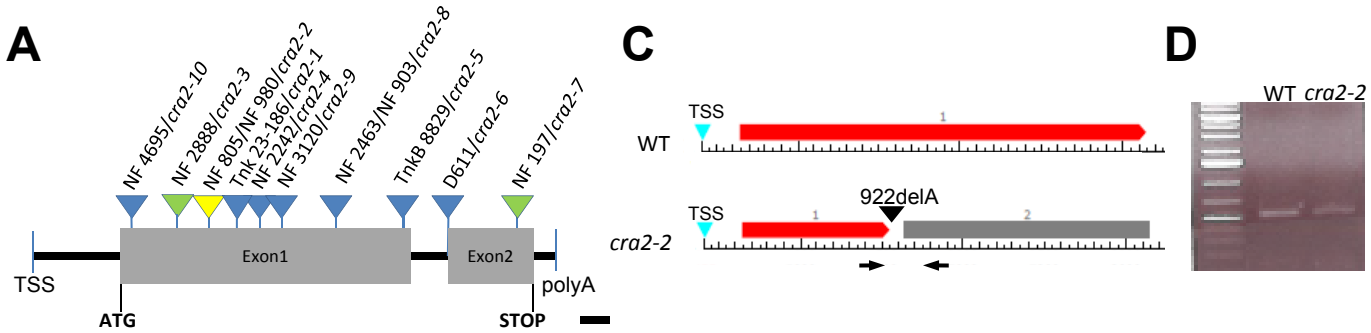

B

```
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
```

T74
ATGAACCATCATCAACCATTCTTCTTCTTTGGACTAATAGTTATTATTTCCATGACCAACATGTCACATGTTATCAGCACAAACCAATCTGAGTTTTTTTCTCTAATGAAAGAGTCTCTCTCAG
GAAACTACCCTTTTGATTGGGGTGTAAGCAAAGTGGATAAACCAATCTGCGACTTCACCGGAATCACCTGTGACAACAAAGGAGACATCATAAGTCTTGATTTTTCAGGTTGGTCATCACTTTC
CGGAAACTTCCCGTCAAATATTTGCTCTTACCTTCCAAATTTACGTGTCCTAAACCTCGGTAATACAAAATTCAAGTTCCCAACAAACAGCATAATCAACTGTTCTCACTTAGAACTACTCAAC
ATGAACAAGATGCACCTGTCAGGAACACTTCCTGATTTCTCGTCTTTAAAATATCTCAGAGTCCTCGACTTATCATACAACTCCTTCACCGGCGATTTTCCCATGTCTGTTTTCAACCTCACCA
ATCTTGAAATTCTCAACTTCAACGAAAACAGTAAACTCAATCTTTGGGAATTACCAAAAAGCTTTGTAAGATTGAGAAGTCTTAAATCCATGATTTTATCAACATGCATGTTGCATGGTCAAAT

T705
TCCACCATCCATATCAAATATAACAACACTCATTGATCTTGAATTAAGTGGAAATTTCCTCACAGGTCAAATTCCAAAAGAACTTGGATTGTTGAAAAACTTGCAACAGCTTGAACTTTACTAT
AACTACTTCCTTGTTGGCAGCATCCCTGAAGAGCTTGGAAATTTAACAGAACTTGTCGATTTAGATATGTCGGTAAACAAGTTAACTGGAACAATCCCTTCTTCTGTTTGTAAACTTCCAAAGC

922delA G925
TTCAGGTTCTCCAATTCTACAATAACAGTCTCACAGGTGAAATTCCAAAATCAATTGAAAACTCAAAAACACTTAGAATATTGTCACTTTACGACAATTTCTTAAGTGGACACGTTCCTGCGAA
ACTAGGACAATCCTCCGGAATGGTTGTTCTTGATTTGTCCGAAAACAAGTTGTCTGGCCCTTTGCCAGAACATGTCTGTCAAGGAGGTAAACTGTTATATTTTCTTGTTTTGGATAACTTTTTC

G1163
TCTGGTGTTATACCAGAAAGTTATTCAAATTGCATGTTTCTGTTAAGGTTTCGTGTCAGTAATAACCGTTTGGAAGGTTCTGTTCCAAAAGGACTCTTGAGTTTATCACATGTTTCAATCATTG

T1317
ATTTGAGCAGCAACAATCTGACCGGTCCGATTCCCGAGATCAACGGAAATTCTAGAAACTTGTCTGAGCTTTTCCTTCAGAGGAACAAGATTTCAGGACAAATAACACCAACAATCTCTAGTGC
TTATAACCTTGTCAAAATTGATTTCAGTTATAACTTTCTTTCTGGTCCAATTCCTTCTGAGATTGGTAACCTTAGAAAGCTTAACTTGCTTATGCTACAAGCAAACAAGCTGAACTCTTCTATT
CCTGATTCATTCTCTTCCTTGGAATCACTCAACCTTCTAGATCTTTCCAGTAATCTCTTAACCGGAAACATCCCTGAAAGCCTCTCTGTGTTGTTGCCTAATTCTATAAACTTTTCACACAATC
TTCTTTCTGGTCCTATTCCTCCTAAATTGATTAAAGGAGGGTTAGTTGAAAGCTTTGCAGGGAATCCTGGTTTATGTGTGATGATGCCGGTTAATGCTAATTCATCTGATCAAAGAAACTTTCC

G1785
ATTATGTTCACATGGTTACAAGAGTAAGAAGATGAACACTATATGGGTGGCCGGTGTATCTGTGATTTTGATTTTTGTAGGTGCTGCTTTGTTCTTGAAAAAAAGGTGTGGCAAAAATGTGTCT
GCGGTTGAACACGAATACACACTCTCTTCATCATTTTTCTCTTATGATGTTAAGAGTTTTCACATGATTAGTTTTGATCAAAGAGAGATTGTTGAGTCTTTGGTTGATAAGAATATAATGGGAC
ATGGAGGGTCAGGGACAGTGTACAAAATCGAGCTGAAAACCGGAGATGTTGTGGCGGTTAAGAGGCTTTGGAGTCGAAGTTCGAAGGATTCTTCGCCAGAAGATGCATTATTTGTGGACAAGGC
ATTGAAAGCTGAGGTGGAAACACTTGGAAGCATAAGACACAAGAACATTGTTAAGTTGTATTGTTGCTTCTCAAGTTTGGATTGTAGCTTGTTGGTTTATGAGTATATGCCAAATGGTACTTTG
TATGATTCTCTTCACAAGGGTTGGATTCATTTGGATTGGCCTACAAGGTATAGGATTGCACTTGGAATTGCTCAGGGTGTTGCATACCTTCACCATGATTTGGTTTTTCCTATTATCCATAGAG

A2388
ATATTAAGTCAACAAATATTCTCTTGGATGAAGATTACCATCCTAAGGTTGCAGATTTTGGGATTGCTAAGGTTTTGCAGGCAAGAGGAGCAAAGGATTCCACAACCACGGTCATTGCTGGAAC
TTATGGTTACCTAGCCCC*AGGTAAATAAATTCTCAATTTAACCTATTAATGTCATCGACCAAATCACGAGAATTAAGAAAGTTGACCTTAATATGAAATTTGTTGCCAATTAATATTGTTTTAC*
AAGCTTATCCTTTAGAGAACACGTTTAATTTATATTTTCATCATAGGACTTACTTATTACAGGTTGCAGAAAACTAAATATAATTAGAAGTATGTTGGTAAAATAATAATTAACACAGTTAAAA
CTTCAAAAGGATTATATATTTAGGGACAGAGGTAGGATTTGATTAAACAGTATTTTGTATTAGGGTATTAGTGATGTGGTTAACTTAAGCGGTAAGTAATCTTATTATCATATTGTAAGCTTGT
*TTAAATCTATTTAATTTGTTTTGATGGATGGTGTGACAGA*ATATGCATATTCACCAAGAGCAACCACAAAGTGTGATGTGTACAGTTTTGGTGTGATTTTGTTGGAATTATTAACTGGGAGGAA

G3006 T3045
GCCAATTGAGTCAGAATTTGGAGAAAACAGAAACATTGTATTTTGGGTTGCAAACAAAGTTGAAGGCAAAGAAGGAGCTAGACCAAGTGAGGTTTTTGATCCAAAGTTATCATGTTCATTCAAA
GATGATATGGTTAAAGTATTAAGGATTGCCATTAGATGCAGCTACAAAGCACCAGCTAGTAGACCAACCATGAAAGAAGTTGTTCAGCTTCTGATTGAGGCAGAGCCAAGAAAATCTGATTCTT
GCAAGTTGTCAACCAAAGACGTATCGACAAATGTCACCTTAGTAAAGAAATCATTTGAATTATAA

E

```
MNHHQPFFFFGLIVIISMTNMSHVISTNQSEFFSLMKESLSGNYPFDWGVSKVDKPICDFTGITCDNKGDIISLDFSGWSSLSGNFPSNICSYLPNLRVLNLGNTKFKF
PTNSIINCSHLEQLNMNKMHLSGTLPDFSSLKYLRVLDLSYNSFTGDFPMSVFNLTNLEILNFNENSKLNLWELPKSFVRLKSLKSMILSTCMLHGQIPPSISNITTLI
DLELSGNFLTGQIPKELGLLKNLQQLELYYNYFLVGSIPEELGNLTELVDLDMSVNKLTGTIPSSVCKLPKLQVLQFYNNSLTGEIPKSIENSKTLRILSLYDNFLSGH
                                                                                                LKTQKHLEYCHFTTIS*

VPAKLGQSSGMVVLDLSENKLSGPLPEHVCQGGKLLYFLVLDNFFSGVIPESYSNCMFLLRFRVSNNRLEGSVPKGLLSLPHVSIIDLSSNNLTGPIPEINGNSRNLSE
LFLQRNKISGQLTPTISRAYNLVKIDFSYNFLSGPIPSEIGNLRKLNLLMLQANKLNSSIPDSFSSLESLNLLDLSSNLLTGNIPESLSVLLPNSINFSHNLLSGPIPP
KLIKGGLVESFAGNPGLCVMMPVNANSSDQRNFPLCSDGYKSKKMNTIWVAGVSVILIFVGAALFLKKRCGKNVSAVEHEYTLSSSFFSYDVKSFHMISFDQREIVESL
VDKNIMGHGGSGTVYKIELKTGDVVAVKRLWSRSSKDSSPEDALFVDKALKAEVETLGSIRHKNIVKLYCCFSSLDCSLLVYEYMPNGTLYDSLHKGWIHLDWPTRYRI
ALGIAQGVAYLHHDLVFPIIHRDIKSTNILLDEDYHPKVADFGIAKVLQARGAKDSTTTVIAGTYGYLAPGK*
```

Supplementary Figure 5. *CRA2* gene structure and mutant allele location

A, Predicted *CRA2* gene model (FGenesh) and localization of the 10 mutant alleles that were identified by forward or reverse genetic screens. The blue arrowheads are alleles that are tagged by the *Tnt1* retro-element insertion; the green arrowheads are alleles that are tagged by another insertional element; and the yellow arrowhead is an allele containing a deletion of one nucleotide. Bar = 250 nucleotides; TSS= predicted Transcription Start Site; polyA: predicted polyadenylation site.

B, Nucleotide sequence of the *CRA2* genomic region (from the predicted initial ATG start codon to the stop codon) locating the 10 mutant alleles (arrowheads; position related to the predicted ATG).

C, Prediction (FGenesh) of a splicing site variant mutation in the *cra2-2* allele carrying a single-nucleotide deletion. Red box (1): WT Exon 1; Grey box (2): new exon that was predicted from the new splicing site. The arrows represent the primers that were used for the RT-PCR as shown in (**D**).

D, RT-PCR analysis of the region containing the predicted splicing site in the *cra2-2* allele. No differential splicing was detected including after sequncing of the PCR product.

E, Sequence of the CRA2 protein. The arrowhead shows the truncated protein that was generated by a frameshift in the *cra2-2* allele carrying a single-nucleotide deletion.