SUPPORTING INFORMATION

The genetic basis of composite spike form in barley and 'Miracle-Wheat'

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SI FIGURES

Figure S1 Additional mutant alleles of spike branching in barley. Different *com2* mutant alleles identified by resequencing of the *COM2* ORF region. The *irregular spike25* mutant shows a higher degree of spikelet infertility. *HOR14427* is a double mutant of *com2*/hooded, see Table S1.

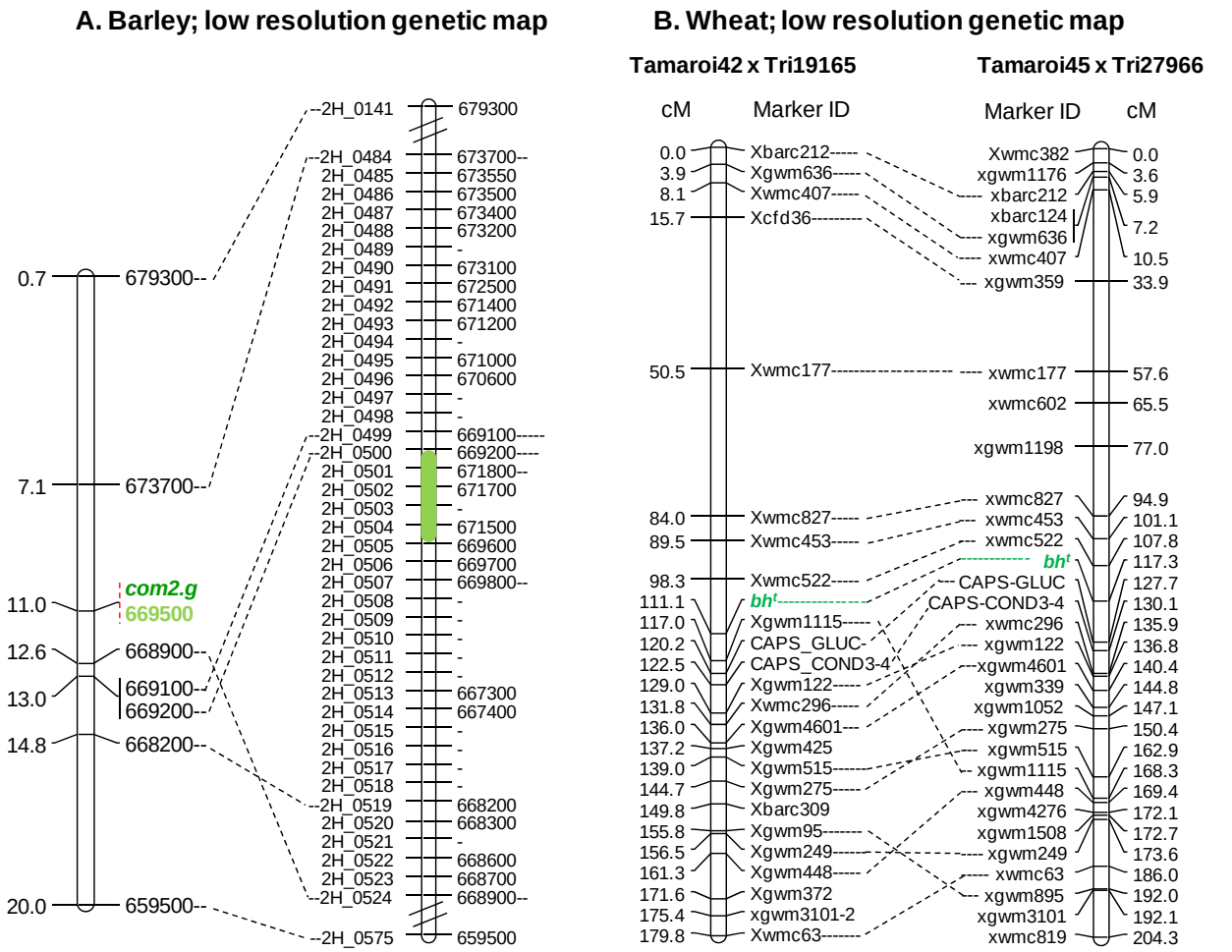

Figure S2 Low resolution linkage map of *com2.g* and *bh*^{t} *locus in* barley and tetraploid wheat. (A) Genetic linkage mapping of *com2.g* in barley. The genome zipper (GZ) model of barley chromosome 2H was considered as resource for marker development. Predicted order of rice genes along this barley chromosome is shown, all gene identifiers start originally with Os07g0. The virtual position for candidate gene ortholog *(Os07g0669500)* was not initially provided; a position (green area) was assumed for the *Os07g0669500* gene according to the gene identifier. Barley BW 192 (com2.g) and barley cv. Haruna Nijo were used as mutant and the wild type parents of the population, respectively. **(B)** Genetic linkage mapping of the *branched head*^{t} (*bh*^{t}) locus in two different tetraploid wheat F_2 mapping populations. Connected markers represent those used in both populations. cM stands for centiMorgan. Tetraploid wheat Tamaroi45 and Tamaroi42 were used as wild type while TRI 19165 and TRI 27966 were mutant parents of the corresponding population.

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Bowman                MSIRSSSGGSGGGQTSQMMAFSEHSLPKPIAGHPQPQPSPPSSPSERPAPRGRRRAQEPG 60
Irregular spike 25    MSIRSSSGGSGGGQTSQMMAFSEHSLPKPIAGHPQPQPSPPSSPSERPAPRGRRRAQEPG 60
Foma                  MSIRSSSGGSGGGQTSQMMAFSEHSLPKPIAGHPQPQPSPPSSPSERPAPRGRRRAQEPG 60
Donaria               MSIRSSSGGSGGGQTSQMMAFSEHSLPKPIAGHPQPQPSPPSSPSERPAPRGRRRAQEPG 60
Morex                 MSIRSSSGGSGGGQTSQMMAFSEHSLPKPIAGHPQPQPSPPSSPSERPAPRGRRRAQEPG 60
Optic                 MSIRSSSGGSGGGQTSQMMAFSEHSLPKPIAGHPQPQPSPPSSPSERPAPRGRRRAQEPG 60
Haruna Nijo           MSIRSSSGGSGGGQTSQMMAFSEHSLPKPIAGHPQPQPSPPSSPSERPAPRGRRRAQEPG 60
brc1.5                MSIRSSSGGSGGGQTSQMMAFSEHSLPKPIAGHPQPQPSPPSSPSERPAPRGRRRAQEPG 60
com2.k                MSIRSSSGGSGGGQTSQMMAFSEHSLPKPIAGHPQPQPSPPSSPSERPAPRGRRRAQEPG 60
Freak                 MSIRSSSGGSGGGQTSQMMAFSEHSLPKPIAGHPQPQPSPPSSPSERPAPRGRRRAQEPG 60
HOR14427              MSIRSSSGGSGGGQTSQMMAFSEHSLPKPIAGHPQPQPSPPSSPSERPAPRGRRRAQEPG 60
BM-NIL (BW192)com2.g  MSIRSSSGGSGGGQTSQMMAFSEHSLPKPIAGHPQPQPSPPSSPSERPAPRGRRRAQEPG 60
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Bowman                RFLGVRRRPWGRYAAEIRDPTTKERHWLGTFDTAQEAALAYDRAALSMKGAQARTNFVYA 120
Irregular spike 25    RFLGVRRRPWGRYAAEIRDPTTKERHWLGTFDTAQEAALAYDRAALSMKGAQARTNFVYA 120
Foma                  RFLGVRRRPWGRYAAEIRDPTTKERHWLGTFDTAQEAALAYDRAALSMKGAQARTNFVYA 120
Donaria               RFLGVRRRPWGRYAAEIRDPTTKERHWLGTFDTAQEAALAYDRAALSMKGAQARTNFVYA 120
Morex                 RFLGVRRRPWGRYAAEIRDPTTKERHWLGTFDTAQEAALAYDRAALSMKGAQARTNFVYA 120
Optic                 RFLGVRRRPWGRYAAEIRDPTTKERHWLGTFDTAQEAALAYDRAALSMKGAQARTNFVYA 120
Haruna Nijo           RFLGVRRRPWGRYAAEIRDPTTKERHWLGTFDTAQEAALAYDRAALSMKGAQARTNFVYA 120
brc1.5                RFLGVRRRPWGRYAAEIRDPTTKERHWLGTFDTAQEAALAYDRAALSMKGAQARTNFVYA 120
com2.k                RFLGVRRRPWGRYAAEIRDPTTKERHWLGTFDTAQEAALAYDRAALSMKGAQARTNFVYA 120
Freak                 RFLGVRRRPWGRYAAEIRDPTTKERHWLGTFDTAQEAALAYDRAALSMKGAQARTNFVYA 120
HOR14427              RFLGVRRRPWGRYAAEIRDPTTKERHWLGTFDTAQEAALAYDRAALSMKGAQARTNFVYA 120
BM-NIL BW192com2.g    RFLGVRRRPWGRYAAEIRDPTTKERHWLGTFDTAQEAALAYDRAALSMKGAQARTNFVYA 120
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Bowman                HAAYNNYPPFLAPFHAQPAYASSTMPYGGQQHAGAAPPHIGSYHSHGGVGYHQQGPGAGA 180
Irregular spike 25    HAAYNNYPPFLAPFHAQPAYASSTMPYGGQQHAGAAPPHIGSYHSHGGVGYHQQGPGAGA 180
Foma                  HAAYNNYPPFLAPFHAQPAYASSTMPYGGQQHAGAAPPHIGSYHSHGGVGYHQQGPGAGA 180
Donaria               HAAYNNYPPFLAPFHAQPAYASSTMPYGGQQHAGAAPPHIGSYHTHGGVGYHQQGPGAGA 180
Morex                 HAAYNNYPPFLAPFHAQPAYASSTMPYGGQQHAGAAPPHIGSYHTHGGVGYHQQGPGAGA 180
Optic                 HAAYNNYPPFLAPFHAQPAYASSTMPYGGQQHAGAAPPHIGSYHTHGGVGYHQQGPGAGA 180
Haruna Nijo           HAAYNNYPPFLAPFHAQPAYASSTMPYGGQQHAGAAPPHIGSYHTHGGVGYHQQGPGAGA 180
brc1.5                HAAYNNYPPFLAPFHAQPAYASSTMPYGGQQHAGAAPPHIGSYHTHGGVGYHQQGPGAGA 180
com2.k                HAAYNNYPPFLAPFHAQPAYASSTMPYGGQQHAGAAPPHIGSYHTHGGVGYHQQGPGAGA 180
Freak                 HAAYNNYPPFLAPFHAQPAYASSTMPYGGQQHAGAAPPHIGSYHTHGGVGYHQQGPGAGA 180
HOR14427              HAAYNNYPPFLAPFHAQPAYASSTMPYGGQQHAGAAPPHIGSYHTHGGVGYHQQGPGAGA 180
BM-NIL BW192com2.g    HAAYNNYPPFLAPFHAQPAYASSTMPYGGQQHAGAAPPHIGSYHTHGGVGYHQQGPGAGA 180
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                                                                  (L228H)

Bowman                GECSMPVPNAADHAASPMDVRSSGHDFLFPSADDNSGYLSSVVPESCLRPRGGDLQDARR 240
Irregular spike 25    GECSMPVPNAADHAASPMDVRSSGHDFLFPSADDNSGYLSSVVPESCHRPRGGDLQDARR 240
Foma                  GECSMPVPNAADHAASPMDVRSSGHDFLFPSADDNSGYLSSVVPESCLRPRGGDLQDARR 240
Donaria               GECSMPVPNAADHAASPMDVRSSGHDFLFPSADDNSGYLSSVVPESCLRPRGGDLQDARR 240
Morex                 GECSMPVPNAADHAASPMDVRSSGHDFLFPSADDNSGYLSSVVPESCLRPRGGDLQDARR 240
Optic                 GECSMPVPNAADHAASPMDVRSSGHDFLFPSADDNSGYLSSVVPESCLRPRGGDLQDARR 240
Haruna Nijo           GECSMPVPNAADHAASPMDVRSSGHDFLFPSADDNSGYLSSVVPESCLRPRGGDLQDARR 240
brc1.5                GECSMPVPNAADHAASPMDVRSSGHDFLFPSADDNSGYLSRVVPESCLRPRGGDLQDARR 240
com2.k                GECSMPVPNAADHAASPMDVRSSGHDFLFPSADDNSGYLSRVVPESCLRPRGGDLQDARR 240
Freak                 GECSMPVPNAADHAASPMDVRSSGHDFLFPSADDNSGYLSRVVPESCLRPRGGDLQDARR 240
HOR14427              GECSMPVPNAADHAASPMDVRSSGHDFLFPSADDNSGYLSRVVPESCLRPRGGDLQDARR 240
BM-NIL BW192 com2.g   GECSMPVPNAADHAASPMDVRSSGHDFLFPSADDNSGYLSRVVPESCLRPRGGDLQDARR 240
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                                                                  (S221R)

Bowman                YSVSDADAYGLGLREDVDDLASMVAGFWGGADAAYGGFAPANGGGHDMVASSQGSDNGYS 300
Irregular spike 25    YSVSDADAYGLGLREDVDDLASMVAGFWGGADAAYGGFAPANGGGHDMVASSQGSDNGYS 300
Foma                  YSVSDADAYGLGLREDVDDLASMVAGFWGGADAAYGGFAPANGGGHDMVASSQGSDNGYS 300
Donaria               YSVSDADAYGLGLREDVDDLASMVAGFWGGADAAYGGFAPANGGGHDMVASSQGSDNGYS 300
Morex                 YSVSDADAYGLGLREDVDDLASMVAGFWGGADAAYGGFAPANGGGHDMVASSQGSDNGYS 300
Optic                 YSVSDADAYGLGLREDVDDLASMVAGFWGGADAAYGGFAPANGGGHDMVASSQGSDNGYS 300
Haruna Nijo           YSVSDADAYGLGLREDVDDLASMVAGFWGGADAAYGGFAPANGGGHDMVASSQGSDNGYS 300
brc1.5                YSVSDADAYGLGLREDVDDLASMVAGFWGGADAAYGGFAPANGGGHXMVASSQGSDNGYS 287
com2.k                YSVSDADAYGLGLREDVDDLASMVAGFWGGADAAYGGFAPANGGGHDMVASSQGSDNGYS 300
Freak                 YSVSDADAYGLGLREDVDDLASMVAGFWGGADAAYGGFAPANGGGHDMVASSQGSDNGYS 300
HOR14427              YSVSDADAYGLGLREDVDDLASMVAGFWGGADAAYGGFAPANGGGHDMVASSQGSDNGYS 300
BM-NIL BW192com2.g    YSVSDADAYGLGLREDVDDLASMVAGFWGGADAAYGGFAPANGGGHDMVASSQGSDNGYS 300
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Bowman                PFSFLSH 307
Irregular spike 25    PFSFLSH 307
Foma                  PFSFLSH 307
Donaria               PFSFLSH 307
Morex                 PFSFLSH 307
Optic                 PFSFLSH 307
Haruna Nijo           PFSFLSH 307
brc1.5                PFSFLSH 307
com2.k                PFSFLSH 307
Freak                 PFSFLSH 307
HOR14427              PFSFLSH 307
BM-NIL BW192com2.g    PFSFLSH 307
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Figure S3 COM2 protein sequence alignment of different mutant alleles: Mutated positions between parents of the population BW-NIL(*com2.g*) and Haruna Nijo as well as other identified mutants that either shared the same mutation observed in the mutant parent of *com2.g* (S221R) (four mutants; *brc1.5*, *com.k*, Freak , HOR14427) or showed a different mutation (L228H) (one mutant; the *irregular spike 25*). The remaining cultivars represent the donor lines; see Table S1.

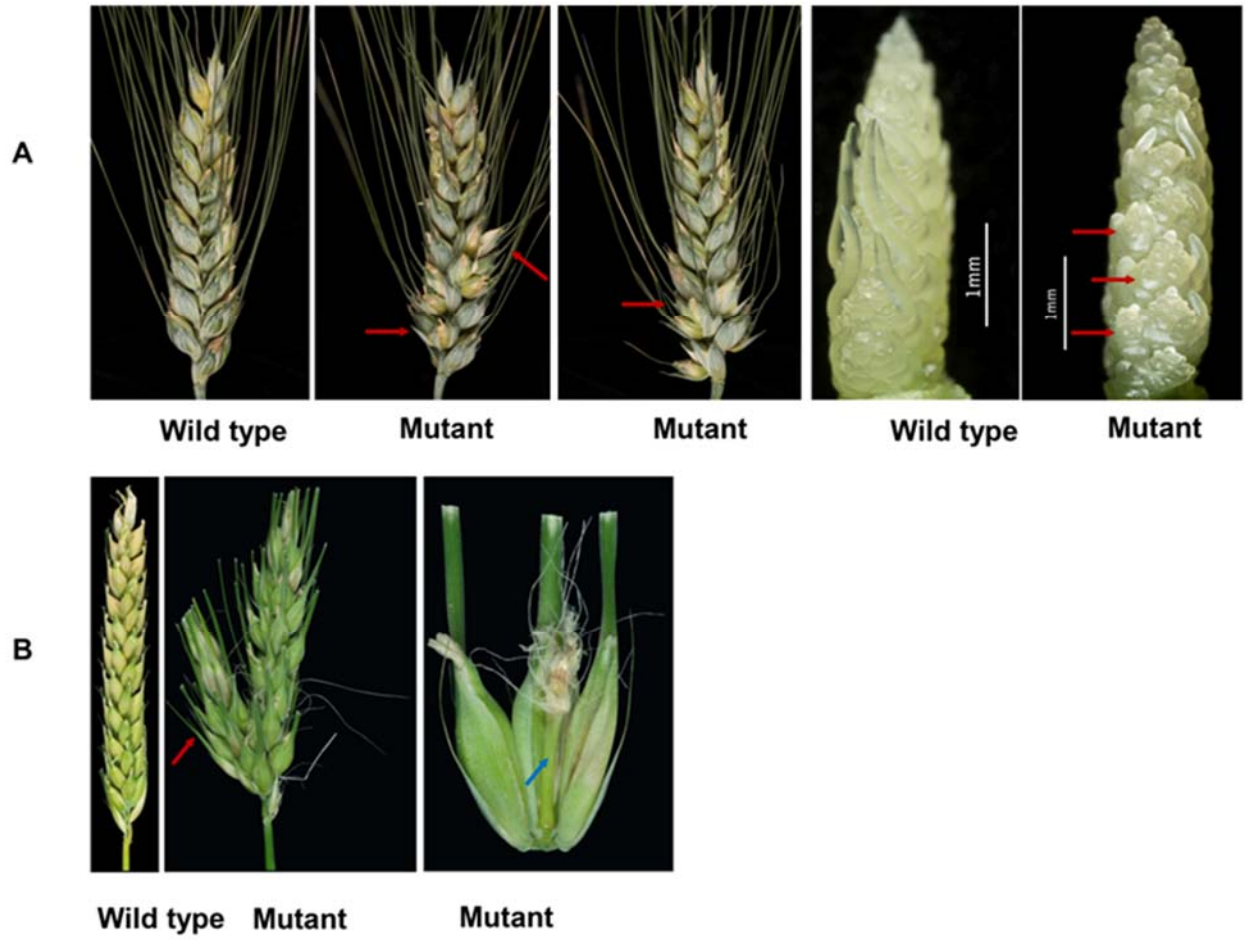
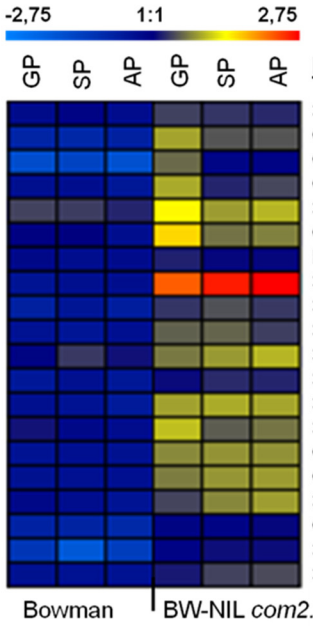


Figure S4 Phenotype of the tetraploid wheat and barley TILLING lines. (A). Supernumerary spikelet formation at the wheat homozygous TILLING plant T4-2447-7 (Mutant). The donor cultivar is tetraploid wheat cv. Kronos (Wild type). The images at the far right showed ectopic branch formation (red arrows) at early stage of development of cv. Kronos (Wild type) and the T4-2447-7 (Mutant). **(B)** Branch formation of the two barley TILLING mutant plants derived from barley cv. Morex (Wild type). Branch formation showed a range of severity from formation of a small-sized secondary spike (red arrows) to an extended rachilla at the central spikelet (blue arrows).

A



HarvEST-ID	ATH_SEQ_DESC	FUNCAT
35_4012	IAA8 (indoleacetic acid-induced protein 8); transcription factor	hormone metabolism,auxin,induced-regulated-responsive-activated
Contig10299_at	gibberellin 2-oxidase / GA2-oxidase (GA2OX2)	hormone metabolism,gibberelin,synthesis-degradation,GA2 oxidase
Contig602_at	sucrose synthase, putative / sucrose-UDP glucosyltransferase,	major CHO metabolism,degradation,sucrose,Susy
Contig481_at	sucrose synthase / sucrose-UDP glucosyltransferase (SUS1)	major CHO metabolism,degradation,sucrose,Susy
35_14480	sucrose synthase/ transferase, transferring glycosyl groups	major CHO metabolism,degradation,sucrose,Susy
Contig481_s_at	sucrose synthase, putative / sucrose-UDP glucosyltransferase,	major CHO metabolism,degradation,sucrose,Susy
HU05I05u_x_at	sucrose synthase type I	major CHO metabolism,degradation,sucrose,Susy
35_13498	no description	not assigned,unknown
35_49587	AGP9 (ARABINOGALACTAN PROTEIN 9)	not assigned,unknown
35_29657	F-box family protein (FBL10)	not assigned,unknown
35_35664	no description	not assigned,unknown
35_50591	zinc finger (MYND type) family protein / F-box family protein	not assigned,unknown
35_16272	BTB-POZ AND MATH DOMAIN 1; protein binding	protein,degradation,ubiquitin,E3,BTB/POZ Cullin3,BTB/POZ
35_4488	BRASSINOSTEROID-RESPONSIVE RING-H2; protein binding	protein,degradation,ubiquitin,E3,RING
Contig12668_s_at	E2F transcription factor-3 (E2F3)	RNA,regulation of transcription,E2F/DP transcription factor family
Contig12668_at	E2F transcription factor-3 (E2F3)	RNA,regulation of transcription,E2F/DP transcription factor family
35_20248	E2F3 (E2F TRANSCRIPTION FACTOR-3)	RNA,regulation of transcription,E2F/DP transcription factor family
Contig15953_at	F-box family protein / tubby family protein	RNA,regulation of transcription,TUB transcription factor family
35_15379	DNA-directed RNA polymerase II, putative	RNA,transcription
35_16422	DNA-directed RNA polymerase I, II, and III, putative	RNA,transcription

B

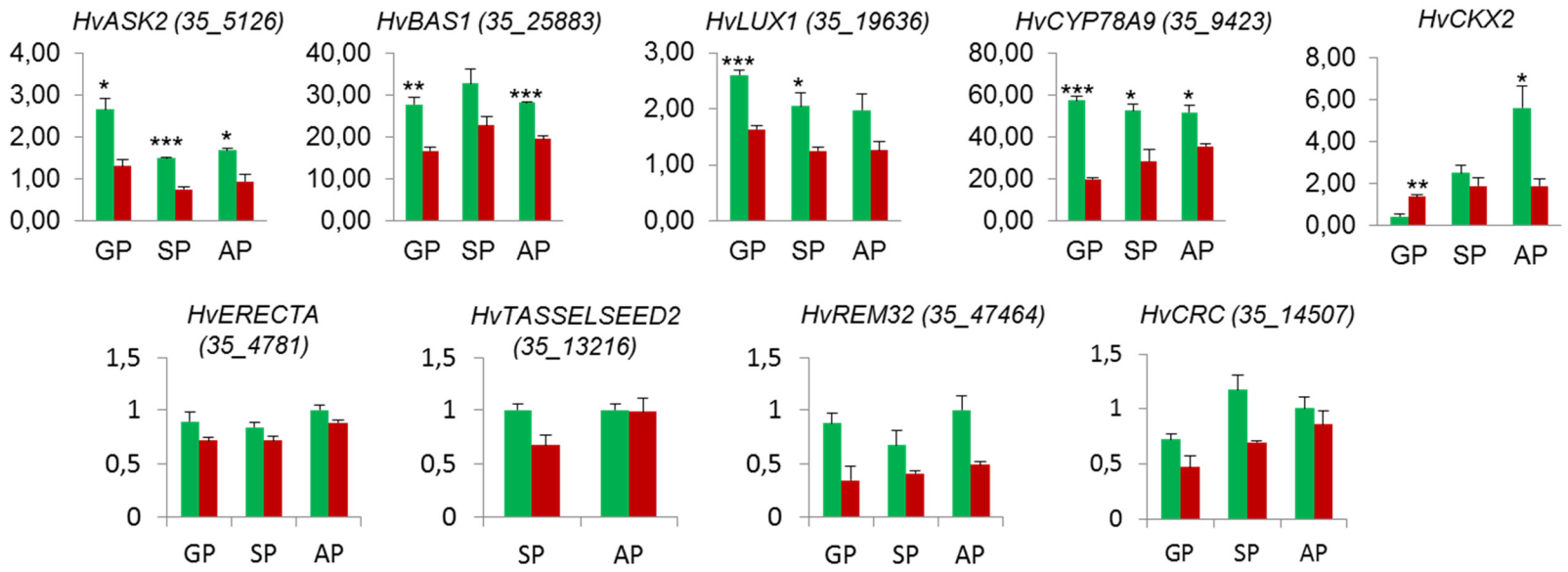


Figure S5 Transcriptome analysis of *com2.g* using microarray experiments and independent qRT PCR validations. (A) Heat map of genes conjointly up-regulated in the BW-NIL(*com2.g*) as compared to the corresponding wild type cv. Bowman. For down-regulated genes in the mutant; see Figure S5A. The scale bar at the top of the heat map indicates the transcript level of differentially regulated genes observed between wild type and mutant (blue color indicates down-regulation while red shows up-regulation). (**B**) qRT-PCR analysis performed for validation of down-regulated genes identified in the BW-NIL*com2.g* (red) as compared to the corresponding wild type cv. Bowman (green). Only highly relevant genes (9 genes) were picked up for qRT-PCR validation. Of these genes, five randomly selected genes were validated using three different biological replicates (B; upper panel) while the remaining four genes were validated using one biological, (with four technical replicates). The mean ± SE of three biological or technical replicates is shown. Expression values were log10 transformed. Asterisks show the significance level calculated by Student's t-test, (no asterisk corresponds to $p > 0.05$. While, single, double and triple asterisks stand for $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively). The Y-axis value shows the expression relative to *HvActin*. For the description of the genes; see Figure 4A and File S1. Corresponding unigene IDs or barley MLOC IDs are given in parentheses. Mean ± S.E of one biological replicate is shown. Y axis is the relative expression of the corresponding gene to *HvActin*. The developmental stages analyzed during microarray and qRT PCR experiments include TM: triple mound, GP: glume primordium, SP: stamen primordium and AP: awn primordium.

Figure S6 Branch formation in *vrs4* mutant (*mul1.a*). (**A**) Mature spike of wild type progenitor cv. Montcalm with determinate triple spikelet meristem. (**B-D**) Mature spikes of *vrs4* mutant MC (*mul1.a*) showing various levels of branch proliferation at the spike base and middle portion of the spike.

SI TABLES

Table S1 Complementary information of the BW-NIL(*com2.g*) allelic mutants.

Branched (*compositum*) Barleys	**origin**
BW-NIL BW 192 (*com2.g*)	EMS or neutrons; branched spike; pedigree: BOWMAN *8/4/ 7.1 / 3ND8670 // ND7015 / CIM; com2.g allele
HOR 14427	according to M. Stanca: double mutant mk: m=branched/k=hooded; *m* and *k* old symbols, have to be *mul* and *Kap*:
FREAK	dense branched ?? ; cv. Freak; 1961; FREAK is not a cultivar but a selection of CIMMYT collection.
com.k	spontaneous mutant probaly in Atlas (PI 539108) isolated by C.A. Suneson
Irregular spike 25	EMS; induced mutant in Foma (CIho 11333, NGB 14659) isolated by U. Lundqvist
brc1.5	A naturally occurring variant in barley from the Braunschweig seed collection

Table S2 Different barley TILLING plants and the corresponding positions of different amino acid substitution.

Mutant ID	population	SNP position	SNP	SNP allele	aa substitiution	Domain position	Conservation
10782-1	Barke	151	C→T	Heterozygote	R → C	-	-
10607-1	Barke	155	G→A	Heterozygote	G → D	-	-
12171-1	Barke	176	C→T	Heterozygote	P → L	-	conserved region
2723-1	Barke	308	G→A	Homozygote	R → H	within AP2/ERF domain	conserved region
3919-1	Barke	320	C→T	Heterozygote	S → F	within AP2/ERF domain	conserved region
6816-1	Barke	529	G→A	Homozygote	G → S	-	-
11023-1	Barke	541	G→A	Homozygote	G → S	-	-
11359-1	Barke	572	C→T	Homozygote	A → V	-	-
4913-1	Barke	587	G→A	Homozygote	S → N	-	-
13679-2	Barke	605	G→A	Homozygote	S → N	-	-
6872-1	Barke	631	A→T	Homozygote	S → C	-	-
9662-1	Barke	662	G→A	Homozygote	S → N	-	conserved region
6893-1	Barke	695	G→A	Homozygote	G → D	-	-
9624-1	Barke	748	G→T	Heterozygote	G → W	-	-
48	Morex	329	G→A	Heterozygote	G → D (110)	within AP2/ERF domain	conserved region
5865	Morex	286	G→A	Homozygote	E → K (96)	within AP2/ERF domain	conserved region
AP2/ERF domain				184 - 357 bp			
phylogenetically highly conserved domain				638 - 691 bp			

Table S3 Barley accessions used for *COM2* haplotype detection and the respective haplotype identified

Accession ID	216bp	300bp	414bp	494bp	536bp	642bp	663bp	696bp	822bp	873bp	Haplotype category
MUT2201 com2.f	G	C	C	C	G	C	**C**	C	A	MISSING	?
GM 1E Nudinka	G	C	C	C	G	C	C	C	A	A	I
GM713 Morex	G	C	C	C	G	C	C	C	A	A	I
GM712 Donaria	G	C	C	C	G	C	C	C	A	A	I
Haruna Nijo	G	C	C	C	G	C	**C**	C	A	A	I
Barke	G	C	C	C	G	C	**C**	C	A	A	I
3167	G	C	C	C	G	C	**C**	C	A	A	I
3906	G	C	C	C	G	C	**C**	C	A	A	I
GM 504 vulg. hybernum vikayarvi	G	C	C	C	G	C	C	C	A	A	I
GM 505 vulg. hybernum tystofte korsby	G	C	C	C	G	C	C	C	A	A	I
GM 506 vulg. parallelum montafon	G	C	C	C	G	C	C	C	A	A	I
GM 507 vulg. parallelum sechszeilige	G	C	C	C	G	C	C	C	A	A	I
GM 508 vulg. hybernum arrecife	G	C	C	C	G	C	C	C	A	A	I
GM 511 vulg. hybernum poliarnyj 14	G	C	C	C	G	C	C	C	A	A	I
GM 513 vulg. hybernum oberbrucker	G	C	C	C	G	C	C	C	A	A	I
GM 516 vulg. subviolaceum abessinien	G	C	C	C	G	C	C	C	A	A	I
GM 527 dist. nutans sarah	G	C	C	C	G	C	C	C	A	A	I
GM 528 dist. nutans loosdorfer	G	C	C	C	G	C	C	C	A	A	I
GM 529 dist. nutans proskowetz gerste	G	C	C	C	G	C	C	C	A	A	I
GM 530 dist. nutans triumf	G	C	C	C	G	C	C	C	A	A	I
GM 531 dist. nutans carbonera	G	C	C	C	G	C	C	C	A	A	I
GM 532 dist. nutans martonvasari	G	C	C	C	G	C	C	C	A	A	I
GM 533 dist. nutans saratov	G	C	C	C	G	C	C	C	A	A	I
GM 537 dist. erectum hokudai no. 1	G	C	C	C	G	C	C	C	A	A	I
GM 542 vulg. hybernum lyallpur	G	C	C	C	G	C	C	C	A	A	I
GM 543 vulg. wisconsin H42 (linie)	G	C	C	C	G	C	C	C	A	A	I
GM 550 fap1 2158 L	G	C	C	C	G	C	C	C	A	A	I
GM 558 dist. glabrierectum sanalta	G	C	C	C	G	C	C	C	A	A	I
GM 561 dist. nutans pfaelzer land	G	C	C	C	G	C	C	C	A	A	I
GM 562 dist. nutans szekacs linie II	G	C	C	C	G	C	C	C	A	A	I
GM 563 dist. nutans maiamana	G	C	C	C	G	C	C	C	A	A	I
GM1050C brc1.5	G	C	C	C	G	C	**A**	C	A	G	II
GM1116 com2.g	G	C	C	C	G	C	**A**	C	A	G	II
GM1118 com2.g introg bow	G	C	C	C	G	C	**A**	C	A	G	II
GM570 Optic	G	C	C	C	G	C	C	C	A	G	III
GM 500 Wild agriocrithon	G	C	C	C	G	C	C	C	A	G	III
GM 518 vulg. trifurcatum aegypten	G	C	C	C	G	C	C	C	A	G	III

GM 545 fap1 ooo8a	G	C	C	C	G	C	C	C	A	G	III
GM 548 fap1 2158 B	G	C	C	C	G	C	C	C	A	G	III
GM 564 hexastichon hybernum abarik	G	C	C	C	G	C	C	C	A	G	III
GM1087 Bowman	G	A	G	G	G	C	C	T	A	G	IV
GM702 Bowman	G	A	G	G	G	C	C	T	A	G	IV
GM2I Proctor	G	A	G	G	G	C	C	T	A	G	IV
GM 569 Golden Promise	G	A	G	G	G	C	C	T	A	G	IV
BM-NIL-flo-a.5 (BW369)	G	A	G	G	G	C	**C**	T	A	G	IV
Igri	G	A	G	G	G	C	**C**	T	A	MISSING	IV
GM 502 sp11 085–50	G	A	G	G	G	C	C	T	A	G	IV
GM 503 vulg. coeleste kleine nacktgerste	G	A	G	G	G	C	C	T	A	G	IV
GM 514 vulg. hybernum estanzuela	G	A	G	G	G	C	C	T	A	G	IV
GM 515 vulg. hybernum elses	G	A	G	G	G	C	C	T	A	G	IV
GM 517 vulg. hybernum marokkanische	G	A	G	G	G	C	C	T	A	G	IV
GM 519 vulg. hybernum algerian	G	A	G	G	G	C	C	T	A	G	IV
GM 522 vulg. himalayense tibet	G	A	G	G	G	C	C	T	A	G	IV
GM 523 vulg. horsfordianum weihenstephan	G	A	G	G	G	C	C	T	A	G	IV
GM 524 dist. nudiforcatum erfurt	G	A	G	G	G	C	C	T	A	G	IV
GM 525 dist. nutans kenia	G	A	G	G	G	C	C	T	A	G	IV
GM 526 dist. nutans spratt archer	G	A	G	G	G	C	C	T	A	G	IV
GM 534 dist. nutans swannek	G	A	G	G	G	C	C	T	A	G	IV
GM 535 dist.medicum anatolien	G	A	G	G	G	C	C	T	A	G	IV
GM 536 dist. nigricans mandschurei	G	A	G	G	G	C	C	T	A	G	IV
GM 538 dist. nutans australische fruche	G	A	G	G	G	C	C	T	A	G	IV
GM 541 vulg. hybernum aegyptische	G	A	G	G	G	C	C	T	A	G	IV
GM 546 dist. nutans bannerts	G	A	G	G	G	C	C	T	A	G	IV
GM 547 fap 1 0266C	G	A	G	G	G	C	C	T	A	G	IV
GM 554 ucnw c177	G	A	G	G	G	C	C	T	A	G	IV
GM 555 npc 0006	G	A	G	G	G	C	C	T	A	G	IV
GM 556 siglah	G	A	G	G	G	C	C	T	A	G	IV
GM 557 siglah	G	A	G	G	G	C	C	T	A	G	IV
GM 560 dist. nutans agio	G	A	G	G	G	C	C	T	A	G	IV
GM 565 hexastichon hybernum chilean	G	A	G	G	G	C	C	T	A	G	IV
GM 566 MPI 2	G	A	G	G	G	C	C	T	A	G	IV
GM 501 ucnw016	T	C	G	G	A	A	C	T	G	G	V
GM 509 vulg. nigroibericum otello	T	C	G	G	A	A	C	T	G	G	V
GM 549 fap 1 2158 H	T	C	G	G	A	A	C	T	G	G	V
GM 540 deficiens steudelii abessinien	T	C	G	G	A	A	C	T	G	G	V
GM 551 deficiens erythraeum foa II	T	C	G	G	A	A	C	T	G	G	V
GM 559 deficiens deficiens fehlgerste	T	C	G	G	A	A	C	T	G	G	V

GM 539 intermedium gymnanomalum	T	C	G	G	A	C	C	T	G	G	VI
GM 510 vulg. hybernum isthmos	G	C	G	G	G	C	C	C	A	G	VII
GM 512 vulg. rikotense brant	G	C	G	G	G	C	C	C	A	G	VII
GM 520 vulg. hybernum parallelum samsun	G	C	G	G	G	C	C	C	A	G	VII
GM 521 vulg. parallelum libanon	G	C	G	G	G	C	C	C	A	G	VII
GM 544 ucnwc72a	G	C	G	G	G	C	C	C	A	G	VII
GM 552 vulg. dundar-beyi nippon	G	C	G	G	G	C	C	C	A	G	VII
GM 553 intermedium horlani arlington	G	C	G	G	G	C	C	C	A	G	VII
AP2/ERF domain	184 - 357 bp										
phylogenetically highly conserved domain	638 - 691 bp										

Table S4 Tetraploid 'Miracle Wheat' used for allelism test and the corresponding F_1 phenotype.

Cross			Resulted Progeny	plant number	spikes per plant	branched spikes
Tri 3261	*	Tri 9652	plant 1	XIX-2012-1	3	1
Tri 3261	*	Tri 9652	plant 2	XIX-2012-2	3	no branched spikes
Tri 3261	*	Tri 9652	plant 3	XIX-2012-3	2	2
Tri 3261	*	Tri 9652	plant 4	XIX-2012-4	4	3
Tri 3261	*	Tri 9652	plant 5	XIX-2012-5	2	2
Tri 3261	*	Tri 9652	plant 6	XIX-2012-6	2	2
Tri 3261	*	Tri 9652	plant 7	XIX-2012-7	4	4
Tri 3261	*	Tri 9652	plant 8	XIX-2012-8	3	3
Tri 3261	*	Tri 9652	plant 9	XIX-2012-9	3	3
Tri 3261	*	Tri 9652	plant 10	XIX-2012-10	3	3
Tri 9652	*	Tri 3261	plant 1	XIX-2012-11	4	4
Tri 9652	*	Tri 3261	plant 2	XIX-2012-12	2	2
Tri 9652	*	Tri 3261	plant 3	XIX-2012-13	3	3
Tri 9652	*	Tri 3261	plant 4	XIX-2012-14	3	2
Tri 9652	*	Tri 3261	plant 5	XIX-2012-15	3	3
Tri 9652	*	Tri 3261	plant 6	XIX-2012-16	3	3
Tri 9652	*	Tri 3261	plant 7	XIX-2012-17	4	4
Tri 9652	*	Tri 3261	plant 8	XIX-2012-18	2	2
Tri 9652	*	Tri 5283	plant 1	XIX-2012-22	2	2
Tri 9652	*	Tri 5283	plant 2	XIX-2012-23	2	2
Tri 9652	*	Tri 5283	plant 3	XIX-2012-24	3	3
Tri 9652	*	Tri 5283	plant 4	XIX-2012-25	4	3
Tri 9652	*	Tri 5283	plant 5	XIX-2012-26	4	4
Tri 9652	*	Tri 5283	plant 6	XIX-2012-27	3	3
Tri 9652	*	Tri 5283	plant 7	XIX-2012-28	3	3
Tri 9652	*	Tri 5283	plant 8	XIX-2012-29	3	3
Tri 9652	*	Tri 5283	plant 2	XIX-2012-31	3	2
Tri 9652	*	Tri 5283	plant 3	XIX-2012-32	3	3
Tri 9652	*	Tri 5283	plant 4	XIX-2012-33	3	3
Tri 3261	*	Tri 5283	plant 1	XIX-2012-34	3	3
Tri 3261	*	Tri 5283	plant 2	XIX-2012-35	3	3
Tri 3261	*	Tri 5283	plant 3	XIX-2012-36	5	5
Tri 3261	*	Tri 5283	plant 4	XIX-2012-37	3	3
Tri 3261	*	Tri 5283	plant 5	XIX-2012-38	3	3
Tri 984	*	Tri 5283	plant 1	XIX-2012-39	4	3

Table S5 Mutant and wild type Tetraploid 'Miracle Wheat' accessions used for resequencing of the *bh*^{t} locus.

				A genome	
Tt-WheatID	**Country of origin**	**Growth habit**	**Spike type**	**SNP (T287C; Tamaroi45 as Reference)**	**corresponding aa substitution (L96P; Tamaroi45 as Reference)**
KALKA	AUS	spring type	Wild type	Not Found	Not Found
BELLAROI	AUS	spring type	Wild type	Not Found	Not Found
TAMAROI	AUS	spring type	Wild type	Not Found	Not Found
FLORADUR	AUT	spring type	Wild type	Not Found	Not Found
WOLLAROI	AUS	spring type	Wild type	Not Found	Not Found
TRI11066	UZB	spring type	Wild type	Not Found	Not Found
TRI17236	TUR	spring type	Wild type	Not Found	Not Found
TRI2230	USA	spring type	Wild type	Not Found	Not Found
TRI3504	POR	spring type	Wild type	Not Found	Not Found
TRI758	TUR	spring type	Wild type	Not Found	Not Found
TD24	GER	winter type	Wild type	Not Found	Not Found
TD97	GER	winter type	Wild type	Not Found	Not Found
AURADUR	AUT	winter type	Wild type	Not Found	Not Found
LOGIDUR	AUT	winter type	Wild type	Not Found	Not Found
LUNADUR	AUT	winter type	Wild type	Not Found	Not Found
LUPIDUR	AUT	winter type	Wild type	Not Found	Not Found
ELSADUR	AUT	winter type	Wild type	Not Found	Not Found
TRI 13541	ITA	winter type	Wild type	Not Found	Not Found
TRI 1669	ALB	winter type	Wild type	Not Found	Not Found
TRI 19273	TUR	winter type	Wild type	Not Found	Not Found
TRI 3023	ALB	winter type	Wild type	Not Found	Not Found
TRI 3720	ESP	winter type	Wild type	Not Found	Not Found
TRI 4292	TUR	winter type	Wild type	Not Found	Not Found
TRI 4522	CHN	winter type	Wild type	Not Found	Not Found
TRI 4886	UK	winter type	Wild type	Not Found	Not Found
TRI 7021	POL	winter type	Wild type	Not Found	Not Found
TRI 7056	FRA	winter type	Wild type	Not Found	Not Found
TRI 9546	ARM	winter type	Wild type	Not Found	Not Found
TRI 9547	ARM	winter type	Wild type	Not Found	Not Found
TRI 9629	CSFR	winter type	Wild type	Not Found	Not Found
TRI 984	EUR	spring type	Mutant	Found as Homozygote	Found as Homozygote
TRI 3261	ESP	spring type	Mutant	Found as Homozygote	Found as Homozygote
TRI 5283	CHN	spring type	Mutant	Found as Homozygote	Found as Homozygote

TRI 18959	FRA	spring type?	Mutant	Found as Homozygote	Found as Homozygote
CITr 13712	USA	spring type	Mutant	Found as Homozygote	Found as Homozygote
CITr 13713	USA	spring type	Mutant	Found as Homozygote	Found as Homozygote
PI 225308	IRAN	spring type	Mutant	Found as Homozygote	Found as Homozygote
PI 349056	ARM	spring type	Mutant	Found as Homozygote	Found as Homozygote
PI 438971	KAZ	spring type	Mutant	Found as Homozygote	Found as Homozygote
TRI 3411	SU	spring type	Mutant	Found as Homozygote	Found as Homozygote
TRI 4045	EUR	spring type	Mutant	Found as Homozygote	Found as Homozygote
TRI 4341	EUR	spring type	Mutant	Found as Homozygote	Found as Homozygote
TRI 5911	IRAN	spring type	Mutant	Found as Homozygote	Found as Homozygote
TRI 9548;W1420	ARM	spring type	Mutant	Found as Homozygote	Found as Homozygote
TRI 27966	-	spring type	Mutant	Found as Homozygote	Found as Homozygote
TRI 1781	GER	winter type	Mutant	Found as Homozygote	Found as Homozygote
TRI 1782	GER	winter type	Mutant	Found as Homozygote	Found as Homozygote
TRI 3365	CHN	winter type	Mutant	Found as Homozygote	Found as Homozygote
TRI 4270	ITA	winter type	Mutant	Found as Homozygote	Found as Homozygote
TRI 4446	HUN	winter type	Mutant	Found as Homozygote	Found as Homozygote
TRI 4461	EUR	winter type	Mutant	Found as Homozygote	Found as Homozygote
TRI 4653	AUS	winter type	Mutant	Found as Homozygote	Found as Homozygote
TRI 9628; ;W1529	IND	winter type	Mutant	Found as Homozygote	Found as Homozygote
TRI 19165	-	winter type	Mutant	Found as Homozygote	Found as Homozygote
TRI 19292	FRA	winter type	Mutant	Found as Homozygote	Found as Homozygote
TRI 28396	ITA	winter type	Mutant	Found as Homozygote	Found as Homozygote
TRI 24012	-	winter type	Mutant	Found as Heterozygote	Found as Heterozygote
TRI 4448	EUR	winter type	Mutant	Found as Heterozygote	Found as Heterozygote
TRI 9652;W1554	CSR	spring type	Mutant	Found as Heterozygote	Found as Heterozygote

Table S6 Primer information

PrimerID	Orien-tation	Experiment	Sequence	Tm (◦C)	PCR product_bp	Restriction enzyme for CAPS marker development
HvCOM2	Forward	qRT-PCR	CGCACATTGGGTCGTACCA	57.9	107	-
HvCOM2	Reverse	qRT-PCR	GTGATCGGCGGCATTGG	57.2	-	-
HvCKX2	Forward	qRT-PCR	GTTCGTGGAGCGGAAGAGGAAG	60	107	-
HvCKX2	Reverse	qRT-PCR	CGGGGCCAATCGATTAACTTCGTG	60	-	-
HvBAS1	Forward	qRT-PCR	AGACGCCAGATCATCACCTGTG	59.1	101	-
HvBAS1	Reverse	qRT-PCR	ACATGGTCATTTCGGCCTGTCAT	62.9	-	-
HvIDS1	Forward	qRT-PCR	CTAGCTCTCTGCTTACTCCCATGGAC	60	134	-
HvIDS1	Reverse	qRT-PCR	CATGGCTCGGCAATGTTATCTCTCTC	59.2	-	-
HvASK2	Forward	qRT-PCR	GGTTGACTCTGCGACGCGA	60.3	76	-
HvASK2	Reverse	qRT-PCR	CGTTAACGGCTGCTCCCAGG	60.9	-	-
HvCYP78A9	Forward	qRT-PCR	CCCATTCGGCCTAAACGCGA	60.4	96	-
HvCYP78A9	Reverse	qRT-PCR	AGAAACGTACAGCAGCCAGCC	60.1	-	-
HvCRC	Forward	qRT-PCR	ATGGATGTGCTCCTGGGTGTG	59.7	82	-
HvCRC	Reverse	qRT-PCR	TGATGTCCCGGTGGATGATCG	59.1	-	-
HvLUX1	Forward	qRT-PCR	CAGAGTTGCAGAGAGTGTGTGC	58.6	107	-
HvLUX1	Reverse	qRT-PCR	TCTTGCCACTGCCCAAAATGG	58.1	-	-
HvERECTA	Forward	qRT-PCR	TGAAGTCGAATGGGCTGACCG	59.6	66	-
HvERECTA	Reverse	qRT-PCR	GCGTCTTAATCGACGAGCAGTCC	59.7	-	-
HvTASSELSEED2	Forward	qRT-PCR	ACTGCGCCTAGTTGTTCGAG	57.4	97	-
HvTASSELSEED2	Reverse	qRT-PCR	TCCTGCACACTTCAAACCACA	57.1	-	-
com2_p11	Forward	ForRecombinantScreen_distal proximal(ortholog of Os07g0673700)	CCCTTCCTCGTTAGGTACGTGTG	66	1147	HpaII
com2_p12	Reverse	ForRecombinantScreen_distal proximal(ortholog of Os07g0673700)	CTGGTGATTTCCCAAACCTGAAG	63	-	-
com2_p19	Forward	ForRecombinantScreen_flank proximal(ortholog of	GTCAACACACCAACGGGTCTTC	64	968	EcoRV

		Os07g0668900)				
com2_p20	Reverse	ForRecombinantScreen_flank proximal(ortholog of Os07g0668900)	AGGCCTATGGCATCATGGAAAT	60	-	-
M1 (com2g_p31)	Forward	Flank marker M1 (morex_contig_1566969 CAJW011566969 carma=2HS)	CGCTACTTGGCACATTCTCA	60	1041	HinfI
M1 (com2g_p32)	Reverse	Flank marker M1 (morex_contig_1566969 CAJW011566969 carma=2HS)	CAGTTAGCTTTCGGGCTTTG	60	-	-
M2 (2HS_3D)	Forward	Flank marker M2 (ortholog of Os07g0669200)	GATCCCACCTCCAGTAACGA	60	1061	BsmI
M2 (2HS_3D)	Reverse	Flank marker M2 (ortholog of Os07g0669200)	GATGCACTGCCTCAACTCAA	60	-	-
Insitu	Forward	mRNA in-situ hybridization (cDNA isolated from cv. Bonus)	CAACGGCTACTCACCCTTCA	60.5	444	-
Insitu	Reverse	mRNA in-situ hybridization (cDNA isolated from cv. Bonus)	GTGCGTACTACATTCTCGAG	60.5	-	-
TtBH-A1	Forward	tetraploid wheat TILLING screen	GCTAGGCGGGAGCAGTAGTA	60.82	1011	
TtBH-A1	Reverse	tetraploid wheat TILLING screen	GTGGGCACAGCAGACCAC	60.98	-	
TtBH-B1	Forward	tetraploid wheat TILLING screen	TCCCCTCCCCTACCCAAG	59.21	1218	
TtBH-B1	Reverse	tetraploid wheat TILLING screen	TGAGTACGTAAGAGGCTAAGATCG	59.49	-	
TILL_FZP_7_rev	Forward	Barley TILLING screen	CAGTGGGAGAGGAAGCTGAA	60.5	1044	
TILL_FZP_7_for	Reverse	Barley TILLING screen	GAAGCTCACAGCAACCACCT	60.5	-	

Available for download as Excel files at www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.176628/-/DC1

Table S7 TILLING analysis in wheat

File S1 Detailed information of the up and down regulated genes in microarray experiment.

File S2: SI TEXT

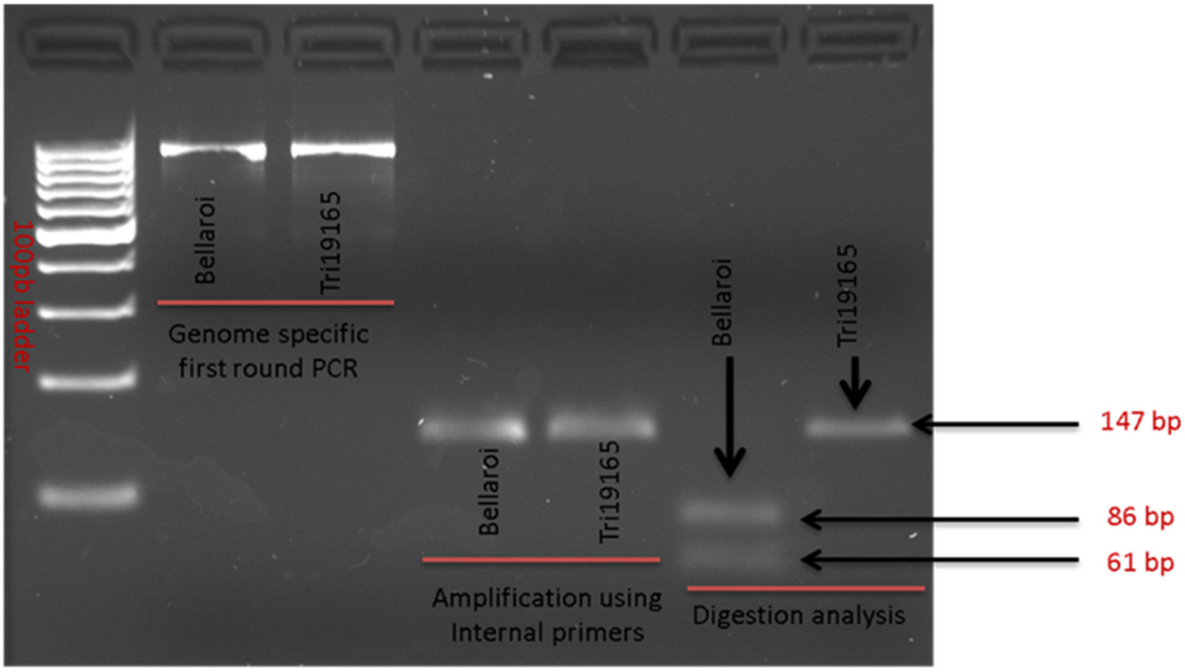
Candidate gene specific marker development in barley and tetraploid wheat

The orthologs of maize *BD1* and rice *FZP/BFL1* (CHUCK *et al.* 2002; KOMATSU *et al.* 2003; ZHU *et al.* 2003) were considered as candidate genes underlying *bh*^{t} and *com2.g* phenotypes in wheat and barley, respectively. To map the barley ortholog, the *COM2* gene, in barley the corresponding specific gene-based markers were developed. The two CAPS markers (table below) are both on the basis of SNP A873G found between the two parents of the population (Haruna Nijo x BW 192 *com2.g*). Barley marker information is provided in the table below. Each of the two markers could be utilized for mapping.

primer name	orientation	primer seq	Restriction Enzyme	Product size	Digested Product size_wild	Digested Product size_mutant	Digested Product size_heterozygote	Tm (°C)
HN_SNP2for	Forward	AACTCCGGGTACCTGAGCA	BtgZI	327 bp	219 bp; 108 bp	327 bp	327 bp; 215 bp; 112 bp	60,66
HN_SNP2rev	Reverse	CAGATCGGCCATTAAGTGAG						58,33
GW_3UTR_For3	Forward	GAGGACGTGGACGACCTG	Hpy99I	207 bp	10bp; 31bp; 168 bp	10bp; 31bp; 59 bp; 108 bp	10bp; 31bp; 59 bp; 108 bp; 168 bp	57,7
HN_SNP2rev	Reverse	CAGATCGGCCATTAAGTGAG						58,33

Wheat marker information for the candidate gene underlying *bh*^{t} is presented in the following table and exemplary image. This CAPS marker is developed on the basis of SNP T287C found between parents of the corresponding mapping populations. Two-step PCR reactions were followed. In the first round, any of the A genome specific primers 1 or 2 could be used. Second, the internal primer pair *(*TdFZP2A_in_F and TdFZP2A_in_R*)* was used to amplify a short fragment using the first round PCR product as template. This short fragment was used for CAPS marker development. At the image below, amplification of the candidate gene using genome-specific primers (left), amplification of region of interest using internal primers (middle), and digestion analysis (right) are depicted.

primer name	Product ID	primer seq	Restriction Enzyme	Product size	Digested Product size _ wild	Digested Product size _ mutant	Digested Product size _ heterozygote
Tafzp_2A_Forward 1	A geneome specific 1	AGCCAACCTCACTTCACTTC	-	946 bp	946 bp	946 bp	946 bp
Tafzp_2A_Reverse 1		GAGCAATGCCAGCGCGTCCGT					
Tafzp_2A_Foward 2	A geneome specific 2	CTAGGCGGGAGCAGTAGTA	-	963 bp	963 bp	963 bp	963 bp
Tafzp_2A_Reverse 2		AGCGCGTCCGTTTCAGTGG					
TdFZP2A_in_F	Internal for A.G. Specific 1 and 2	GACCCGACCACCAAGGAG	BstNI	147 bp	61 bp; 86 bp	147 bp	61 bp; 86 bp; 147 bp
TdFZP2A_in_R		GTAGTTGTTGTAGGCGGCGT					



Genetic mapping in barley

To newly map the phenotype in barley, we developed an F_2 mapping population comprising 286 individuals between the parental mutant BW 192 *com2.g* and barley cv. Haruna Nijo. The parental mutant BW 192 *com2.g* is the Bowman Near Isogenic Line of *com2.g* and was previously developed until BC7≤ generation (DRUKA *et al.* 2010). The segregation pattern of the phenotype among the corresponding F2 barley plants fitted well with a 3:1 ratio typical for a monogenic recessive trait. The syntenic information reported in the form of barley chromosome 2H Genome Zipper (MAYER *et al.* 2011) was explored to genetically localize the phenotype and to develop further markers surrounding the locus (Figure S2). This low resolution genetic mapping localized the *com2.g* phenotype in an interval of 5.5 cM along the barley chromosome 2H short arm. The interval was flanked by two barley gene based markers orthologous of rice genes Os07g0673700 (barley CAPS marker com2_p11/com2_p12) and Os07g0668900 (barley CAPS marker com2_p19/com2_p20), respectively. The two aforementioned flanking markers were used for screening a larger population consisting of 1750 F_2 plants from which 52 recombinant plants were identified.

While fine-mapping *com2.g*, we discovered that the observed genotypic alleles for the *COM2*-specific marker failed to fully match the *com2.g* phenotypic score since six out of the 52 F_2 recombinants showed a discrepant phenotype. While genotypically heterozygous for *COM2*, they showed the supposedly recessive branched phenotype. F_3 offsprings (17 to 28

progenies per F_2 totaling 128 F_3 plants) segregated for the respective spike-branching phenotype at both phenotypic and genotypic levels thus confirming the heterozygosity of the parental F_2 plants. However, also F_3 plants showed inconsistencies between genotypic and phenotypic data. While all 36 genotypically homozygous mutant plants showed clear spike-branching, 33 of the 70 heterozygous and three out of 22 homozygous wild type plants showed a faintly branched spike. The deviant F_3 plants were progenies from three out of six F_2 plants which suggest that additional genetic and/or environmental factors regulate spike-branching. This is supported by observations in maize that found an effect of genotypic background on the expressivity of the inflorescence branching (COLOMBO *et al.* 1998).

Genetic mapping in tetraploid wheat

A linkage map for *bh*^{t} in tetraploid wheat was established in parallel to genetic mapping of *com2.g* in barley. Two different mapping populations consisting of 279 and 159 F_2 plants were developed using a set of published microsatellite markers (RODER *et al.* 1998) (Figure S2), the phenotype could be genetically mapped to a region spanning ~20 cM on wheat chromosome 2A short arm (2AS) (Figure S2) that confirmed the previous finding of *bh*^{t} genetic mapping (KLINDWORTH *et al.* 1997). Both mutant parents TRI 27966 and TRI 19165 carry the same recessive allele of *bh*^{t} showing only a single amino acid substitution of leucine to proline at position 96 (L96P) as compared to the wild type. However, further genetic mapping for positional cloning of the gene was not followed in wheat.

SI MATERIALS AND METHODS

Marker development in barley

Barley chromosome 2H genome zipper (GZ) (MAYER *et al.* 2011) was utilized for initial marker development. Barley sequence information, the homologs of the rice genes ordered along the 2H-GZ was used for primer design. Barley sequence information was obtained from IPK Barley Blast Server (http://webblast.ipk-gatersleben.de/barley/viroblast.php). Corresponding rice genes were extracted from respective genome browser server (http://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice/). Rice gene sequences were blasted against the IPK barley blast server (http://webblast.ipk-gatersleben.de/barley/viroblast.php) to obtain barley sequences. Sequences were amplified in parental lines of the mapping population using primers designed to detect SNP polymorphisms. Identified SNPs were converted to restriction enzyme based CAPS (http://nc2.neb.com/NEBcutter2/). The barley ortholog of rice *FZP/BFL1* gene sequence (*Os07g0669500)* was used for candidate gene marker development. In case of wheat, publicly available SSR markers (RODER *et al.* 1998) were used for genetic mapping.

TILLING analysis

Barley

For identifying further mutant alleles of *COM2* in barley, a TILLING population consisting of 10,279 EMS (Ethyl methanesulfonate) treated plants of cv. Barke was screened. A primer combination (Table S6) was used to amplify the single exon of the *COM2* gene. The product was subjected to standard procedure of AdvanCE™ TILLING kit as described in (GAWRONSKI *et al.* 2014). Amplified products were digested with dsDNA cleavage kit followed by analysis via mutation discovery kit and gel - dsDNA reagent kit. These were performed on the AdvanCE™ FS96 system according to manufacturer's guidelines (advanced analytical, IA, USA). Amplified ORF was also re-sequenced by Sanger sequencing on all accessions carrying polymorphism identified to confirm SNPs. In addition to the Barke population, a different TILLING population, TillMore (in the cv. Morex) (TALAME *et al.* 2008) was also screened for additional mutant alleles of *COM2.* A set of TillMore lines with branched phenotype was screened. From these lines, the *COM2* single exon was re-sequenced for detection of causal SNPs.

Wheat

To identify *TtBH-1* mutants in wheat, a tetraploid TILLING population consisting of 1,139 EMS plants of cv. Kronos was screened (Uauy et al 2009). Homoeologue-specific primers were designed (Table S6) and tested for specificity; betaine was added at 1M final concentration to the PCR reactions. A 1,011 bp fragment of *TtBH-A1* and a 1,218 bp fragment of *TtBH-B1* were screened. Mutant detection was performed with *Cel*1 digestion followed by analysis on a capillary ABI3730 sequencer (Applied Biosystems, Foster City, California, USA) using published protocols (LE SIGNOR *et al.* 2009). Individual DNAs from positive pools were Sanger sequenced to identify the nature of the mutations.

Haplotype analysis

Genomic DNA was extracted using mixed alkyl trimethyl ammonium bromide method (SALLAUD *et al.* 2003) from a diverse set of barley accessions (Table S3) using standard protocols and the full coding sequence of the barley *COM2* gene was PCR-amplified using primers **Ptp #56:** GCATGCATGTCACTCGAACT (upstream of ATG) and **Ptp #67:** CTAGGGCACCGAAACAAGCC (downstream of stop codon). PCR reactions (15 μL) contained 40-50 ng genomic DNA, 0.3 μM of each primer, dNPT mix 0.5 mM, DMSO 5%, herculase buffer 1x, Herculase Hotstart DNA polymerase 0.74 U (Stratagene #600310). Thermal cycling protocol consisted of: 1 cycle (95°/5'), 4 cycles (95°/20", 65°/10" [with 0.5°reduction per cycle], 72°/45"), 2 cycles (95°/20", 62°/20",72°/45"), followed by 34 cycles (95°/20", 60°/30",72°/1') and the last step of 1 cycle (72°/10'). PCR products were purified using the ExoSAP-IT (Exo-nucleases) PCR clean-up protocol (Applied Biosystems®) following the manufacturer's instructions and sequenced using the Sanger method with BigDye™ Terminator v3.1 Matrix Standard Sequencing Kit (Applied Biosystems®, ABI PRISM® 3700 DNA Analyzer) using primers **Ptp** #56 and **Ptp** #23 ACCAACTTCGTCTACGCGCA.

Sequence annotation

Unpublished sequence information for the two BAC contigs (44575 and 47813; spanning the interval between M1 and M2) was made available from the international barley sequencing consortium (through Dr. Nils Stein). First, barley BAC contig overlap detection was performed by an all against-all alignment with megablast (ZHANG *et al.* 2000). This was to confirm the overlap between the two BAC contigs initially identified. The criteria were only BLAST hits longer than 2 kb and 99.5% sequence identity. Sequence annotation was performed as described by (MASCHER *et al.* 2014). Sequences were first subjected to k-mer-based repeat masking using Kmasker algorithm (SCHMUTZER *et al.* 2014). *Augustus* was implemented for structural gene annotation of repeat-masked contigs using the maize model. Finally, predicted protein sequences were functionally annotated with the AHRD pipeline. This included parses the description of BLASTP hits against the TAIR, Uniprot/trEMBL, and Uniprot/SwissProt databases as utilized by (ZHANG *et al.* 2000). Genes annotated as unknown proteins or transposable elements were excluded from further analysis.

File S3 TtBH and COM2 DNA sequence

Available for download at www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.176628/-/DC1

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