages and different tissues. The graph shows the Transcripts Per Million (TPM) of the CsCCR4 in the pool of RNA retrieved from <http://bar.utoronto.ca/efp_camelina/cgi-bin/efpWeb.cgi>

**Supplementary data 1-b**



The heat map indicates the expression level of CsCCR genes during different developmental stages and tissues retrieved from atlas of Camelina transcriptome at ([http://bar.utoronto.ca/efp\_camelina/cgi-bin/efpWeb.cgi). The CCRs are grouped by their Transcript Per Million(TPM). two distinct groups containing a low and high level of expression](#_ENREF_14) based on the parsimony trees on left, representing differents among genes, and top, representing differential expression among tissues and developmental stages, are shown in dark blue to cherry-red. Abbreviations: R: Root, S: stem F: flower, C: cotyledon YL: young leaf OL: old leaf, IF inflorescence, GS: germinating seed, ESD: early seed development, EMSD: early-mid seed development, LMSD: late-mid seed development and LSD: late seed development.

**Supplementary data 2-a**

**Genomic DNA sequence of *AtCCR4* and *CsCCR4***

> At5g58490, *AtCCR4*

ATCTGAAACTTAGAGAGACAGAGAGAGAGAAAGCTCAAATTCAATCATCAATGTTAACGGACGAGAGAGAAGTAGTCTGTGTCACCGGCGCCAGTGGCTGCATCGGCTCGTGGCTGGTCCATCAGCTCCTCCTCCGCGGCTACTCCGTCCACGCCACCGTGAAAAACCTCCGTAATCTTCACAAATTTCTCTCTTCGATTACGCTTCTGGTTACCCTATCACCTCCTAATTCCCTTACATTTCTGTGTTGCAGAGGATGAGAAAGAGACGAAACATCTAGAAGGTCTCGAAGGTGCAGCCACACGCCTCCATCTATTCGAGATGGATCTCCTACAATACGACACCGTTTCCGCCGCCATCAATGGTTGCTCCGGCGTATTCCACCTCGCATCACCTTGTATCGTCGATGAAGTCCAAGATCCCCAGGTTCTAATCTCCACTCCCAAAAAGGACCAATAGTGAATTTGATCACTTCAAATGTATATAAATAAATGAGTGTATTCACAGAAGCAACTACTTGACCCGGCGGTTAAAGGAACCATAAATGTTCTGACGGCGGCAAAAGAAGCCAGTGTTAAGAGAGTTGTTGTGACGTCTTCGATATCGGCGATTACTCCAAGTCCCAACTGGCCTGCTGATAAGATCAAGAATGAGGAATGTTGGGCTGCTGAAGACTACTGCAGGCAAAATGGAGTATGTTATTCTCTAATTTAAGATTTGTTACTGCAAAACATCTTATTGGTTATCTTTATAGATGATGTGTTGTTGTTGGTAGTTGTGGTATCCACTGTCGAAGACGCTTGCTGAGAAGGCAGCTTGGGAATTTGCAGAGGAGAAAGGATTGGATGTGGTTGTGGTGAATCCAGGCACTGTCATGGGGCCTGTGATTCCTCCGTCTCTTAACGCTAGCATGCACATGCTTCTACGCCTTCTTCAGGGTATTTTGGTTCTCTATGAATGTTTGATTGAATATGTCTTGAGTTCCATGGAACATCTTTGAATTTGAATAGATATGGATGCTTAGGGTGCACGGAGACATACGAGAACTTCTTTATGGGGTCTGTGCATTTCAAGGATGTGGCTTTAGCTCATATTCTTGTATACGAGGATCCATATTCGAAAGGAAGGCACTTGTGCGTTGAGGCTATCTCTCACTACGGTGATTTTGTAGCCAAAGTTGCTGAGCTCTATCCCAATTACAATGTCCCCAAGTAAGCCACAAATTTTTCTTTGAAACAGACGTTATATATAGAGAGAGAGATTTCAATAACTTGAATTGGAAAAATCTCTTAGGTTACCGAGAGAGACTCAACCGGGTTTACTTCGAGATAAGAATGCATCAAAGAAGCTCATAGATTTGGGGTTAAAGTTCATTTCCATGGAGGAAATCATCAAGGAAGGTGTGGAGAGTCTTAAAAGCAAAGGATTTATCTCTTAATACCTACCTCTGTAACTCTGTTTGATTGAGCAATTAACACAATCTCTAGGCAGCAGAAACATTTCATATGATATACGATTTTGCACTTTTGTAATAAATAAGCTGGCCTATGGTATGGTATGAACATCCAATTAATATTTATT

> Csa02g064110

TCTGAAACTTAAGAGAGAGAGAGAGAGAGAGCTCTAAAATCAATTCATCAATGTCGATAGAACGAGAAGTAGTCTGTGTCACCGGAGCCAGTGGCTGCATCGGCTCGTGGCTGGTCCATCTCCTCCTCCACCGCGGCTACTCCGTCCACGCCACCGTGAAAAACCTCCGTAATCTTCACAAATATTCCTCTCTTCGATTTTGTTACAATATCTCCTTAACATTTGTTAAATTCGTGTTGCAGAGGATGAGAAAGAGACGAAACATCTAGAAGCTCTCGAAGGTGCAGCCACACGCCTCCATCTATTCGAGATGGATCTCCTCCAATACGACACCGTTTCCGCTGCCATCAACGGATGCTCCGGCGTATTTCACCTCGCATCGCCCTGTATTGTCGACGAAGTTCAAGATCCCCAGGTTCTTATACATGATCCACTCCCAAAACCTTTAGCTTCAAAAGGAAGGAACGATAGTGAATTTGATTACTTCATATGTGTGTTTTAACAGAAGCAACTACTTGACCCGGCGGTTAAAGGAACCATAAATGTACTGACGGCAGCTAAAGAAGCAGGTGTTAAGAGGGTTGTTGTGACGTCATCTATATCAGCGATAACTCCAAGTCCCAACTGGCCTGCTGATAAGATCAAGAATGAGGAGTGTTGGGCTGACCAAGACTACTGCAAACAGAATGAAGTATATGGTTCTCTAAGTTTAAGATCTAATCTTCTTGATTTGTCTTATAGATGATGTTTGATTCTTGTGTTGTTGGTTGGTAGTTATGGTATCCACTGTCGAAGACGCTTGCTGAGAAGGCAGCTTGGGAGTTTGCAGAGGAGAAAGGATTGGACGTGGTTGTGGTGAATCCAGGCACTGTCATGGGCCCTGTCATTCCCCCATCTATCAACGCTAGCATGCTCATGCTTCAACGTCTTCTTGAAGGTAATTTGCTTCTCTGTGATTAAATTAGTCTGAGTTCCATGGAACATTTTAGGATTTGAATCGACATGAATGCTTAGGATGTACGGAGACGTATGAGAACTTCTTTATGGGGTTGGTTCATTTCAAGGACGTGGCCTTAGCCCATATTCTTGTATATGAGAACACAGCTGCGAAAGGAAGGCACTTGTGTGTTGAGGCTATCTCTCACTACGGTGATTTTGTAGCCAAAGTTGCTGAGCTCTATCCCAATTACAGTGTTCCCAAGTAAGTCTCTCAAACTATGTTTTAAACACATGTGTGTGTGTGTGTGTGTGTGTGTGTAGATTTCAATTAGCTTTATTGGTAATTCTCTTAGGTTACCAAGAGAGACTCAACCTGGTTTACTCCGAGCCAAGAATGCATCAAAGAAGCTGATGGAATTGGGGTTAGAGTTCAGTTCCATGGAGGAGATCATCAAGGAAGGTGTGGAGAGTCTTAAAAGCAAAGGATTTATCTCT

> Csa11g092190

GAAACAGAGAGAGAGAGCTCTTTTATCAATTCATCAATGTCGATAGAACGAGAAGTAGTCTGTGTCACCGGAGCCAGTGGCTGCATCGGCTCGTGGCTGGTCCATCTACTCCTCCACCGCGGCTACTCCGTCCACGCCACCGTGAAAAACCTCCGTAATCTTCACAAATTCCTCTCTTCGATTTTGTTACAATATCTCCTACTACTAATTGCCTTAACATTTGTTGAAAATTCGTGTTGCAGAGGATGAGAAAGAGACGAAACATCTAGAAGCTCTCGAAGGTGCAGCCACACGCCTCCATCTATTCGAGATGGATCTCCTGCAATCCGACACCGTTTCCGCCGCCATCAACGGATGCTCCGGTGTATTTCACCTCGCATCGCCCTGTATCGTAGACGAAGTTCAAGATCCCCAGGTTCTTCTTATACATGATCCACTCCCAAAAACCTTTAGCTTCAAAAGGACCGATCAATAGTGAATTTGATCACTTCATTCATATGTGTTTAACAGAAGCAACTACTTGACCCGGCGGTTAAAGGAACCATAAATGTACTGACGGCAGCTAAAGAAGCAGGTGTTAAGAGGGTTGTTGTGACTTCTTCTATATCAGCGATAACGCCAAGTCCCAACTGGCCTGCTGATAAGATCAAGAATGAGGAGTGTTGGGCTGACCAAGACTACTGCAAACAAAATGAAGTATATGGTTTTTTAGTTTTAAGATCTATAGCAAAACTAATCTTCTTGATTTGTCTTATAGATGATGTGTTATTCTTGTGTTGGTTGGTAGTTATGGTATCCACTGTCGAAGACGCTTGCTGAGAAGGCATCTTGGGAGTTTGCAGAGCAGAAAGGATTGGACGTGGTTGTGGTGAATCCAGGCACTGTCATGGGCCCTGTCATTCCCCCATCTATCAACGCTAGCATGCTCATGCTTCTACGCCTTCTTGAAGGTAATTTGCTTCTCTGTGAATACTCTGACTTCCATGGAACATCTTAGGATTTGAATGGACATGTTATGCTCAGGGTGCACAGAGACATACGAGAACTTCTTCATGGGATCTGTTCATTTCAAGGACGTGGCCTTAGCACATATTCTTGTATATGAGAACCCATCTGCGAAAGGAAGGCACTTGTGCGTTGAGGCCATATCTCACTACGGTGATTTTGTAGCCAAAGTTGCTGAGCTCTATCCCAATTACAATGTTCCCAAGTGAGTCTCTCAAACTATCTTTCAAATACATGTGTGTGTATATAGATTTCAGTAAGCTTCATTGGTAATTCTCTTAGGTTACCGAGAGAGACTCAACCTGGTTTACTCCGAGCCAAGAATGCATCAAAGAAGCTGATGGAATTGGGGTTAGAGTTCAGTTCCATGGAGGAGATCATCAAGGAAGGTGTGGAGAGTCTTAAAAGCAAAGGATTTATCTCT

> Csa18g031650

GAGAGAGAGAGCTCTTATCAATTCATCAATGTCGATAGAACGAGAAGTAGTCTGTGTCACCGGCGCTAGTGGCTGCATCGGCTCGTGGCTGGTCCATCTGCTCCTCCACCGCGGCTACTCCGTCCACGCCACCGTGAAAAACCTCCGTAATCTTCACAAATATTCCTCTCTTCGATTCGTTACAATATCTCCTTCTAATTAATTGCCTTAACATTTGTTAAAAATTCGTGTTGCAGAGGATGAGAAAGAGACGAAACATCTAGAAGCTCTCGAAGGTGCAGCCACACGCCTCCATCTATTCGAGATGGATCTCCTGCAATACGACACCGTTTCCGCCGCCATCAACGGATGCTCCGGCGTATTTCACCTCGCATCGCCCTGTATCGTCGACGAAGTTCAAGATCCCCAGGTTCTTATACATGATCCACTCCCAAAAACCTCTAGCTTCAAAAGGACCGAACGATAGTGAATTTGATCACTTCATTCATATGTGTTTAACAGAAGCAACTACTTGACCCGGCGGTTAGAGGGACCATGAATGTACTGACGGCGGCGAAAGAAGCAGGTGTTAAGAGGGTTGTTGTGACGTCTTCTATATCAGCGATAACTCCAAGTCCCAACTGGCCTGCCGATAAGATCAAGAATGAAGATTGTTGGGCTGACCAAGACTACTGCAAACAGAATGAAGTATATGTTTCTCTAGTATTAAGATCTAATCTTCTTGATTTATCTTTTATAGATGATGTGTGATTCTTGTGTTGTTGGTTGGTAGTTATGGTATCCACTGTCAAAGACGCTTGCTGAGAAGGCAGCTTGGGAGTTTGCAGAGCAGAAAGGATTGGACTTGGTTGTGGTGAATCCAGGCACTGTCATGGGCCCTGTTATTCCCCCATCTATCAACGCTAGCATGCTCATGCTTCTACGCCTTCTTGAAGGTATTTGCTTCTCTGTGATTAATTAGTCTGAGTTTCATGGAACGTCTTAGGATTTGAATCGACATGTTATGCTCAGGGTGCACAGAGACATACGAGAACTTCTTCATGGGGTTGGTTCATTTCAAGGACGTGGCCTTAGCCCATATTCTTGTATATGAGAACCCATCTGCCAAAGGAAGGCACTTGTGCGTCGAGGCCATCTCTCACTACGGTGATTTTGTAGCCAAAGTTGCTGAGCTCTATCCCAATTACAATGTCCCCAAGTAAGTCTCTCAAACACATGTGTGTGTGTGTGTTTATATATTTCAATAAGCTTCATTGGTAATTCTCTTAGGTTACCGAGAGAGACTCAACCTGGTTTACTCCGAGCCAAGAAAGCATCAAAGAAGCTGATGGAATTGGGGTTAGAGTTCAGTTCCATGGAGGAGATCATCAAGGAAGGTGTGGAGAGTCTTAAAAGCAAAGGATTTATCTCT

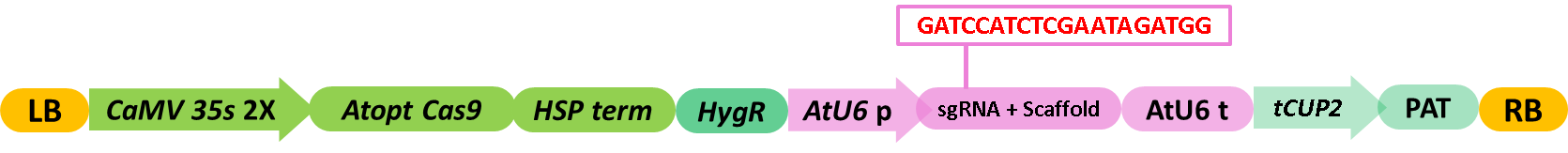
**supplementary data 2-b**



Phylogenetic relationships among CsCCR4 homeologs on chromosomes 2 (Csa02g064110), 11 (Csa11g092190) and 18 (Csa18g031650) in Camelina and its corresponding paralogue gene in Arabidopsis (At5g58490). This tree is created by CLC Genomic Workbench. The numbers on each branch show the rate of substitutions in each nucleotide.

**supplementary data 3**

CRISPR/Cas9 Final construct preparation



Order of the modules on the T-DNA. Left and right border are in orange. Module of Cas9 expression CaMV 35s 2X, the promoter of the Arabidopsis codon optimized Cas9 and the Heat shock protein’s terminator are in green, gRNA module including AtU6 regulatory sequences and its scaffold are shown in purple. PAT gene, resistance gene to glyphosate for T0 screening, are in light green.

**gRNA expression construct based on Golden Gate cloning**

Green= *AtU6-26* promoter and terminator

Black= *Bsa*I Recognition site

*lacZα* = blue(*lac Z α* is replaced with synthesized oligo gRNA)

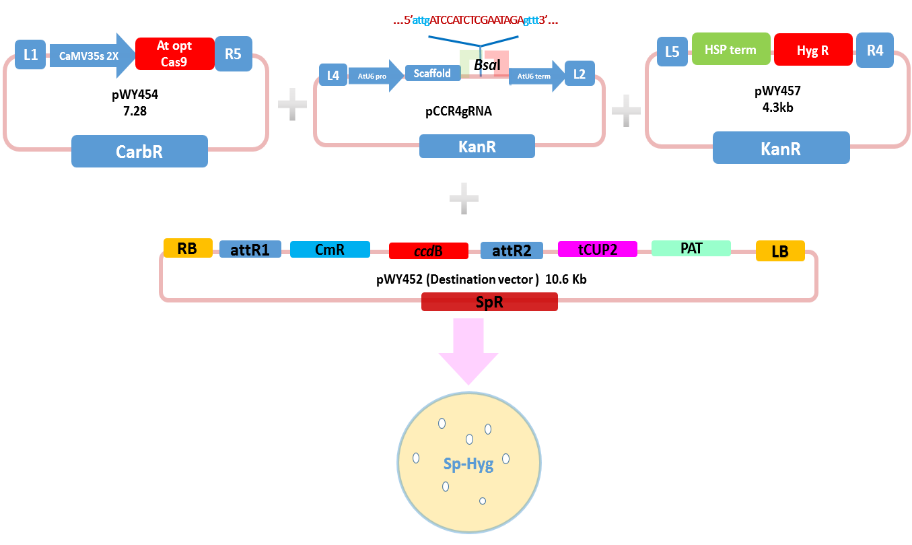
Red= scaffold for gRNA

**ACTTTCCATTCGGAGTTTTTGTATCTTGTTTCATAGTTTGTCCCAGGATTAGAATGATTAGGCATCGAACCTTCAAGAATTTGATTGAATAAAACATCTTCATTCTTAAGATATGAAGATAATCTTCAAAAGGCCCCTGGGAATCTGAAAGAAGAGAAGCAGGCCCATTTATATGGGAAAGAACAATAGTATTTCTTATATAGGCCCATTTAAGTTGAAAACAATCTTCAAAAGTCCCACATCGCTTAGATAAGAAAACGAAGCTGAGTTTATATACAGCTAGAGTCGAAGTAGTGATTGGGAGACCGCACGTGTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACAAGGTGAGGAACTAACTCATGACCATGATTACGGATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGCCGATACGTAAGCCTAGGCCAAAGCCCGCCGAAAGGCGGGCTTTTCTGTATCTGGACTAGTAGGTCTC**A**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTTTGCAAAATTTTCCAGATCGATTTCTTCTTCCTCTGTTCTTCGGCGTTCAATTTCTGGGTTTTTCTCTTCGTTTTCTGTAACTGAAT**

DNA sequence of Atopt*Cas9* including large T-antigen (green), 3xFLAG epitope tag (purple), Nuclear Localization Sequence (NLS) from nucleoplasmin (brown) and *S. pyrognenes* Cas9 (grey highlighted).

Underlined sequence (110 nucleotides) was considered as the target DNA for T-DNA copy number experiment. Forward and reverse primers and T-DNA target probe is represented on red and blue color respectively

**ATGGCTCCTAAGAAAAAGCGTAAAGTGGGTGGCAGTGGAGGAGATTACAAAGATCACGATGGGGATTACAAAGACCATGACATCGACTACAAGGACGATGATGACAAAGGTGGTAGCGCTGGGTCTGGAGCTGCTGATAAGAAATACTCTATCGGACTCGATATTGGAACGAATTCAGTAGGGTGGGCTGTGATCACCGACGAATACAAAGTACCCTCCAAGAAATTCAAGGTCCTAGGAAATACTGATCGCCACTCTATCAAGAAGAACCTCATCGGAGCATTACTTTTTGACTCTGGCGAGACTGCTGAGGCTACTAGATTGAAGAGGACCGCACGTAGAAGATACACCCGAAGGAAAAACAGAATATGCTATTTGCAAGAGATTTTCAGCAATGAGATGGCTAAAGTTGACGATTCTTTCTTCCATCGACTTGAAGAGTCATTCCTAGTTGAGGAGGACAAAAAGCATGAACGGCATCCGATTTTTGGGAATATAGTGGATGAAGTTGCTTATCACGAAAAGTATCCCACGATCTACCACCTCCGTAAAAAGTTGGTGGATAGTACCGATAAAGCGGATCTCAGACTCATATATCTGGCTCTTGCTCACATGATTAAGTTTCGTGGACACTTCCTCATAGAAGGAGATCTTAACCCAGATAATAGCGACGTTGATAAGTTGTTTATACAATTGGTGCAAACGTACAACCAGCTTTTCGAGGAAAATCCTATTAACGCCAGTGGAGTCGATGCCAAAGCAATTCTGTCAGCAAGATTGTCTAAGTCAAGACGCCTTGAAAATCTAATCGCCCAATTGCCAGGTGAAAAGAAGAACGGATTGTTCGGCAATCTTATTGCACTTTCGCTTGGTTTGACGCCGAACTTTAAGTCAAACTTCGATTTGGCGGAGGATGCTAAACTTCAGTTATCCAAAGACACTTATGACGATGATCTCGACAATCTGCTTGCCCAAATTGGAGATCAATATGCAGACCTGTTCCTGGCTGCTAAAAACTTGAGTGATGCTATCCTGCTCAGTGACATCCTCAGAGTTAATACTGAGATCACTAAAGCACCACTCTCAGCAAGTATGATAAAGAGATACGATGAGCATCATCAAGACCTTACACTCTTGAAGGCTCTTGTAAGGCAACAGCTACCAGAGAAGTATAAGGAAATCTTTTTCGATCAATCCAAAAACGGTTACGCTGGATACATCGACGGGGGAGCATCTCAAGAGGAGTTCTACAAGTTCATCAAGCCAATCCTTGAGAAAATGGATGGGACTGAAGAGTTATTGGTGAAGCTCAACAGAGAGGATCTTCTAAGGAAACAGAGGACCTTTGATAATGGATCAATCCCACACCAAATCCATTTGGGTGAATTACATGCCATTTTGCGCCGTCAGGAGGATTTCTACCCTTTCTTAAAAGATAACAGAGAGAAAATTGAAAAGATTTTGACTTTCCGTATTCCGTATTACGTTGGACCCCTAGCAAGGGGTAACTCCCGATTTGCATGGATGACCAGGAAGTCTGAGGAAACGATTACACCTTGGAATTTTGAGGAAGTAGTCGATAAAGGCGCTTCTGCACAGTCTTTCATCGAGCGAATGACAAACTTCGACAAAAATCTGCCCAATGAGAAGGTGCTTCCTAAGCACTCTTTACTCTATGAATACTTTACCGTTTACAACGAGCTTACAAAGGTGAAGTACGTCACAGAGGGTATGCGGAAACCTGCTTTCCTTTCTGGCGAACAGAAAAAGGCGATTGTTGATTTGTTGTTCAAGACTAATCGGAAAGTGACAGTTAAACAACTTAAAGAAGATTACTTCAAAAAGATCGAATGTTTTGACTCAGTGGAAATATCAGGTGTAGAGGATCGTTTCAATGCGTCTCTTGGGACTTATCACGATTTGCTGAAGATCATTAAGGATAAGGACTTTTTAGACAATGAGGAAAATGAGGACATCCTCGAAGATATTGTATTGACTCTCACGCTATTTGAAGATCGAGAGATGATCGAGGAGAGGCTTAAAACGTATGCACATCTTTTCGATGACAAGGTGATGAAGCAACTGAAACGACGCAGATATACCGGTTGGGGAAGGCTCTCTCGGAAGTTGATCAATGGAATACGTGATAAGCAGTCTGGAAAGACAATCCTCGACTTTTTGAAAAGTGATGGATTTGCTAATCGAAATTTCATGCAACTTATTCACGACGACTCACTCACGTTCAAAGAGGACATTCAAAAGGCACAAGTATCAGGGCAGGGAGATTCCCTCCATGAACATATTGCAAATCTGGCCGGTTCTCCCGCAATTAAGAAAGGCATACTTCAAACAGTCAAAGTGGTCGATGAATTAGTTAAAGTAATGGGTCGTCATAAGCCAGAGAATATTGTTATTGAAATGGCTAGGGAAAACCAGACCACTCAGAAAGGACAGAAAAACTCAAGAGAAAGGATGAAACGAATCGAGGAGGGAATCAAGGAGCTTGGTAGCCAAATCTTAAAAGAGCATCCTGTCGAGAATACCCAACTTCAAAACGAGAAATTGTATCTTTACTACCTCCAAAATGGTAGGGATATGTATGTAGATCAGGAATTAGACATTAATCGGCTCTCGGATTATGATGTTGACCATATCGTTCCTCAGTCTTTCCTTAAAGATGATTCGATAGACAATAAGGTCCTGACTAGATCCGATAAGAACAGAGGAAAGAGTGATAATGTTCCCAGTGAGGAAGTTGTTAAGAAGATGAAAAACTACTGGAGACAGTTGCTTAACGCTAAACTGATTACTCAAAGGAAATTTGATAACCTCACTAAGGCTGAACGAGGTGGACTGAGCGAATTGGATAAAGCTGGATTCATCAAAAGACAATTGGTCGAGACAAGGCAGATTACCAAGCATGTGGCACAAATACTTGATTCAAGGATGAATACCAAATATGATGAGAATGATAAACTTATTAGAGAGGTTAAAGTAATTACACTGAAATCTAAGTTGGTGTCTGATTTCCGTAAGGATTTCCAGTTTTACAAAGTGCGAGAAATCAATAACTACCACCACGCGCACGACGCATATCTCAACGCTGTTGTCGGGACAGCCCTCATTAAGAAGTATCCTAAGCTCGAATCAGAGTTCGTTTATGGTGATTACAAAGTTTATGATGTCCGCAAAATGATTGCAAAATCAGAGCAAGAGATTGGTAAAGCGACAGCCAAATACTTTTTCTATTCTAACATTATGAACTTTTTCAAGACTGAAATAACTCTGGCGAATGGGGAAATTCGTAGAGGCCTTTGATTGAGACAAACGGTGAGACTGGAGAGATAGTATGGGACAAAGGCCGCGATTTTGCTACTGTTAGGAAGGTTTTGAGTATGCCGCAAGTAAACATCGTTAAGAAAACAGAAGTTCAGACTGGAGGTTTTAGTAAGGAGAGCATCCTGCCAAAGAGGAACTCCGATAAGCTCATCGCTCGTAAAAAGGATTGGGACCCGAAAAAGTATGGCGGTTTCGACTCTCCTACTGTTGCCTATAGCGTACTTGTCGTGGCCAAGGTCGAGAAAGGAAAAAGCAAAAAGCTCAAGAGCGTTAAGGAACTCCTTGGTATCACTATAATGGAAAGATCGTCATTCGAGAAAAACCCGATAGACTTCCTAGAAGCTAAAGGGTATAAAGAGGTCAAAAAGGATCTCATTATCAAACTGCCTAAGTATTCGCTATTTGAATTGGAGAATGGTAGAAAGAGAATGCTTGCAAGTGCTGGAGAACTTCAGAAGGGAAATGAGCTCGCTTTGCCGTCAAAATACGTGAATTTCCTTTATCTCGCTTCACATTATGAGAAATTGAAAGGTTCACCAGAGGATAACGAGCAGAAACAGTTATTTGTGGAACAACACAAACATTACCTCGATGAGATAATAGAGCAGATAAGCGAGTTTTCGAAGAGGGTGATTTTGGCTGATGCAAATCTCGATAAAGTGCTCTCGGCATATAACAAGCATAGAGATAAGCCTATAAGGGAGCAAGCGGAGAACATTATTCACCTTTTTACCCTAACAAACTTGGGCGCACCAGCCGCTTTTAAGTACTTTGACACAACTATCGACCGAAAAAGATACACAAGTACCAAAGAGGTCTTGGACGCTACTTTAATCCATCAATCCATCACGGGACTTTATGAAACCAGAATTGATTTGAGCCAGTTAGGAGGAGATGGCGCCGGTTCTGGTACCGGCAAACGTCCAGCAGCGACTAAAAAGGCGGGTCAGGCCAAAAAGAAGAAATGA**



The schematic view of multisite Gate-Way cloning to make destination vector carrying modules of CRISPR-mediated CsCCR4 gene knock out.

**Supplementary data 4**

**Drop Digital PCR Protocol**

Time considerations:

Prepare samples (~1.5 hours)

Prepare droplets (~0.5 hour)

Cycling (~2.5 hours)

Data Analysis

1. **RESTRICTION DIGEST**

100 ng of genomic DNA was digested in a 20 μl reaction for 1-2 hours. 10 x over-digestion is recommended (1μl enzyme per 1000ng DNA).



1. **PREPARATION OF ddPCR MASTER MIX**

\*\* Reagents were allowed to equilibrate to room temperature before use and were mixed by vortexing and spinning down

Maximum concentration of 50ng/μl digested/fragmented DNA should be used.

**We ordered primers & probes mixed together.** 

1. **PREPAREATON OF DROPLETS**

* ddPCR plate (blue)
* Foil for sealing PCR plate
* Droplet maker cassettes/cartridge
* Gaskets for droplet maker holder
* Rainin 8 channel pipette (20, 50 & 200μl volumes)
* Rainin filter tips (red & green)
* Droplet oil (check it is not control DNA labels very similar)
* Thin sharpie for labeling
* Small volume reservoir for oil

**Procedure of droplet generation was done the same following order:**

1. Turn on droplet maker
2. Turn on plate sealer warm up to 105C (takes ~10 minutes)
3. Locate cassette holder (there are 2)
4. Label droplet cassette.
5. Take a droplet maker cassette and add 20μl (Rainin 20μl pipettor) of sample to well (smallest of the 3 in the center
6. Add 70μl of oil to the oil reservoir (medium size well).
7. Place cassette in to cassette holder (A is at small end of cassette) and snap close
8. Add gasket (single use) on hooks
9. Place assembled unit in to droplet generator and close lid.
10. Droplets are produced in the third and larges well
11. Transfer all droplets using multichanel pipette and tips about 40 μl in to the PCR plate.
12. Start assembling the next cassette. Repeat until all samples are done
13. Seal the filled PCR plate with a single foil
14. Carefully carry the sealed plate to a PCR machine. The droplets are fragile do not carry on a cart.
15. Run PCR program CAMDDPCR about 2 ½ hours.

CAMDDPCR: (worked for our experiment)

Heated lid 105 C

95 c for 10 min

94 C for 30 sec X50 using 2 C ramp per cycle

60 C for 1 min

98 C for 10 min

12 C forever

\*\*Basic was as follow:

95 C 10 minutes

45-50 cycles of:

94 C 30 seconds

60 C 60 seconds

98 C 10 minutes

4 C forever

50% ramp (2-3 C/second)

Heated lid 105 C

**Quanta Soft Template Was Created in the software**

**Reading the Cycled PCR Plate**

1. Remove the cycled PCR plate from the thermocycler Turn on the plate reader. Check reader oil and waste containers levels
2. Turn on Computer open Quanta soft
3. Open lid of plate reader
4. Remove plate holder
5. Open cassette place plate in to holder.
6. Close black levers on the left and right of the plate holder
7. Open the template file
8. Select run- window opens check dye combination Hex/Fam
9. OK- Run takes 10 min per 8 samples.
10. After Run close software, remove plate from reader.
11. Copy folder generated by Quant soft from C drive onto a network drive.
12. Turn off reader and computer
13. Analyse the data.

**supplementary data 5**

Table 1- **primers and probes for TDNA Copy number estimation**

|  |  |
| --- | --- |
| cas9 int1 | Sequence |
| probe | 5'-/56-FAM/CGCCACTCT/ZEN/ATCAAGAAgaaCCTCATCG/3IABkFQ/-3' |
|  |  |
| primer1 | 5'-CAATCTAGTAGCCTCAGCAGTC-3' |
| primer2 | 5'-CCTCCAAGAAATTCAAGGTCCTA-3' |
| CS ALS 9830 (on sub-genome 2) | |
| probe | 5'-/5HEX/CAACTCTGC/ZEN/ACTATCTCCAATGCCTGA/3IABkFQ/-3' |
| primer1 | 5'-TTCTGACTTTGTAGCCGATGTT-3' |
| primer2 | 5'-CTCGGTTCATGTCTCTCCAATC-3' |

*Table 2*-**drop-off experiment primers and probes**

|  |  |
| --- | --- |
| F primer | TTCGTGTTGCAGAGGATGAG |
| R primer | GGCGATGCGAGGTGAAATA |
| Ref probe(FAM) | 5'-/FAM/CTAGAAGCT/ZEN/CTCGAAGGTGCAGCC/3IABkFQ/-3' |
| Target probe(HEX) | 5'-/5HEX/ACGCCTCCA/ZEN/TCTATTCGAGATGGATCT/3IABkFQ/-3' |

*Table 3-* primers used in TOPO TA cloning for sequencing

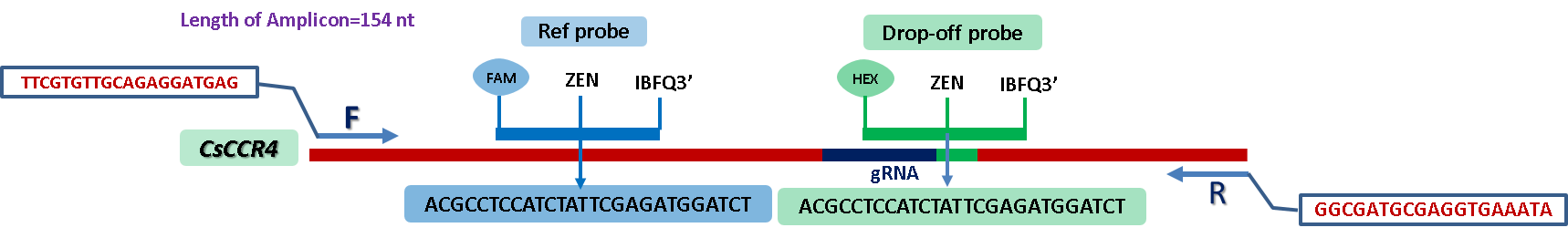
|  |  |
| --- | --- |
| **primer** | **sequence** |
| M13 F | 5´-GTAAAACGACGGCCAG-3´ |
| M13 R | 5´-CAGGAAACAGCTATGAC-3´ |
| T3 | 5´-ATTAACCCTCACTAAAGGGA-3´ |
| T7 | 5´-TAATACGACTCACTATAGGG-3´ |

**supplementary data 6-a**

E:\Dana caroll presentation papers\CAMELLINA ATTICLE\COPY NUMBER.tif

1. Schematic view of primers and probe designed to identify the copy number of T-DNA amongst T1 events. The reference probe is bound to an amplicon with the origin of the *CsAls* gene in which its amplification is considered one copy as compared with the *Cas9* intron probe (FAM) which represents the copy of T-DNA. The graph show T-DNA CN in the T1 population.

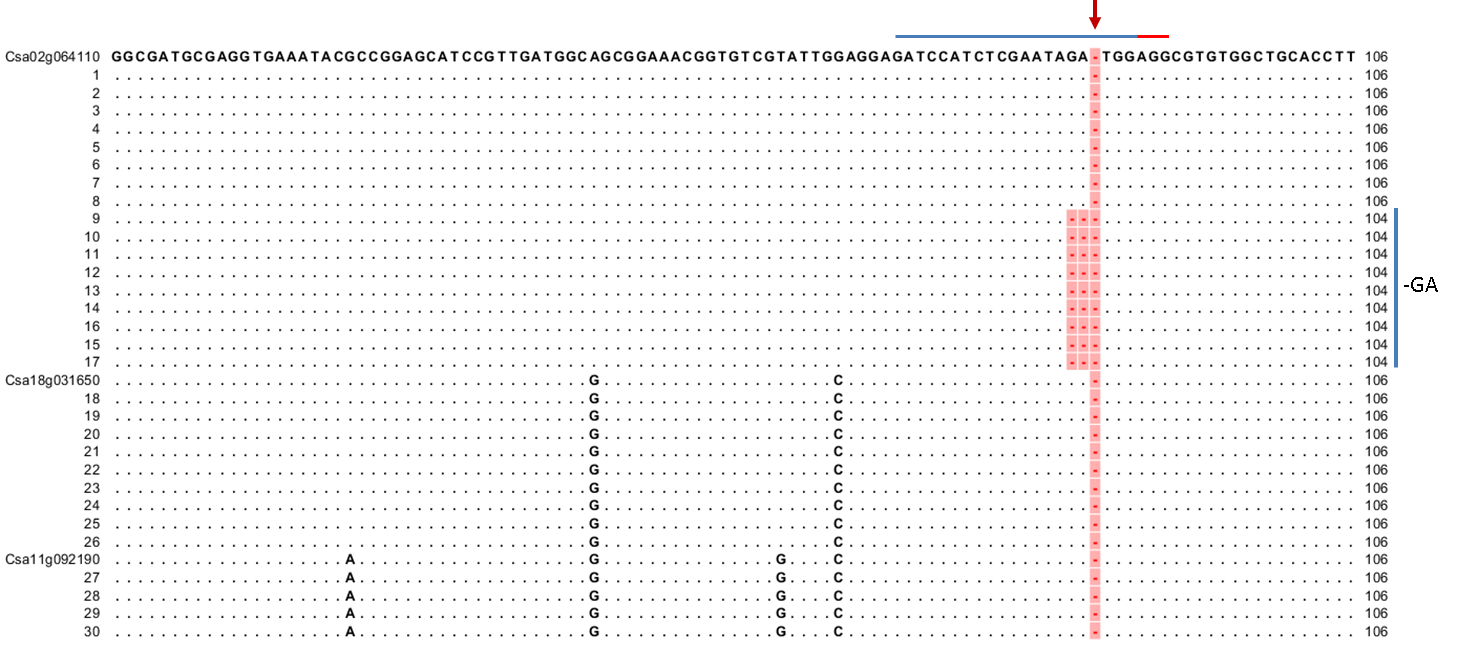
**supplementary data 6-B**



B - Schematic representation of primers and probes designed for mutation detection via drop-off assay. Forward and reverse primers generate an amplicon in which both reference (FAM) and drop-off probes bind to their targets during PCR reaction in the thermal cycler.

**supplementary data 7**

Sequencing results for 30 TOPO TA cloned of three T2 generation plants around the interest cut site of CsCCR4 mutation are represented. The wild type name of each version of *CsCCR4* are shown in the left of the picture. The WT versions are used as reference to assign sequencing results to each sub-genome based on the SNPs on CsCCR4 genes. Same nucleotides are indicated as dots. SNPs, G----C and A…G…G…C for chromosome 18 and 11 respectively, are bolded in the left region of the cut site. The position of gRNA is shown in blue line on the top and PAM is represented in small red line.

**T2 plant 10**

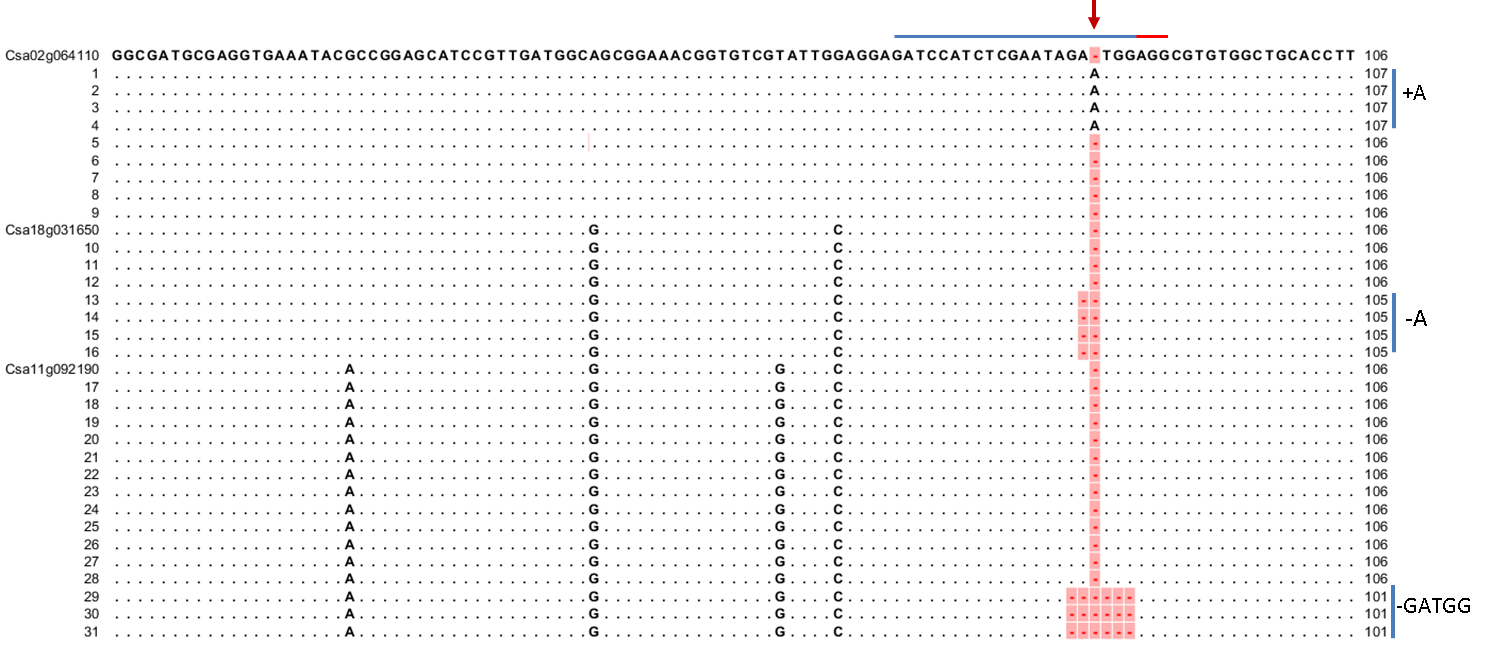
**PAM**

**gRNA**

**T2 plant 15**

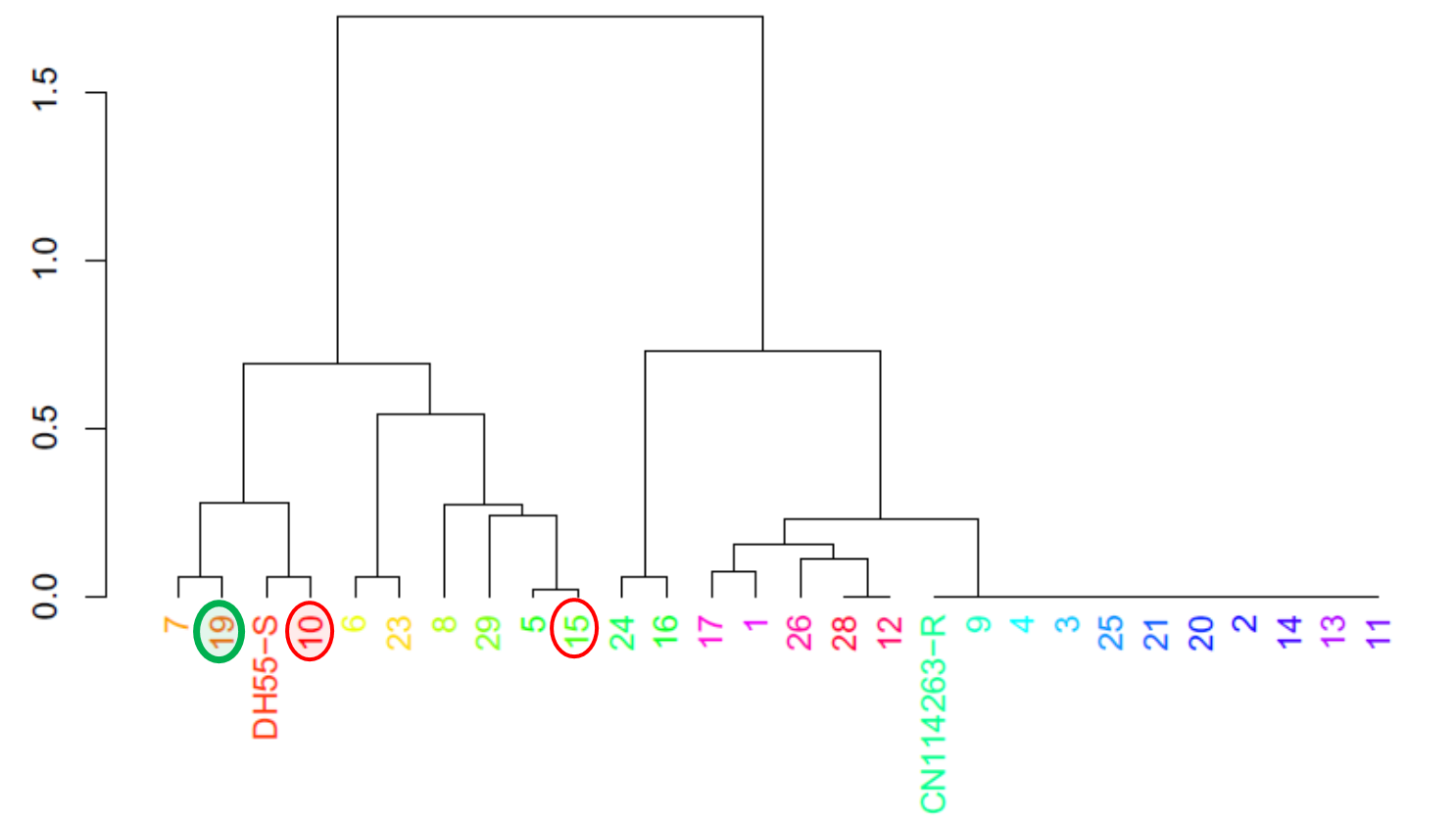


**T2 plant 19**



**Supplementary data 8**

T3 population’s response to Sclerotinia inoculation. Some events show resistance- to the pathogen- in left- while some of them are similar to susceptible lines in the right.



T3 clustering base on the level of the lesion using the Neighbour-Joining method.