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**Figure S1.** Field plot design. Control field plot with non-transformed Golden Promise (**A**) and the cisgenic field plot (**B**). Total area per plot is 6 x18 m (108 m2). Plots were surrounded by a border of 1.5 m non-transformed Golden Promise barley (Grey) for pollen catching, leaving the actual experimental plots to 3 x 15 m = 45 m2. The cisgenic plot was divided into three areas, which were harvested separately.

**A**

CAGTGCCAGTATCCAGTGAGGCTGGCCGGCCGTGTCCCGCACTGTCGTCGCCGCCGCCGGCAGAACAAATCGCTAGGAAGGAAGGAACACCGGCTGGCGAATGGGGCTGGAGGGGGCGAATTTGGGGGGAGAAATCTGCGAGGAGACAAGGCGACGGGAAGGATGAACGTTGGAAACGGGGCAAATCCACGATTTTATTAGTCGGTTTATATAGGAGACACCAGCACTAATGGCTGTTCTACCGAATTGGCCGGGAGAAGGCCCCGACGTCTCCCGTCACACACAGTCTGCTGAAAACCCTCACCTTTGAGTAGATTTTAGATTTAGGGGTGTCTGACTCTTGTCTAGATAAGAAATACTGTAATTATGTCTTGTGTAACTCCTACACCATTGAATTTTACGTGGAGATTCATAGGATCTTTTTTCTTTTGCCTTTTGTTTGGTTAGAATATTACAGTATATAGGTTAGATGAGACTTTGTTAATTGTCATTTACTGCCTTCGTTCGTAGATGTAAGTTTTTTTTTAAAATTACACTATTAAACAACATACGGATGTACATAGATATATTTTAAAATATAAATTTATTTATTTTATTTTGTA*TGCAGCCTCCTGTTAAAATGTCCAA*AAAGGTTTATACTATTTAGGAAGTGAGAGAGTAGATGGGAATTAACAACTTCATTGAATTTAACCTTGAAATGCAATACGTACTGTTTAGTGTGTGTATGGCCGATAGATA

*atattgtggtgtaaac*gttcctgcggccgc

atcttgggcaacatatcaggggcagcgccattgccctgcgactgacggcggcggtggaggagcttggggcagacatgagctgagaacgacgagagagaggagtggtggcgggcgagacagaggagcgacatgattgaagaagagcagcgggattgaggattagggattcctgcgattttacacttgacctctccataaaagattggcctaatcgaagctgagaacgtggaggtcaacaagtggtcaaacgagcctgtacgcaccgcatacgagcaacagtgatcggattttcacgtcacatcgtatatagtgatcgtaaaagccatattctaaagttggatgaccgtattgtgcttccat*gtcaactgcaaggaccgtgagtgt*atttatctctaaaat

**B**

AGTGGTAGATAGGACTCGAGAAAATATTAGTCTTACCTTTAGCTCCTTATGGGTTCGACACTTTATACTTATCACTTCCATCTTCGGAAAGTTCTACGATGATTCCTTGCACTTGGGAATTATCAAAGGGTCGTCGAGGCCTGTCACGCGGAGGAAATGGTGGCGACAGAGGCATCTGATGCGGCGGCGCGGGCGACCGGAGAGAAGGAAGCCATTCGCGTCCGTATTTTGAAGAAACGTATTTTGAAGAAACAGCAACGGAGGAACACGCGCGGCCTCGCCCGAGATCAGAA*TCGTGCGGTCCGCGAAATGG*CTGGATTGCCACAAAAGGAGGAGGAGGAGGTGAGCGACGATGAGAACAGCTCCGATGACAAGAAGATCCGGTTGGATCCATATTAGGTCTTGGACTGGTACTTAGGCGAGAAAGACAGCAAGGTCTCCGGGAAGGACAAGGAGAGTCGTGGCTAATGTCCACCGTAGTCATACATGTTAAATTTTGATAGTCCAATGAGATGTTAATATTAATGGAGTAGCCGGATGATATAT

*atatattgtggtgtaaac*gttcctgcggccgc

atcttgggcaacatatcaggggcagcgccattgccctgcgactgacggcggcggtggaggagcttggggcagacatgagctgagaacgacgagagagaggagtggtggcgggcgagacagaggagcgacatgattgaagaagagcagcgggattgaggattagggattcctgcgattttacacttgacctctccataaaagattggcctaatcgaagctgagaacgtggaggtcaacaagtggtca*aacgagcctgtacgcaccgc*atacgagcaacagtgatcggattttcacgtcacatcgtatatagtgatcgtaaaagccatattctaaagttggatgaccgtat

**C**

GATCATATATTTTATATTACTAGATAAATGAGCATTCTTTGCAATGGGGTACAAATATTTTGTACACAATTGATACTTCTCCGACATATCTATATATCTTTTAATATTTCATGCTATTATCATACCACTTCACATATTTTTTAGCAACAATTTATGTTATTTTTATTTGGATTAACATATT*TGATAGAGGCGAAGGTGTCC*CGATATTTCAATGAGATGACAACTATCGATTTGGTGGAGACGAATTTGACGTTCCGACTACAAACGTGCACGACGTTGCGCCTTAGCAATCGCTAAACCAACTCCAAGAGGTTATGGACCACGCCAGAGCACGATCAACCTGACCACGAAAGTCTATTCCTGCAAGCAATCGAAGAACAAGTAAGAATATGATATTGCAATCTGAATATTGTGAATATAGATAAAGTATTGATAAGGGTGGGGATCCGAAAGCGGTCTTAGTCTGGTC

*gtaaac*gttcctgcggccgc

atcttgggcaacatatcaggggcagcgccattgccctgcgactgacggcggcggtggag*gagcttggggcagacatgag*ctgagaacgacgagagagaggagtggtggcgggcgagacagaggagcgacatgatt

**Figure S2.** Left-border flanking sequences of the three *HvPAPhy\_a* inserts.

The whole LB-flanking sequence of each insert showed 99 % homology to a corresponding sequence in Barke using the Webblast ipk-gatersleben.de/barley and for each flanking area no other homologies were found for the whole sequences. The LB-flanking sequence identified for inserts in PAP07 (**A**), PAP 05 (**B**) and PAP03 (**C**) showed 99 % homology to a sequence on chromosome 3HS, 3HL and 2HL, respectively. Capital letters: LB-flanking sequences; LB-sequences integrated (grey), multiple cloning site (yellow). Lower case letters: Sequences of the first part of the *HvPAPhy\_a* promoter. Primers used to amplify the flanking sequences in the current study are shown in red (Table S1).

**Table S1.** Sequences of forward and reverse primers, PCR-product size and conditions for the PCR reactions for the Left T-DNA border flanking areas of the *HvPAPhy\_a* inserts in PAP07, PAP05 and PAP03.

|  |  |  |  |
| --- | --- | --- | --- |
| Primers | Sequences | PCR-products | Conditions for PCR reactions |
| Primers for the amplification of the Left border (LB) of PAP07 | Forward: 5’-TGCAGCCTCCTGTTAAAATGTCCAA-3’Reverse: 5’-ACACTCACGGTCCTTGCAGTTGAC-3’ | 550 bp | An initial step of 95°C for 5 min, then 35 cycles of 95°C for 30 s, 60°C for 30s, 72°C for 30s and then 72°C for 7 min  |
| Primers for the amplification of the LB of PAP05 | Forward: 5’-TCGTGCGGTCCGCGAAATGG-3’Reverse: 5’-GCGGTGCGTACAGGCTCGTT-3’ | 551 bp | Do  |
| Primers for the amplification of the LB of PAP03  | Forward: 5’- TGATAGAGGCGAAGGTGTCC-3’Reverse: 5’- CTCATGTCTGCCCCAAGCTC-3’ | 387 bp | Do  |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Generations | Parents or donor material forDH-production | Crosses or DH-production | Plants selected for further crossing /selfing or DH-production | Selected for MGPA analysis |
| Expected genotype of crosses | Identified genotypes by PCR |
| First cross | PAP07 (AAbb) | PAP05 (aaBB) | All AaBb | Nine F1 seeds germinatedAll AaBb  | Two randomly chosen F1-plants  | Seeds from four F1-plants.  |
| Second cross | Two F1-plants (AaBb) | PAP03 (aabbCC) | 1: AaBbCc; 1: AabbCc;1: aaBbCc; 1: aabbCc | Eight F1 seeds3: AaBbCc; 3: AabbCc;0: aaBbCc; 2: aabbCc  | One plant (AbBbCc) selfed and seeds used for DH-production | Seeds of all eight F1-plants  |
| DH-production | Twenty five F2-seeds (from self-pollinated AaBbCc) germinated and genotyped for A-,B-,C- by PCR of flanking sequences. Seven plants containing all three inserts used for anther cultures. Spikes with anthers for DH-production were randomly chosen among these seven plants. | If all combinations present in the seven plants then with random ratios:AABBCCAABBccAAbbCCAAbbccaaBBCCaaBBccaabbCCaabbcc  | Total green plants10: AABBCC14: AABBcc 5: AAbbCC  0: AAbbcc 0: aaBBCC 0: aaBBcc 0: aabbCC 0: aabbcc | Plants setting seeds 3: AABBCC11: AABBcc 3: AAbbCC |  3: AABBCC11: AABBcc 3: AAbbCC | Seeds of the 17 DH plants  |

**Table S2.** Crosses and production of DH-lines used for the stacking of the three *HvPAPhy\_a* inserts A-, B- and C- from the plants PAP07, PAP05 and PAP03, respectively.