Fig. S1 Schematic representation of the experimental design

EXPERIMENTAL WORKFLOW

Identification of early cytokinin response proteins

Identification of early cytokinin response phosphoproteins

Contribution of individual cytokinin receptors to phosphoproteome regulation

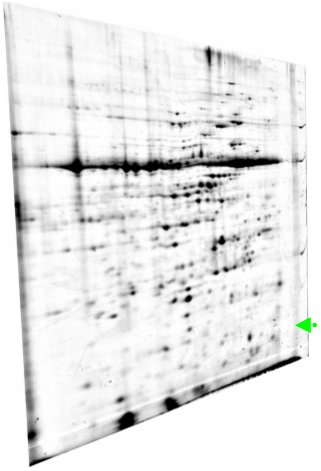
Effect of calcium signaling inhibitors on phosphoproteome response to cytokinins

Identification of early cytokinin response proteins

Arabidopsis thaliana (ecotype Columbia) seedlings were cultivated for 7 days on MS medium supplemented with (5.10^{-4} % v/v) DMSO at standard light intensity (~90 μmol.m^{-2}.s^{-1}).

Seedlings were transferred on liquid MS medium supplemented with DMSO (mock; 5.10^{-4} % v/v) or 5 μM cytokinin (BA, iP, TDZ or *t*-Z) dissolved in DMSO and incubated for 15 min.

Seedlings were rapidly harvested, frozen and ground in liquid nitrogen



Total *Arabidopsis* protein was extracted by standard acetone/TCA extraction and resolved by large (18 cm IPG strips, 500 μg of protein) 2-DE.

Identification of early cytokinin response proteins

2-DE

3 technical replicates for each biological replicate

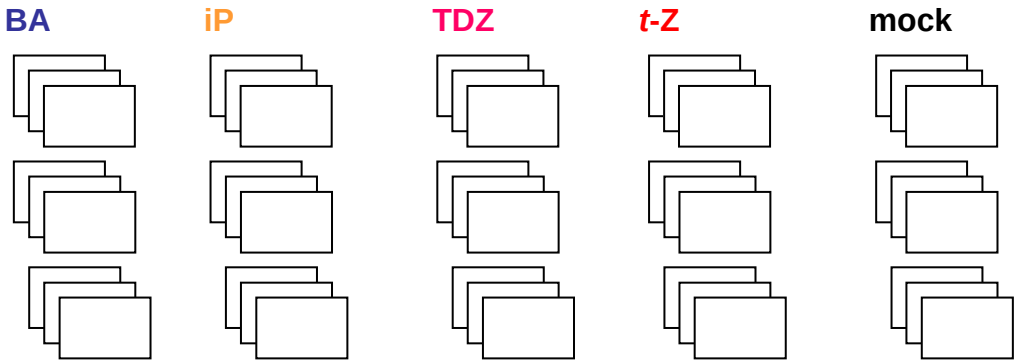
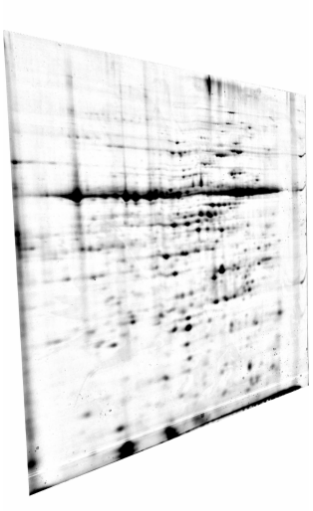


image analysis

45 2D gels



protein digest

53 protein spots reproducibly significant in at least 2 biological replicates

MALDI-TOF/TOF analysis

MASCOT search

67 identified proteins

Identification of early cytokinin response phosphoproteins

Arabidopsis thaliana (ecotype Columbia) seedlings were cultivated for 7 days on MS medium supplemented with (5.10^{-4} % v/v) DMSO at standard light intensity (~90 μmol.m^{-2}.s^{-1}).

Seedlings were transferred on liquid MS medium supplemented with DMSO (mock; 5.10^{-4} % v/v) or 5 μM cytokinin (BA, iP, TDZ or *t*-Z) dissolved in DMSO and incubated for 15 min.

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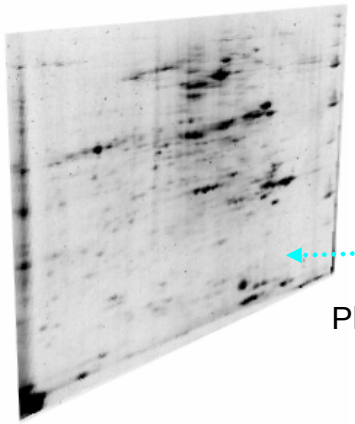


For phosphoproteome analysis, an isolation procedure was established based on PhosphoProtein Purification Kit (Qiagen). Collected phosphoprotein fractions were then resolved by 2-DE (7 cm IPG strip, 150 μg of protein per strip).

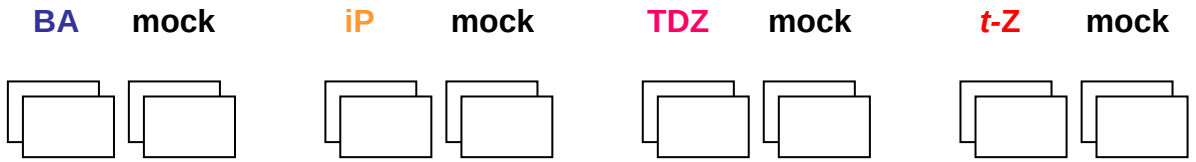
Identification of early cytokinin response phosphoproteins

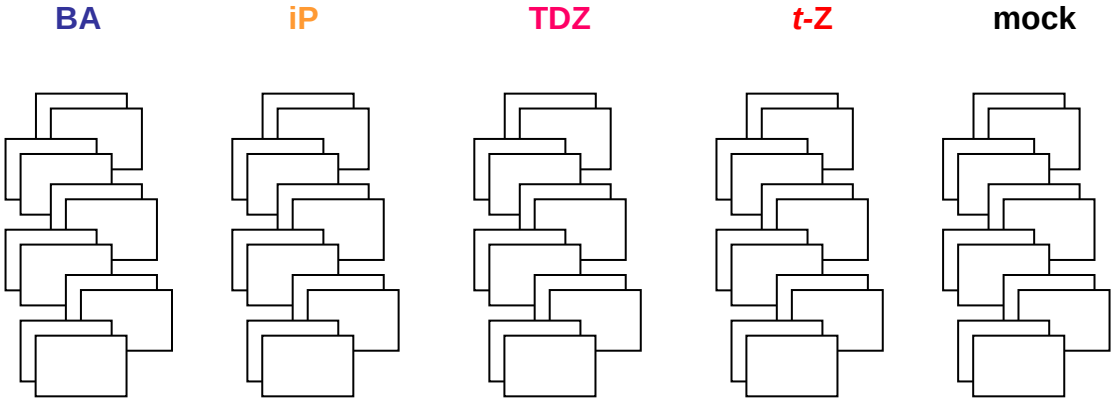
Pilot experiments

Main experiments

2-DE

2 technical replicates for each biological replicate





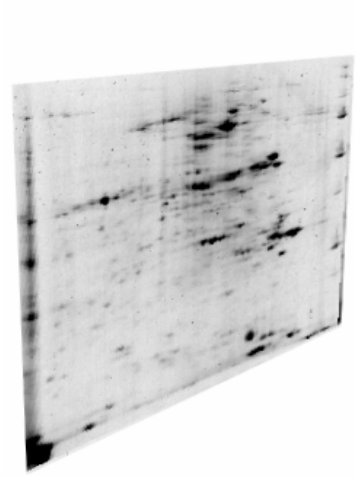


image analysis

76 2D gels

protein digest

31 protein spots reproducibly significant in at least 3 biological replicates

MALDI-TOF/TOF analysis

MASCOT search

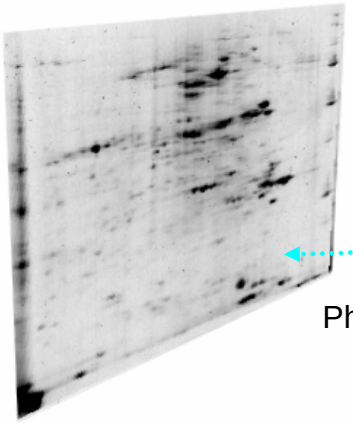
29 identified proteins

Contribution of individual cytokinin receptors to phosphoproteome regulation

Arabidopsis thaliana ***ahk2ahk3***, ***ahk2cre1*** and ***ahk3cre1*** double mutants seedlings were cultivated for 7 days on MS medium supplemented with (5.10^{-4} % v/v) DMSO at standard light intensity (~90 μmol.m^{-2}.s^{-1}).

Seedlings were transferred on liquid MS medium supplemented with DMSO (mock; 5.10^{-4} % v/v) or 5 μM *t*-Z dissolved in DMSO and incubated for 15 min.

Seedlings were rapidly harvested, frozen and ground in liquid nitrogen

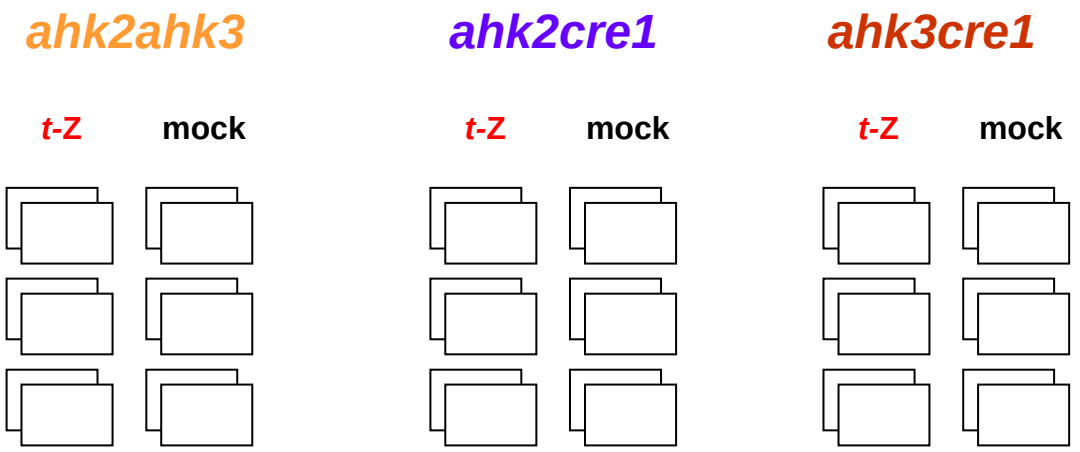


For phosphoproteome analysis, an isolation procedure was established based on PhosphoProtein Purification Kit (Qiagen). Collected phosphoprotein fraction was then resolved by 2-DE (7 cm IPG strip, 150 μg of protein per strip).

Contribution of individual cytokinin receptors to phosphoproteome regulation

2-DE

2 technical replicates for each biological replicate



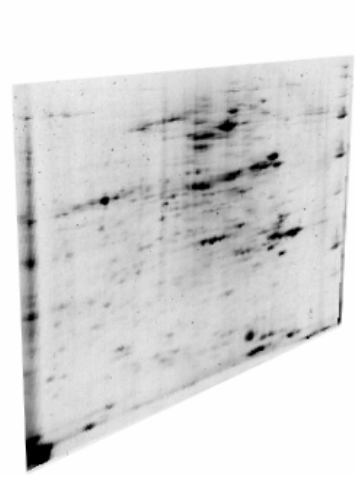


image analysis

36 2D gels

Comparison with early cytokinin response phosphoproteins

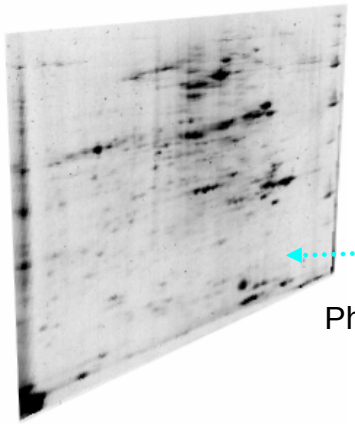
significant differences for 28 out of 31 proteins

Effect of calcium signaling inhibitors on phosphoproteome response to cytokinins

Arabidopsis thaliana (ecotype Columbia) seedlings were cultivated for 7 days on MS medium supplemented with (5.10^{-4} % v/v) DMSO at standard light intensity (~90 μmol.m^{-2}.s^{-1}).

Seedlings were transferred on liquid MS medium supplemented with inhibitors **INH**: 60 μM La^{3+}, 30 μM D600 and DMSO (5.10^{-4} % v/v), ***t*-Z:** 5 μM *t*-Z dissolved in DMSO and ***t*-Z+INH** (inhibitors and cytokinins simultaneously) and incubated for 15 min.

Seedlings were rapidly harvested, frozen and ground in liquid nitrogen



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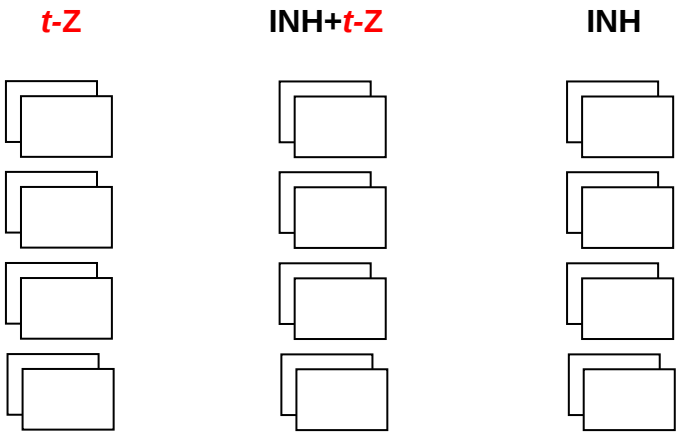
Effect of calcium signaling inhibitors on phosphoproteome response to cytokinins

2-DE

2 technical replicates for each biological replicate



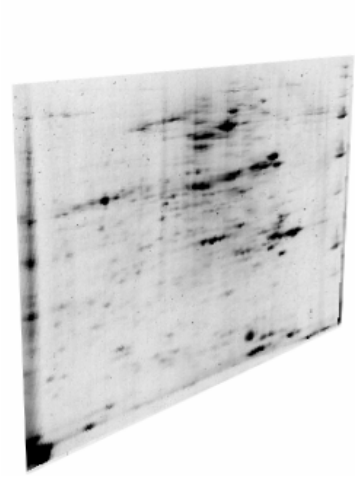


image analysis

24 2D gels

Comparison with early cytokinin response phosphoproteins

significant differences for 5 out of 31 proteins