

Article

Rice Momilactones and Phenolics: Expression of Relevant Biosynthetic Genes in Response to UV and Chilling Stresses

Supplementary Materials:

Table S1. Primer sequences of the tested genes

| Gene | Gene description | Primer sequence |
|----------------|---------------------------------------|--|
| <i>PAL</i> | Phenylalanine ammonia-lyase | Forward: GAGGATCTCGAACCGGATGC Reverse: GTGGATCCTCAACTGCATCGC |
| <i>OsCPS4</i> | Syn-copalyl diphosphate synthase-like | Forward: TGGAGGACGGCATGTTTTGA Reverse: CCGTACTTTCCCCATTCTGG |
| <i>OsKSL4</i> | Syn-pimara-7,15-diene synthase-like | Forward: AAGAGGCTAAAGAGGCGA Reverse: TCAGTGGCTCAAAGATTACTC |
| <i>CYP99A3</i> | 9-Beta-pimara-7,15-diene oxidase-like | Forward: CGTCAAGGCAATTATACTGGAT Reverse: CATGAGCCACTCGGTGGTAG |
| <i>OsMAS</i> | Momilactone A synthase-like | Forward: AGCACTTCTCGGACAGCAAAT Reverse: CTTGCCCACCAGCTTCCTT |
| <i>OsMAS2</i> | Momilactone A synthase-like | Forward: GAAAGAGACCCATCGTTCGTCGT Reverse: CCACGCAAACATTTGGGAAACCA |
| <i>Actin</i> | Housekeeping gene | Forward: CTCCCCCATGCTATCCTTCG Reverse: TGAATGAGTAACCACGCTCCG |
| <i>eIF-4A</i> | Housekeeping gene | Forward: TTGTGCTGGATGAAGCTGATG Reverse: GGAAGGAGCTGGAAGATATCATAGA |

Table S2. Expression (Ct value) of candidate housekeeping genes (*eIF-4A* and *actin*) in rice under UV and chilling stresses

| Treatment | Sample | Ct value | |
|-----------|--------|---------------|--------------|
| | | <i>eIF-4A</i> | <i>Actin</i> |
| UV | UVC | 31.35 ± 0.07 | 28.72 ± 0.15 |
| | UV2 | 28.23 ± 0.33 | 29.16 ± 0.10 |
| | UV4 | 28.24 ± 0.15 | 29.05 ± 0.17 |
| Chilling | ChiC | 27.46 ± 0.07 | 26.95 ± 0.25 |
| | Chi4 | 31.64 ± 0.28 | 26.74 ± 0.28 |
| | Chi8 | 29.40 ± 0.12 | 23.46 ± 0.16 |

Data express mean ± standard deviation (SD). UVC, control rice seedlings without UV treatments; UV2, UV-treated rice seedlings for 2 h per day; UV4, UV-treated rice seedlings for 4 h per day; ChiC, control rice seedlings without chilling treatments; Chi4, chill-treated rice seedlings for 4 h per day; Chi8, chill-treated rice seedlings for 8 h per day.

Table S3. Relative quantification (RQ) of relevant genes to the biosynthesis of phenolics and momilactones in rice response to UV and chilling stresses

| Gene | UV treatment | | | Chilling treatment | | |
|----------------|----------------------------|------------------------------|------------------------------|----------------------------|----------------------------|----------------------------|
| | UVC | UV2 | UV4 | ChiC | Chi4 | Chi8 |
| <i>PAL</i> | 1.000 ± 0.000 ^c | 2.047 ± 0.233 ^b | 2.582 ± 0.105 ^a | 1.000 ± 0.000 ^a | 0.230 ± 0.062 ^b | 0.021 ± 0.002 ^c |
| <i>OsCPS4</i> | 1.000 ± 0.000 ^b | 3.710 ± 0.884 ^a | 3.852 ± 0.520 ^a | 1.000 ± 0.000 ^a | 0.026 ± 0.006 ^b | 0.008 ± 0.001 ^c |
| <i>OsKSL4</i> | 1.000 ± 0.000 ^c | 5.851 ± 1.110 ^b | 17.887 ± 0.765 ^a | 1.000 ± 0.000 ^a | 0.054 ± 0.136 ^b | 0.004 ± 0.000 ^c |
| <i>CYP99A3</i> | 1.000 ± 0.000 ^b | 48.217 ± 26.324 ^a | 58.781 ± 35.049 ^a | 1.000 ± 0.000 ^a | 0.012 ± 0.005 ^b | 0.002 ± 0.000 ^c |
| <i>OsMAS</i> | 1.000 ± 0.000 ^b | 54.932 ± 19.724 ^a | 48.148 ± 22.926 ^a | 1.000 ± 0.000 ^a | 0.010 ± 0.004 ^b | 0.006 ± 0.002 ^b |
| <i>OsMAS2</i> | 1.000 ± 0.000 ^b | 33.265 ± 4.426 ^a | 42.267 ± 9.562 ^a | 1.000 ± 0.000 ^a | 0.061 ± 0.016 ^b | 0.007 ± 0.001 ^c |

The relative quantification (RQ) value is expressed as mean ± standard deviation (SD). Different superscript letters in a row within a treatment indicate significant differences at $p < 0.05$. UVC, control rice seedlings without UV treatments; UV2, UV-treated rice seedlings for 2 h per day; UV4, UV-treated rice seedlings for 4 h per day; ChiC, control rice seedlings without chilling treatments; Chi4, chill-treated rice seedlings for 4 h per day; Chi8, chill-treated rice seedlings for 8 h per day; *PAL*, gene encoding phenylalanine ammonia-lyase; *OsCPS4*, gene encoding syn-copalyl diphosphate synthase-like; *OsKSL4*, gene encoding syn-pimara-7,15-diene synthase-like; *CYP99A3*, gene encoding 9-beta-pimara-7,15-diene oxidase-like; *OsMAS*, gene encoding momilactone A synthase-like; *OsMAS2*, gene encoding momilactone A synthase-like.

Table S4. Pearson's correlation coefficients between antioxidant activity, chemical profiles, and relevant gene expressions of rice seedlings under UV stress

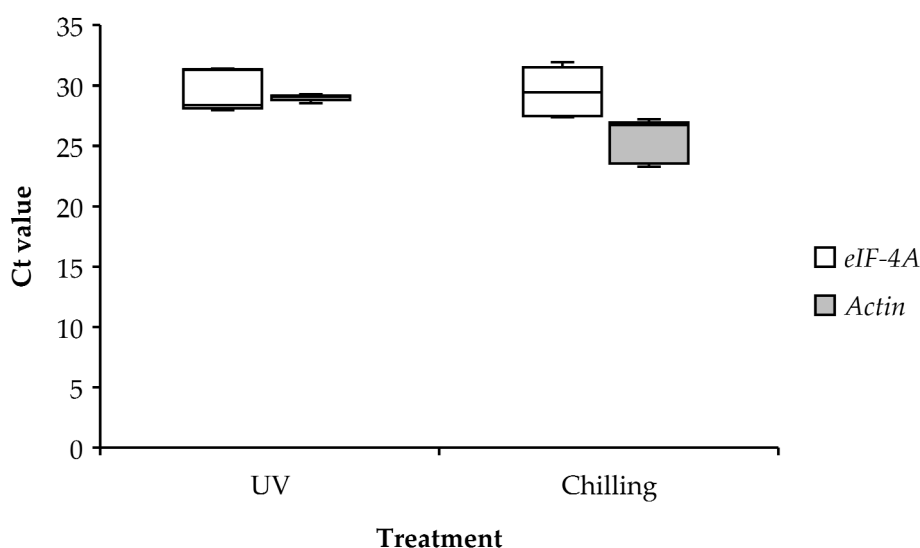
| | DPPH | ABTS | PAL | Cat | ChA | HyA | SaA | Esc | Rut | Fis | CPS | KSL | CYP | MAS | MAS2 | MA |
|-------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| ABTS | 0.24 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| PAL | -0.95 | -0.21 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Cat | -0.28 | -0.38 | 0.19 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| ChA | -0.64 | -0.33 | 0.58* | 0.90* | - | - | - | - | - | - | - | - | - | - | - | - |
| HyA | -0.27 | -0.39 | 0.20 | 1.00* | 0.90* | - | - | - | - | - | - | - | - | - | - | - |
| SaA | -0.95 | -0.23 | 0.93* | 0.50* | 0.81* | 0.50* | - | - | - | - | - | - | - | - | - | - |
| Esc | -0.81 | -0.28 | 0.74* | 0.78* | 0.96* | 0.76* | 0.93* | - | - | - | - | - | - | - | - | - |
| Rut | -0.73 | -0.23 | 0.67* | 0.79* | 0.93* | 0.78* | 0.87* | 0.97* | - | - | - | - | - | - | - | - |
| Fis | -0.32 | -0.36 | 0.20 | 1.00* | 0.90* | 0.99* | 0.52* | 0.79* | 0.81* | - | - | - | - | - | - | - |
| CPS | -0.95 | -0.24 | 0.88* | 0.44 | 0.72* | 0.42 | 0.94* | 0.87* | 0.78* | 0.47 | - | - | - | - | - | - |
| KSL | -0.81 | -0.16 | 0.80* | -0.11 | 0.25 | -0.12 | 0.68* | 0.43 | 0.26 | -0.09 | 0.79* | - | - | - | - | - |
| CYP | -0.85 | -0.27 | 0.72* | 0.26 | 0.52* | 0.23 | 0.76* | 0.67* | 0.50* | 0.30 | 0.91* | 0.89* | - | - | - | - |
| MAS | -0.83 | 0.02 | 0.78* | 0.56* | 0.82* | 0.55* | 0.93* | 0.92* | 0.87* | 0.59* | 0.86* | 0.54* | 0.68* | - | - | - |
| MAS2 | -0.90 | 0.01 | 0.94* | 0.30 | 0.66* | 0.31 | 0.94* | 0.81* | 0.79* | 0.32 | 0.84* | 0.63* | 0.60* | 0.91* | - | - |
| MA | -0.92 | 0.03 | 0.94* | -0.01 | 0.40 | -0.01 | 0.85* | 0.61* | 0.52* | 0.01 | 0.84* | 0.88* | 0.76* | 0.77* | 0.90* | - |
| MB | -0.95 | -0.06 | 0.97* | -0.20 | 0.59* | 0.20 | 0.94* | 0.76* | 0.67* | 0.22 | 0.91* | 0.84* | 0.79* | 0.86* | 0.95* | 0.98* |

*, a significance at $p < 0.05$; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); PAL, gene encoding phenylalanine ammonia-lyase; Cat, catechol; ChA, chlorogenic acid; HyA, *p*-hydroxybenzoic acid; SaA, salicylic acid; Esc, esculetin; Rut, rutin; Fis, fisetin; CPS, gene encoding syn-copalyl diphosphate synthase-like (*OsCPS4*); KSL, gene encoding syn-pimara-7,15-diene synthase-like (*OsKSL4*); CYP, gene encoding 9-beta-pimara-7,15-diene oxidase-like (*CYP99A3*); MAS, gene encoding momilactone A synthase-like (*OsMAS*); MAS2, gene encoding momilactone A synthase-like (*OsMAS2*); MA, momilactone A; MB, momilactone B.

Table S5. Pearson's correlation coefficients between antioxidant activity, chemical profiles, and relevant gene expressions of rice seedlings under chilling stress

| | DPPH | ABTS | PAL | TPC | TFC | SyA | SaA | BeA | CiA | CoA | Que | Tri | CPS | KSL | CYP | MAS | MAS2 | MA |
|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| ABTS | 0.92* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| PAL | 0.20 | 0.31 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| TPC | 0.85* | 0.79* | -0.25 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| TFC | 0.91* | 0.94* | 0.45 | 0.75* | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| SyA | 0.79* | 0.79* | -0.25 | 0.96* | 0.72* | - | - | - | - | - | - | - | - | - | - | - | - | - |
| SaA | 0.93* | 0.88* | -0.03 | 0.94* | 0.84* | 0.92* | - | - | - | - | - | - | - | - | - | - | - | - |
| BeA | 0.63* | 0.74* | 0.85* | 0.27 | 0.83* | 0.26 | 0.46 | - | - | - | - | - | - | - | - | - | - | - |
| CiA | 0.47 | 0.57* | 0.95* | 0.05 | 0.70* | 0.04 | 0.25 | 0.96* | - | - | - | - | - | - | - | - | - | - |
| CoA | 0.64* | 0.74* | 0.85* | 0.29 | 0.85* | 0.28 | 0.46 | 0.99* | 0.97* | - | - | - | - | - | - | - | - | - |
| Que | 0.60* | 0.68* | 0.89* | 0.19 | 0.78* | 0.19 | 0.38 | 0.96* | 0.98* | 0.98* | - | - | - | - | - | - | - | - |
| Tri | 0.68* | 0.76* | 0.83* | 0.33 | 0.87* | 0.32 | 0.50* | 0.98* | 0.96* | 0.99* | 0.98* | - | - | - | - | - | - | - |
| CPS | 0.37 | 0.48 | 0.98* | -0.06 | 0.61* | -0.07 | 0.14 | 0.93* | 0.99* | 0.94* | 0.96* | 0.92* | - | - | - | - | - | - |
| KSL | 0.35 | 0.46 | 0.98* | -0.09 | 0.59* | -0.10 | 0.11 | 0.92* | 0.99* | 0.93* | 0.95* | 0.91* | 1.00* | - | - | - | - | - |
| CYP | 0.38 | 0.49 | 0.98* | -0.05 | 0.62* | -0.06 | 0.15 | 0.93* | 0.99* | 0.94* | 0.96* | 0.92* | 1.00* | 1.00* | - | - | - | - |
| MAS | 0.39 | 0.49 | 0.98* | -0.05 | 0.62* | -0.06 | 0.16 | 0.94* | 1.00* | 0.94* | 0.96* | 0.92* | 1.00* | 1.00* | 1.00* | - | - | - |
| MAS2 | 0.35 | 0.46 | 0.99* | -0.09 | 0.59* | -0.10 | 0.11 | 0.92* | 0.99* | 0.93* | 0.95* | 0.91* | 1.00* | 1.00* | 1.00* | 1.00* | - | - |
| MA | 0.34 | 0.43 | 0.99* | -0.12 | 0.56* | -0.13 | 0.09 | 0.90* | 0.98* | 0.91* | 0.95* | 0.90* | 1.00* | 1.00* | 1.00* | 1.00* | 1.00* | - |
| MB | 0.67* | 0.77* | 0.84* | 0.29 | 0.84* | 0.27 | 0.48 | 0.99* | 0.96* | 0.99* | 0.97* | 0.98* | 0.93* | 0.91* | 0.93* | 0.93* | 0.91* | 0.90* |

*, a significance at $p < 0.05$; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); PAL, gene encoding phenylalanine ammonia-lyase; TPC, total phenolic content; TFC, total flavonoid content; SyA, syringic acid; SaA, salicylic acid; BeA, benzoic acid; CiA, cinnamic acid; CoA, p -coumaric acid; Que, quercetin; Tri, tricin; CPS, gene encoding syn-copalyl diphosphate synthase-like (*OsCPS4*); KSL, gene encoding syn-pimara-7,15-diene synthase-like (*OsKSL4*); CYP, gene encoding 9-beta-pimara-7,15-diene oxidase-like (*CYP99A3*); MAS, gene encoding momilactone A synthase-like (*OsMAS*); MAS2, gene encoding momilactone A synthase-like (*OsMAS2*); MA, momilactone A; MB, momilactone B.

**Figure S1.** A boxplot for the Ct values of candidate housekeeping genes (*eIF-4A* and *actin*) in rice under UV and chilling conditions. The line inside the box indicates the median. The top and bottom lines of the box display the first and third quartiles, respectively. Whiskers present the minimum and maximum Ct values.