SUPPLEMENTARY DATA

Table S1. Frequency of leafy/shoot-like structures and naked gaps in spikes of various barley double mutant lines cultivated under field conditions

Cross-combination	In F_7					In F_8	
	n	Variation, %				n	Variation
		A	B	Others	Sum		sum, %
Hv-tw$_2$ × Hv-Lh	178	16.8	9.5	33.2	59.5 ± 3.6	962	34.3 ± 1.5
Hv-tw$_2$ × Hv-Mf	504	22.0	0.4	41.5	63.9 ± 2.1	307	48.2 ± 2.9
Hv-tw$_2$ × Hv-Hcs	112	0	17.0	1.8	18.8 ± 3.7	210	31.9 ± 3.2
Hv-tw$_2$ × Hv-H	391	4.1	6.4	15.4	25.9 ± 2.3	785	20.5 ± 1.4
Hv-tw$_2$ × Hv-Dwh	66	0	4.5	1.5	6.0 ± 2.9	512	7.6 ± 1.2
Hv-tw$_2$ × Hv-Br	89	2.2	48.3	13.5	64.0 ± 5.1	722	18.8 ± 1.5

Hv-tw$_2$ – Hv-tweaky spike 2; *Hv-Mf – Hv-Multiflorous*; *Hv-Hcs – Hv-Hooded on center spikelet*; *Hv-H – Hv-Hooded* (*Colsess II*); *Hv-Dwh – Dense wing hood*; *Hv-Br – Hv-Brittle rachis*; variation types: A – leafy-like phenotype with long naked gaps in spike (see Fig. 1A–F); B – shoot-like structures within inflorescence (see Fig. 1H, I, K, L); others – with long (see Fig. 1F) or short (see Fig. 1G) spike gaps, but without leafy/shoot-like structures or with multiple bract-lemma structures forming 'bulbs' (see Fig. 1O).

Table S2. PCIB and HFCA effects on basic flower structures of barley single and double mutants cultivated under greenhouse conditions

Mutant/hybrid	Inhibitor	n	Spectrum of basic flowers, %				
			Normal	Lt	SO (1)	Lt+SO (2)	Sum (1+2)
Hv-tw_2 (-)	0	80	37.5 ± 5.4	62.5 ± 5.4	0	0	0
	PCIB	210	66.2 ± 3.3^{c}	25.7 ± 3.0^{c}	3.3 ± 1.2	4.8 ± 1.5	8.1 ± 1.9
	HFCA	120	65.8 ± 4.3^{c}	10.8 ± 2.8^{c}	0	23.3 ± 3.9^{c}	23.3 ± 3.9^{c}
Hv-Lemma hooded (Hv-Lh) (-)	0	46	97.8 ± 2.2^{3}	0	2.2 ± 2.2	0	2.2 ± 2.2
	PCIB	76	97.4 ± 1.8^{3}	0	2.6 ± 1.8	0	2.6 ± 1.8^{3}
	HFCA	64	98.4 ± 1.6^{3}	0	1.6 ± 1.6	0	1.6 ± 1.6^{2}
Hv-$tw_2 \times Hv$-Lh N13 (-)	0	65	60.0 ± 6.1^{2}	16.9 ± 4.7^{3}	18.5 ± 4.9^{3}	4.6 ± 2.6	23.1 ± 5.3^{3}
	PCIB	92	78.3 ± 4.3^{a1}	3.3 ± 1.9^{b3}	12.0 ± 3.4^{1}	6.5 ± 2.6	18.5 ± 4.1
	HFCA	101	96.0 ± 2.0^{c3}	0	4.0 ± 2.0^{b1}	0	4.0 ± 2.0^{c}
Hv-$tw_2 \times Hv$-Lh N11 (+)	0	64	26.6 ± 5.6	18.8 ± 4.9^{3}	18.8 ± 4.9^{3}	35.9 ± 6.0^{3}	54.7 ± 6.3^{3}
	PCIB	96	59.4 ± 5.0^{c}	24.0 ± 4.4	8.3 ± 2.8	8.3 ± 2.8^{c}	16.7 ± 3.8^{c1}
	HFCA	91	82.4 ± 4.0^{c2}	11.0 ± 3.3	5.5 ± 2.4^{a1}	1.1 ± 1.1^{c3}	6.6 ± 2.6^{c3}
Hv-$tw_2 \times Hv$-Lh N19 (+)	0	111	96.4 ± 1.8^{3}	0	2.7 ± 1.5	0.9 ± 0.9	3.6 ± 1.8
	PCIB	109	78.9 ± 3.9^{c1}	2.8 ± 1.6^{3}	13.8 ± 3.3^{b2}	4.6 ± 2.0	18.3 ± 3.7^{c1}
	HFCA	111	82.0 ± 3.7^{c2}	0.9 ± 0.9^{3}	9.9 ± 2.8^{a3}	7.2 ± 2.5^{a3}	17.1 ± 3.6^{c}
Hv-$tw_2 \times Hv$-Hooded N6 (+)	0	74	93.2 ± 2.9^{3}	0	5.4 ± 2.6^{1}	1.4 ± 1.4	6.8 ± 2.9^{1}
	PCIB	139	91.4 ± 2.4^{3}	1.4 ± 1.0^{3}	5.0 ± 1.9	2.2 ± 1.2	7.2 ± 2.2
	HFCA	100	95.0 ± 2.2^{3}	0	5.0 ± 2.2^{1}	0	5.0 ± 2.2^{3}

Flower variations: Lt – lodicule transformation into sexual organs; SO – only the number of flower organs is changed; (+, -) – formation of leafy/shoot-like structures: (+) – present, (-) – absent, (+-) – present under field conditions, absent in greenhouse; n – the number of tested flowers. In *WT* (cv. 'Auksiniai II'), mutant *Hv-Hooded* and in hybrid *Hv-tw_2 × Hv-Hooded* N21 – all flowers of *WT* phenotype with respective n for 0, PCIB, HFCA: 78-88-126; 86-138-146; 71-119-85; a, b, c – in comparison with the respective control (0): a – $P < 0.05$; b – $P < 0.01$; c – $P < 0.001$; 1, 2, 3 – in comparison with the respective test-condition of mutant *Hv-tw_2*: 1 – $P < 0.05$; 2 – $P < 0.01$; 3 – $P < 0.001$.

Table S3. Effects of PCIB and HFCA on the development of ectopic flower structures in barley single and double mutants cultivated under greenhouse conditions

Mutant/ hybrid	Inhibitor	Ectopic flower structures, %	n	Spectrum of ectopic flowers with sexual organs, %							Without sexual organs, %
				L+S+P	S+P	L+S	L+P	S	P	Others	
Hv-Hooded	0	100	103	39.8 ± 4.8	28.2 ± 4.5	8.7 ± 2.8	0	6.8 ± 2.5	0	12.6 ± 3.3	3.9 ± 1.9
(*Hv-H*) (-)	PCIB	100	180	3.9 ± 1.4[c]	1.1 ± 0.8[c]	70.0 ± 3.4[c]	0	9.4 ± 2.2	0	5.6 ± 1.7	10.0 ± 2.2[a]
	HFCA	100	155	21.3 ± 3.3[b]	8.4 ± 2.2[c]	53.5 ± 4.0[c]	1.3 ± 0.9	10.3 ± 2.4	0	2.6 ± 1.3[b]	2.6 ± 1.3
Hv-tw$_2$ ×	0	100	83	55.4 ± 5.5	13.3 ± 3.7	10.8 ± 3.4	0	6.0 ± 2.6	0	0	14.5 ± 3.9
Hv-H N6	PCIB	98.6 ± 1.0	141	14.2 ± 3.0[c]	39.0 ± 4.1[c]	1.4 ± 1.0[b]	0.7 ± 0.7	9.2 ± 2.4	1.4 ± 1.0	2.1 ± 1.2	31.9 ± 3.9[b]
(+)	HFCA	96.3 ± 1.8[a]	103	37.9 ± 4.8[a]	30.1 ± 4.5[c]	4.9 ± 2.1	2.9 ± 1.7	1.9 ± 1.4	1.0 ± 1.0	0	21.4 ± 4.1
Hv-Lemma	0	48.6 ± 4.3	67	0	0	0	0	0	0	0	100
hooded	PCIB	69.7 ± 3.1[c]	159	0	0	0	0	0.6 ± 0.6	0	0	99.4 ± 0.6
(*Hv-Lh*) (-)	HFCA	71.9 ± 3.3[c]	138	0	0	0	0	0	0	0	100
Hv-tw$_2$ ×	0	95.7 ± 1.6	155	0	0	0	0	0	0	0	100
Hv- Lh N13	PCIB	98.1 ± 1.0	202	0	0	0	0	0	0	0	100
(-)	HFCA	98.6 ± 0.7	273	0	0	0	0	0	0.4 ± 0.4	0	99.6 ± 0.4
Hv-tw$_2$ ×	0	87.1 ± 3.5	81	0	0	0	0	1.2 ± 1.2	0	0	98.8 ± 1.2
Hv-Lh N11	PCIB	77.1 ± 4.3	74	0	1.4 ± 1.4	0	0	0	0	0	98.6 ± 1.4
(+)	HFCA	93.6 ± 2.4	102	0	0	0	0	0	0	0	100
Hv-tw$_2$ ×	0	96.6 ± 1.7	112	26.8 ± 4.2	19.6 ± 3.8	5.4 ± 2.1	0	3.6 ± 1.8	0.9 ± 0.9	0	43.8 ± 4.7
Hv-Lh N19	PCIB	100	111	7.2 ± 2.5[c]	12.6 ± 3.2	0.9 ± 0.9[a]	1.8 ± 1.3	3.6 ± 1.8	1.8 ± 1.3	4.5 ± 2.0[a]	67.6 ± 4.5[c]
(+)	HFCA	82.5 ± 3.5[c]	99	6.1 ± 2.4[c]	5.1 ± 2.2[c]	4.0 ± 2.0	4.0 ± 2.0[a]	3.0 ± 1.7	1.0 ± 1.0	3.0 ± 1.7	73.7 ± 4.4[c]

Flower organs and variations: L – lodicules; S – stamens; P – pistils; Others – chimeric or not fully developed organs; (+, -) – as in Table S2; n – number of tested flowers; a, b, c – in comparison with the respective control (0): a – $P < 0.05$, b – $P < 0.01$, c – $P < 0.001$.

Table S4. Effects of 2,4-D on the basic and ectopic flower structures of selected barley single and double mutant lines (F_{10}) cultivated under field conditions

Mutant/ hybrid	Treat- ment	n	Basic flower structures, %				Ectopic flower structures[1], %					
			Normal	Lt	Lt+SO	Sc	Common frequency	L+S+P	S+P	L+S	L+P	Without sexual organs
Hv-tw₂ (-)	0	101	22.8 ± 4.2	68.3 ± 4.7	8.9 ± 2.8	0	0	0	0	0	0	0
	2,4-D	100	33.0 ± 4.7	33.0 ± 4.7^c	34.0 ± 4.8^c	0	0	0	0	0	0	0
Hv-Hooded (*Hv-H*) (-)	0	102	100	0	0	0	100	15.7 ± 3.6	23.5 ± 4.2	9.8 ± 3.0	0	15.7 ± 3.6
	2,4-D	128	100	0	0	0	100	25.0 ± 3.8	35.9 ± 4.3^a	13.3 ± 3.0	0	21.9 ± 3.7
Hv-tw₂ × Hv-H N6 (+)	0	51	68.6 ± 6.6	21.6 ± 5.8	9.8 ± 4.2	0	100	19.6 ± 5.6	0	0	9.8 ± 4.2	52.9 ± 7.1
	2,4-D	60	71.7 ± 5.9	8.3 ± 3.6	16.7 ± 4.9	3.3 ± 2.3	100	41.5 ± 6.8^a	0	0	15.1 ± 5.0	39.6 ± 6.8
Hv-tw₂ × Hv-H N21 (+)	0	96	88.5 ± 3.3	3.1 ± 1.8	8.3 ± 2.8	0	0	0	0	0	0	0
	2,4-D	100	65.0 ± 4.8^c	18.0 ± 3.9^c	17.0 ± 3.8	0	0	0	0	0	0	0
Hv-Lemma hooded (*Hv-Lh*) (-)	0	65	100	0	0	0	57.7 ± 3.5	0	0	0	0	100
	2,4-D	100	93.0 ± 2.6^b	1.0 ± 1.0	6.0 ± 2.4^a	0	56.3 ± 3.0	0	0	0	0	100
Hv-tw₂ × Hv-Lh N1 (+)	0	97	48.5 ± 5.1	44.3 ± 5.1	7.2 ± 2.6	0	100	5.5 ± 2.4	12.1 ± 3.4	0	0	80.2 ± 4.2
	2,4-D	100	39.0 ± 4.9	44.0 ± 5.0	15.0 ± 3.6	2.0 ± 1.4	100	35.6 ± 5.1^c	0^c	0	10.0 ± 3.2^b	54.4 ± 5.3^c
Hv-tw₂ × Hv-Lh N2 (+)	0	98	58.2 ± 5.0	16.3 ± 3.8	25.5 ± 4.4	0	93.4 ± 2.6	0	7.1 ± 2.8	0	0	92.9 ± 2.8
	2,4-D	91	34.1 ± 5.0^c	23.1 ± 4.4	39.6 ± 5.2^a	3.3 ± 1.9	96.4 ± 2.0	1.2 ± 1.2	0^a	0	22.2 ± 4.6^c	76.5 ± 4.7^b
Hv-tw₂ × Hv- Lh N11 (+)	0	185	23.2 ± 3.1	8.6 ± 2.1	64.3 ± 3.5	3.8 ± 1.4	61.9 ± 3.6	0	0	0	0	100
	2,4-D	102	21.6 ± 4.1	3.9 ± 1.9	59.8 ± 4.9	14.7 ± 3.5^b	61.2 ± 4.8	0	0	0	0	100
Hv-tw₂ × Hv-Lh N17 (+ -)	0	113	38.1 ± 4.6	22.1 ± 3.9	39.8 ± 4.6	0	86.5 ± 3.6	0	0	0	0	88.3 ± 3.7
	2,4-D	104	38.5 ± 4.8	12.5 ± 3.3	43.3 ± 4.9	5.8 ± 2.3^a	90.0 ± 3.2	0	0	0	0	98.8 ± 1.2^b
Hv-tw₂ × Hv- Lh N19 (+)	0	127	66.1 ± 4.2	3.1 ± 1.5	30.7 ± 4.1	0	93.5 ± 2.2	6.0 ± 2.2	30.2 ± 4.3	0	0	48.3 ± 4.7
	2,4-D	100	65.0 ± 4.8	4.0 ± 2.0	28.0 ± 4.5	3.0 ± 1.7	98.0 ± 1.4	22.4 ± 4.2^c	0^c	0	21.4 ± 4.2^c	53.1 ± 5.1

[1]Only part of variations in the spectrum is shown; (+, -) – as in Table S2; n – the number of tested flowers; flower organs: L – lodicule; S – stamen; P – pistil; variations in basic flowers: Lt –transformation of lodicules into stamens and/or pistils; Lt+SO – flowers with lodicule transformation and altered number of flower organs (L, S or P); Sc – chimeric stamens with partially developed carpel structures; a, b, c – in comparison with respective 2,4-D untreated plants (0): a – $P < 0.05$, b – $P < 0.01$, c – $P < 0.001$.