

Genes *ScBx1* and *ScIgl* – Competitors or Cooperators?

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Table S1. The content of BX in plants of three inbred lines, L318, D33, and D39 measured after the period of natural vernalization [16].

| Line | BX content [$\mu\text{g}/\text{mg}$ d.m.] | | | | | |
|------|--|---------|---------|---------|--------|--------|
| | HBOA | GDIBOA | DIBOA | GDIMBOA | DIMBOA | MBOA |
| L318 | 0.01297 | 0.02594 | 1.17881 | 0 | 0 | 0 |
| D33 | 0.0153 | 0.1736 | 0.84587 | 0.0019 | 0 | 0.0015 |
| D39 | 0.0099 | 0.1755 | 0.4760 | 0.00003 | 0 | 0.0012 |

Table S2. Parameters of mass spectrometry analysis.

| Compound | RT [min] | <i>m/z</i> of the parent [M-H] ⁻ ion | <i>m/z</i> of fragment ions | Collision energy [eV] | Cone voltage [V] |
|----------|-------------|--|---------------------------------|-----------------------------|------------------------|
| IbG (IS) | 3.70 | 294 | quantifier 131 qualifier 174 | 15 | 20 |
| HBOA | 4.15 | 164 | quantifier 108 qualifier 136 | 15 | 30 |
| DIBOA | 4.45 | 180 | quantifier 134 qualifier 124 | 6 | 20 |
| GDIBOA | 5.10 | 342 | quantifier 162 qualifier 180 | 15 | 25 |
| DIMBOA | 6.30 | 210 | quantifier 149 qualifier 195 | 6 | 15 |
| GDIMBOA | 6.70 | 372 | quantifier 149 qualifier 210 | 15 | 35 |
| MBOA | 8.00 | 164 | quantifier 149 qualifier 121 | 15 | 20 |

Table S3. The values of r coefficients for correlations between the expression level of the genes *ScBx1* and *ScIgl*, and the content of BXs (based on Spearman's rho).

| IL | BX gene | Time point | | | | | | | | | | | |
|------|------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| | | I | | II | | III | | IV | | V | | VI | |
| | | <i>ScBx1</i> | <i>ScIgl</i> | <i>ScBx1</i> | <i>ScIgl</i> | <i>ScBx1</i> | <i>ScIgl</i> | <i>ScBx1</i> | <i>ScIgl</i> | <i>ScBx1</i> | <i>ScIgl</i> | <i>ScBx1</i> | <i>ScIgl</i> |
| L318 | HBOA | 0.83** | 0.00 | -0.10 | -0.40 | 0.00 | 0.63 | anpo | 0.65 | anpo | 0.50 | anpo | -0.03 |
| | GDIBOA | -0.77 | 0.54 | 0.09 | 0.46 | -0.58 | 0.29 | anpo | 0.41 | anpo | 0.52 | anpo | -0.03 |
| | DIBOA | 0.6 | -0.14 | 0.77 | -0.87** | -0.93** | 0.7 | anpo | 0.5 | anpo | 0.44 | anpo | 0.03 |
| | GDIMBOA | -0.77 | 0.54 | 0.54 | -0.52 | -0.52 | -0.35 | anpo | anpe | anpo | 0.46 | anpo | 0.17 |
| | DIMBOA | -0.75 | -0.23 | 0.6 | -0.46 | -0.52 | -0.23 | anpo | anpe | anpo | 0.46 | anpo | -0.03 |
| | MBOA | -0.75 | -0.23 | 0.43 | -0.84** | -0.93** | 0.46 | anpo | anpe | anpo | 0.52 | anpo | 0.4 |
| D33 | HBOA | -0.81 | 0.62 | -0.65 | -0.65 | -0.39 | 0.39 | 0.44 | -0.50 | anpo | 0.52 | anpo | -0.49 |
| | GDIBOA | 0.46 | -0.03 | -0.70 | -0.67 | -0.93** | 0.70 | -0.93** | 0.99** | anpo | 0.52 | anpo | -0.60 |
| | DIBOA | 0.52 | -0.77 | -0.94** | -0.43 | -0.43 | 0.09 | -0.46 | 0.52 | anpo | -0.41 | anpo | -0.60 |
| | GDIMBOA | 0.06 | -0.60 | -0.43 | -0.94** | -0.94** | 0.71 | -0.43 | 0.43 | anpo | -0.06 | anpo | 0.14 |
| | DIMBOA | 0.52 | -0.60 | -0.94** | -0.43 | -0.49 | -0.09 | 0.37 | -0.49 | anpo | -0.50 | anpo | -0.89** |
| | MBOA | -0.46 | 0.83** | -0.26 | -0.94** | -0.43 | 0.09 | -0.03 | -0.03 | anpo | 0.52 | anpo | -0.54 |
| D39 | HBOA | -0.50 | -0.97** | anpe | anpe | anpo | anpe | anpo | anpe | anpo | 1.00 | anpo | -0.46 |
| | GDIBOA | -0.46 | -0.99** | 0.87** | -0.49 | anpo | 0.46 | anpo | -0.89** | anpo | 0.54 | anpo | -0.43 |
| | DIBOA | -0.37 | -0.94** | -0.93** | 0.70 | anpo | 0.41 | anpo | -0.37 | anpo | 1.00** | anpo | 0.37 |
| | GDIMBOA | 0.43 | 0.89** | 0.37 | 0.26 | anpo | 0.93** | anpo | -0.49 | anpo | 0.43 | anpo | -0.77 |
| | DIMBOA | 0.85** | 0.78 | -0.93** | 0.70 | anpo | anpe | anpo | anpe | anpo | 0.09 | anpo | -0.03 |
| | MBOA | 0.09 | -0.03 | -0.31 | 0.54 | anpo | anpe | anpo | anpe | anpo | anpe | anpo | -0.94** |

* – significant at $p < 0.1$

** – significant at $p < 0.05$

anpo - analysis not possible because of undetectable expression of *ScBx1* gene

anpe - analysis not performed because of the equal values of BX contents

Table S4. Proteins bound to the promoter sequences of the *ScBx1* and *ScIgl* genes annotated in the *in vivo* assay.

| <i>ScBx1</i> Prs-treated | <i>ScIgl</i> Prs-treated | <i>ScBx1</i> untreated | <i>ScIgl</i> untreated |
|---|--|--|---|
| HMG-I/Y protein (HMGa) [<i>Triticum aestivum</i>] AAM22691.1 | Methyl-CpG-binding domain-containing protein 9 (MBD9); PKA61759.1 | HMG-I/Y protein (HMGa); AAM22691.1 | <u>NAC domain containing transcription factor (NAC042); ANR02348.1</u> |
| <u>germin-like protein (GLP1) AEN02469.1</u> | <u>germin-like protein (GLP1); AEN02469.1</u> | KIX_2 domain-containing protein; ABK26292.1 | Integrase catalytic domain-containing protein (TCM_012637); EOY21236.1 |
| histone deacetylase 2 (HDAC2); ABG43091.1 | Integrase catalytic domain-containing protein (TCM_012637); EOY21236.1 | HMG-Y-related protein A (TRIUR3_33243); EMS65335.1 | <u>Zinc finger protein (ZAT1_1); PWZ38869.1</u> |
| KIX_2 domain-containing protein; ABK26292.1 | hypothetical protein (CFOL_v3_04877), partial; GAV61350.1 | Integrase catalytic domain-containing protein (TCM_012637); EOY21236.1 | PREDICTED: uncharacterized protein (LOC103500952) isoform X2; XP_008462641.1 |
| H15 domain-containing protein; AFK35979.1 | NusB domain-containing protein (Ahy_B04g070543) isoform B; RYR13675.1 | DNA/RNA helicase, DEAD/DEAH box type, N-terminal (Ccrd_016929); KVI04750.1 | <u>RING-H2 finger protein ATL8-like; XP_020242355.1</u> |
| predicted protein BAK00355.1 | <u>protein EXORDIUM-like 2; XP_002960721.1</u> | <u>protein EXORDIUM-like 2; XP_002960721.1</u> | LOW QUALITY PROTEIN: DNA-directed RNA polymerase 3B, chloroplastic; XP_020980201.1 |
| unnamed protein product (TRAES_3BF060500360CFD_c1); CDM80146.1 | PREDICTED: uncharacterized protein (LOC103500952) isoform X2; XP_008462641.1 | PREDICTED: pre-mRNA-splicing factor ATP-dependent RNA helicase (PRP16); XP_004148974.1 | |
| Integrase catalytic domain-containing protein (TCM_012637); EOY21236.1 | PREDICTED: sister chromatid cohesion protein (PDS5) homolog A-like isoform X1; XP_011040159.1 | PREDICTED: serine/arginine-rich splicing factor RSZ22-like; XP_008337095.1 | |
| HMG1/2-like protein; P40621.1 | LOW QUALITY PROTEIN: DNA-directed RNA polymerase 3B, chloroplastic; XP_020980201.1 | PREDICTED: uncharacterized protein (LOC103500952) isoform X2; XP_008462641.1 | |
| MYND-type domain-containing protein (CHLRE_17g740000v5) PNW70945.1 | | PREDICTED: sister chromatid cohesion protein (PDS5) homolog A-like isoform X1; XP_011040159.1 | |
| <u>NIM1-interacting TFIIF subunit (TorRG33x02_009350); POO02874.1</u> | | <u>RING-H2 finger protein ATL8-like; XP_020242355.1</u> | |
| TOP4c domain-containing protein (GQ55_9G036200); PUZ36416.1 | | LOW QUALITY PROTEIN: DNA-directed RNA polymerase 3B, chloroplastic; XP_020980201.1 | |
| <u>Zinc finger protein (ZAT1_1); PWZ38869.1</u> | | structural maintenance of chromosomes protein 5-like (PHYPA_028643); XP_024364315.1 | |
| Nucleosome assembly protein 1;3-like (C5167_004537), partial; RZC57230.1 | | <u>agamous-like MADS-box protein (AGL62); XP_027364274.1</u> | |
| PREDICTED: uncharacterized protein (LOC103500952) isoform X2; XP_008462641.1 | | | |
| DNA repair protein RAD5B; XP_010918825.1 | | | |
| sister chromatid cohesion protein (PDS5) homolog A; XP_021902104.1 | | | |
| SpoU_methylase domain-containing protein (LOC112275201); XP_024361104.1 | | | |
| <u>probable transcription factor (KAN4) myb-like DNA- binding domain; XP_026398713.1</u> | | | |
| <u>agamous-like MADS-box protein (AGL62); XP_027364274.1</u> | | | |
| No. of proteins bound to the promoter sequences | | | |
| 20 | 9 | 14 | 6 |

Prs - Puccinia recondita f.sp. *secalis* (Roberge ex Desmaz), stress related transcription factors (TFs) are marked in bold growth and development related TFs are marked in bold and underlined by a single line

stress related and growth and development related TFs are marked in bold and underlined by a dotted line

Table S5. Normalized expression level of *ScBx1* and *ScIgl* genes in leaves of rye cv. Stach F1 inoculated with BSMV:*ScBx1* on the 14th dpi.

| Plant | Normalized expression level of <i>ScBx1</i> | Normalized expression level of <i>ScIgl</i> |
|--------------|--|--|
| # 1 | 0.00 | 0.89 |
| # 3 | 0.06 | 1.16 |
| # 5 | 0.06 | 0.21 |
| # 7 | 0.00 | 1.47 |
| # 9 | 0.02 | 6.26 |

The relative expression level of *ScBx1* and *ScIgl* gene was normalized to their expression level in plants inoculated with BSMV: $\alpha, \beta^{(-)}, \gamma^{(PDS)}$ vector assumed as 1.

Table S6. Normalized expression level of *ScBx1* and *ScIgl* genes in leaves of rye cv. Stach F1 inoculated with BSMV:*ScBx1* on the 21th dpi.

| Plant | Normalized expression level of <i>ScBx1</i> | Normalized expression level of <i>ScIgl</i> |
|--------------|--|--|
| # 2 | 0.00 | 0.31 |
| # 5 | 0.00 | 0.14 |
| # 6 | 0.01 | 0.13 |
| # 8 | 0.02 | 0.74 |
| # 10 | 0.05 | 0.32 |
| # 11 | 0.00 | 0.32 |
| # 12 | 0.03 | 0.98 |
| # 13 | 0.00 | 1.89 |
| # 14 | 0.12 | 1.15 |

The relative expression level of *ScBx1* and *ScIgl* gene was normalized to their expression level in plants inoculated with BSMV: $\alpha, \beta^{(-)}, \gamma^{(PDS)}$ vector assumed as 1.