

Supplementary Material

Alternative oxidase gene family in *Hypericum perforatum* L.: characterization and expression at the post-germinative phase

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1 Supplementary Figures and Tables



1.1 Supplementary Figures



Figure S1. Alignment of the four isolated sequences corresponding to 5'end of *HpAOX1* gene. The sequences correspond to the region from the primer adapter until ATG codon (included in the alignment). **a**) Alignment of the nucleotide sequences; **b**) Alignment of the corresponding putative translated peptide where no alternative ATG codon was identified.





Figure S2. Alignment of the four isolated sequences corresponding to 5'end of *HpAOX2* gene. The sequences correspond to the region from the primer adapter until ATG codon (included in the alignment). **a**) Alignment of the nucleotide sequences; **b**) Alignment of the corresponding putative translated peptide where an alternative ATG codon was identified in the sequence HpAOX2_cl1 (region in blue).



| | 20 | 40 | eo | 80 | |
|----------------------------|---|--------------------|-------------------------|---------------------------------------|------------|
| HpAOX1 ORF | TAA | | | | 3 |
| Hp3UTR cl1 | TAAAATGGAAGTCATTACTCATCAGTACACTGC | TCGTTGGAGAGAGAT | CAAATTAGTGACCTTTGTATTC | AATACGACC | 80 |
| Hp3UTR_cl2 | TAAAATGGAAGTCATTACTCATCAGTACTTTGA | TTCGTTGGAGAGAGAT | CAAATTAGTGACCTTTGTATTC | AATACGACT | 80 |
| Hp3UTR_cl3 | TAAAATGGAAGTCATTACTCATCAGTACTTTGA | TTCGTTGGAGAGAGAT | CAAATTAGTGACCTTTGTATTC | AATACGACT | 80 |
| Hp3UTR_cl4 | TAAAATGGAAGTCATTGCTCATCAGTACACTGC | TTCGTTGGAGAGAGAT | CAAATTAGTGACCTTTGTATTC | AATACGACC | 80 |
| Hp3UTR_cl5 | TAAAATGGAAGTCATTACTCATCAGTACTTTGA | TTCGTTGGAGAGAGAT | CAAATTAGTGACCTTTGTATTC | AATACGACC | 80 |
| Hp3UTR_cl6 | TAAAATGGAAGTCATTACTCATCAGTACACTGC | TTCGTTGGAGAGAGAT | CAAATTAGTGACCTTTGTATTC | AATACGACC | 80 |
| Hp3UTR_cl7 | TAAAATGGAAGTCATTACTCATCAGTACACTGC | TTCGTTGGAGAGAGAT | CAAATTAGTGACCTTTGTATTC | AATACGACC | 80 |
| Hp3UTR_cl8 | TAAAATGGAAGTCATTACTCATCAGTACACTGC | TTCGTTGGAGAGAGAT | CAAATTAGTGACCTTTGTATTC | AATACGACC | 80 |
| Hp3UTR_cl9 | TAAAATGGAAGTCATTACTCATCAGTACACTGC | TTCGTTGGAGAGAGAT | CAAATTAGTGACCTTTGTATTC | AATACGACC | 80 |
| Hp3UTR_cl10 | TAAAATGGAAGTCATTACTCATCAGTACACTGC | TTCGTTGGAGAGAGAT | CAAATTAGTGACCTTTGTATTC | AATACGACC | 80 |
| Hp3UTR_cl11 | TAAAATGGAAGTCATTACTCATCAGTACACTGC | TTCGTTGGAGAGAGAA | CAAATTAGTGACCTTTGTATTC | AATACGACC | 80 |
| Hp3UTR_cl12 | TAAAATGGAAGTCATTACTCATCAGTACACTGC | TTCGTTGGAGAGAGAT | CAAATTAGTGACCTTTGTATTC | AATACGACC | 80 |
| Hp3UTR_cl13 | TAAAATGGAAGTCATTACTCATCAGTACACTGC | TTCGTTGGAGAGAGAT | CAAATTAGTGACCTTTGTATTC | AATACGACC | 80 |
| Hp3UTR_cl14 | TAAAATGGAAGTCATTACTCATCAGTACACTGC | TTCGTTGGAGAGAGAT | CAAATTAGTGACCTTTGTATTC | AATACGACC | 80 |
| Hp3UTR_cl15 | TAAAATGGAAGTCATTACTCATCAGTACACTGC | TTCGTTGGAGAGAGAGAT | CAAATTAGTGACCTTTGTATTC | AATACGACC | 80 |
| Hp3UTR_cl16 | TAAAATGGAAGTCATTACCCATCAGTACACTGC | TTCGTTGGAGAGAGAGA | CAAATTAGTGACCTTTGTATTC | AATACGACC | 80 |
| Hp3UTR_cl17 | TAAAATGGAAGTCATTACTCATCAGTACACTGC | TTCGTTGGAGAGAGAGAT | CAAATTAGTGACCTTTGTATTC | AATACGACC | 80 |
| Hp3UTR_cl18 | TAAAATGGAAGTCATTACTCATCAGTACACTGC | TTCGTTGGAGAGAGAGAT | CAAATTAGTGATCTTTGTATTC | AATACGACC | 80 |
| Hp3UTR_cl19 | TAAAATGGAAGTCATTACTCATCAGTACATTGA | TCGTTGGAGCGAGAT | CAAATTAGTGACCTTTGTATTC | AATACGACC | 80 |
| Hp3UTR_cl20 | TAAAATGGAAGTCATTACTCATCAGTACATTGA | TCGTTGGAGCGAGAT | CAAAITAGIGACCIIIGIAIIC | AATACGACC | 80 |
| Hp3UTR_cl21 | TAAAATGGAAGTCATTACTCATCAGTACACTGC | TCGTTGGAGAGAGAGAT | CAAATTAGTGACCTTTGTATTC | AATACGACC | 80 |
| Hp3UTR_cl22 | TAAAATGGAAGTCATTACTCATCAGTACACTGC | TEGTIGGAGAGAGAGA | CAAAITAGIGACCITIGIAITC | AATACGACC | 80 |
| Hp3UTR_CI23 | TAAAAIGGAAGICATTACICATCAGIAGACIGC | | | AATACGACC | 80 |
| Hp3UTR_CI24 | TRAREIGGREGICETTECICATCAGIAGECICO | I COLLOGEOROAGEO | | AATACGACC | 80 |
| | 100 | 120 | 140 | 160 | |
| HpAOX1_ORF | | | | | 3 |
| Hp3UTR cl1 | ATAC - GAGTGCTTAAATTGTTACTACCGTCTTG | ACCGTTCATTGTAGA | CAATTTACATTTAGTGTAAGAA | AATTAAGAA | 159 |
| Hp3UTR_cl2 | ATACCGAGTGCTTAAATTGTTACTACCGTCTTG | ACCGTTCATTGTAGA | CAATTTACATTTAGTGTAAGAA | AATTAAGAA | 160 |
| Hp3UTR_cl3 | ATACC GAGT GC TTA AAT TG TTAC TACC GT CT TG | ACCGTTCATTGTAGA | CAATTTACATTTAGTGTAAGAA | AATTAAGAA | 160 |
| Hp3UTR_cl4 | ATAC - GAGTGCTTAAATTGTTACTACCGTCTTG | ACCGTTCATTGTAGA | CAATTTACATTTAGTGTAAGAA | AATTAAGAA | 159 |
| Hp3UTR_cl5 | ATAC - GAGTGCTTAAATTGTTACTACCGTCTTG | ACCGTTCATTGTAGA | CAATTTACATTTAGTGTAAGAA | AATTAAGAA | 159 |
| Hp3UTR_cl6 | ATAC - GAGTGCTTAAATTGTTACTACCGTCTTG | ACCGTTCATTGTAGA | CAATTTACATTTAGTGTAAGAA | AATTAAGAA | 159 |
| Hp3UTR_cl7 | ATAC - GAGTGCTTAAATTGTTACTACCGTCTTG | ACCGTTCATTGTAGA | CAATTTACATTTAGTGTAAGAA | AATTAAGAA | 159 |
| Hp3UTR_cl8 | ATAC - GAGTGCTTAAATTGTTACTACCGTCTTG | ACCGTTCATTGTAGA | CAATTTACATTTAGTGTAAGAA | AATTAAGAA | 159 |
| Hp3UTR_cl9 | ATAC - GAGTGCTTAAATTGTTACTACCGTCTTG | ACCGTTCATTGTAGA | CAATTTACATTTAGTGTAAGAA | AATTAAGAA | 159 |
| Hp3UTR_cl10 | ATAC - GAGTGCTTAAATTGTTACTACCGTCTTG | ACCGTTCATTGTAGA | CAATTTACATTTAGTGTAAGAA | AATTAAGAA | 159 |
| Hp3UTR_cl11 | ATAC - GAGTGCTTAAATTGTTACTACCGTCTTG | AACCGTTCATTGTAGA | CAATTTACATTTAGTGTAAGAA | AATTAAGAA | 159 |
| Hp3UTR_cl12 | ATAC - GAGTGCTTAAATTGTTACTACCGTCTTG | AACCGTTCATTGTAGA | CAATTTACATTTAGTGTAAGAA | AATTAAGAA | 159 |
| Hp3UTR_cl13 | ATAC - GAGTGCTTAAATTGTTACTACCGTCTTG | ACCGTTCATTGTAGA | CAATTTACATTTAGTGTAAGAA | AATTAAGAA | 159 |
| Hp3UTR_cl14 | ATAC - GAGTGCTTAAATTGTTACTACCGTCTTG | ACCOTTCATTGTAGA | CAATTTACATTTAGTGTAAGAA | AATTAAGAA | 159 |
| Hp3UTR_cl15 | ATAC - GAGTGCTTAAATTGTTACTACCGTCTTG | AACCGTTCATTGTAGA | CAATTTACATTTAGTGTAAGAA | AATTAAGAA | 159 |
| Hp3UTR_cl16 | ATAC - GAGTGCTTAAATTGTTACTACCGTCTTG | AACCGTTCATTGTAGA | CAATTTACATTTAGTGTAAGAA | AATTAAGAA | 159 |
| Hp3UTR_cl17 | ATAC - GAGTGCTTAAATTGTTACTACCGTCTTG | ACCGTTCATTGTAGA | CAATTTACATTTAGTGTAAGAA | AATTAAGAA | 159 |
| Hp3UTR_cl18 | ATAC-GAGIGETTAAATIGTTACTACCGTCTTG | ACCGITCATIGIAGA | CAATITACATTTAGTGTAAGAA | AATTAAGAA | 159 |
| Hp3UTR_cl19 | ATAC - GAGAAATTAAATTGTTATTCCCGTCTTG | ACCGITCATIGIAGA | CAATTTACATTTAGGGTAAGAA | AAATAAGAA | 159 |
| Hp3UTR_CI2U | | ACCOLLCATIONAC | CAATTTACATTTAGGGTAAGAA | AATAAGAA | 109 |
| Hp3UTR_cl21 | ATAC - GAGIGCTTAAATIGTTACTACCGTCTTG | ACCGITCATIGIAGA | CAATITACATTTAGTGTAAGAA | AATTAAGAA | 159 |
| Hp3UTR_CI22 | | ACCOLLEGE | AATTTACATTTACTCTAACAA | ATTAAGAA | 109 |
| Hp3UTR_CI23 | ATAC CACTCOTTAAATIGTTACTACCOTTTC | ACCOLLCATIONAC | AATTTACAT TTACTCTAACAA | AATTAACAA | 100 |
| Hpsolik_di24 | | ACCOLLCATION ACA | | | 100 |
| | 180 | 200 | 220 | 240 | |
| HpAOX1_ORF | · · · · · · · · · · · · · · · · · · · | | | | 3 |
| Hp3UTR_cl1 | ACGAATTGGAGACATATTATTAATCAGATCAAG | TATTGTGCTGAAACTC | TTTTTATCAGCACGTCTATAGT | AAAAAAAAA | 239 |
| Hp3UTR_cl2 | ACGGATTG-AGACATATTATTAATCAGATCAAG | ATTGTGCTGAAACTC | TTTTAATCATCACGTTTATAGT | AAAAAAAAA | 239 |
| Hp3UTR_cl3 | ACGGATTG-AGACATATTATTAATCAGATCAAG | TATTGTGCTGAAACTC | TTTTAATCATCACGTTTATAGT. | AAAAAAAA | 239 |
| Hp3UTR_cl4 | ACGAATTGGAGAGACATATTATTAATCAGATCAAG | ATTGTGCCG | | AAAAAAAAA | 211 |
| Hp3UTR_cl5 | ACGAATTGGAGACATATTATTAATCAGATCAAG | TATTGTGCTG | | AAAAAAAAA | 211 |
| Hp3UTR_cl6 | ACGAATTGGAGACATATTATTAATCAGATCAAG | ATTGTGCTG | | AAAAAAAAA | 211 |
| Hp3UTR_cl7 | ACGAATTGGAGACATATTATTAATCAGATCAAG | ATTGTGCT | | <u>AAAAAAAAA</u> | 210 |
| Hp3UTR_cl8 | ACGAATIGGAGAGAGATATTATTATTAATGAGATGAAG | | | <u>AAAAAAAA</u> A | 210 |
| Hp3UTR_cl9 | ACGAATTGGAGACATATTATTAATC | | | <u>AAAAAAAAA</u> | 193 |
| Hp3UTR_cl10 | ACGAAIIGGAGAGAGATAIIAIIAAIG | | | ********* | 193 |
| Hp3UTR_d11 | | | | 88888888AAA | 193 |
| Hp3UTR_012 | | | | AAAAAAAAAA | 193 |
| Hp3UTR_013 | | | | ********* | 193 |
| Hp3UTR_014 | | | | A A A A A A A A A A A A A A A A A A A | 183 |
| Hpsuik_clib | | | | | 183 |
| HISUIK_CIIO | | | | | 103 |
| Hp3UTR_017 | | | | | 161 |
| Ho2UTP allo | | | | | 101 |
| Host ITP also | | | | | 101 |
| Ho2UTP d24 | | | | | 174 |
| Hostine data | | | | | 174 |
| INDOUR CIZZ | | | | | |
| Ho3UTR d23 | | | | | 171 |
| Hp3UTR_cl23 Hp3UTR_cl24 | A CC | | | | 171 171 |



Figure S3. Alignment of the twenty four isolated sequences corresponding to 3'end of *HpAOX1* gene. The sequences correspond to the region from the stop codon (TAA, included in the alignment) to the poly(A)-tail (only nine common adenines are represented).



ACATGGGGATCCCCAAGAAACACGAAGCAAAAAAAAAACCCTAGAAGACAAAACCTAACTGCTCTCCTAAAAAG AAATTAAG 80 TGAAAACAAAGAAATTGTAGAGATTATTGAATCGAGATGATAAGCCGCGGCGGTACTAGGCTGGCGAGTTCG GCGGTTGC 160

MISRGGTRLASSAVA

GGCGGCCGCTGCCAATGCTCGGCGCTTCTCGACTGCCGGAGCAACGGAGAAGTCTGTTCTCAGATACTATGC TACTCCGG 240 A A A A N A R R F S T A G A T E K S V L R Y Y A T P

CAAAGGGCGGAAACAGTGCGGTGTCGCCGTGGGCGAGGGGCCCGATCTTGACGAGGAGTGCAACGACGATG

AGCTTGGGA 320 A K G G N S A V S P W A R G P I L T R S A T T M S L G

GAGAAGCAAGTGAAGGAGGAGGCTGAAGAGAATAAGGTTCCTTCGGAGCGTTCCACCGGCGCCGGAGGGAA CAAGGATGG 400 E K Q V K E E A E E N K V P S E R S T G A G G N K D G

CGGCGATGAGAAGGCCGTTGTTAGCTACTGGGGGTATTAACCCCGGGAAGATCACTAAGGAAGACGGCACCGT TTGGACCT 480

G D E K A V V S Y W G I N P G K I T K E D G T V W T

GGACTTGCTTTAGGCCATGGGAAGCATACCAACCAACCATGTCGATTGATCTGAAGAAGCATCACGCTCCGA CTACTTTC 560 W T C F R P W E A Y Q P N M S I D L K K H H A P T T F

TTGGACAAACTGGCTTTTCGGACCGTTAAGTCTCTCCGATGGCCCACCGACATATTCTTTCAGAGGAGGTATG GTTGTCG 640

L D K L A F R T V K S L R W P T D I F F Q R R Y G C R

GGCAATGATGCTCGAAACAGTGGCCGCGGGTGCCAGGCATGGTAGGAGGAATGCTCCTCCACTGCAAGTCACT GAGGCGCT 720

A M M L E T V A A V P G M V G G M L L H C K S L R R

TCGAGCACAGCGGAGGATGGATCAAAACCCTACTGGACGAGGCAGAGAACGAGCGGATGCACTTGATGACA TTCATGGAG 800

FEHSGGWIKTLLDEAENERMHLMTFME

GTTGCCAAGCCCAAATGGTACGAGCGTGCTCTGGTCTTCACAGTGCAGGGCGCCTTCTTCAACATCTACTTTC TGGGGTA 880 VAKPKWYERALVFTVQGAFFNIYFLGY

TCTCATTTCCCCCAAGTTCGCGCATAGGGTGGTTGGATACCTCGAGGAAGAAGCAATCCACTCCTACACCGA GTTTCTCA 960 ▼ LISPKFAHRVVGYLEEEAIHSYTEFL

AGGAGTTGGACAATGGCAACATCGAGAATGTGCCTGCTCCTGCAATTGCTGTCGACTACTGGCGCCTACCTC CCAACTCC 1040

K E L D N G N I E N V P A P A I A V D Y W R L P P N S

ACTCTAAAGGATGTGGTTCAGGTTGTCAGGGCCGACGAGGCTCATCACCGCGACGTCAATCATTTTGCATCG GACATACA 1120 TLKDVVOVVPADEAHHPDVNHEASDIH

T L K D V V Q V V R A D E A H H R D V N H F A S D I H

CTACCAAGGACGTGAGCTGAGGGAATGCCCTGCTCCACTGGGTTATCACTAAAATGGAAGTCATTGCTCATC AGTACACT 1200



Y Q G R E L R E C P A P L G Y H *

Figure S4. Nucleotide and deduced amino acid sequences of cDNA encoding *Hypericum perforatum* L. alternative oxidase 1 (*HpAOX1*, acc. no. EU330415.1). \checkmark indicates the position of the three introns, * indicates stop codon.



ACATGGGAAGCAGTGGTATCAACGCAGAGTACATGGGGGAAAAGGAGCAGAAACATAGAAAGAGGGAGAGAG **AGAAAAAA** 80 ATATATTAGGAGAAGGGGGAATTTGTGGAGAAATGTACCGCCACGTGGCAAGGTCAGTGATCAGGGGGACTCCT GGTCAACG 160 MYRHVARSVIRGLLVN CACCTCCTC 240 G S A R Q A G A G I R L L P A S T A A A R T T D H L L TTGAGCAG 320 L A R P F G R M D G G F P S S L G A A S Y L R M L S S CTCATCGTCGTCGGAGGGGAAGCCGGTGGCGGAGGAGGAGGAGGAGGAGGCGGTGGCGTCCAGTTACTGGGGAA **TCTCGAGGC** 400 S S S E G K P V A E E E K K A V A S S Y W G I S R CGAAGGTCACTAGGGAGGACGGCACCGACTGGCCTTGGAACTGCTTCATGCCTTGGGAGTCTTATAGGACAG **ATACGTCA** 480 P K V T R E D G T D W P W N C F M P W E S Y R T D T S ATAGATCTGACGAAGCACCACGTTCCAAAGACATTTCTTGACAAGTTTGCTTACTGGACGGTCAAGATCCTTC GAATTCC 560 I D L T K H H V P K T F L D K F A Y W T V K I L R I P TACAGATATATTCTTTCAGAGACGGTACGGCTGCCGCGCAATGATGTTGGAAACAGTTGCAGCTGTACCGGG TATGGTTG 640 T D I F F Q R R Y G C R A M M L E T V A A V P G M V GTGGGATGCTTCTGCACTTGAGGTCGCTCCGCAAGTTCGAGCAAACTGGGGGGTTGGGTCAAGGCCTTGCTCG AAGAAGCA 720 G G M L L H L R S L R K F E Q T G G W V K A L L E E A GAGAACGAAAGAATGCACTTGATGACAATGGTAGAGCTCGTTCAGCCAAAATGGTACGAGAGGATGCTGGTT **CTTATCGT** 800 ENERMHLMTMVELVQPKWYERMLVLIV **CTATTTGG** 880 Q G L F F N A Y F V I Y L V S P K L A H R V T G Y L AAGAAGAGGCCATTCATTCATATACCGAGTACCTCAAAGACATAAGGAGTGGGAAAATCGAGAATGTCAAGG **CTCCCGCT** 960 E E E A I H S Y T E Y L K D I R S G K I E N V K A P A ATTGCGATCGACTACTGGAGGTTGCCTAAGGAAGCTACACTCGAGGATGTCATAACTGTGATCCGAGCCGAT GAAGCACA 1040 I A I D Y W R L P K E A T L E D V I T V I R A D E A H TCACCGTGATGTCAACCACTTTGCTTCTGATGTTCACTATCAGGGCAAGAAACTGAAAGAGGCACCTGCTCCT **ATTGGCT** 1120 HRDVNHFASDVHYQGKKLKEAPAPIG ATCATTGAGGGGTTTTTTTTTTTTTTTTAACTTATGTTTTCAGAGTCAAGTAATATCGAAATTTATTCGAGACTT

ATTTAG 1200

YH *



TTCTTACTTCAGCTTGTGTTGTATATGCTATGTATGTAAACGTATATTATTGTAAAATATAAGGCGCATATAA TATACA 1280 TTGTTTTGTTGACATAAAAAAAAAAAAAAA 1311

Figure S5. Nucleotide and deduced amino acid sequences of cDNA encoding *Hypericum perforatum* L. alternative oxidase 2 (*HpAOX2*, acc. no. EU330413.1). \checkmark indicates the position of the three introns, * indicates stop codon.



| EU330415.1 | MIS RGGTRI ASSAVA AAAAAAAREESTAGATEKSVI RYYA TPA KGGNSAVSPWARGPI I TRSATTM | 65 |
|--|---|---|
| AT3G22370 | MMIT RGGAKAAKSI I VAAGPRI ESTVRTVSSHEALSASHII KRGVTSAWIWTRAPT I GGMREASTI | 66 |
| AT3G22360 | | 47 |
| AT3022300 | | 40 |
| AT3027020 | MITTELERSLEDASKC-ATSTRCTEF | 40 |
| AT1G32350 | MSTRSTRTLRPVLSSSVUS | 34 |
| TC03G031300 | MMS RGGIRLASSM - LAFVGPRY HSTA - SARILSNEGM - SGWIRAPAVG - VRCRSIL | 52 |
| EU330413.1 | <u>MY - RHVARSVIRGLLVNG SARQ</u> AGAGIRLLPASTAAARTTDHLLLARPFGRMDGGFPSSLGAA | 62 |
| AT5G64210 | M-SQLITKAALRVLLVCGRGNCNMFVSSVSSTSVMKSPYEITAPMRIHDWCGGFGDFKIGSKHVQGNF | 67 |
| CP00042G00490 | M-NRFVARSVIRALMVNSRSCGGSNRMLYAATTATVRPWEISDVGRGGDFGAF | 52 |
| ME10292G00060 | MMNNLVIRSVVRGLLVNGSRGGRYISTATAATTAGVLKPTEVLTEFRSRNGASVGVL | 57 |
| RC30063G00030 | M - N RVV I RSVL RGLQVNGG - GGRC I STAA SGGGAAA VAAAAV E IWSRE LR SKNGAY SYGVC | 59 |
| TC02G011670 | M-NRFLVRSVMQGL-INGRSYSGSSISNGHMYRAAIVGRDVWLSGQQNGMPFGGF | 53 |
| | | |
| | | |
| EU330415.1 | SLGEKQVKEEAEENKVPSERSTGAGGNKDGGDEKAVVSYWGINPGKITKEDGTV | 119 |
| AT3G22370 | TLGEKTPMKEEDANQKKTENESTGGDAAGGNNKGDKGIASYWGVEPNKITKEDGSE | 122 |
| AT3G22360 | TFEKKKTTEEKGSSGGKADQGNKGEQLIVSYWGVKPMKITKEDGTE | 93 |
| AT3G27620 | TFEKKKTSEEEEGSGDGVKVNDQGNKGEQLIVSYWGVKPMKITKEDGTE | 97 |
| AT1G32350 | HLPNVRLLSSDTSSPVSGNNQPENPIRTADGKVISTYWGIPPTKITKPDGSA | 86 |
| TC03G031300 | ALGDKEQEEKQAVDGGPAKDEKGIVSYWGLEPTKVTKEDGSP | 94 |
| EU330413.1 | SYLRMLSSSSSSEGKPVAEEEKKAVASSYWGISRPKVTREDGTD | 106 |
| AT5G64210 | NLRWMGMSSASAM EKKDENLTVKKGQN GGGSVAVPSYWGIETAKMKITRKDGSD | 121 |
| CP00042G00490 | N WRRMMS - SQESSAVE EVKKEKSAVEQ AKKDSSGDKMMLSSYWGI SRPKITRKDGTE | 108 |
| ME10292G00060 | Y WRRMMSTSAETAVAEKETTEKSVGKQGE KEVASLKTSDG - SV ISSYWGI SRPK I L REDGTE | 118 |
| RC30063G00030 | C WRRMMST SOLT FOSE KAVAKKE FASEVKGVE SVKVND SKAVVS SVWG L VRPKVL REDGSE | 120 |
| TC02G011670 | E - WRRMMN SAP AWTEKVPIEKEEKKENPATGEKK - GTEMMVS SYWGI SRPK I TKEDGTD | 110 |
| | 160 180 200 | |
| | | |
| EU330415.1 | WTWT ©F R PWEAY Q PNMSIDLKKHHAPTTFLDKLAFRTVKSLRWPTDIFFQRRYGCRAMMLJETVAAV PGMV | 189 |
| AT3G22370 | WKWN©FR PWETYKAD IT ID LKKHHVPTTFLDRIAWTVKSLRWPTDLFFQRRYGCRAMMLETVAAVPGMV | 192 |
| AT3G22360 | WKWSCFR PWETYKSDLTIDLKKHHVPSTLPDKLAYWTVKSLRWPTDLFFQRRYGCRAMMLJETVAAVPGMV | 163 |
| AT3G27620 | WKWSCFR PWETYKADLTIDLKKHHVPSTLPDKIAYWWVKSLRWPTDLFFQRRYGCRAIMLETVAAVPGMV | 167 |
| AT1G32350 | WKWNCFQPWDSYKPDVSIDVTKHHKPSNFTDKFAYWTVQTLKIPVQLFFQRKHMCHAMLLETVAAVPGMV | 156 |
| TC03G031300 | WKWTCFR PWEAYKADLSIDLKKHHAPVTVMDKMAYWTVKALRWPTDLFFQRRYGCRAMMLETVAAVPGMV | 164 |
| EU330413.1 | WPWNCFMPWE SYRTDTSIDLTKHHVPKTFLDKFAYWTVKILRIPTDIFFQRRYGCRAMMLETVAAVPGMV | 176 |
| AT5G64210 | WPWN CFMPWETYQANLSIDLKKHHVPKNIADKVAYRIVKLLRIPTDIFFQRRYGCRAMMLETVAAVPGMV | 191 |
| CP00042G00490 | WPWNCFMPWEAYGTDISIDLTKHHVPKTFLDKVAYRTVKLLRIPTDIFFQRRYGCRAMMLETVAAVPGMV | 178 |
| ME10292G00060 | WPWNCFMPWETYRANTSIDLSKHHVPKTFLDKVAYRTVKLLRLPTDLFFQRRYGCRAMMLETVAAVPGMV | 188 |
| RC30063G00030 | WPWN CEMPWETY OSN TALDLSKHHVPKTELDKWAYRTYKLLRYPTDLEER RYGCRAMMUETYAAY PGMM | 190 |
| TC02G011670 | WOWN CENDWE TVK AD LOLKKHHVDK NEVDKEAVD TVK LLDLDTDLEECOD DVCCD ANNU LETVA AV DONY | |
| 10020011010 | WPWNUF MPWEITRADLSIDLRRINVPRNFVDRFATRIVRILRIPIDLFFURRTGURAMMLIEIIVAAV PGMV | 180 |
| 10020011010 | 220 240 250 260 280 280 280 280 280 280 280 280 280 28 | 180 |
| 10020011010 | | 180 |
| EU330415.1 | 220 1 COMLLHCKSLRR FEHSGGWIKTLLDEAENERMHLMTFMEVAKPKWYERALVFTVQGAFFNIYFLGYLISP | 180 259 |
| EU330415.1 AT3G22370 | 220 1 1 1 1 1 1 1 1 1 1 1 1 1 | 180 259 262 |
| EU330415.1 AT3G22370 AT3G22360 | 20 20 20 20 20 20 20 20 20 20 | 180 259 262 233 |
| EU330415.1 AT3G22370 AT3G22360 AT3G27620 | 20 20 20 20 20 20 20 20 20 20 | 180 259 262 233 237 |
| EU330415.1 AT3G22370 AT3G22360 AT3G27620 AT1G32350 | 20 20 20 20 20 20 20 20 20 20 | 180 259 262 233 237 226 |
| EU330415.1 AT3G22370 AT3G22360 AT3G27620 AT1G32350 TC03G031300 | 200 200 200 200 200 200 200 200 | 180 259 262 233 237 226 234 |
| EU330415.1 AT3G22370 AT3G22360 AT3G27620 AT1G32350 TC03G031300 EU330413.1 | 20 20 20 20 20 20 20 20 20 20 | 180 259 262 233 237 226 234 246 |
| EU330415.1 AT3G22370 AT3G22360 AT3G27620 AT1G32350 TC03G031300 EU330413.1 AT5G64210 | 20 20 20 20 20 20 20 20 20 20 | 180 259 262 233 237 226 234 246 261 |
| EU330415.1 AT3G22370 AT3G22360 AT3G27620 AT1G32350 TC03G031300 EU330413.1 AT5G64210 CP00042G00490 | 20 20 20 20 20 20 20 20 20 20 | 180 259 262 233 237 226 234 246 261 248 |
| EU330415.1 AT3G22370 AT3G22360 AT3G22560 AT1G32350 TC03G031300 EU330413.1 AT5G64210 CP00042G00490 ME10292G00060 | 210 210 210 201 201 201 201 201 | 180 259 262 233 237 226 234 246 261 248 258 |
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Figure S6. Multiple alignment of translated amino acid sequences of previously reported AOX proteins from, AtAOX1b AT3G22360, *Arabidopsis* thaliana (AtAOX1a_AT3G22370, AtAOX1c_AT3G27620, AtAOX1d AT1G32350 and AtAOX2 AT5G64210), Theobroma cacao (TcAOX1 TC03G031300 and TcAOX2_TC02G011670) Carica papaya (CpAOX2_CP00042G00490), Manihot esculenta (MeAOX2_ME10292G00060), Ricinus communis (RcAOX2_RC30063G00030) and AOX from Hypericum perforatum (HpAOX1 EU330415.2 and HpAOX2 EU330413.2). The alignment was performed using CLC Main Workbench 6.7.1 software. The data were retrieved from public web-based database Plaza v2.5, freely available at http://bioinformatics.psb.ugent.be/plaza/versions/plaza v2 5/. Amino acids residues differing are shown in red, deletions are shown by minus signs. The putative mitochondrial transit peptides (mTP) are shown in blue boxes. The sites of two conserved cysteins (CysI and CysII) involved in dimerization of the AOX protein by S–S bond formation (Umbach and Siedow, 1993) are indicated in dark grey boxes. Helices α1 and α 4, which form the hydrophobic region on the AOX molecular surface and are involved in membrane binding, are shown in red; helices $\alpha 2$, $\alpha 3$, $\alpha 5$ and $\alpha 6$, which form the four-helix bundle accommodating the diiron center, are shown in green (Moore et al. 2013). Amino acids residues that coordinate the diiron center (E, glutamate and H, histidine) and those that interact with the inhibitor are in yellow and light pink boxes, respectively.



ATGATAAGC CG C G C G G T A C T A G G C T G C G A G T T C G G C G G C T G C C G C T G C C A T G C T C G G T G C T TCTCGACGGCCGGAGCCACGGAGAGGTCTGTTCTCAGATACCATGCTACTCCGGCGAAGGGCGGAAACAG TGCGGTGTCGCCGTGGGCGAGGGGCCCCGATCTTGACGAGGAGTGTAACGACGATGAGCTTGGGAGAGAAG CAAGTGAAGGAGGAGGCTGAAGAGAATAAGGTTCCTTCGGAGCGTTCCACCGGCGCCGGAGGGAACAAGG ATGGCGGCGATGAGAAGGCCGTTGTTAGCTACTGGGGTATTAACCCCGGGAAGATCACTAAGGAAGACGG CACCGTTTGGACGTGGATCTGTTTTAGGGTAAGCTTGATTTTATTTTATTTTTCTTATTTTTTTGGT AGTAATATTTTATTTTTTTTTCTTATTTCTTGACCTTCGTCAATAGAGATTTTGACTTCCTTTTGTCACGC GGC GTT TGAAT CTTTGACTTTGTTATTTAG GGATTTTTTTGTTGTGGATTAAT TAATTGATAT GATTTAGATGTTCTAATATATAGGGAATTTTTGTTTTTGTGATGTATATTTAATTAAAATGTATTGGTA AATGTGAAGCCATGGGAAGCATACCAACCAACATGTCGATTGATCTGAAGAAGCATCACGCTCCGACTA CTTTCTTGGACAAACTGGCTTTTTGGACCGTTAAGTCTCTCCGATGGCCCACCGACATATTCTTTCAGGT AATATTTAATTTCTTCAATATTATTTTTTCGCATATCTTCTTCTAGGTTGAGATTACTACTGGGAGACTT TTTTACTGAGAAAAATCTAAATAACATTGTTATAAAATTTTGGAGTGAACATGAAAATTTGAGTTTGGTA ATCTGACGCCATTTTCCTGGTTCTGTTTTTTTTGGTGTAATCCTGGTTCTGTTGTTGTTACTACTT TCCCCCAAAAAATTGTAATGTGAATTAATGAGAAAAATTCTCGTTAAGTCATCGAAAGAAGGATTTTTT TTAAATCTTCCTTCAAGAGGAGATCTATATTATTAGAAAGAGGAGGACAATGGGTGGCAGCTTAATTGGT TCCTGGCGCCACCTTTTCTTGCCCTCTTTTACGTGAATCACTTGTCCATCAGTTGAGAACAGAAGGCTTT GCTGTACCATGTAAGTCTCTAGTTAATGCAAAAAATTCGAGAAACTTTGGGTTGAATATGAAACCCCAAGT TATACTTAGATGAGAATCTGGTTTATTGTTTGTGTTTTATCACAAACTCTATGGACTTAAAAGACTTCCA TTTAGAGTATGA TTGTAGATAATTCTCTCTGATTAATCTCTGGCATTATGTCATGCAGAGGAGGTATGG TTGTCGGGCAATGATGCTCGAAACAGTGGCCGCGGTGCCAGGCATGGTAGGAGGAATGCTCCTCCACTGC AAGT CA C T G AGG C G C T T C G AG C A C A G C G A G G A T G G A T C A A A A C C C T A C T G G A C G A G G C A G A G A A C G A G C GGATGCACTTGATGACATTCATGGAGGTTGCCAAGCCCCAAATGGTACGAGCGTGCTCTGGTCTTCACAGT GCAGGGCGCCTTCTTCAACATCTACTTTCTGGGGTATCTCATTTCCCCCCAAGTTCGCGCATAGGGTGGTT GGATACCTCGAGGAAGAAGCAATCCACTCCTACACCGAGTTTCTCAAGGAGTTGGACAATGGCAACATCG AGAATGTGCCTGCTCCTGCAATTGCTGTCGACTACTGGCGCCTACCTCCCAACTCCACTCTAAAGGATGT GGTT CAGGTTGT CAGGGCCGACGAGGCT CATCACCGCGACGT CAAT CATTTTG CATCGGT AAAT CAAGTT CCCTTGTTTTGTTTTCCCCTAACAACCTGTGGTTTCTGCAGATTCTCGATGTGATACAAACGGTTGATAC TAT CGT C T T A A T G C A G G A C A T A C A A C C A A G G A C G T G A G G G A A T G C C C T G C T C C A C T G G G T T A TCACTAA



Figure S7. Genomic sequence of *Hypericum perforatum* L. alternative oxidase 1 (HpAOX1, acc. no. KU674355). Exons are in yellow, stop codon in pink, repetitive sequences in red, pre-miRNA sequences in grey. In blue there is the second predicted pre-miRNA sequence which is two base pairs shorter and was predicted as in reverse sequence.



ATGTACCGC CACGTGGCAAGGT CAGTGAT CAGGGGACTC CTGGTCAACGGGAGTGCC CGCCAGGCTGGCG CCGGGATCCGGCTGCTTCCAGCTTCCACAGCCGCCGCGCGGACGACCACCTCCTCCTCGCGAGGCC TTTCGGCCGGATGGACGGTGGATTCCCGTCCTCGCTCGGCGCGGCGAGTTACTTGAGGATGTTGAGCAGC TCTCGAGGCCGAAGGTCACTAGGGAGGACGGCACCGACTGGCCTTGGAACTGCTTCATGGTATTAACATA TCTTAGAAAAGCTTGATACGATGTGATTAAATGATTTGATTGTTATTATCGATATTAACAAGTTTAGCGT GGTTAGCCTCGGATAATTTTAGAATGGATAATCTGAGAAGTTGTTAGGGTGTGTGAACGAGGACAAAACA CGCTGGAAAAAGTTATCTAGATTTGTGTAGACACTCGGTCAGATCGCGGGATGTTACAGTATTAATAGT AGAAATTTGAATTACATGCTACAAGCTTTACATGTTGTTTCGTTACGCAGTAGATCAGGTTCATAGCAGA ATGCAGAAATCTTATTAGTTCAATTCCGTGCTTGTATGAATAACGCATGCGATCAGCCGGACAATTATAT TTCGAGTTCAACAAGTATGGGAATGATGAAGTCTGTACTAGTTCAAAACTGCTTTATCAGATGTTAAACA ACTTTTTTAGTTACGTGGAAATTTTCTTATTAGAAATGAACGAATTAAGTACATGGATGTGGATTTCTTT CATTGTCTGAGACAGTTGGCCTACGATCTTGACGAGTCCTAGAGTATCGGGTTTGTCCTAAATCTTTGT GGCTACTTTGCCTGCTAAACCAATATGTAGCTTATATGAATTGCTCGTTTAACTAAGTGTATGTGTTTTG TGGGGAACTTTGATTAAGAAGTGGAGTTGATTGAAGTTATGTTTATGGGTGTTGGTAGCCTTGGGAGTCT TATAGGACAGATACGTCAATAGATCTGACGAAGCACCACGTTCCAAAGACATTTCTTGACAAGTTTGCTT ACTGGACGGT CAAGAT C C T C GAA T T C C T A C AGAT A T A T T C T T C AGG T C AGG T T T G T T A A T C T T C AT G GGTTGTTCAAAATTTGGCATGAGTATTGGTGGTCTGGATAATCAATACAGAGCAAAGACTGTTTTCGATG ATTGTCGTTAACTTGTGCCGCTGAATTAGTTTTGATCATCCACTGCAAAGTTTACTACACTTTGTTCCTC TTGTCAAAGTTTTCACAAATCTTGTGAATCGTGTCTCAG<mark>AGACGGTACGGCTGCCGCGCAATGATGTTGG</mark> AAACAGTTGCAGCTGTACCGGGTATGGTTGGTGGGATGCTTCTGCACCTGAGGTCACTCCGCAAGTTCGA GCAAACCGGGGGTTGGGTCAAGGCCTTGCTCGAAGAAGCCGAGAACGAAGAATGCACTTGATGACAATG GTAGAGCTCGTTCAGCCAAAGTGGTACGAGAGGATGCTGGTTCTTATCGTGCAGGGGCTGTTCTTCAACG CCTACTTTGTGATCTATCTCGTGTCCCCGAAGCTCGCTCACAGGGTAACAGGCTATTTGGAAGAAGAGGC CATTCATTCATATACCGAGTACCTCAAAGACATAAGGAGTGGGAAAATCGAGAATGTCAAGGCTCCCGCT ATTGCGATCGACTACTGGAGGTTGCCTAAGGAAGCTACACTCGAGGATGTCATAACTGTGATCCGAGCCG ATGAAGCACATCACCGTGATGTCAACCACTTTGCTTCTGTAAGTATTAGCTCTCAATCTGAATTTATATT TGGCATGCCAGTTACGAGATCGATAAGATTGATATACCAAACTTTCTTCGTGAACCAGTATTTGTTGTAA AAAGCGATTCTTTGATTAAACAGAATTCATTTCAGATGGAATTCGATTTTACTCAATTCTACTCGGTCTT TGGGTGCAGGATGTTCACTATCAGGGCAAGAAACTGAAAGAGGCACCTGCTCCTATTGGCTATCATTGA



Figure S8. Genomic sequence of HpAOX2 (acc. no. KU674356). Exons are in yellow, stop codon in pink, repetitive sequences in red, predicted LTR region homologous of a *Cassandra* MT-LTR retrotransposon sequence in green.



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| | 49.673.750 | | _ | | 49,675 | .000 | 49.675.65 | 49,676,250 | | 49 | .677,500 | |
| O User Bland | t Results | | | | | | your.seq | BLAST Chr06 featu | re1 (e-Value = 1.2e | -99] | | |
| ③ Transcrip | t | | | | orai.00 | 5G253600.1 | | | | | • | |
| | | | | | | | | | | | | |

Figure S9. Genome mapping showing the location of the pre-miRNA sequences that encode the homologous miRNAs.





Figure S10. Expression stability analysis of candidate reference genes by GeNorm algorithm. (A) Average expression stability M. (B) Determination of the optimal number of reference genes for normalization by pairwise variation (V).







Figure S11. Melting curves of the genes analyzed showing specific peaks.



1.2 Supplementary Tables

| Gene | Aim | Primer sequence (5'-3') | Ta (°C) |
|--------|------------|---|---------|
| | 3'RACE-PCR | Fw: GCAGGGCGCCTTCTTCAACATCTAC | 58 |
| HpAOX1 | 5'RACE-PCR | Rev: CATCAAGTGCATCCGCTCGTTCTCT | 68 |
| | Whole gene | Fw: CCTAACTGCTCTCCTAAAAAGA Rev: CTCTCTCCAACGAAGCAATG | 55 |
| | 3'RACE-PCR | Fw: AAGCTCGCTCACAGGGTAACAGG | 55 |
| HpAOX2 | 5'RACE-PCR | Rev: GAACAGCCCCTGCACGATAAGAACC | 68 |
| | Whole gene | Fw: AGAAGGGGAATTTGTGGAG Rev: CAACACAAGCTGAAGTAAGAAC | 60 |

 Table S1. Primer sequences used in HpAOX genes isolation.

Ta: annealing temperature, Fw: forward, Rev: reverse.



| 0 | F | Species | Accession number |
|----------|----------------|-----------------------|-----------------------------|
| | | | AL1G33660 |
| | | | AL3G24680 |
| | | Arabidopsis lyrata | AL3G24690 |
| | | 1 7 | AL5G06730 |
| | | | AL0G08000 |
| | | | AL8G30860 |
| | | | AT1G32350 |
| | | | AT3G22360 |
| | | Arabidonsis thaliana | AT3G22370 |
| | | indotaopsis manana | AT3G27620 |
| | | | AT5G64210 |
| | | | Bostr 19424s0421 1 |
| | | Boechera stricta | Bostr 19424s0422.1 |
| | | bocchera siricia | Bostr 3359s0124 1 |
| | | | Bostr 0568s0383 1 |
| | в | | Bro010153 |
| | ea: | | Bra001865 |
| les | cat | Brassica rana | Bra031351 |
| ica | ssi | Brassica rapa | Bra023835 |
| ass | 3ra | | Bra027768 |
| Br_{i} | I | | Cagra 1180:0011 1 |
| | | Capsella grandiflora | $C_{agra} = 1189s0011.1$ |
| | | | Cagra $5575_{\circ}0007.1$ |
| | | | Cagra $2057_{2}0010.1$ |
| | | | $Cagra 0.248 \pm 0.102.1$ |
| | | | Cagia.024650102.1 |
| | | Canaalla mikalla | Caruby10014005111 |
| | | Capsella rubella | Caruby10019337III |
| | | | Caruby 10009739III |
| | | | Calubv10020081111 |
| | | Futnoma calcusinoum | Think 10021015 m |
| | | Eutrema saisugineum | The 1000934211 |
| | | | TD2C20120 |
| | | Thellungiella parvula | TP2C20140 |
| | | Theilungiella parvula | TP3G20140 |
| | | Carica nanana | avm model supersontia 8 20 |
| | C | Carica papaya | evin.model.supercontig_8.29 |
| | | Citerally I an atom | cl 10C07420 |
| tbi | 5 | Curulus lanalus | CL10007420 |
| urcu | Cura | Cucumis melo | CM00136G00010 |
| ũ | - | Cucumis sativus | Cucsa.398150.1 |
| | | | GM04G14800 |
| | | Glycine max | GM08G07690 |
| | | | GM08G07700 |
| | | | GM05G24455 |
| | | | LJ2G020780 |
| S | ae | Lotus japonicus | LJ4G005280 |
| ale | nce | | LJ4G005290 |
| dp | aba | | MT5G026620 |
| F | F_{ϵ} | Medicago truncatula | MT5G070680 |
| | | | MT5G070870 |
| | | | MT5G070880 |
| | | | Phvul.002G127100.1 |
| | | Phaseolus vulgaris | Phvul.002G209100.1 |
| | | | Phyul.002G209200.1 |

Table S2. Eudicot plant species used in the NJ analysis.



| 0 | F | Species | Accession number |
|--------------|---------|------------------------------|--|
| | | | Gorai.008G296600.1 |
| Malvales | ae | Gossypium raimondii | Gorai.012G142200.1 |
| | nce | | Gorai.005G220400.1 |
| | ilve | | Gorai.005G220500.1 |
| | Ma | | TC03G031300 |
| | | Theobroma cacao | TC02G011670 |
| | ı | | Lus10035670 |
| | ina | Linum usitatissimum | Lus10005372 |
| | Ι | | Lus10020523 |
| | | | PT03G09340 |
| | | Populus trichocarpa | PT12G01430 |
| es | | | PT12G01440 |
| <i>iia</i> | sae | | PT15G01960 |
| iel | ace | | SapurV1A.1470s0080.1 |
| alp | ılic | | SapurV1A.0346s0170.1 |
| Μ | Se | Salix purpurea | SapurV1A.0377s0140.1 |
| | | | SapurV1A.0377s0150.1 |
| | | | SapurV1A.3352s0030.1 |
| | | | SapurV1A.0894s0160.1 |
| | E | Manihot esculenta | ME10292G00060 |
| | | Ricinus communis | RC30063G00030 |
| nc | | | Aquca_105_00003.1 |
| nu | R | Aquilegia coerulea Golasmith | Aquca_043_00024.1 |
| Ra | | | Aquea 022 00110 1 |
| | | | Aquca_055_00110.1 |
| | | Fragaria vesca | FV5C21050 |
| | | - | MD00C028680 |
| | eae | Malus domestica | MD000028080 MD00G081720 |
| les | | manas aomestica | MD13G026910 |
| <u> 2</u> 20 | sac | | MD16G016620 |
| R | R_{O} | | Prupe 5G018700 1 |
| | | Prunus persica | Prupe.1G061800.1 |
| | | 1 | Prupe.1G061900.1 |
| | | | Prupe.1G061400.1 |
| | | Citrus clementina | Ciclev10001766m |
| les | ne | | Ciclev10003687m |
| tda | ncer | | Ciclev10028835m |
| nia | uta | | orange1.1g037339m |
| Sa | R | Citrus sinensis | orange1.1g019765m |
| | | | orange1.1g020532m |
| | | | Solyc08g005550 |
| | 0 | Solanum lycopersicum | Solyc08g075540 |
| les | sea | | Solyc08g0/5550 |
| una | nac | | Solyc01g105220 |
| olo | ola | | PGSC0003DM1400019708 |
| S | S | Solanum tuberosum | PGSC0003DM1400019707 |
| | | | PGSC0003DM1400047502 PGSC0003DMG400012558 |
| | | | ATR 00038G01180 |
| P | a | Amborella trichopoda | ATR 00048G01570 |
| | | | BV5G19180 |
| B | q | Beta vulgaris | BV9G03180 |
| _ | | | Kalax.0453s0008.1 |
| | | | Kalax.1476s0004.1 |
| Ċ | S | Kalanchoe laxiflora | Kalax.0496s0017.1 |
| | | U U | Kalax.0907s0011.1 |
| | | | Kalax.0414s0014.1 |



| d | d | Mimulus guttatus | Migut.J01127.1 Migut.E01358.1 Migut.N01067.1 |
|----------|---|--------------------|--|
| Ε | е | Eucalyptus grandis | Eucgr.E01214.1 Eucgr.E01213.1 Eucgr.I02663.1 |
| H | f | Vitis vinifera | VV02G09030 VV02G09050 VV00G00110 |

O: order, F: family; C: *Caricaceae*, Curcubi: *Curcubitales*, Curc: *Curcubitaceae*, Lina: *Linaceae*, E: *Euphorbiaceae*, Ranun: *Ranunculales*, R: *Ranunculaceae*, A: *Amborellales*, B: *Caryophyllale*, C: *Saxifragales*, D: Lamiales, E: Myrtales, F: *Vitales*, a: *Amborellaceae*, b: *Amarantaceae*, c: *Crassulaceae*, d: *Phrymaceae*, e: *Myrtaceae*, f: *Vitaceae*.

In grey the AOX2 sequences.



Table S3. Monocot plant species used in the NJ analysis.

| Species | Accession number |
|-------------------------|--|
| Brachypodium distachyon | BD3G52505 BD5G20540 BD5G20547 BD5G20557 |
| Hordeum vulgare | CAJW010038523 CAJW011587016 CAJW010099492 |
| Musa acuminata | GSMUA_Achr5G03810_001 GSMUA_Achr6G01170_001 GSMUA_Achr6G01300_001 GSMUA_Achr1G27800_001 |
| Oryza brachyantha | OB02G22630 OB02G36280 OB04G30980 OB04G30990 |
| Oryza glaberrima | ORGLA02G0249500 ORGLA04G0206000 ORGLA04G0206100 |
| Oryza sativa | BGIOSGA008063 BGIOSGA005788 BGIOSGA014421 BGIOSGA014422 |
| Sorghum bicolor | SB04G030820 SB06G027410 SB06G027420 SB06G027430 |
| Zea mays | ZM02G05480 ZM02G05490 ZM02G05500 ZM05G37570 |