# Supplementary Figures & Tables

VE pathway

**Figure S1. Simplified model of the vitamin E biosynthesis pathway in barley.**

Compound and pathway names are given in the boxes and gene names are indicated in italic. HPT1/HPT2 (homogentisate phytyltransferase) and HGGT (homogentisate geranylgeranyl transferase) are key enzymes in the vitamin E biosynthesis pathway. DMGGBQ, 2,3-dimethyl-5-geranylgeranyl-1,4-benzoquinol; DMPBQ, 2,3-dimethyl-5-phytyl-1,4-benzoquinone; GGDP, geranylgeranyl diphosphate; GGDR, GGDP reductase; HGA, Homogentisate; HPP, 4-Hydroxyphenylpyruvate; HPPD, 4-hydroxyphenylpyruvate dioxygenase; MEP, methylerythritol 4-phosphate; MGGBQ, 2-methyl-6-geranylgeranyl-1,4-benzoquinol; MPBQ, 2-methyl-6-phytyl-benzoquinone; MT, methyltransferase; PDP, phytyl diphosphate; TC, tocopherol cyclase; VTE5/VTE6, phytol kinase; γ-TMT, γ-tocopherol methyltransferase.

**Table S1. 62 *HPT* homologous genes in 22 *Poales* species.** *HPT2s* are highlighted in red.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Species name** | **Gene IDs** | |
| ***HPT*** | ***HGGT*** |
| *Pooideae* | *Hordeum vulgare* | HORVU.MOREX.r2.2HG0173050.1 | HORVU.MOREX.r2.7HG0616750.1 |
| HORVU.MOREX.r2.7HG0614050.1 |
| *Aegilops tauschii* | AET7Gv21206900.1 | AET7Gv21252600.7 |
| *Triticum urartu* | TuG1812G0700005336 | TuG1812G0700005515 |
| *Triticum dicoccoides* | TRIDC7Av2G224380.1  TRIDC7Bv2G199050.1  TRIDC2Bv2G208200.1 | TRIDC7Av2G229100.1  TRIDC7Bv2G205810.1 |
| *Triticum aestivum* | TraesLAC7A01G533600.1 | TraesLAC7A01G552400.1 |
| TraesLAC7B01G434500.1 | TraesLAC7B01G456800.1 |
| TraesLAC7D01G532600.1  TraesLAC2B01G490500.1 | TraesLAC7D01G551600.1 |
| *Thinopyrum intermedium* | Thint.19G0629100.1.v2.1  Thint.20G0876300.1.v2.1  Thint.21G0611900.1.v2.1  Thint.V1245000.1.v2.1 | Thint.19G0590500.1.v2.1  Thint.20G0932000.1.v2.1 |
| *Secale cereale* | ScWN2R01G006800.1 | ScWN1R01G103600.1  ScWN6R01G537600.1 |
| *Avena eriantha* | AE039967.mRNA1  AE040009.mRNA1 | AE040113.mRNA1 |
| *Nardus stricta* | TR95660 | TR66908 |
| *Stipa lagascae* | TR78164 |  |
| *Melica nutans* | TR82291 |  |
| *Brachypodieae* | *Brachipodium distachyon* | Bradi1g31380 | Bradi1g30809 |
| *Brachypodium hybridum* | Brahy.D01G0415300.1 | Brahy.S07G0253700.1 |
| Brahy.S07G0247200.1 | Brahy.D01G0405900.1 |
| *Brachypodium mexicanum* | Brame.07PG044700.1 | Brame.07UG026600.1 |
| Brame.07UG033300.1 |  |
| *Brachypodium stace* | Brast07G222200.1 | Brast07G228800.1 |
| *Brachypodium sylvaticum* | Brasy7G207000.1 | Brasy7G216000.1 |
| *Panicoideae* | *Panicum hallii* | PAN22995 | PAN23104 |
| *Sorghum bicolor* | Sobic.010G215600 | Sobic.010G207900 |
| *Setaria italica* | Si006528m | Si006839m |
| *Zea mays* | Zm00001eb389370 | Zm00001eb121230 |
|  | Zm00001eb386720 |
|  |  |
|  | *Oryza sativa* | LOC\_Os06g44840.1 | LOC\_Os06g43880.1 |
|  | *Ananas comosus* | Aco008264.1 | Aco027576.1  Aco008193.1 |

**Table S2. Amino acid sequence similarity comparison of HvHPT1 and HvHPT2 with other monocot HPTs.** Pa\_HPTs: *Panicoideae* (see species names in Table S1) HPTs. Tr\_HPTs: Triticeae (see species name in Table S1) HPT1.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Identity (%)** | HvHPT2 | OsHPT | Pa\_HPTs | Tr\_HPT1s |
| HvHPT1 | 75.63 | 81.36 | 83.29 (± 0.63) | 94.89 (± 2.42) |
| HvHPT2 |  | 78.39 | 75.39 (± 0.82) | 75.63 (± 0.68) |
| OsHPT |  |  | 84.38 (± 0.88) | 81.95 (± 0.47) |
| Pa\_HPTs |  |  |  | 83.12 (± 1.05) |

**Table S3. Genotyping results of HPT2 in 113 bread wheat accessions.**

|  |  |  |
| --- | --- | --- |
| **WheatAccessionID** | **HPT2** | **Country** |
| Aus20272 | present | AUSTRALIA |
| CCIV(AGG90664) | present | AUSTRALIA |
| CornerstoneMutantTimgalea | present | AUSTRALIA |
| Lancer | present | AUSTRALIA |
| LG Gold | present | AUSTRALIA |
| LRPB Havoc | present | AUSTRALIA |
| Naparoo | present | AUSTRALIA |
| RecessiveMaleSterileFs20 | present | AUSTRALIA |
| RGT Zanziba | present | AUSTRALIA |
| Taigu/6\*Sun276A | present | AUSTRALIA |
| Taigu/6\*Sun290B | present | AUSTRALIA |
| Einstein | present | UNITED KINGDOM |
| Aus90666 | absent | AUSTRALIA |
| Beckom | absent | AUSTRALIA |
| Calingiri | absent | AUSTRALIA |
| Catapult | absent | AUSTRALIA |
| Catpulta | absent | AUSTRALIA |
| CCIV(AGG90665) | absent | AUSTRALIA |
| Condo | absent | AUSTRALIA |
| Coolah | absent | AUSTRALIA |
| Corack | absent | AUSTRALIA |
| CornerstoneMutantGamenga | absent | AUSTRALIA |
| CornerstoneMutantZenis | absent | AUSTRALIA |
| Cosmick | absent | AUSTRALIA |
| Cutlass | absent | AUSTRALIA |
| Devil | absent | AUSTRALIA |
| DS Bennett | absent | AUSTRALIA |
| DS Faraday | absent | AUSTRALIA |
| DS Pascal | absent | AUSTRALIA |
| DS Tull | absent | AUSTRALIA |
| EG Jet | absent | AUSTRALIA |
| EG Titanium | absent | AUSTRALIA |
| EGA Gregory | absent | AUSTRALIA |
| Elmore CL Plus | absent | AUSTRALIA |
| Emu Rock | absent | AUSTRALIA |
| Grenade CL Plus | absent | AUSTRALIA |
| HeteroGeneticMaleSterile | absent | AUSTRALIA |
| Illabo | absent | AUSTRALIA |
| Kinsei | absent | AUSTRALIA |
| Kord CL Plus | absent | AUSTRALIA |
| Longsword | absent | AUSTRALIA |
| LRPB Beaufort | absent | AUSTRALIA |
| LRPB Flanker | absent | AUSTRALIA |
| LRPB Hellfire | absent | AUSTRALIA |
| LRPB Impala | absent | AUSTRALIA |
| LRPB Kittyhawk | absent | AUSTRALIA |
| LRPB Mustang | absent | AUSTRALIA |
| LRPB Nighthawk | absent | AUSTRALIA |
| LRPB Nyala | absent | AUSTRALIA |
| LRPB Oryx | absent | AUSTRALIA |
| LRPB Parakeet | absent | AUSTRALIA |
| LRPB Reliant | absent | AUSTRALIA |
| LRPB Scout | absent | AUSTRALIA |
| LRPB Spitfire | absent | AUSTRALIA |
| LRPB Trojan | absent | AUSTRALIA |
| Manning | absent | AUSTRALIA |
| Mitch | absent | AUSTRALIA |
| Ms3present7\*QT4646 | absent | AUSTRALIA |
| Ms3present7\_LLI\_\*Hartog | absent | AUSTRALIA |
| Ms3present7\_LLI\_BWSN50 | absent | AUSTRALIA |
| Ninja | absent | AUSTRALIA |
| Razor CL Plus | absent | AUSTRALIA |
| RecessiveMaleSterileFs2 | absent | AUSTRALIA |
| RecessiveMaleSterileFs24 | absent | AUSTRALIA |
| RecessiveMaleSterileFs3 | absent | AUSTRALIA |
| RGT Calabro | absent | AUSTRALIA |
| RockStar | absent | AUSTRALIA |
| Scepter | absent | AUSTRALIA |
| SEA Condamine | absent | AUSTRALIA |
| Sheriff CL Plus | absent | AUSTRALIA |
| SQP Revenue | absent | AUSTRALIA |
| Sunchaser | absent | AUSTRALIA |
| Sunmax | absent | AUSTRALIA |
| Sunprime | absent | AUSTRALIA |
| Sunprime -W | absent | AUSTRALIA |
| Suntop | absent | AUSTRALIA |
| Taigu/6\*Genero\_T8present | absent | AUSTRALIA |
| Taigu/7\*Jang | absent | AUSTRALIA |
| Taigupresent7\*Seri\_M82 | absent | AUSTRALIA |
| Tungsten | absent | AUSTRALIA |
| Vixen | absent | AUSTRALIA |
| Wedin | absent | AUSTRALIA |
| Westonia | absent | AUSTRALIA |
| Wyalkatchem | absent | AUSTRALIA |
| Wyalkatchem | absent | AUSTRALIA |
| Yitpi | absent | AUSTRALIA |
| Zen | absent | AUSTRALIA |
| Xiaomairs26 | absent | CHINA |
| Xiaomairs28 | absent | CHINA |
| Xiaomairs4 | absent | CHINA |
| 93MSC\*480\_4 | absent | FRANCE |
| 93MSC\*Briscand | absent | FRANCE |
| DMS\*R37 | absent | FRANCE |
| Magenta | absent | ITALY |
| ANZa | absent | MEXICO |
| Borlaug present00 | absent | MEXICO |
| Buckbuck\_DMS | absent | MEXICO |
| Opata\_M85\_DMS | absent | MEXICO |
| Superkang\_DMS | absent | MEXICO |
| Thornbird\_DMS | absent | MEXICO |
| Wheaton\_DMS | absent | MEXICO |
| Probus\_ms | absent | SWITZERLAND |
| Chris | absent | UNITED STATES |
| D6647 | absent | UNITED STATES |
| D6654 | absent | UNITED STATES |
| D6659 | absent | UNITED STATES |
| DominentChrisOutcrossing | absent | UNITED STATES |
| ks87upg | absent | UNITED STATES |
| Len | absent | UNITED STATES |
| Mace | absent | UNITED STATES |
| UC44 | absent | UNITED STATES |
| Dominent (Secale cereale) | absent | unknown |
| DominentMaleSterileFs6 | absent | unknown |

**Table S4. The content of tocochromanol isomers in Tpresent leavesand T2 grains of transgenic lines.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Tissues** | **Isomers** | **WT** | ***35S:HPT2***  **\_2#** |  | ***35S:HPT2***  **\_present6#** |  | ***35S:HPT2***  **\_27#** |  |
| Leaf | δ-T | 0.present3±0.0present | 0.09±0.0present | \* | 0.06±0.0present | \*\* | 0.05±0.02 | \* |
| β-T | 0.59±0.02 | present.present2±0.0present | \*\* | present.present2±0.0present | \*\* | present.03±0.04 | \*\* |
| γ-T | 2.02±0.present6 | 4.35±0.06 | \*\* | 4.99±0.03 | \*\* | 3.80±0.present6 | \*\* |
| α-T | 90.00±3.44 | present08.28±present.8present | \*\* | presentpresent6.79±3.82 | \*\* | present89.89±present3.82 | \*\* |
| T total | 92.74±3.30 | presentpresent3.84±present.89 | \*\* | present22.97±3.85 | \*\* | present94.78±present4.02 | \*\* |
|  |  |  |  |  |  |  |  |  |
| Grain | δ-T | 0.27±0.04 | 0.39±0.04 |  | 0.20±0.04 |  | 0.33±0.08 |  |
| β-T | 0.32±0.0present | 0.49±0.0present | \*\* | 0.46±0.04 |  | 0.44±0.04 |  |
| γ-T | 3.86±0.37 | 4.99±0.6present |  | 3.63±0.48 |  | 4.54±0.78 |  |
| α-T | 8.4present±0.2present | present2.present2±0.92 | \* | present3.42±0.26 | \*\* | presentpresent.60±0.56 | \*\* |
| T total | present2.87±0.22 | present7.99±present.57 | \* | present7.72±0.79 | \*\* | present6.9present±present.43 | \* |
| δ-T3 | 0.42±0.02 | 0.27±0.03 | \*\* | 0.40±0.00 |  | 0.4present±0.00 |  |
| β-T3 | present.37±0.28 | 0.65±0.03 | \* | 0.66±0.08 | \* | 0.57±0.02 | \*\* |
| γ-T3 | 6.05±0.present0 | 2.69±0.39 | \*\* | 4.present5±0.present9 | \*\* | 3.62±0.08 | \*\* |
| α-T3 | 32.4present±4.present5 | 24.62±2.20 |  | 22.present4±present.present3 | \* | 2present.65±present.8present | \* |
| T3 total | 40.26±4.55 | 28.23±2.6present | \* | 27.36±present.22 | \* | 26.26±present.74 | \* |
| T+T3 total | 53.present3±4.77 | 46.22±4.present8 |  | 45.08±present.88 |  | 43.present7±present.23 |  |

The leaves of two-month old transgenic lines and their mature grains were used for HPLC analysis. The data (means ± standard errors, mg/kg DW) are derived from at least three independent samples. Asterisks (\* or \*\*) indicate a significant difference between wild-type and transgenic lines at *P* < 0.05 or *P* < 0.0present, as determined by Student's *t* tests. T, Tocopherol; T3, Tocotrienol.

**Table S5. Conserved elements in the promoter of *HvHPTpresent* and *HvHPT2.***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene name** | **Classfication** | Cis-acting element | **Sequence** | **Distance from ATG** | **Description** |
| ***HvHPTpresent*** | **Defense and stress response** | MBS | CAACTG | +present805 | MYB binding site involved in drought-inducibility |
|  | DRE core | GCCGAC | +present3present, +333 | dehydration, low-temp, salt stresses response |
|  | ARE | AAACCA | +2present84 | cis-acting regulatory element essential for the anaerobic induction |
|  | **Growth** | ACE | GCGACGTACC | +present557 | cis-acting element involved in light responsiveness |
|  | Box 4 | ATTAAT | -8present7 | part of a conserved DNA module involved in light responsiveness |
|  | G-Box | CACGTG/CACGTT | -489, -2302 | cis-acting regulatory element involved in light responsiveness |
|  | GTpresent-motif | GGTTAA | +present53 | light responsive element |
|  | GATA-motif | GATAGGA | +70, +present2present7 | part of a light responsive element |
|  | TCT-motif | TCTTAC | +2present78 | part of a light responsive element |
|  | L-box | ATCCCACCTAC | +225present | part of a light responsive element |
|  | Sppresent | GGGCGG | -2present, -96, -27present, -2376 | light responsive element |
|  | CAT-box | GCCACT | -204 | cis-acting regulatory element related to meristem expression |
|  | O2-site | GATGATGTGG | -present089 | cis-acting regulatory element involved in zein metabolism regulation |
|  | GC-motif | CCCCCG | +24present, +382, -2present67 | enhancer-like element involved in anoxic specific inducibility |
|  | **Hormone-related** | ABRE | CACGTG/ACGTG | -489, +2302 | cis-acting element involved in the abscisic acid responsiveness |
|  | TATC-box | TATCCCA | -73 | cis-acting element involved in gibberellin-responsiveness |
|  | CGTCA-motif | CGTCA | +presentpresentpresent6, +present673, -present7present5, +present739 | cis-acting regulatory element involved in the MeJA-responsiveness |
| ***HvHPT2*** | **Defense and stress response** | LTR | CCGAAA | +presentpresent7present, +2present42 | cis-acting element involved in low-temperature responsiveness |
|  | DRE core | GCCGAC | -present024, -present226 | dehydration, low-temp, salt stresses response |
|  | ARE | AAACCA | +474 | cis-acting regulatory element essential for the anaerobic induction |
|  | GC-motif | CCCCCG | +present358, -2069 | enhancer-like element involved in anoxic specific inducibility |
|  | **Growth** | AE-box | AGAAACAA | -23present7 | part of a module for light response |
|  | G-box | CACGTG/TACGTG | -29, -569, -present059, +present236, +present269, +present873, +2090 | cis-acting regulatory element involved in light responsiveness |
|  | GTpresent-motif | GGTTAAT | -present22, -present676 | light responsive element |
|  | O2-site | GTTGACGTGA/GATGACATGG | +944, present78present, 2089 | cis-acting regulatory element involved in zein metabolism regulation |
|  | MRE | AACCTAA | -present84, -223 | MYB binding site involved in light responsiveness |
|  | Sppresent | GGGCGG | -present03present, -present073, -present082, -presentpresent75, -presentpresent82 | light responsive element |
|  | TCCC-motif | TCTCCCT | -508 | part of a light responsive element |
|  | MBSI | TTTTTACGGTTA/aaaAaaC(G/C)GTTA | -present669, 2present39 | MYB binding site involved in flavonoid biosynthetic genes regulation |
|  | **Hormone-related** | ABRE | CACGTG/ACGTG | -30, +569, -present237, -present270, +2090, -209present | cis-acting element involved in the abscisic acid responsiveness |
|  | TCA-element | CCATCTTTTT | +22present2 | cis-acting element involved in salicylic acid responsiveness |
|  | TGA-element | AACGAC | -present024, -present266 | auxin-responsive element |

**Table S6. Primers for sequence amplification and genotyping.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene** | **Forward primer (5′-3′)** | **Reverse primer (5′-3′)** | **Product size (bp)** |
| *HvACTIN* | GCTGAGCGGGAAATTGTAAG | GATCATGGATGGCTGGAAGA | present92 |
| *HvHGGT* | TTGCTTCTCTGCCGTCATAG | GCTGTCAACAATATGCTTATGC | present38 |
| *HvHPTpresent* | CGAGTTTCTTTGTCCATCCA | CAGTATCGTGTGCTTCAGTT | present63 |
| *HvHPT2* | TTGTCAGCCATGCAGTCCTT | AGAGAAGCAAGCTCAGCCAG | 223 |
| *HvHGGT\_*CDS | GCGAGGATGCAAGCCGTCAC | AAGGGCCAGCAGATGTGAACTA | present264 |
| *HvHPTpresent\_*CDS | GGAACAGTATGCCGAAACG | GGGTTGCTCGTCGTTGTCG | present23present |
| *HvHPT2\_*CDS | CACGCTGCTCACTCCTAGTC | ATACTGTCCTCGCACCGAAC | present297 |
| *HvHPT2\_*promoter | TGAGCGGACTCTGGTTCAAA | CTAGGAGTGAGCAGCGTGAG | 2327 |
| *TaHPT2* | CGCCCTTTTCATGAACATTT | GATACTATTGCAACCCCAGC | 225 |