

Aromatic cytokinin arabinosides promote PAMP-like responses and positively regulate leaf longevity

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Supporting Information

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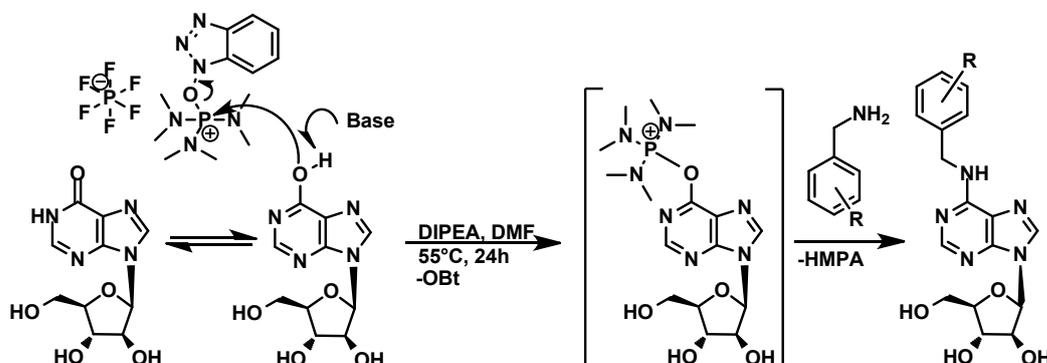
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Supplementary Methods

Synthesis of prepared derivatives

The synthesis of BAPAs was achieved according to the following general reaction scheme given here:



9-(β-D-arabinofuranosyl)hypoxanthine (100 mg, 0.37 mmol) and BOP (196 mg, 0.37 mmol) were mixed together in DMF (2 ml). Subsequently, the appropriate benzylamine (0.37 mmol) and DIPEA (97 μl, 0.44 mmol) were added. The reaction mixture was stirred under an argon atmosphere in an oil bath at 60 °C for 24 h then evaporated using a rotary evaporator. MeOH with a drop of chloroform was added to the distillation residue and the mixture was sonicated. The resulted white solid was isolated by filtration and re-crystallized from EtOH. The identity and purity of the synthesized compounds was confirmed by elemental and melting point analysis, analytical thin layer chromatography, high performance liquid chromatography, ES+MS spectrometry, and ¹H NMR (Supplementary Table 1).

¹H NMR data for the new compounds (DMSO-d₆, 500 MHz) δ ppm:

Compound 1:

3.55-3.75 (m,3H), 4.09 (t,J=4,5Hz,2H), 4.66 (bs,2H), 5.05 (t,J=5,5Hz,1H), 5.48 (d,J=4,5Hz,1H), 5.57 (d,J=5Hz,1H), 6.21 (d,J=4Hz,1H), 7.12-7.32 (m,5H), 8.14 (s,1H), 8.16 (s,1H), 8.32 (bs,1H)

Compound 2:

3.55-3.68 (m,2H), 3.74 (d,J=3,5Hz,1H), 4.10 (s,2H), 4.71 (bs,2H), 5.05 (d,J=4,5Hz,1H), 5.49 (s,1H), 5.58 (d,J=3,5Hz,1H), 6.23 (d,J=3,5Hz,1H), 7.03-7.32 (m,4H), 8.15 (s,1H), 8.18 (s,1H), 8.30 (bs,1H)

Compound 3:

3.55-3.67 (m,2H), 3.74 (d,J=4Hz,1H), 4.10 (s,2H), 4.67 (bs,2H), 5.05 (t,J=4,5Hz,1H), 5.48 (d,J=4Hz,1H), 5.57 (d,J=3,5Hz,1H), 6.22 (d,J=4Hz,1H), 6.98 (t,J=6Hz,1H), 7.06-7.16 (m,2H), 7.25-7.33 (m,1H), 8.15 (s,1H), 8.18 (s,1H), 8.37 (bs,1H)

Compound 4:

3.55-3.66 (m,2H), 3.73 (d,J=4,5 Hz,1H), 4.07-4.11 (m,2H), 4.62 (bs,2H), 5.06 (t,J=5,5 Hz,1H), 5.49 (d,J=4,5 Hz,1H), 5.57 (d,J=5 Hz,1H), 6.22 (d,J=5 Hz,1H), 7.07 (t,J=8,5 Hz,2H), 7.33 (t,J=5,5 Hz,2H), 8.16 (s,2H), 8.34 (bs,1H)

Compound 5:

3.54-3.80 (m,3H), 4.10 (bs,2H), 4.71 (bs,2H), 5.06 (bs,1H), 5.49 (d,J=3,5Hz,1H), 5.60 (d,J=4Hz,1H), 6.23 (d,J=4Hz,1H) 7.21 (d,J=4Hz,3H), 7.37-7.44 (m,1H), 8.13 (s,1H), 8.20 (s,1H), 8.34 (bs,1H)

Compound 6:

3.54-3.65 (m,2H), 3.74 (bs,1H), 4.09 (bs,2H), 4.65 (bs,2H), 5.11 (bs,1H), 5.56 (bs,2H), 6.21 (d, J=4,5 Hz,1H), 7.21-7.35 (m,4H), 8.15 (s,1H), 8.17 (s,1H), 8.37 (s,1H)

Compound 7:

3.56-3.67 (m,2H), 3.73 (bs,1H), 4.09 (bs,2H), 4.63 (bs,2H), 5.05 (t,J=4,5Hz,1H), 5.48 (d,J=3Hz,1H), 5.57 (d,J=4,5Hz,1H), 6.22 (d,J=4Hz,1H), 7.30 (s,4H), 8.14 (s,1H), 8.17 (s,1H), 8.36 (bs,1H)

Compound 8:

3.75-3.79 (m,2H), 4.13 (t,J=5 Hz,3H), 4.64 (bs,2H), 5.23 (bs, 1H), 5.66 (bs,2H), 6.25 (d,J=5,5 Hz,1H), 7.09 (t,J=7,5 Hz,1H), 7.33 (d,J=7,5 Hz,1H), 7.55 (d,J=8 Hz,1H), 7.68 (s,1H), 8.18 (s,1H), 8.21 (s,1H), 8.36 (bs,1H)

Compound 9:

3.54-3.65 (m,2H), 3.72 (d,J=4Hz,1H), 4.09 (t,J=4,5Hz,2H), 4.58 (bs,2H), 5.04 (t,J=5Hz,1H), 5.48 (d,J=4Hz,1H), 5.56 (d,J=4,5Hz,1H), 6.21 (d,J=4Hz,1H), 7.09 (d,J=8,5Hz,2H), 7.59 (d,J=8Hz,2H), 8.13 (s,1H), 8.16 (s,1H), 8.34 (bs,1H)

Compound 10:

3.55-3.68 (m,2H), 3.74 (d,J=4Hz,1H), 3.79 (s,3H), 4.10 (s,2H), 4.62 (bs,2H), 5.07 (bs,1H), 5.50 (s,1H), 5.59 (s,1H), 6.23 (d,J=4Hz,1H), 6.79 (t,J=8Hz,1H), 6.93 (d,J=7,5Hz,1H), 7.05 (d,J=6,5Hz,1H), 7.16 (t,J=8Hz,1H), 8.06 (s,1H), 8.11 (s,1H), 8.17 (bs,1H)

Compound 11:

3.56-3.79 (m,6H), 4.10 (s,2H), 4.63 (bs,2H), 5.06 (s,1H), 5.49 (s,1H), 5.58 (s,1H), 6.22 (d,J=2,5Hz,1H), 6.72 (d,J=6Hz,1H), 6.86 (d,J=8Hz,2H), 7.15 (t,J=8Hz,1H), 8.15 (s,1H), 8.17 (s,1H), 8.30 (bs,1H)

Compound 12:

3.52-3.75 (m,6H), 4.03-4.12 (m,2H), 4.56 (bs,2H), 5.00-5.09 (m,1H), 5.44-5.59 (m,2H), 6.15-6.24 (m,1H), 6.79 (d,J=8,5Hz,2H), 7.16-7.26 (m,2H), 8.12 (s,1H), 8.14 (s,1H), 8.24 (bs,1H)

Compound 13:

3.54-3.67 (m,2H), 3.74 (d,J=3,5Hz,1H), 4.10 (s,2H), 4.57 (bs,2H), 5.06 (s,1H), 5.49 (s,1H), 5.58 (d,J=3,5Hz,1H), 6.22 (d,J=3Hz,1H), 6.54 (d, J=7,5Hz,1H), 6.65-6.74 (m,2H), 7.02 (t,J=7,5Hz,1H), 8.14 (s,1H), 8.16 (s,1H), 8.26 (bs,1H), 9.20 (s,1H)

Compound 14:

3.54-3.76 (m,6H), 4.03-4.15 (m,2H), 4.62 (bs,1H), 5.04 (t,J=5,5Hz,1H), 5.49 (d,J=4,5Hz,2H), 5.59 (d,J=6Hz,2H), 6.12-6.25 (m,1H), 7.01-7.18 (m,2H), 7.99 (s,1H), 8.10 (s,1H), 8.12 (s,1H), 8.23 (bs,1H)

Compound 15:

3.55-3.78 (m,6H), 4.06-4.15 (m,2H), 4.62 (bs,2H), 5.14 (t,J=5,5HZ,1H), 5.56 (d,J=4Hz,1H), 5.62 (d,J=5Hz,1H), 6.22 (d,J=4Hz,1H), 6.96 (d,J=7Hz,1H), 7.04-7.15 (m,3H), 8.14 (s,1H), 8.16 (s,1H), 8.26 (bs,1H)

Compound 16:

3.54-3.77 (m,6H), 4.09 (s,2H), 4.61 (bs,2H), 5.05(t,J=5,5Hz,1H), 5.48 (d,J=3,5Hz,1H), 5.57 (d,J=4,5Hz,1H), 6.21 (d,J=4Hz,1H), 7.04 (d,J=7,5Hz,2H), 7.17 (d,J=7,5Hz,2H), 8.13 (s,1H), 8.15 (s,1H), 8.26 (bs,1H)

Compound 17:

3.57-3.70 (m,2H), 3.76 (d,J=4 Hz,1H), 4.12 (s,2H), 4.51 (bs,2H), 5.09 (t,J=5 Hz,1H), 5.19 (s,2H), 5.52 (d,J=4 Hz,1H), 5.59 (d,J=5 Hz,1H), 6.25 (d,J=4 Hz,1H), 6.45 (t,J=7 Hz,1H), 6.59 (d,J=8 Hz,1H), 6.90 (t,J=7 Hz,1H), 7.07 (d,J=7,5 Hz,1H), 8.20 (s,3H)

Compound 18:

3.55-3.76 (m,3H), 4.06-4.13 (m,2H), 4.73 (bs,2H), 5.05 (t,J=5Hz,1H), 5.48 (d,J=4,5Hz,1H), 5.56(d,J=5,5Hz,1H), 6.22 (d,J=5Hz,1H), 7.46-7.68 (m,4H), 8.15 (s,1H), 8.18 (s,1H), 8.44 (bs,1H)

Compound 19:

3.56-3.76 (m,3H), 4.05-4.14 (m,2H), 4.68 (bs,2H), 5.05 (t,J=5,5Hz,1H), 5.48 (d,J=4,5Hz,1H), 5.57 (d,J=5,5Hz,1H), 6.22 (d,J=4,5Hz,1H), 7.15 (d,J=8Hz,1H), 7.25-7.42 (m,3H), 8.15 (s,1H), 8.18 (s,1H), 8.40 (bs, 1H)

Compound 20:

3.54-3.76 (m,3H), 4.03-4.15 (m,2H), 4.68 (bs,2H), 5.05 (t,J=5,5Hz,1H), 5.48 (d,J=4Hz,1H), 5.56 (d,J=5Hz,1H), 6.23 (d,J=4,5Hz,1H), 7.02-7.22 (m,3H), 8.16 (s,1H), 8.19 (s,1H), 8.34 (bs,1H)

Compound 21:

3.40-3.53 (m,2H), 3.60 (d,J=2,5 Hz,1H), 3.95 (s,2H), 4.52 (bs,2H), 4.91 t,J=5Hz,1h), 5.35 (d,J=2Hz,1H), 5.42 (d,J=4Hz,1H), 6.09 (d,J=3Hz,1H), 6.79-6.92 (m,3H), 8.02 (s,1H), 8.05 (s,1H), 8.26 (bs,1H)

General procedures

Elemental analyses were performed using an EA1112 CHN analyzer (Thermo Finnigan, ThermoFisher Scientific). Melting points were determined on a Buchi Melting Point B-540 apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was carried out using silica gel 60 WF₂₅₄ plates (Merck) with CHCl₃/MeOH (4:1, v/v) as the solvent. The purity of the synthesized compounds was determined as described previously ¹. Briefly, samples were dissolved in methanol (1 mg ml⁻¹) and then diluted to a concentration of 10 µg ml⁻¹ in the initial mobile phase. 10 µl of the resulting solution was injected onto a thermostated (25 °C) RP column (150 mm × 2.1 mm, 5 µm C18 Symmetry, Waters) and analyzed using an Alliance 2695 Separations Module high-performance liquid chromatograph coupled to a PDA 2996 detector (Waters) with detection at wavelengths of 210–400 nm. The chromatograph's effluent was directed into the ion source of a benchtop quadrupole orthogonal acceleration time-of-flight Q-TOF Micro tandem mass spectrometer (Waters). HPLC elution was performed at a flow rate of 0.3 ml min⁻¹ using a linear gradient of 15 mM ammonium formate at pH 4.0 (mobile phase A) and pure methanol (mobile phase B). The proportion of B in the mobile phase was initially 10% and was increased linearly to 90% over 24 min, after which isocratic elution was performed with 90% B. The column was then re-equilibrated under the initial conditions (10% B) for 10 min. The electrospray source was maintained at 120 °C with a capillary voltage of +3.0 kV, a cone voltage of +20 V, and a desolvation temperature of 300 °C. Nitrogen was used as both the desolvation gas (500 l h⁻¹) and the cone gas (50 l h⁻¹). The mass spectrometer was operated in positive (ESI+) ionization mode and data were acquired in the 50–1000 *m/z* range. The mass spectrometer was directly coupled to a MassLynx 4.1 data system. ¹H NMR spectra were measured on a JEOL 500 ECA instrument at 500 MHz. Samples were prepared by dissolving the compounds in DMSO-*d*₆. Tetramethylsilane (TMS) was used as the internal standard.

KEGG pathway over-representation analysis

For the visual interpretation of transcriptomic data from 3-methoxy-BAPA treatments, the KEGG database was employed (<https://www.kegg.jp>). KEGG Mapper was used for processing of KO annotation data ².

Bacterial cytokinin assay

E. coli strain KMI001 (*ΔrcsC*, *cps::lacZ*), harboring either the plasmid pIN-III-AHK4 or pSTV28-AHK3, which express the Arabidopsis histidine kinases CRE1/AHK4 or AHK3 ^{3, 4} was used in the experiments. Activity of β-galactosidase was measured using 4-methylumbelliferyl β-D-galactopyranoside as a substrate after overnight growth in the presence of CK ⁵. In addition, live-cell cytokinin-binding assay with radiolabeled *tZ* as a competitor was performed as previously described ⁶. Homogenous bacterial suspensions with OD₆₀₀ values of 0.8 and 1.2 were found to be optimal for CRE1/AHK4 and AHK3 cultures, respectively. The competition reaction was allowed to proceed with 2 nM [2-³H]*tZ* and various concentrations of the tested compounds for 30 min at 4 °C. When binding equilibrium was reached, the suspension was centrifuged (6000 x g), the supernatant was removed, and the bacterial pellet was resuspended in the scintillation cocktail (Beckman).

Effect of 3-methoxy-BAPA and 3-hydroxy-BAPA on infection level in field trial experiments

An effect of 3-methoxy-BAPA and 3-hydroxy-BAPA was tested in field trials on winter wheat (cv. Turandot; Selgen, Czech Republic) and spring barley (cv. Francin; Selgen, Czech Republic) performed near Olomouc, Czech Republic (GPS: 49.5748542N, 17.2851261E; altitude 218 m) in a growth season 2017/2018. The 5 μM solution of 3-methoxy-BAPA or 3-hydroxy-BAPA was applied as a foliar spray on wheat at a growth stage of tillering (BBCH 25 ⁷) and on barley at stage of beginning of stem elongation (BBCH 30-33 ⁷) or start of flag leaf extending (BBCH 39-41 ⁷) in amount of 300 ml per 10 m². A level of infection was evaluated at a growth stage of beginning of heading (BBCH 51 ⁷) in the case of wheat and leaf rusts and barley and powdery mildew or at growth stage of early milk ripeness (BBCH 73 ⁷) in the case of barley and foot rot diseases. The evaluation of each variant was carried out on 5 plots (n = 5), 20 plants were evaluated from each plot. The level of infection was evaluated according the following scales:

- i) for wheat and leaf rusts (namely *Puccinia striiformis* syn. *Puccinia glumarum*, *Puccinia recondita* f. sp. *tritici*, *Puccinia graminis*): 1 – without infection; 2 – a few clusters of chlorosis; 3 – clusters to 1 % of leaf area; 4 – clusters from 1 to 3 % of leaf area; 5 - clusters from 3 to 5 % of leaf area, beginning of strips; 6 – clusters and strips from 5 to 15 % of leaf area;
- ii) for barley and powdery mildew (*Blumeria graminis* syn. *Erysiphe graminis*): 1 – without symptoms; 2 – mycelium at max. 1 % of leaf area; 3 – mycelium at 1 – 5 % of leaf area; 4 – mycelium at 5 – 10 % of leaf area; 5 - mycelium at 10 – 30 % of leaf area;
- iii) for barley and foot rot diseases (*Gaeumannomyces graminis* syn. *Ophiobolus graminis*, *Ramulispora herpotrichoides* syn. *Pseudocercospora herpotrichoides*, teleomorpha *Tapesia yallundae*, *Fusarium* spp., *Rhizoctonia* spp.): 1 – without symptoms; 3 – brown spots to 5 % of stem perimeter; 5 – brown spots from 5 to 25 % of stem perimeter.

In the end of each experiment, a number of tillers per plant was determined with 50 randomized plants of the variant (10 plants from each plot). A number of spikes per 0.25 m² was counted and converted to 1 m². After harvest, grain yield was determined.

Supplementary Tables

Supplementary Table 1. Elemental analysis, ES+ mass spectrometry analysis, melting points and HPLC purity of prepared BAPA derivatives.

Compound	Elemental analysis calculated/found			Mp (°C)	ES-MS [M+H ⁺]	HPLC (%)
	% of C	% of H	% of N			
1	57.1/55.9	5.3/4.9	19.6/19.0	191-193	358	>98
2	54.4/52.6	4.8/4.7	18.7/18.0	191-193	376	>98
3	54.4/54.0	4.8/4.7	18.7/18.8	224-225	376	>98
4	54.4/52.9	4.8/4.9	18.7/18.0	190-192	376	>98
5	52.1/51.7	4.6/4.7	17.9/17.6	226-229	393	>99
6	52.1/51.9	4.6/4.6	17.9/17.4	240-242	393	>98
7	52.1/52.3	4.6/4.4	17.9/17.2	181-183	393	>99
8	42.3/41.8	3.8/3.6	14.5/14.1	221-223	484	>98
9	42.3/42.2	3.8/3.8	14.5/13.8	182-184	484	>98
10	55.8/55.6	5.5/5.5	18.1/17.9	224-225	388	>98
11	55.8/55.4	5.5/5.4	18.1/17.9	236-238	388	>99
12	55.8/55.7	5.5/5.5	18.1/17.9	182-183	388	>99
13	54.7/53.9	5.1/5.0	18.8/18.3	235-238	374	>98
14	58.2/57.9	5.7/5.4	18.9/18.8	224-227	372	>98
15	58.2/57.4	5.7/5.3	18.9/18.9	216-218	372	>99
16	58.2/58.0	5.7/5.4	18.9/18.6	173-177	372	>98
17	54.8/54.2	5.4/5.3	22.6/21.8	193-196	373	>98
18	50.8/49.9	4.3/3.9	16.5/15.8	182-184	426	>98
19	49.0/48.6	4.1/3.9	15.9/15.4	175-176	442	>98
20	51.9/51.2	4.4/4.3	17.8/17.6	214-216	394	>98
21	51.9/51.4	4.4/3.7	17.8/17.3	236-237	394	>98

Supplementary Table 2. Overview of DEGs regulated by 3-methoxy-BAPA. The table lists the 50 most strongly upregulated and downregulated genes (adjusted P-value < 0.05), respectively, that showed the highest absolute log₂ fold change (log₂FC) after 6 h dark-incubation of detached Arabidopsis leaves with 10 μM 3-methoxy-BAPA.

UPREGULATED DEGs		
AGI number	Description	log ₂ FC
<i>At2g47040</i>	Vanguard 1/Pectinesterase 5 [Source:Uniprot/SWISSPROT 3BAcc:Q5MFV8]	2.80
<i>At3g01270</i>	Probable pectate lyase 7 [Source:Uniprot/SWISSPROT 3BAcc:Q9SRH4]	2.79
<i>At2g47050</i>	Plant invertase/pectin methylesterase inhibitor superfamily protein [Source:TAIR 3BAcc:47050]	2.31
<i>At3g07820</i>	Pectin lyase-like superfamily protein [Source:TAIR 3BAcc:07820]	2.22
<i>At3g28750</i>	Hypothetical protein	2.21
<i>At2g47030</i>	Plant invertase/pectin methylesterase inhibitor superfamily protein (VGDH1) [Source:Uniprot/SWISSPROT 3BAcc:O80722]	2.16
<i>At4g35010</i>	Beta-galactosidase 11 (BGAL11) [Source:Uniprot/SWISSPROT 3BAcc:Q9SCV1]	2.03
<i>At3g62230</i>	F-box protein At3g62230/DUO1-ACTIVATED F-BOX 1 [Source:Uniprot/SWISSPROT 3BAcc:Q9M1Q1]	1.95
<i>At3g05610</i>	Probable pectinesterase/pectinesterase inhibitor 21 [Source:Uniprot/SWISSPROT 3BAcc:Q8GX86]	1.80
<i>At5g44430</i>	PLANT DEFENSIN 1.2C (PDF1.2C)/Defensin-like protein 17 [Source:Uniprot/SWISSPROT 3BAcc:Q9FI22]	1.75
<i>At1g55560</i>	SKU5 similar 14/SKS14 [Source:TAIR 3BAcc:55560]	1.71
<i>At1g02790</i>	Exopolygalacturonase 4 (PGA4) [Source:Uniprot/SWISSPROT 3BAcc:P49062]	1.67

<i>At5g39880</i>	Transmembrane protein [Source:TAIR 3Bacc:39880]	1.64
<i>At5g44420</i>	PLANT DEFENSIN 1.2A/Defensin-like protein 16 [Source:Uniprot/SWISSPROT 3Bacc:Q9FI23]	1.58
<i>At5g07430</i>	Pectin lyase-like superfamily protein/Probable pectinesterase 50 [Source:Uniprot/SWISSPROT 3Bacc:Q9LY17]	1.57
<i>At1g07260</i>	UDP-glycosyltransferase 71C3 (UGT71C3) [Source:Uniprot/SWISSPROT 3Bacc:Q9LML7]	1.54
<i>At2g26020</i>	PLANT DEFENSIN 1.2B/Putative defensin-like protein 15 [Source:Uniprot/SWISSPROT 3Bacc:O80994]	1.52
<i>At5g19580</i>	Glyoxal oxidase-related protein [Source:TAIR 3Bacc:19580]	1.51
<i>At2g26010</i>	PLANT DEFENSIN 1.3/Defensin-like protein 14 [Source:Uniprot/SWISSPROT 3Bacc:O80995]	1.47
<i>At5g45880</i>	Pollen Ole e 1 allergen and extensin family protein [Source:TAIR 3Bacc:45880]	1.45
<i>At5g50030</i>	Plant invertase/pectin methylsterase inhibitor superfamily protein [Source:TAIR 3Bacc:50030]	1.42
<i>At3g01240</i>	Splicing regulatory glutamine/lysine-rich-like protein	1.35
<i>At3g62710</i>	Glycosyl hydrolase family protein [Source:TAIR 3Bacc:62710]	1.35
<i>At5g04180</i>	Alpha carbonic anhydrase 3 (ATACA3) [Source:Uniprot/SWISSPROT 3Bacc:Q9FYE3]	1.33
<i>At5g12960</i>	Proline-tRNA ligase (DUF1680) [Source:TAIR 3Bacc:12960]	1.33
<i>At5g26700</i>	RmlC-like cupins superfamily protein/Probable germin-like protein subfamily 2 member 5 [Source:Uniprot/SWISSPROT 3Bacc:O65252]	1.26
<i>At1g05580</i>	Cation/H(+) antiporter 23 2C chloroplastic (CHX23) [Source:Uniprot/SWISSPROT 3Bacc:Q8VYD4]	1.26
<i>At5g58390</i>	Peroxidase superfamily protein [Source:Uniprot/SWISSPROT 3Bacc:Q9LVL2]	1.23
<i>At1g61566</i>	Protein RALF-like 9 (RALFL9) [Source:Uniprot/SWISSPROT 3Bacc:Q3ECL0]	1.19
<i>At1g62760</i>	Pectin methylsterase inhibitor (ATPMEI10) [Source:TAIR 3Bacc:AT1G62760]	1.18
<i>At1g06160</i>	Ethylene-responsive transcription factor ERF094/Ethylene responsive factor 59 [Source:Uniprot/SWISSPROT 3Bacc:Q9LND1]	1.16
<i>At2g04460</i>	Transposable element gene [Source:TAIR 3Bacc:04460]	1.16
<i>At5g61160</i>	Agmatine coumaroyltransferase/Anthocyanine 5-aromatic acyltransferase 1 [Source:Uniprot/SWISSPROT 3Bacc:Q9FNP9]	1.13
<i>At3g20470</i>	Glycine-rich protein 5 [Source:TAIR 3Bacc:AT3G20470]	1.09
<i>At1g59950</i>	NAD(P)-linked oxidoreductase superfamily protein [Source:TAIR 3Bacc:59950]	1.08
<i>At1g75830</i>	PLANT DEFENSIN 1.1 (PDF1.1)/Defensin-like protein 13 [Source:Uniprot/SWISSPROT 3Bacc:P30224]	1.06
<i>At4g16260</i>	Putative beta-1,3-endoglucanase [Source:TAIR 3Bacc:16260]	1.01
<i>At2g41180</i>	Sigma factor binding protein 2 2C chloroplastic (SIB2) [Source:Uniprot/SWISSPROT 3Bacc:O80669]	0.98
<i>At3g49110</i>	Peroxidase 33 (PRX33) [Source:Uniprot/SWISSPROT 3Bacc:P24101]	0.98
<i>At2g43590</i>	Chitinase family protein [Source:TAIR 3Bacc:43590]	0.95
<i>At3g28153</i>	Transposable element gene [Source:TAIR 3Bacc:28153]	0.92
<i>At2g43580</i>	Chitinase family protein [Source:TAIR 3Bacc:43580]	0.91
<i>At1g61563</i>	Protein RALF-like 8 (RALFL8) [Source:Uniprot/SWISSPROT 3Bacc:Q1ECR9]	0.90
<i>At3g43828</i>	Transposable element gene [Source:TAIR 3Bacc:43828]	0.90
<i>At1g15415</i>	Protein phosphatase 2A (PP2A) B'gamma subunit [Source:TAIR 3Bacc:15415]	0.88
<i>At2g31141</i>	Hypothetical protein	0.88
<i>At2g28210</i>	Alpha carbonic anhydrase 2 (ATACA2) [Source:TAIR 3Bacc:28210]	0.85
<i>At3g13400</i>	SKU5 similar 13/SKS13 [Source:TAIR 3Bacc:13400]	0.82
<i>At2g22320</i>	Hypothetical protein	0.82
<i>At1g66390</i>	Transcription factor MYB90 [Source:Uniprot/SWISSPROT 3Bacc:Q9ZTC3]	0.81

DOWNREGULATED DEGs

<i>At4g36850</i>	PQ-loop repeat family protein / transmembrane family protein [Source:TAIR 3Bacc:At4g36850]	-2.16
<i>At1g29920</i>	Chlorophyll a/b binding protein 2 (LHCB1.2) [Source:Uniprot/SWISSPROT 3Bacc:Q8VZ87]	-1.78

<i>At2g34430</i>	Light-harvesting chlorophyll-protein complex II subunit B1 [Source:TAIR 3BAcc:At2g34430]	-1.64
<i>At3g27690</i>	Photosystem II light harvesting complex gene 2.3 [Source:TAIR 3BAcc:At3g27690]	-1.40
<i>At2g05100</i>	Photosystem II light harvesting complex gene 2.1 [Source:TAIR 3BAcc:At2g05100]	-1.32
<i>At1g29910</i>	Light-harvesting chlorophyll a/b binding protein 1.2 [Source:TAIR 3BAcc:At1g29910]	-1.29
<i>At2g05070</i>	Photosystem II light harvesting complex gene 2.2 [Source:TAIR 3BAcc:At2g05070]	-1.29
<i>At3g19390</i>	Granulin repeat cysteine protease family protein [Source:TAIR 3BAcc:At3g19390]	-1.20
<i>At3g19710</i>	Methionine aminotransferase BCAT4 [Source:Uniprot/SWISSPROT 3BAcc:Q9LE06]	-1.18
<i>At1g62510</i>	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein [Source:TAIR 3BAcc:At1g62510]	-1.16
<i>At4g21650</i>	Subtilase 3. 13 (SBT3.13) [Source:TAIR 3BAcc:At4g21650]	-1.11
<i>At5g65690</i>	Phosphoenolpyruvate carboxykinase 2 (PCK2) [Source:TAIR 3BAcc:At5g65690]	-1.09
<i>At5g24490</i>	30S ribosomal protein 2C putative [Source:TAIR 3BAcc:At5g24490]	-1.08
<i>At1g29930</i>	Chlorophyll a/b binding protein 1 2C chloroplastic/Light-harvesting chlorophyll a/b protein 1.3 [Source:Uniprot/SWISSPROT 3BAcc:P04778]	-1.08
<i>At5g54270</i>	Light-harvesting chlorophyll B-binding protein 3 [Source:TAIR 3BAcc:At5g54270]	-1.08
<i>At5g38420</i>	Ribulose biphosphate carboxylase small chain 2B 2C chloroplastic (RBCS2B) [Source:Uniprot/SWISSPROT 3BAcc:P10797]	-1.06
<i>At4g25580</i>	CAP160 protein [Source:TAIR 3BAcc:At4g25580]	-1.05
<i>At1g16410</i>	Dihomomethionine N-hydroxylase/Cytochrome P450 79F1 (CYP79F1) [Source:Uniprot/SWISSPROT 3BAcc:Q949U1]	-1.05
<i>At4g26260</i>	Myo-inositol oxygenase 4 (MIOX4) [Source:Uniprot/SWISSPROT 3BAcc:Q8H1S0]	-1.05
<i>At3g26060</i>	Peroxiredoxin Q 2C chloroplastic [Source:Uniprot/SWISSPROT 3BAcc:Q9LU86]	-1.03
<i>At5g02160</i>	FTSH5 interacting protein [Source:TAIR 3BAcc:At5g02160]	-1.03
<i>At5g64040</i>	Photosystem I reaction center subunit N 2C chloroplastic [Source:Uniprot/SWISSPROT 3BAcc:P49107]	-1.02
<i>At3g22740</i>	Homocysteine S-methyltransferase 3 [Source:Uniprot/SWISSPROT 3BAcc:Q8LAX0]	-1.01
<i>At2g10940</i>	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein [Source:TAIR 3BAcc:AT2G10940]	-0.99
<i>At3g08940</i>	Chlorophyll a-b binding protein CP29.2 2C chloroplastic/LHCB4.2 [Source:Uniprot/SWISSPROT 3BAcc:Q9XF88]	-0.98
<i>At4g01330</i>	Protein kinase superfamily protein [Source:TAIR 3BAcc:At4g01330]	-0.97
<i>At1g30280</i>	Chaperone DnaJ-domain superfamily protein/Auxilin-like 7 [Source:TAIR 3BAcc:At1g30280]	-0.96
<i>At2g20670</i>	Putative sugar phosphate exchanger (DUF506) [Source:TAIR 3BAcc:At2g20670]	-0.96
<i>At2g34420</i>	Photosystem II light harvesting complex gene B1B2 [Source:TAIR 3BAcc:At2g34420]	-0.95
<i>At1g68560</i>	Alpha-xylosidase 1 (ATXYL1)/Altered xyloglucan 3 (AXY3) [Source:Uniprot/SWISSPROT 3BAcc:Q9S7Y7]	-0.94
<i>At4g08870</i>	Arginine amidohydrolase 2 2C mitochondrial [Source:Uniprot/SWISSPROT 3BAcc:Q9ZPF5]	-0.93
<i>At5g23010</i>	Methylthioalkylmalate synthase 1 2C chloroplastic [Source:Uniprot/SWISSPROT 3BAcc:Q9FG67]	-0.93
<i>At3g50800</i>	Hypothetical protein	-0.93
<i>At2g46740</i>	L-gulonolactone oxidase 5 [Source:Uniprot/SWISSPROT 3BAcc:O81030]	-0.92
<i>At5g63450</i>	Cytochrome P450 94B1 [Source:Uniprot/SWISSPROT 3BAcc:Q9FMV7]	-0.92
<i>At4g03060</i>	Alkenyl hydroxalkyl producing 2 (AOP2) [Source:TAIR 3BAcc:At4g03060]	-0.91
<i>At5g55150</i>	F-box SKIP23-like protein (DUF295) [Source:Uniprot/SWISSPROT 3BAcc:Q9FLP7]	-0.90
<i>At1g12900</i>	Glyceraldehyde-3-phosphate dehydrogenase GAPA2 2C chloroplastic [Source:Uniprot/SWISSPROT 3BAcc:Q9LPW0]	-0.89
<i>At3g02020</i>	Aspartokinase 3 2C chloroplastic [Source:Uniprot/SWISSPROT 3BAcc:Q9S702]	-0.88
<i>At1g68010</i>	Glycerate dehydrogenase HPR 2C peroxisomal [Source:Uniprot/SWISSPROT 3BAcc:Q9C9W5]	-0.86
<i>At3g46780</i>	Plastid transcriptionally active 16 [Source:TAIR 3BAcc:AT3G46780]	-0.86

<i>At5g14740</i>	Beta carbonic anhydrase 2 2C chloroplastic [Source:Uniprot/SWISSPROT 3BAcc:P42737]	-0.85
<i>At1g32060</i>	Phosphoribulokinase 2C chloroplastic [Source:Uniprot/SWISSPROT 3BAcc:P25697]	-0.85
<i>At4g10340</i>	Light-harvesting complex of Photosystem II 5 (LHCB5) [Source:Uniprot/SWISSPROT 3BAcc:Q9XF89]	-0.85
<i>At4g28040</i>	Nodulin MtN21-like transporter family protein/WAT1-related protein [Source:Uniprot/SWISSPROT 3BAcc:Q9SUD5]	-0.85
<i>At1g01620</i>	Aquaporin PIP1;3 [Source:Uniprot/SWISSPROT 3BAcc:Q08733]	-0.84
<i>At5g57550</i>	Probable xyloglucan endotransglucosylase/hydrolase protein 25 [Source:Uniprot/SWISSPROT 3BAcc:Q38907]	-0.84
<i>At2g13360</i>	L-Serine: glyoxylate aminotransferase (SGAT) [Source:Uniprot/SWISSPROT 3BAcc:Q56YA5]	-0.84
<i>At2g46820</i>	Curvature thylakoid 1B 2C chloroplastic (CURT1B) [Source:Uniprot/SWISSPROT 3BAcc:Q8LCA1]	-0.84
<i>At5g13630</i>	Magnesium-chelatase subunit ChlH 2C chloroplastic/ABA-binding protein (ABAR) [Source:Uniprot/SWISSPROT 3BAcc:Q9FNB0]	-0.84

Supplementary Table 3. The most strongly affected gene ontology (GO) terms assigned to upregulated or downregulated differentially expressed genes (adjusted *P*-value < 0.05) in Arabidopsis leaves treated with 3-methoxy-BAPA for 6 h.

GO number	Domain	Description	Total #	Affected genes (%)
Up-regulated				
GO:0009861	BP	jasmonic acid and ethylene-dependent systemic resistance	13	23.1
GO:0016653	MF	oxidoreductase activity, acting on NAD(P)H, heme protein as acceptor	9	22.2
GO:0047893	MF	flavonol 3-O-glucosyltransferase activity	11	18.2
GO:0004089	MF	carbonate dehydratase activity	14	14.3
GO:0006026	BP	aminoglycan catabolic process	24	12.5
GO:0046348	BP	amino sugar catabolic process	24	12.5
GO:1901072	BP	glucosamine-containing compound catabolic process	24	12.5
GO:0006032	BP	chitin catabolic process	24	12.5
GO:0080043	MF	quercetin 3-O-glucosyltransferase activity	24	12.5
GO:0016998	BP	cell wall macromolecule catabolic process	27	11.1
GO:0071456	BP	cellular response to hypoxia	26	7.7
GO:0071804	BP	cellular potassium ion transport	43	7.0
GO:0009817	BP	defense response to fungus, incompatible interaction	44	6.8
GO:0004364	MF	glutathione transferase activity	54	5.6
GO:0019722	BP	calcium-mediated signaling	55	5.5
Down-regulated				
GO:0030076	CC	light-harvesting complex	26	61.5
GO:0009522	CC	photosystem I	42	59.5
GO:0010196	BP	nonphotochemical quenching	7	57.1
GO:0019253	BP	reductive pentose-phosphate cycle	20	55.0
GO:0009765	BP	photosynthesis, light harvesting	39	53.9
GO:0016168	MF	chlorophyll binding	38	52.6
GO:0019685	BP	photosynthesis, dark reaction	21	52.4
GO:0015977	BP	carbon fixation	22	50.0
GO:0042549	BP	photosystem II stabilization	6	50.0
GO:0009533	CC	chloroplast stromal thylakoid	8	50.0
GO:0008187	MF	poly-pyrimidine tract binding	18	44.4
GO:0008266	MF	poly(U) RNA binding	18	44.4
GO:0009773	BP	photosynthetic electron transport in photosystem I	48	41.7
GO:0018298	BP	protein-chromophore linkage	41	41.5

GO:0009523

CC

photosystem II

68

39.7

GO, gene ontology; BP, biological process; CC, cellular component; MF, molecular function.

Detached *Arabidopsis* leaves were treated with 10 μ M 3-methoxy-BAPA or an equivalent volume of buffer containing 0.1% DMSO (negative control) for 6 h in darkness. The most strongly affected GO terms at level 6 or higher are shown. Percentages of DEGs are calculated based on the total number of genes in *Arabidopsis thaliana* genome annotated with the same GO number (total #).

Supplementary Table 4. Effect of the treatment with 3-methoxy-BAPA (3MeOBAPA) and 3-hydroxy-BAPA (3OHBAPA) on the grain yield, number of tillers and number of spikes in winter wheat / spring barley plants. The same plants were used in field-plot experiments evaluating the level of plant infection by fungal pathogens (see Figure 7). The 5 μ M solutions were applied as a foliar spray at different plant growth stages (tillering, the beginning of stem elongation, the beginning of flag leaf extending). The values in bold italics show statistically significant difference between treated and non-treated plants ($P < 0.01$). n.d., not determined.

	Compound (growth stage during application)	Grain yield		Number of tillers per plant		Number of spikes per m ²	
		[t ha ⁻¹]	[%]		[%]		[%]
wheat	non-treated	6.72	100	5.9	100	432	100
	3MeOBAPA (tillering)	6.48	96	6.3	107	470	109
barley	non-treated	8.53	100	3.8	100	698	100
	3MeOBAPA (stem elongation)	8.78	103	4.7	124	748	107
	3MeOBAPA (flag leaf extending)	8.67	102	n.d.	n.d.	721	103
	3OHBAPA (stem elongation)	8.62	101	4.2	111	688	99
	3OHBAPA (flag leaf extending)	8.86	104	n.d.	n.d.	686	98

Supplementary Table 5. Primers for expression profiling of *A. thaliana* MAPK genes.

Gene	AGI No.	Primers 5' - 3'
Housekeeping genes (Endogenous controls)		
Act2	<i>At3g18780</i>	TGGTCGTACAACCGGTATTGTG ATCAGTAAGGTCACGTCCAGCAA
Act7	<i>At5g09810</i>	CTAGAGACAGCCAAGAGCAGTTC GTTTCATGGATTCCAGGAGCTTC
EF1 α	<i>At5g60390</i>	TGAGCACGCTCTTCTTGCTTCA GGTGGTGGCATCCATCTTGTTACA
MAPK/PTI marker genes		
MPK3	<i>At3g45640</i>	GACAGAGTTGCTTGGCACACCGA GGCTGACGTGGGAAGTTGGGA
MPK4	<i>At4g01370</i>	TGTCGGCTGGTGCAGTCGATTT TGGCACAACGCCTCATCAACTGT
MPK6	<i>At2g43790</i>	ACAGCTTCCACCTTATCCTCGCCA TGGGCAATGCGTCTAAAAGTGTG
MPK11	<i>At1g01560</i>	TTCTTAAGAAGCGACAACGCTAG ATTGACCGACATGTTTGGGAATC
FRK1	<i>At2g19190</i>	GAGACTATTTGGCAGGTAAGGTT AGGAGGCTTACAACCATTGTG
WRKY33	<i>At2g38470</i>	GGGAAACCCAAATCCAAGA GTTCCCTTCGTAGGTTGTGA
WRKY53	<i>At4g23810</i>	CGGAAGTCCGAGAAGTGAAG TCTGACCACTTTGGTAACATCTTT

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