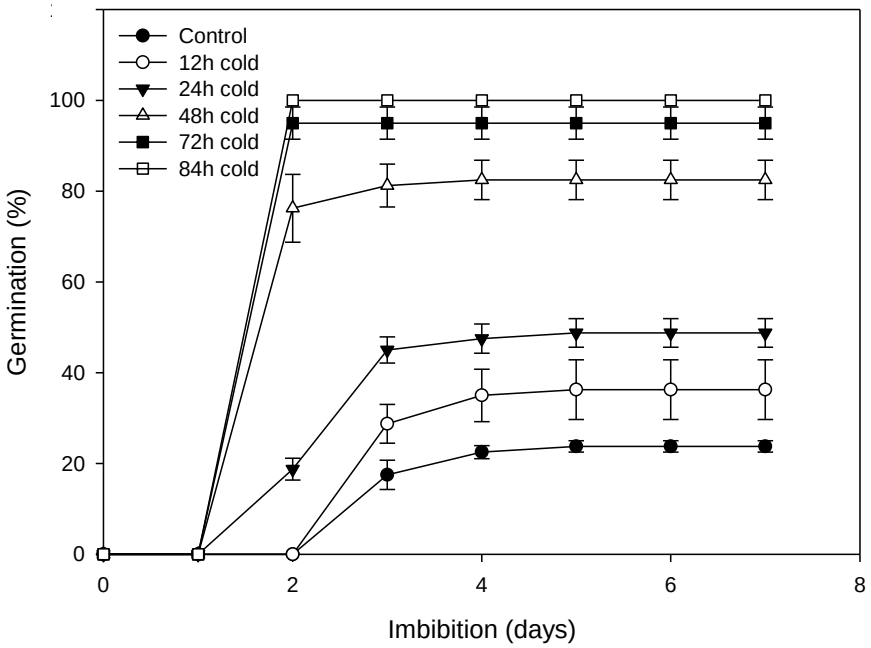
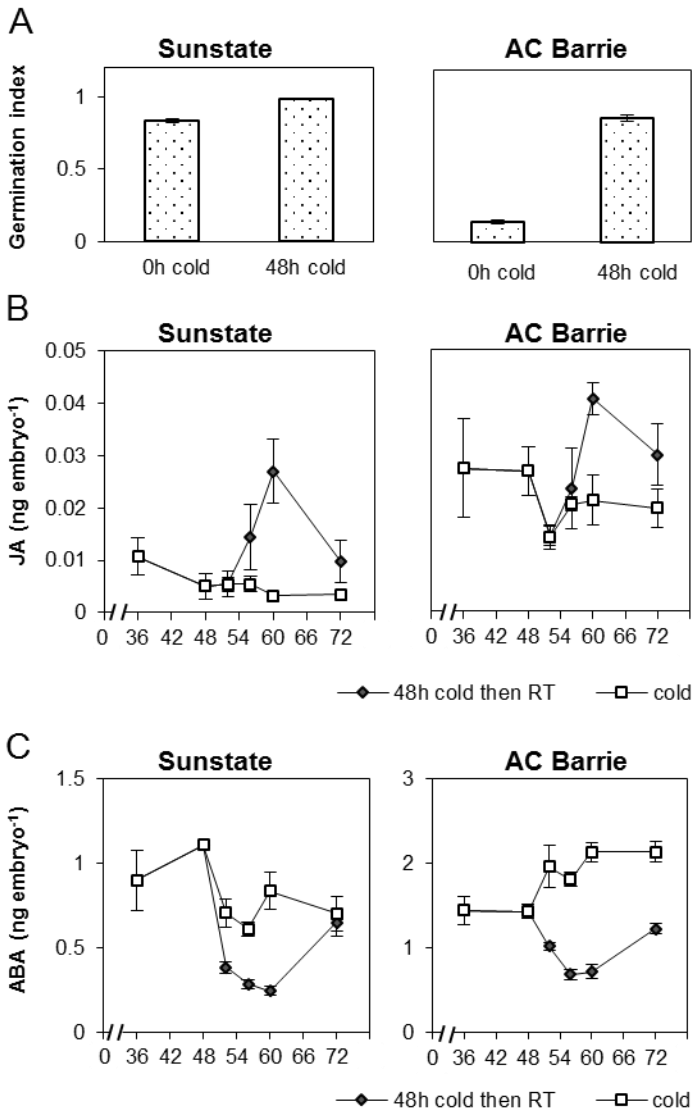
## Supplemental Figure S1



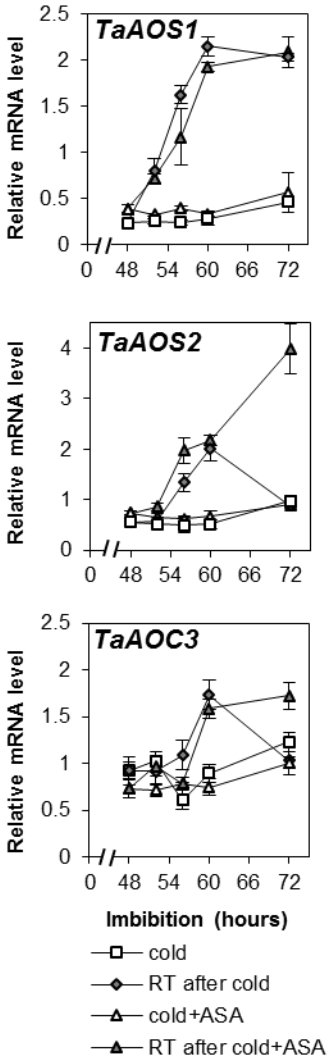

**Supplemental Figure S1.** The effect of stratification on the germination of dormant grain. The germination was measured over 7 days' imbibition at 20°C following different periods of cold treatment (0 to 84 h).

## Supplemental Figure S2



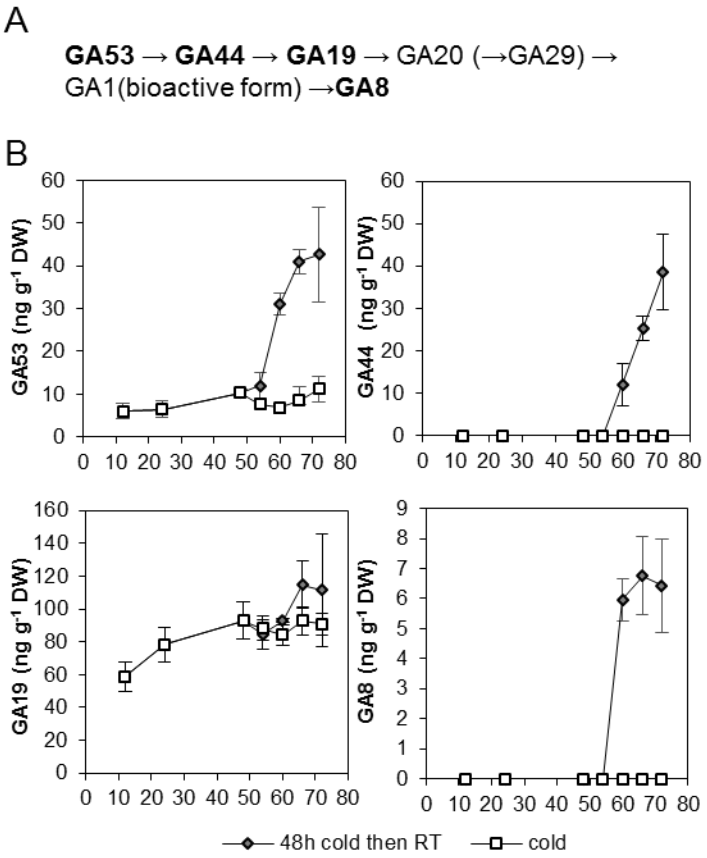

**Supplemental Figure S2**. Changes in JA and ABA content in embryos of imbibed wheat grains in response to stratification in darkness. (A) The effect of 48 h stratification on the germination of dormant cv. Sunstate and cv. AC Barrie grains in darkness. (B) Changes in JA content in embryos isolated from cv. Sunstate and cv. AC Barrie wheat grains in response to 48 h stratification in dark. Grains were imbibed at 4$^{o}$C for 48 h in dark and then transferred to RT in dark or kept at 4$^{o}$C in dark. (C) Changes in ABA content in embryos isolated from cv. Sunstate and cv. AC Barrie wheat grains in response to 48 h stratification in dark. Grains were imbibed at 4$^{o}$C for 48 h in dark and then transferred to RT in dark or kept at 4$^{o}$C in dark. Values are means ±SE (n=4) and each replicate was obtained from 20 grains (A) and 40 embryos (B-C).

**Supplemental Figure S3**



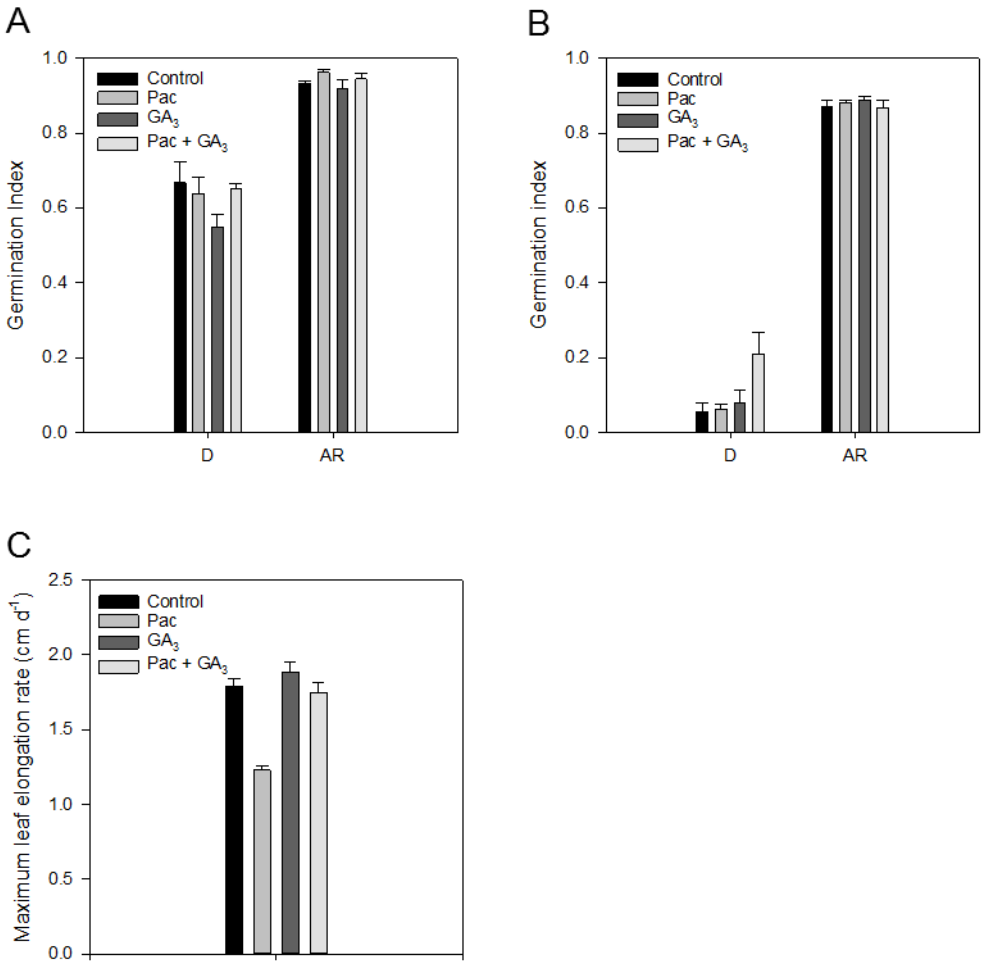

**Supplemental Figure S3**. Effect of ASA on expression of *TaAOC1, TaAOC2* and *TaAOS3* in embryos of imbibed wheat grains in response to 48 h stratification. Cv. Sunstate wheat grains were imbibed for 48 h with or without ASA in BL in the cold before being transferred to RT. For comparison, grains were kept in the cold after the 48 h cold treatment. Embryos were isolated for gene expression analysis after 48 h (end of cold treatment), 52 h, 56 h, 60 h and 72 h imbibition. Values are means ±SE (n=4) and each replicate was obtained from 10 embryos.

## Supplemental Figure S4




**Supplemental Figure S4**. Changes in GA content in embryos of Sunstate wheat grains in response to 48 h stratification. (A) Partial GA metabolic pathway in plants. (B) GA contents in imbibed wheat embryos in response to cold..$GA_{53,}$ $GA_{44}$ and $GA_8$ increase in embryos of cv. Sunstate wheat grains in response to 48 h stratification. Grains were imbibed at 4$^o$C for 48 h under BL and then transferred to RT under BL or kept at 4$^o$C under BL. Values are means ±SE (n=4) and each replicate was obtained from 40 embryos. DW, dry weight.

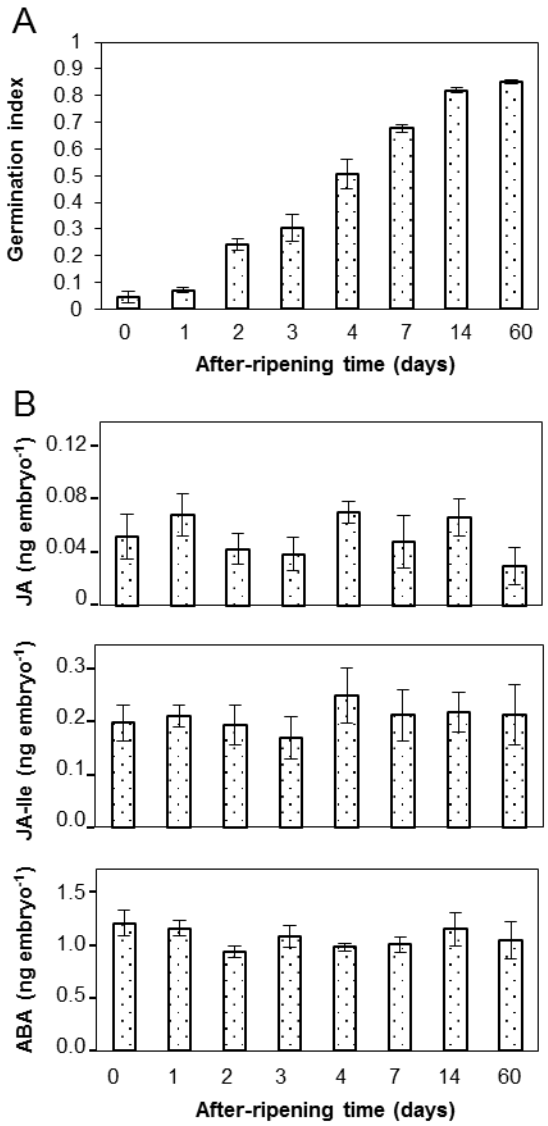
**Supplemental Figure S5**



**Supplemental Figure S5**. Effect of paclobutrazol and gibberellic acid on germination index and leaf 1 elongation rate. (A) The effect of paclobutrazol (Pac) and gibberellic acid ($GA_3$) on germination of dormant (D) and after-ripened (AR) grains following 48 h stratification. At the start of the stratification the grains were imbibed in control, 10 μM paclobutrazol, 50 μM $GA_3$ or 10 μM paclobutrazol and 50 μM $GA_3$. Paclobutrazol was made as a 10 mM stock in DMSO and diluted 1000 fold. Control and $GA_3$ treatments included addition of DMSO at the same concentration as the paclobutrazol treatments. Grains were imbibed at 4°C for 48 h under BL and then transferred to RT for 7 days under BL. Germination index of grains was calculated over the 7 days. Values are means ±SE (n=4) and each replicate was obtained from 20 grains. (B) The effect of paclobutrazol (Pac) and $GA_3$ on germination of D and AR grains imbibed at RT. The grains were imbibed in control, 10 μM paclobutrazol, 50 μM $GA_3$ or 10 μM paclobutrazol and 50 μM $GA_3$. Paclobutrazol was made as a 10 mM stock in DMSO and diluted 1000 fold. Control and $GA_3$ treatments included addition of DMSO at the same concentration as the paclobutrazol treatments. Grains were imbibed for 7 days under BL. Germination index of grains was calculated over the 7 days. Values are means ±SE (n=4) and each replicate was obtained from 20 grains. (C) Effect of paclobutrazol and $GA_3$ on leaf 1 elongation rate. The maximal leaf elongation rate for leaf 1 was determined as described by Chandler and Robertson (1999) except that Sunstate wheat grains were not stratified prior to germination. After-ripened grains were placed in paper envelopes moistened with various treatments: control, 10 μm paclobutrazol (Pac), 50 μM $GA_3$, or 10 μM paclobutrazol and 50 μM $GA_3$. The envelopes were incubated under constant white light for 7 days. Lengths of leaf 1 from 18-25 seedlings were measured daily from day 3 to day 7. Error bars represent standard error.

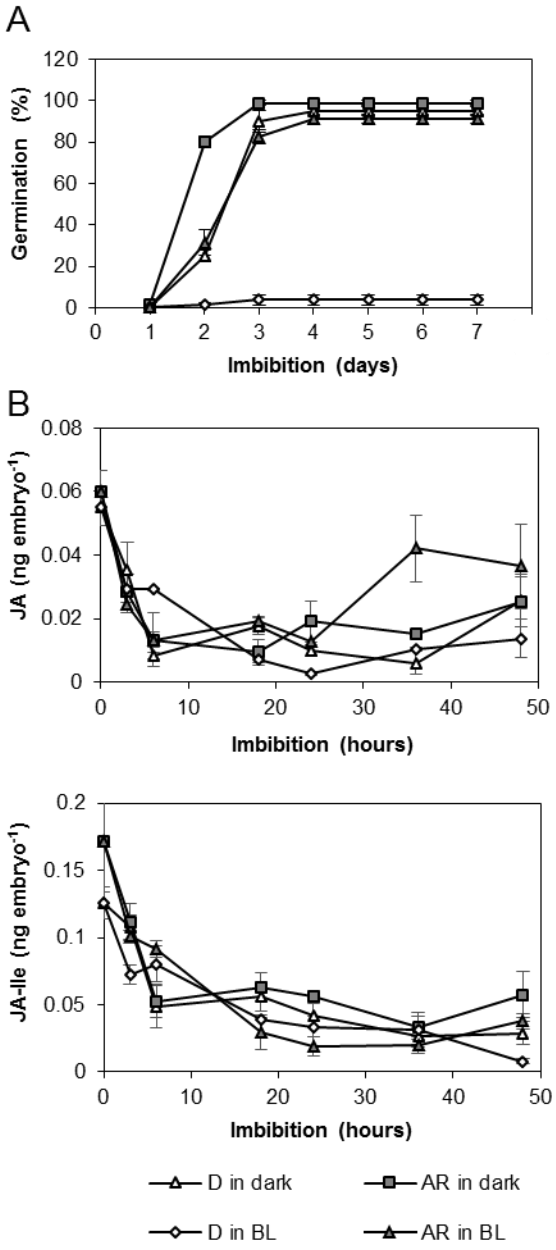
Chandler PM, Robertson M. 1999. Gibberellin dose–response curves and the characterization of dwarf mutants of barley. Plant Physiology 120, 623–632.

**Supplemental Figure S6**



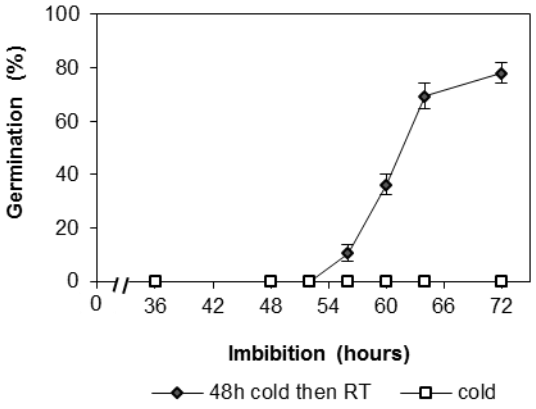

**Supplemental Figure S6**. JAs and ABA contents in dry embryos during after-ripening. (A) After-ripening increases the GI of wheat.GI was calculated over 7 days of imbibition in BL at 20° C following different periods of after-ripening in 37°C . Values are means ±SE (n=4) and each replicate was obtained from 20 grains. (B) JA, JA-Ile and ABA contents in embryo of dry seeds during after-ripening. Embryos were isolated for hormone quantification after different times of after-ripening. Values are means ± SE (n=4) and each replicate was obtained from 25 embryos.

## Supplemental Figure S7



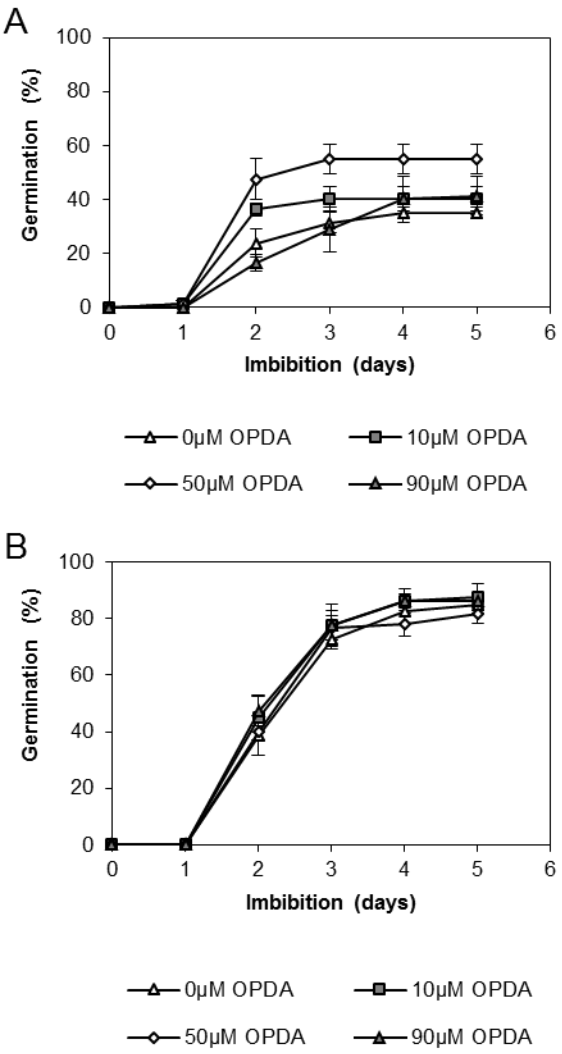

**Supplemental Figure S7**. Time course of JAs content in dormant and after-ripened embryos in BL and darkness. (A) The effect of BL and darkness on germination of dormant and after-ripened wheat. Germination rate of dormant and after-ripened was calculated for 7 days in BL and dark. Values are means ± SE (n=4) and each replicate was obtained from 20 grains. (B) JA and JA-Ile contents in dormant and after-ripened embryos in BL and in dark during imbibition. Embryos were isolated for hormone quantification after different times of after-ripening. Values are means ± SE (n=4) and each replicate was obtained from 40 embryos.

## Supplemental Figure S8




**Supplemental Figure S8**. Time course of germination of Sunstate wheat grains in response to 48 h stratification. Germination rate of dormant wheat grains in response to 48 h stratification. Grains were imbibed at 4$^{o}$C for 48 h under BL and then transferred to RT under BL or kept at 4$^{o}$C under BL. Values are means ±SE (n=4) and each replicate was obtained from 20 grains.

## Supplemental Figure S9




**Supplemental Figure S9**. Effect of ODPA on germination on dormant and after-ripened wheat grains**.** (A) OPDA promotes the germination of dormant wheat. The germination rate of dormant was scored for 5 days in BL ($10\mu mol \cdot m^{-2} \cdot sec^{-1}$ ) and the different concentrations of OPDA. Values are means ±SE (n=4) and each replicate was obtained from 20 embryos. (B) OPDA has no effect on germination of after-ripened wheat. The germination rate of after-ripened grains was scored for 5 days in BL ($10\mu mol \cdot m^{-2} \cdot sec^{-1}$) and different concentration of OPDA. Values are means ±SE (n=4) and each replicate was obtained from 20 embryos.

**Supplementary Table S1.** Validation results for extraction and LC-ESI-MS/MS analytical methodologies for the detection and absolute quantification of plant hormones in wheat embryos

| Analyte | Molecular formula | MW (g $mol^{-1}$) | Parent ion (*m/z*) | CE (eV) | $t_R$ (min) | Base Peak Product ion (*m/z*) | LOD (pg/µg) | LLOQ (pg/µg ) | Linear regression equation | Correlation coefficient ($R^2$) | Recovery efficiency (%) | Optimised ESI-QTOF Ion Polarity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (±)-Jasmonic acid (JA) | $C_{12}H_{18}O_3$ | 210.27 | 209.1163 | 12 | 14.0602 ± 0.2721 | 59.0144 | 0.2647 | 0.4111 | y = 0.8689x + 0.0457 | 0.9902 | 90±0.06 | Negative |
| N-[(-)-Jasmonoyl]-(L)-isoleucine ((-)-Ja-L-Ile)) | $C_{18}H_{29}NO_4$ | 323.21 | 322.2027 | 20 | 16.612 ± 0.2363 | 130.0878 | 0.2186 | 0.3643 | y = 1.2109x + 0.0061 | 0.9915 | 99±0.04 | Negative |
| (±)-9,10-DihydroJasmonic acid (DHJA) | $C_{12}H_{20}O_3$ | 212.28 | 211.1349 | 12 | 15.3150 ± 0.2332 | 59.0163 | 0.0523 | 0.0871 | | | | Negative |
| (±)-*cis,trans*-Abscisic acid (ABA) | $C_{15}H_{20}O_4$ | 264.32 | 263.1297 | 10 | 12.8344 ± 0.2842 | 153.0935 | 0.1801 | 0.3002 | y = 2.4495x + 0.0018 | 0.9974 | 85±0.03 | Negative |
| [$^2H_6$](+)-*cis,trans*-Abscisic acid (D-ABA) | $d_6$-$C_{15}H_{14}O_4$ | 270.35 | 269.1647 | 10 | 12.7952 ± 0.2843 | 159.1294 | 0.4109 | 0.6848 | | | | Negative |
| *Cis*-12-oxo-phytodienoic acid (*cis*-OPDA) | $C_{18}H_{28}O_3$ | 292.42 | 293.2095 | 7 | 20.6835 ± 0.0281 | 275.1994 | 0.9518 | 1.5864 | y = 419.9x + 2.0084 | 0.9974 | N/A | Positive |
| [$^2H_5$]*cis*-12-Oxo-phytodienoic acid (D-OPDA) | $C_{18}H_{23}D_5O_3$ | 297.38 | 298.2252 | 15 | 20.6169 ± 0.0316 | 280.2160 | 2.5930 | 4.3216 | | | | Positive |

Limits of detection (LOD = 3xS/N) and lower limits of quantification (LLOQ = 5xS/N) were calculated according to the calibration curves of individual analyte standards. Standards were run in both negative and positive ion polarities, and the respective ion mode with better detection sensitivity was selected for each analyte. Results for the extraction recovery (%) of each targeted analyte (n=3, means ± %CV); retention times (n=10; means ± SE); optimal collision energies to fragment the parent ions to generate their respective characteristic product ions; calibration curve linearity and correlation coefficients. The presence of internal standards (analogues and isotope-labeled) was necessary to achieve reliable accuracy and precision of the extraction and LC/MS methods.

**Supplementary Table S2**. Primer sequences used for qRT-PCR

| Gene | Forward primer (5'-3') | Reverse primer (5'-3') | Accession |
|---|---|---|---|
| ***TaAOS1*** | TCTCATAGCAGCCGTCAATC | CACATACACAAGGGCACCAT | AY196004 |
| ***TaAOS2*** | ATGGTGCCCTTGTGTATGTG | TACCGTACGCCAAGAGTGAG | BT009396 |
| ***TaAOC1*** | CGTCTTCGAGGGCGTCTACG | GCAGGTCGGGGATGCCCTTGA | KF573524 |
| ***TaAOC2*** | ATTCATTCAACACTGGTACAAGG | ATCTATTATTGCTCCTGCTAGTAG | BJ241555 |
| ***TaAOC3*** | TCCTCGTAGGTGAGGTAGGG | CTACGAGGCCATCTACAGCA | CK163974 |
| ***TaNCED1*** | CCCAGCACTAATCGATTCC | CCGCTAACTGTATCCATGC | CD884104 |
| ***TaNCED2*** | GGAGATGGAAAGAGGAAGTCG | GAAGCAAGTGTGAGCTAAC | CA731387 |

Supplemental Table S3. Changes in GAs content in embryos of Sunstate wheat grains in response to 48 h stratification in BL.

| | | | Gibberellins (ng/g DW) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample information | | weight (mg) | GA1 | GA3 | GA4 | GA7 | GA8 | GA9 | GA19 | GA20 | GA24 | GA29 | GA34 | GA44 | GA51 | GA53 |
| 1A | 12h in 4°C | 52 | n.d. | <3.8 | <3.8 | n.d. | <3.8 | n.d. | 50 | <3.8 | <3.8 | <4.4 | <3.8 | <3.8 | n.d. | 4 |
| 1B | 12h in 4°C | 48 | n.d. | n.d. | n.d. | n.d. | <4.1 | n.d. | 55 | <4.1 | n.d. | n.d. | n.d. | <4.1 | n.d. | 7 |
| 1C | 12h in 4°C | 52 | n.d. | <3.9 | <3.9 | <3.9 | <3.9 | n.d. | 59 | <3.9 | n.d. | n.d. | <3.9 | <3.9 | n.d. | 4 |
| 1D | 12h in 4°C | 52 | n.d. | n.d. | <3.8 | <3.8 | <3.8 | n.d. | 71 | <3.8 | <3.8 | n.d. | <3.8 | <3.8 | n.d. | 8 |
| 2A | 24h in 4°C | 48 | n.d. | n.d. | n.d. | <4.2 | <4.2 | n.d. | 72 | <4.2 | <4.2 | <5.8 | <4.2 | n.d. | n.d. | 5 |
| 2B | 24h in 4°C | 44 | <4.5 | n.d. | <5.4 | <4.5 | <4.5 | n.d. | 91 | <4.5 | <4.5 | n.d. | <4.5 | n.d. | n.d. | 6 |
| 2C | 24h in 4°C | 52 | n.d. | <3.8 | <3.8 | <3.8 | <3.8 | n.d. | 83 | <3.8 | <3.8 | n.d. | <3.8 | <3.8 | n.d. | 9 |
| 2D | 24h in 4°C | 49 | n.d. | <4 | n.d. | <4 | <4 | n.d. | 68 | <4 | <4 | n.d. | n.d. | <4 | n.d. | 5 |
| 3A | 48h in 4°C | 39 | n.d. | <5.1 | n.d. | n.d. | <5.1 | n.d. | 92 | <5.1 | <5.1 | n.d. | <5.1 | <5.1 | n.d. | 10 |
| 3B | 48h in 4°C | 37 | n.d. | <5.5 | n.d. | n.d. | <5.5 | n.d. | 101 | <5.5 | n.d. | n.d. | <5.5 | <5.5 | n.d. | 10 |
| 3C | 48h in 4°C | 47 | n.d. | n.d. | n.d. | n.d. | <4.3 | n.d. | 77 | <4.3 | n.d. | n.d. | <4.3 | <4.3 | <4.3 | 9 |
| 3D | 48h in 4°C | 39 | n.d. | n.d. | n.d. | <5.2 | <5.2 | n.d. | 101 | <5.2 | n.d. | <6.7 | n.d. | n.d. | n.d. | 11 |
| 4A | 54h in 4°C | 31 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 99 | n.d. | n.d. | n.d. | n.d. | <6.4 | n.d. | 7 |
| 4B | 54h in 4°C | 33 | n.d. | <6.1 | <6.1 | n.d. | n.d. | n.d. | 86 | <6.1 | n.d. | n.d. | n.d. | n.d. | n.d. | 8 |
| 4C | 54h in 4°C | 37 | n.d. | n.d. | <5.4 | n.d. | <5.7 | n.d. | 81 | <5.4 | <5.4 | n.d. | <5.4 | n.d. | n.d. | 7 |
| 4D | 54h in 4°C | 41 | n.d. | <4.8 | n.d. | n.d. | n.d. | n.d. | 88 | <4.8 | n.d. | n.d. | n.d. | <4.8 | n.d. | 8 |
| 5A | 60h in 4°C | 49 | n.d. | <4.1 | n.d. | <4.1 | n.d. | n.d. | 78 | <4.1 | <4.1 | n.d. | <4.1 | <4.1 | n.d. | 7 |
| 5B | 60h in 4°C | 35 | n.d. | n.d. | <5.8 | <5.8 | <5.8 | n.d. | 93 | <5.8 | n.d. | <7.9 | <5.8 | <5.8 | n.d. | 8 |
| 5C | 60h in 4°C | 39 | n.d. | n.d. | n.d. | n.d. | <5.1 | n.d. | 86 | <5.1 | n.d. | n.d. | <5.1 | <5.1 | n.d. | 5 |
| 5D | 60h in 4°C | 44 | n.d. | n.d. | <4.6 | <4.6 | <4.6 | n.d. | 80 | <4.6 | n.d. | n.d. | n.d. | <4.6 | n.d. | 7 |
| 6A | 66h in 4°C | 40 | n.d. | n.d. | <5 | n.d. | <5 | n.d. | 91 | <5 | n.d. | n.d. | <5 | <6.6 | <5 | 10 |
| 6B | 66h in 4°C | 45 | n.d. | <4.5 | <4.5 | <4.5 | <4.5 | n.d. | 87 | <4.5 | n.d. | n.d. | <4.5 | <4.5 | n.d. | 9 |
| 6C | 66h in 4°C | 45 | n.d. | n.d. | <4.4 | n.d. | <4.6 | n.d. | 88 | <4.4 | <4.4 | <6.7 | <4.4 | n.d. | n.d. | 8 |
| 6D | 66h in 4°C | 38 | n.d. | n.d. | n.d. | n.d. | <5.2 | n.d. | 105 | <5.2 | n.d. | n.d. | n.d. | <5.2 | <5.2 | 8 |
| 7A | 72h in 4°C | 34 | n.d. | <5.8 | n.d. | n.d. | n.d. | n.d. | 98 | <5.8 | <5.8 | n.d. | n.d. | <5.8 | n.d. | 14 |
| 7B | 72h in 4°C | 36 | n.d. | n.d. | n.d. | n.d. | <5.5 | n.d. | 87 | <5.5 | <5.5 | <7.4 | n.d. | <5.5 | n.d. | 8 |
| 7C | 72h in 4°C | 41 | n.d. | <4.8 | n.d. | n.d. | <4.8 | n.d. | 84 | <4.8 | <4.8 | <10.7 | n.d. | <4.8 | n.d. | 9 |
| 7D | 72h in 4°C | 36 | n.d. | n.d. | <5.6 | n.d. | n.d. | n.d. | 94 | <5.6 | n.d. | n.d. | <5.6 | <5.6 | <5.8 | 14 |
| 8A | 48h in 4°C+ 6h in 20°C | 41 | n.d. | n.d. | n.d. | n.d. | <4.9 | n.d. | 81 | <5.3 | n.d. | n.d. | <4.9 | <4.9 | n.d. | 11 |
| 8B | 48h in 4°C+ 6h in 20°C | 51 | n.d. | n.d. | n.d. | n.d. | <3.9 | n.d. | 75 | <3.9 | n.d. | n.d. | <3.9 | <3.9 | <3.9 | 9 |
| 8C | 48h in 4°C+ 6h in 20°C | 33 | n.d. | n.d. | <6 | n.d. | <6 | n.d. | 96 | <6 | n.d. | n.d. | <6 | <6 | n.d. | 16 |
| 8D | 48h in 4°C+ 6h in 20°C | 38 | n.d. | <5.3 | n.d. | <5.3 | <5.3 | n.d. | 86 | <5.3 | n.d. | n.d. | n.d. | <5.3 | n.d. | 12 |
| 9A | 48h in 4°C+ 12h in 20°C | 44 | <4.6 | n.d. | n.d. | <4.6 | <4.6 | n.d. | 94 | n.d. | <4.6 | n.d. | <4.6 | 8 | n.d. | 28 |
| 9B | 48h in 4°C+ 12h in 20°C | 47 | n.d. | n.d. | n.d. | <4.3 | <4.3 | n.d. | 92 | <4.3 | n.d. | n.d. | n.d. | 7 | n.d. | 30 |
| 9C | 48h in 4°C+ 12h in 20°C | 37 | n.d. | <5.3 | <5.3 | <5.3 | 5 | n.d. | 94 | <5.3 | n.d. | n.d. | n.d. | 15 | <7.2 | 32 |
| 9D | 48h in 4°C+ 12h in 20°C | 37 | n.d. | n.d. | n.d. | n.d. | 6 | n.d. | 92 | <5.5 | n.d. | n.d. | n.d. | 17 | <5.5 | 34 |
| 10A | 48h in 4°C+ 18h in 20°C | 44 | n.d. | n.d. | n.d. | n.d. | 6 | n.d. | 103 | <4.6 | n.d. | n.d. | n.d. | 24 | n.d. | 44 |
| 10B | 48h in 4°C+ 18h in 20°C | 45 | n.d. | n.d. | n.d. | n.d. | 7 | n.d. | 101 | <4.5 | n.d. | n.d. | n.d. | 23 | n.d. | 42 |
| 10C | 48h in 4°C+ 18h in 20°C | 40 | n.d. | n.d. | <5 | <5 | 9 | n.d. | 128 | <5 | <5 | n.d. | <5 | 25 | <5 | 37 |
| 10D | 48h in 4°C+ 18h in 20°C | 48 | n.d. | n.d. | <4.2 | n.d. | 6 | n.d. | 127 | <4.2 | n.d. | n.d. | n.d. | 29 | n.d. | 41 |
| 11A | 48h in 4°C+ 24h in 20°C | 51 | n.d. | n.d. | n.d. | n.d. | 4 | n.d. | 66 | <3.9 | n.d. | n.d. | n.d. | 31 | n.d. | 31 |
| 11B | 48h in 4°C+ 24h in 20°C | 40 | n.d. | n.d. | n.d. | n.d. | 7 | n.d. | 144 | <5 | n.d. | <5.1 | <5 | 50 | n.d. | 58 |
| 11C | 48h in 4°C+ 24h in 20°C | 49 | n.d. | <4.1 | n.d. | <4.1 | 7 | n.d. | 105 | n.d. | n.d. | n.d. | n.d. | 41 | n.d. | 42 |
| 11D | 48h in 4°C+ 24h in 20°C | 50 | n.d. | n.d. | n.d. | n.d. | 8 | n.d. | 131 | <4 | n.d. | n.d. | <4 | 32 | <4 | 40 |

**Values greater than or equal to the limits of quantification (LOQ ≤ 8 ng/g DW) are reported in red. Values in blue indicate concentrations (ng/g DW) that were below the LOQs, but required reporting.**

***n.d.* = not detected (values below LOQs of 3 ng/g DW). LODs = 3xS/N.**

**LEGEND:**

| | | | |
|---|---|---|---|
| A,B,C,D | **Gibberellins** | | |
| four replicates | | GA1 | Gibberellin 1 |
| | | GA3 | Gibberellin 3 |
| 1: 12h in 4°C in BL | | GA4 | Gibberellin 4 |
| 2: 24h in 4°C in BL | | GA7 | Gibberellin 7 |
| 3:48h in 4°C in BL | | GA8 | Gibberellin 8 |
| 4:54h in 4°C in BL | | GA9 | Gibberellin 9 |
| 5:60h in 4°C in BL | | GA19 | Gibberellin 19 |
| 6:66h in 4°C in BL | | GA20 | Gibberellin 20 |
| 7:72h in 4°C in BL | | GA24 | Gibberellin 24 |
| 8:48h in 4°C in BL+ 6h in 20°C in BL | | GA29 | Gibberellin 29 |
| 9:48h in 4°C in BL+ 12h in 20°C in BL | | GA34 | Gibberellin 34 |
| 10:48h in 4°C in BL+ 18h in 20°C in BL | | GA44 | Gibberellin 44 |
| 11:48h in 4°C in BL+ 24h in 20°C in BL | | GA51 | Gibberellin 51 |
| | | GA53 | Gibberellin 53 |