Experiment 6 - Data S4

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Experiment 6 − Genetics of BX-feedbacks on maize

The Experiment 6 was performed with maize using regular potting soil (Klasmann–Deilmann GmbH, Germany), which was conditioned in 3L-pots (13.1 cm depth and 20.2 cm diameter) in the greenhouse. The conditioning was not only done with B73 and bx1(B73) but also with the maize lines W22, bx1(W22), bx2(W22) and bx6(W22). The growth conditions and maize lines were described earlier (Hu et al., 2018; 'Benzoxazinoid pathway experiment'). Different compared to the earlier experiment is that here we utilized potting soil and we only grew B73 plants on B73 and W22 conditioned soils (n = 8–11 replicates; see Data S6 for experimental design). Pots were randomly placed on a greenhouse table (26 °C ± 2 °C, 55% relative humidity, 14:10 h light/dark, 50,000 lm m−2) and re-arranged weekly. Plants were watered three times per week. Ten weeks after planting, the shoot biomass and larval growth were measured.

Table 1: Number of replicates

B73	bx1(B73)	W22	bx1(W22)	bx2(W22)	bx6(W22)
11	10	10	8	9	10

Feedback experiment with maize B73 grown on Changins soil conditioned by maize B73 and W22 (*background* variable), and mutants B73(*bx1*), W22, W22(*bx1*), W22(*bx2*), W22(*bx6*) (*BXgenotype_condition* variable).

Maize fresh weight

Table 2: ANOVA and Tukey test. formula = shoot fresh weight ~ BXgenotype_condition * background

	Sum Sq	Pr(>F)
BXgenotype_condition	239.2	0.0004294
background	0.4423	0.8433
BXgenotype_condition:background	10.08	0.3474
Residuals	582.8	NA

- **BXgenotype_condition**:

	diff	lwr	upr	p adj
bx2-bx1	-1.346	-4.974	2.281	0.7586
bx6-bx1	-0.6652	-4.17	2.839	0.9578
WT-bx1	-4.633	-7.487	-1.779	0.0004156
bx6-bx2	0.6809	-3.402	4.763	0.9707
WT-bx2	-3.287	-6.827	0.2532	0.07773
WT-bx6	-3.968	-7.382	-0.5538	0.01667

- **background**:

	diff	lwr	upr	p adj
W22-B73	0.1545	-1.681	1.99	0.8665

- **BXgenotype_condition:background**:

	diff	lwr	upr	p adj
WT:B73-bx1:B73	-3.7	-8.319	0.9187	0.2063
bx1:W22-bx1:B73	1.321	-3.693	6.335	0.9904
bx2:W22-bx1:B73	-0.7589	-5.616	4.098	0.9996
bx6:W22-bx1:B73	-0.078	-4.805	4.649	1
WT:W22-bx1:B73	-4.426	-9.153	0.3014	0.08191
bx1:W22-WT:B73	5.021	0.1094	9.933	0.04176
bx2:W22-WT:B73	2.941	-1.81	7.692	0.5213
bx6:W22-WT:B73	3.622	-0.9967	8.241	0.2286
WT:W22-WT:B73	-0.726	-5.345	3.893	0.9996
bx2:W22-bx1:W22	-2.08	-7.217	3.056	0.9026
bx6:W22-bx1:W22	-1.399	-6.413	3.615	0.9865
WT:W22-bx1:W22	-5.747	-10.76	-0.7331	0.01436
bx6:W22-bx2:W22	0.6809	-4.176	5.538	0.9998
WT:W22-bx2:W22	-3.667	-8.524	1.19	0.2707
WT:W22-bx6:W22	-4.348	-9.075	0.3794	0.09255
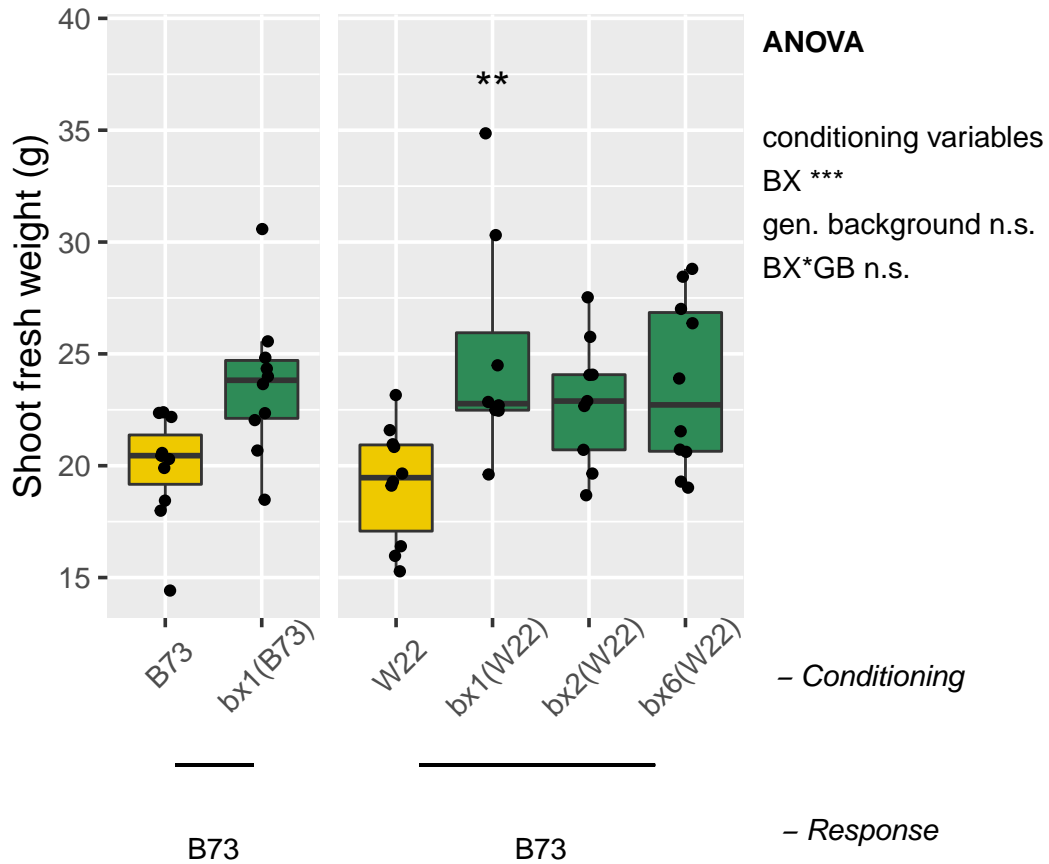
Figure 4 | Genetics of BX-feedback on maize
Potting soil was conditioned with B73 and bx1(B73) as well as with W22, bx1(W22), bx2(W22) and bx6(W22) followed by a feedback phase with only B73 plants (n = 8–11). Ten week old plants were utilized for measuring shoot biomass (fresh weight). The ANOVA results (model: ∼ BX condition (BX) * genetic background condition (GB)) are reported next to the Figure and the pair-wise Tukey-test results inside the panels (pair-wise comparison with wild-type, significance code: P < 0.01 **, P < 0.05 *; not significant = 'n.s.').

Insect growth rate

Table 6: ANOVA and Tukey test. formula = caterpillar growth rate ~ BXgenotype_condition * background

	Sum Sq	Pr(>F)
BXgenotype_condition	11.88	0.001228
background	0.2247	0.5587
BXgenotype_condition:background	3.557e-05	0.9941
Residuals	33.74	NA

- **BXgenotype_condition**:

	diff	lwr	upr	p adj
bx2-bx1	-0.1555	-1.028	0.7173	0.9647
bx6-bx1	-0.08692	-0.9301	0.7562	0.9928
WT-bx1	-0.9968	-1.683	-0.3101	0.001781
bx6-bx2	0.06857	-0.9137	1.051	0.9977
WT-bx2	-0.8413	-1.693	0.0104	0.05402
WT-bx6	-0.9099	-1.731	-0.08852	0.02445

- **background**:

	diff	lwr	upr	p adj
W22-B73	-0.1101	-0.5517	0.3315	0.6189

- **BXgenotype_condition:background**:

	diff	lwr	upr	p adj
WT:B73-bx1:B73	-0.9937	-2.105	0.1175	0.1112
bx1:W22-bx1:B73	-0.1544	-1.361	1.052	0.9999
bx2:W22-bx1:B73	-0.2241	-1.393	0.9444	0.9987
bx6:W22-bx1:B73	-0.1556	-1.293	0.9818	0.9999
WT:W22-bx1:B73	-1.144	-2.282	-0.006931	0.04761
bx1:W22-WT:B73	0.8393	-0.3424	2.021	0.3445
bx2:W22-WT:B73	0.7696	-0.3735	1.913	0.4127
bx6:W22-WT:B73	0.8382	-0.273	1.949	0.2719
WT:W22-WT:B73	-0.1506	-1.262	0.9607	0.9999
bx2:W22-bx1:W22	-0.0697	-1.306	1.166	1
bx6:W22-bx1:W22	-0.001131	-1.208	1.205	1
WT:W22-bx1:W22	-0.9899	-2.196	0.2165	0.1827
bx6:W22-bx2:W22	0.06857	-1.1	1.237	1
WT:W22-bx2:W22	-0.9202	-2.089	0.2484	0.2241
WT:W22-bx6:W22	-0.9888	-2.126	0.1486	0.1323
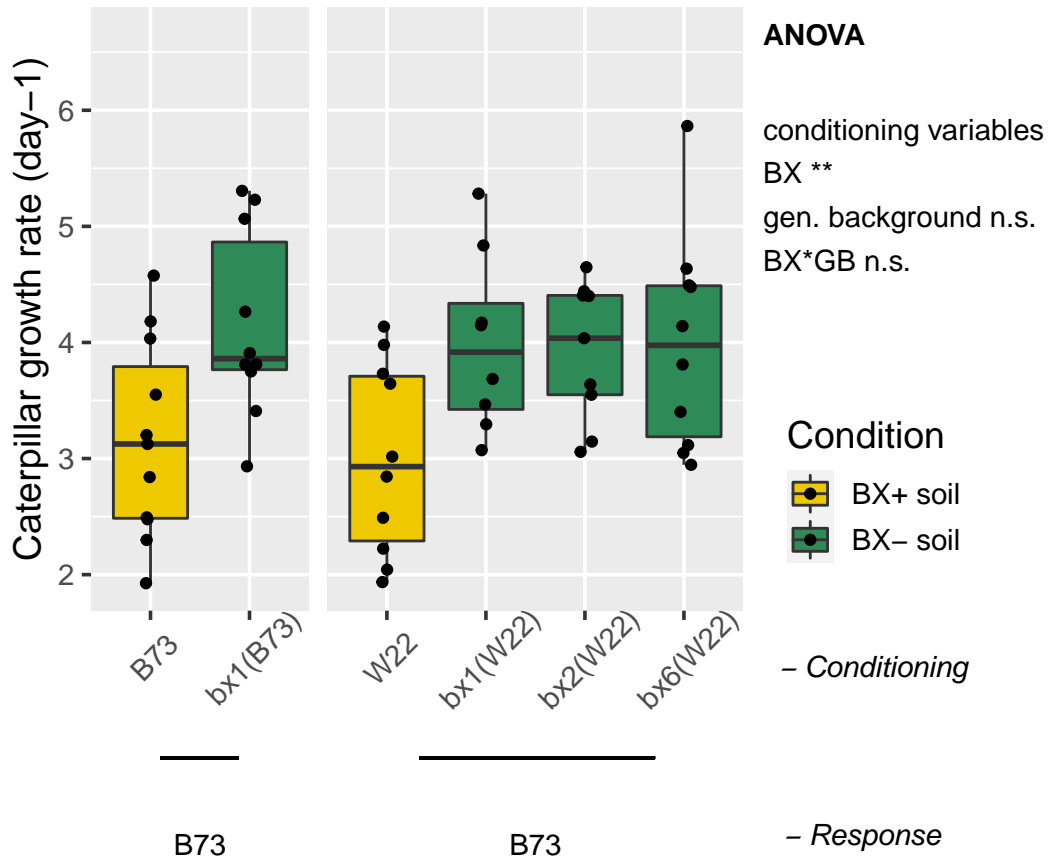
Figure 4 | Genetics of BX-feedback on maize
Potting soil was conditioned with B73 and bx1(B73) as well as with W22, bx1(W22), bx2(W22) and bx6(W22) followed by a feedback phase with only B73 plants (n = 8–11). Ten week old plants were utilized for measuring Spodoptera frugiperda performance. The ANOVA results (model: ~ BX condition (BX) * genetic background condition (GB)) are reported next to the Figure and the pair-wise Tukey-test results inside the panels (pair-wise comparison with wild-type, significance code: P < 0.01 **, P < 0.05 *; not significant = 'n.s.').