**Supplemental Table S1**. The specific primer sequences for Real-time PCR analysis.

|  |  |  |  |
| --- | --- | --- | --- |
| **Spot No.** | **Forward (5'-3')** | **Reverse (5'-3')** | **Tm (℃)** |
| *18S* | GAGGTAGCTTCGGGCGCAACT | GCAGGTTAGCGAAATGCGATAC | 57 |
| Spot.15 | ACCATACCAAAGAGGAGGG | TTACCCTCTCGAGTTCGTTC | 57 |
| Spot.17 | ATGGCCGCTGGAATTATTG | CAACCACCACAACATCAGAC | 57 |
| Spot.18 | GTCCAGCTGATGTTCCTTC | CTAGTCTGCAACTCAACCATT | 57 |
| Spot.22 | GTACCACGTACAGTTGCGTC | GCTTAGCGTCGAAGACAGTG | 59 |
| Spot.32 | GAGTGCTGGTTACAGATC | GCCTTGATAGCGGAAACA | 55 |
| Spot.33 | TAAGAAGGACATCAGCGGCA | TCAATGGTCGTCTGCGTAGT | 57 |
| Spot.36 | CAGGAAAACAAAGAGGGTCT | CACATTCTTCAGACTATCGC | 56 |
| Spot.58 | CCTCAAGGAGGTGAAGTGGT | AACTCCACTTCTGTCCCAGC | 60 |
| Spot 27 | AGCGAGGCTATCTTTACAGG | CACAAGGAGTTGTCCTTCCT | 57 |

**Supplemental Table S2**. The identification of DEPs in C. gigantea by mass spectrometry (MS) analysis during seed germination.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Spot no.** | **Homologous protein** | **Species** | **Accession** | **Theor****pI/Mr** | **Exper****pI/Mr** | **Description** | **score** | **C (%)** | **PN** | **Peptide sequence** |
| **Cytoskeleton** |
| 1 | tr|A0A090ARE9|A0A090ARE9\_TAXCU | *Taxus cuspidata* | BAP59012 | 4.93/50.3 | 5.28/50.3 | alpha tubulin | 153 | 15  | 4 | R.AVFVDLEPTVIDEVR.TR.QLFHPEQLISGKEDAANNFAR.GR.SLDIERPTYTNLNR.LR.FDGALNVDVTEFQTNLVPYPR.I |
| 49 | tr|A0A0A7RG85|A0A0A7RG85\_PINTB | *Pinus tabuliformis* | AJA33569 | 4.80/21.4 | 4.82/35.1 | late embryogenesis abundant protein LEA2-1 | 28 | 5  | 2 | K.DFGSALWDMIR.GK.DFGSALWDMIR.G |
| 52 | tr|Q39483|Q39483\_CALDE | *Macleaya cordata* | OVA14900 | 6.50/57.5 | 6.34/30.1 | 11-S seed storage protein | 162 | 14  | 5 | R.EGLMQQQQSCR.TR.FSNVPEIR.YR.TVAIADASNHQNQLDKR.YR.YRPFFLAGSPATR.ER.YGGNVLAGFDANMLAEALGVR.R |
| 60 | tr|A0A0D6R1T2|A0A0D6R1T2\_ARACU | *Olea europaea var* | XP\_022894432 | 9.36/14.1 | 5.87/28.0 | signal peptidase complex-like protein DTM1 | 31 | 10 | 1 | R.FQILNSTPKYAQR.F |
| 65 | tr|O04689|O04689\_METGY | *Ginkgo biloba* | [CAA90641.1](https://www.ncbi.nlm.nih.gov/protein/CAA90641.1?report=genbank&log$=protalign&blast_rank=9&RID=NTCBRYWW014) | 8.91/57.9 | 6.12/42.4 | 11S-globulin | 228 | 14 | 7 | R.ALFAPSWLSSHAILYATR.GR.VFDGRVQEGQFLVIPQFYAVVK.RR.VQEGQFLVIPQFYAVVK.RR.AGDQGFEWITFTTSHSPIR.SR.WNREHEFLILPPR.QR.EHEFLILPPR.QR.EHEFLILPPR.Q |
| 27 | tr|Q39521|Q39521\_CRYJA | *Ginkgo biloba* | [CAA90641.1](https://www.ncbi.nlm.nih.gov/protein/CAA90641.1?report=genbank&log$=protalign&blast_rank=9&RID=NTCBRYWW014) | 6.84/59.0 | 6.43/45.0 | 11S-globulin | 173 | 10 | 4 | R.QDNDELDCAGVEFIR.ER.FSNVPEIR.YR.TVAIADASNHQNQLDKR.YR.QVVIDIQENNR.E |
| 31 | [AAA68981.1](https://www.ncbi.nlm.nih.gov/protein/AAA68981.1?report=genbank&log$=protalign&blast_rank=9&RID=NTTDC54601N) | *Pseudotsuga menziesii* | [AAA68981.1](https://www.ncbi.nlm.nih.gov/protein/AAA68981.1?report=genbank&log$=protalign&blast_rank=9&RID=NTTDC54601N) | 5.43/52.7 | 5.72/44.2 | legumin-like storage protein | 21 | 1 | 1 | QDNDELDCAGVEFIR |
| 40 | tr|Q39482|Q39482\_CALDE | *Ginkgo biloba* | [CAA90641.1](https://www.ncbi.nlm.nih.gov/protein/CAA90641.1?report=genbank&log$=protalign&blast_rank=9&RID=NTCBRYWW014) | 7.30/57.5 | 6.38/66.1 | 11S-globulin | 280 | 13 | 6 | R.FSNVPEIR.YR.TVAIADASNHQNQLDKR.YR.YRPFFLAGSPATR.EK.YGGNVLAGFDANMLAEALGVR.RR.QVVIDIQENNR.ER.QVVIDIQENNR.E |
| 57 | tr|Q39521|Q39521\_CRYJA | *Ginkgo biloba* | [CAA90641.1](https://www.ncbi.nlm.nih.gov/protein/CAA90641.1?report=genbank&log$=protalign&blast_rank=9&RID=NTCBRYWW014) | 6.84/59.0 | 6.80/30.0 | 11S-globulin | 185 | 8 | 4 | R.QDNDELDCAGVEFIR.ER.QDNDELDCAGVEFIR.ER.TVAIADASNHQNQLDKR.YR.QVVIDIQENNR.E |
| 72 | [XP\_029311928.1](https://www.ncbi.nlm.nih.gov/protein/XP_029311928.1?report=genbank&log$=protalign&blast_rank=5&RID=P71CTEV5014) | *Cottoperca gobio* | [XP\_029311928.1](https://www.ncbi.nlm.nih.gov/protein/XP_029311928.1?report=genbank&log$=protalign&blast_rank=5&RID=P71CTEV5014) | 5.40/26.4 | 5.40/19.2 | 2 inactive rhomboid protein 1-like isoform X1 | 28 | 1 | 1 | RFMGEPGNLNSLQR |
| **Energy metabolism** |
| 14 | tr|A9NUR7|A9NUR7\_PICSI | *wild Malaysian banana* | XP\_009394473 | 6.01/60.4 | 5.37/53.5 | ATP synthase subunit beta | 324 | 13 | 5 | R.EAPSFVEQSTEQEILVTGIK.VK.VVDLLAPYQR.GK.AHGGFSVFAGVGER.TR.FTQANSEVSALLGR.IR.IPSAVGYQPTLATDLGGLQER.I |
| 18 | tr|A0A0D6R7B2|A0A0D6R7B2\_ARACU | *Jatropha curcas* | XP\_012090397 | 5.83/48.3 | 6.21/45.3 | Enolase1 | 131 | 12 | 3 | R.GNPTVEVDIVLSDGFLAR.AK.VNQIGSVTESIEAVK.MR.SGETEDTFIADLAVGLSTGQIK.T |
| 30 | tr|A0A0D6R7B2|A0A0D6R7B2\_ARACU | *Jatropha curcas* | XP\_012090397 | 5.83/48.3 | 5.73/45.7 | Enolase3 | 311 | 18 | 4 | R.GNPTVEVDIVLSDGFLAR.AK.YGQDATNVGDEGGFAPNIQENKEGLELLK.IK.VNQIGSVTESIEAVK.MR.SGETEDTFIADLAVGLSTGQIK.T |
| 68 | tr|A0A0D6R7B2|A0A0D6R7B2\_ARACU | *Jatropha curcas* | XP\_012090397 | 5.83/48.3 | 6.44/45.7 | Enolase2 | 114 | 4 | 1 | R.GNPTVEVDIVLSDGFLAR.A |
| 15 | tr|A0A0C9SAE7|A0A0C9SAE7\_9SPER | *Wollemia nobilis* | [XP\_015073430.1](https://www.ncbi.nlm.nih.gov/protein/XP_015073430.1?report=genbank&log$=protalign&blast_rank=2&RID=NTA8CS1H01N) | 5.84/61.1 | 5.69/58.4 | ATP synthase subunit beta | 676 | 28 | 10 | K.VVDLLAPYQR.GK.AHGGFSVFAGVGER.TK.CALVYGQMNEPPGAR.AR.VGLTGLTVAEHFR.DR.DAEGQDVLLFIDNIFR.FR.FTQANSEVSALLGR.IR.IPSAVGYQPTLATDLGGLQER.IR.QISELGIYPAVDPLDSTSR.MR.MLSPLILGEDHYNTAR.GK.NLQDIIAILGMDELSEDDKLTVAR.A |
| **Transport** |
| 13 | tr|A0A0C9S3N2|A0A0C9S3N2\_9SPER | *Wollemia nobilis* | [XP\_012470371.1](https://www.ncbi.nlm.nih.gov/protein/XP_012470371.1?report=genbank&log$=protalign&blast_rank=1&RID=NT9SGMPE01N) | 5.09/73.9 | 5.38/66.1 | luminal-binding protein | 306 | 14 | 7 | K.IKDAVVTVPAYFNDAQR.QK.DAVVTVPAYFNDAQR.QK.DAGVIAGLNVAR.IR.IINEPTAAAIAYGLDKK.GR.VEIESLFDGVDFSEPLTR.AR.FEELNNDLFR.KK.SQVFTTYQDQQTTVSIQVFEGER.S |
| 62 | [WP\_021626641.1](https://www.ncbi.nlm.nih.gov/protein/WP_021626641.1?report=genbank&log$=protalign&blast_rank=5&RID=NTXH60F001N) | *Atopobium sp. oral taxon 810* | [WP\_021626641.1](https://www.ncbi.nlm.nih.gov/protein/WP_021626641.1?report=genbank&log$=protalign&blast_rank=5&RID=NTXH60F001N) | 5.84/27.5 | 5,86/31.4 | ABC transporter ATP-binding protein | 36 | 1 | 1 | MELISSPFSGSIKR |
| 42 | [WP\_051095206.1](https://www.ncbi.nlm.nih.gov/protein/WP_051095206.1?report=genbank&log$=protalign&blast_rank=2&RID=NTZ9FD9A015) | Cyclotella Nana | [WP\_051095206.1](https://www.ncbi.nlm.nih.gov/protein/WP_051095206.1?report=genbank&log$=protalign&blast_rank=2&RID=NTZ9FD9A015) | 5.45/64.6 | 6.24/62.1 | LysR family transcriptional regulator | 23 | 1 | 1 | KGASAGNLYLEGAR |
| 24 | tr|A0A0C9S5Q8|A0A0C9S5Q8\_9SPER | *Wollemia nobilis* | [XP\_010262211.1](https://www.ncbi.nlm.nih.gov/protein/XP_010262211.1?report=genbank&log$=protalign&blast_rank=1&RID=NTBUTAH301N) | 5.54/79.1 | 5.85/98.0 | far upstream element-binding protein 1-like | 54 | 2 | 1 | R.IQLIPLHLPEGDTSTER.T |
| 53 | tr|D5AAF9|D5AAF9\_PICSI | *Cinnamomum micranthum* f. kanehirae | [RWR94686.1](https://www.ncbi.nlm.nih.gov/protein/RWR94686.1?report=genbank&log$=protalign&blast_rank=2&RID=NTVN5561015) | 7.21/36.0 | 6.48/30.5 | double-stranded RNA-binding protein 2-like protein | 45 | 2 | 1 | R.QQQQQQQR.Q |
| **Amino-acid biosynthesis** |
| 41 | tr|A0A0D6QUB9|A0A0D6QUB9\_ARACU | *Pinus pinaster* | AQX43169 | 8.10/166.5 | 6.37/60.1 | Ferredoxin-dependent glutamate synthase | 25 | 1 | 1 | K.NSPSHGIIQDALTALGCMEHR.G |
| 10 | [MBH87899.1](https://www.ncbi.nlm.nih.gov/protein/MBH87899.1?report=genbank&log$=protalign&blast_rank=3&RID=NT8D7VP9015) | *Pelagibacterales bacterium (marine metagenome)* | [MBH87899.1](https://www.ncbi.nlm.nih.gov/protein/MBH87899.1?report=genbank&log$=protalign&blast_rank=3&RID=NT8D7VP9015) | 5.09/56.9 | 6.42/66.2 | tryptophan synthase subunit alpha | 31 | 1 | 1 | IFIKLEEENK |
| 47 | tr|A0A0C9QMQ9|A0A0C9QMQ9\_9SPER | *Wollemia nobilis* | [NP\_001267644.1](https://www.ncbi.nlm.nih.gov/protein/NP_001267644.1?report=genbank&log$=protalign&blast_rank=1&RID=NTV47W51015) | 5,69/39.6 | 6.01/41.2 | glutamine synthetase cytosolic isozyme-like | 155 | 8 | 3 | K.HKEHIAAYGEGNER.RK.EHIAAYGEGNER.RR.HETADINTFSWGVANR.G |
| **Oxidation reduction** |
| 36 | tr|A0A0D6QRJ4|A0A0D6QRJ4\_ARACU | Handroanthus impetiginosus | PIN25109 | 5.10/66.3 | 5.69/60.6 | Thioredoxin, nucleoredoxin2 | 43 | 3% | 2 | R.AFTPLLTEVYTK.LK.AYPFTDAR.L |
| 0 | tr|A0A0D6QVY9|A0A0D6QVY9\_ARACU | Araucaria cunninghamii | PIN25109.1 | 4.99/66.1 | 5.67/64.8 | Thioredoxin, nucleoredoxin3 | 89 | 5 | 3 | R.AFTPLLTEVYTK.LR.AAQTLESLLVSDER.NK.AYPFTDAR.L |
| **Molecular chaperones** |
| 58 | tr|A9NRZ6|A9NRZ6\_PICSI | *Ananas comosus* | XP\_020090760 | 8.39/27.3 | 6.21/24.8 | 20 kDa chaperonin, chloroplastic-like | 61 | 8 | 2 | K.FTTIKPLGDR.VK.TQVEPSVQLGAK.I |
| 17 | tr|A9NV22|A9NV22\_PICSI | *Amborella trichopoda* | XP\_006838174 | 5.69/65.8 | 5.51/59.5 | ruBisCO large subunit-binding protein subunit beta | 233 | 10 | 4 | K.LADLVGVTLGPK.GK.VVAAGANPIQITR.GK.LSGGVAVIQVGAQTETELKEK.KK.AAVEEGIVVGGGCALLR.L |
| 32 | tr|A0A0C9S9Q0|A0A0C9S9Q0\_9SPER | *Tarenaya hassleriana* | XP\_010542582 | 5.28/63.9 | 4.86/57.7 | ruBisCO large subunit-binding protein subunit alpha | 62 | 2 | 1 | R.GYISPQFVTNPEK.L |
| **Stress response** |
| 9 | tr|B8LM05|B8LM05\_PICSI | *Picea sitchensis* | [TKY71402.1](https://www.ncbi.nlm.nih.gov/protein/TKY71402.1?report=genbank&log$=protalign&blast_rank=7&RID=NT7H1MTK014) | 5.46/75.8 | 4.79/66.0 | Stromal 70 kDa heat shock-related protein | 319 | 8 | 5 | K.QFAAEEISAQVLR.KK.QFAAEEISAQVLR.KK.AVITVPAYFNDSQR.TK.DLDEIILVGGSTR.IK.SEVFSTAADGQTSVEINVLQGER.E |
| 26 | [XP\_006849547.1](https://www.ncbi.nlm.nih.gov/protein/XP_006849547.1?report=genbank&log$=protalign&blast_rank=1&RID=NTDJ1S2F01N) | *Amborella trichopoda* | [XP\_006849547.1](https://www.ncbi.nlm.nih.gov/protein/XP_006849547.1?report=genbank&log$=protalign&blast_rank=1&RID=NTDJ1S2F01N) | 5.56/16.2 | 5.97/42.6 | activator of 90 kDa heat shock protein ATPase homolog | 31 | 1 | 1 | IQAVEKVEGEAYVNIR |
| 37 | tr|A0A0C9S652|A0A0C9S652\_9SPER | *Wollemia nobilis* | [XP\_010273662.1](https://www.ncbi.nlm.nih.gov/protein/XP_010273662.1?report=genbank&log$=protalign&blast_rank=1&RID=NTUAEBG2014) | 5.21/71.3 | 5.73/66.2 | heat shock 70 kDa protein | 197 | 7 | 4 | R.TTPSYVAFTDTER.LK.DAVITVPAYFNDSQR.QK.EQVFSTYSDNQPGVLIQVYEGER.AK.EQVFSTYSDNQPGVLIQVYEGER.A |
| 22 | tr|A0A0C9S652|A0A0C9S652\_9SPER | *Trema orientale* | PON89345 | 5.21/71.3 | 5.52/66.2 | Heat shock protein 70 family | 91 | 2 | 1 | R.TTPSYVAFTDTER.L |
| 33 | tr|A0A0C9S652|A0A0C9S652\_9SPER | *Trema orientale* | PON89345 | 5.21/71.3 | 5.54/66.2 | Heat shock protein 70 family | 386 | 145 | 5 | R.TTPSYVAFTDTER.LK.DAVITVPAYFNDSQR.QK.ATAGDTHLGGEDFDNR.MR.TLSSTAQTTIEIDSLYEGIDFYSTITR.AK.EQVFSTYSDNQPGVLIQVYEGER.A |
| **Abscisic acid signaling pathway** |
| 16 | [AQP49145.1](https://www.ncbi.nlm.nih.gov/protein/AQP49145.1?report=genbank&log$=protalign&blast_rank=4&RID=NTAZHBCM015) | *Tessaracoccus aquimaris* | [AQP49145.1](https://www.ncbi.nlm.nih.gov/protein/AQP49145.1?report=genbank&log$=protalign&blast_rank=4&RID=NTAZHBCM015) | 5.45/44.9 | 6.29/65.9 | AraC family transcriptional regulator | 15 | 1 | 1 | EPAQVLSVGQR |

Spot no, corresponds to spots in Fig. 3.; Panel B, Homologous protein; Species and Accession, species and accession of the matched protein from the NCBI database; Theor pI/Mr, pI and molecular weight (kDa) annotated in the NCBI database; Exper pI/Mr, pI and molecular weight (kDa) found from the gel and analyzed using Image Master and Melanie software; Description, the name of proteins; Score, the score obtained from the NCBI database for each match; C (%), percentage of the conserved region of the identified peptide sequences in the matched protein; PN, number of matched peptides; Peptide sequence, sequences of the matched peptides.

**Supplemental Table S3.** Expression levels of DEPs in C. gigantean during seed germination.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Spot no.** | **Description** | **Period B** | **Period C** | **Period D** |
| **Cytoskeleton** |
| 1 | alpha tubulin | -0.33731±0.265721 | 0.145254±0.545347 | 2.08754±0.0287106 |
| 49 | late embryogenesis abundant protein LEA2-1 | - | 0.430892±0.37712 | 1.77424±0.010891 |
| 52 | 11-S seed storage protein | 0.071248±0.696649 | -1.79902±0.040105 | -0.85748±0.194726 |
| 60 | signal peptidase complex-like protein DTM1 | -0.56753±0.220258 | 1.08927±0.0945818 | -2.02327±0.0147 |
| 25 | 11S-globulin | 0.647824±0.44136 | -4.92752±0.001080 | -0.150785±0.77554 |
| 27 | 11S-globulin | 2.87568±0.0080288 | -1.15154±0.068294 | -0.150785±0.77554 |
| 31 | legumin-like storage protein | 0.867205±0.163262 | -1.73417±0.024568 | 0.334062±0.650637 |
| 40 | 11S-globulin | 0.614935±0.606139 | -14.8817±0.001356 | 0.215243±0.37271 |
| 57 | 11S-globulin | -0.107749±0.88675 | -0.885972±0.14687 | 2.34441±0.00359 |
| 72 | inactive rhomboid protein 1-like isoform X1 | 0.334983±0.303691 | 0.910469±0.257211 | -7.14315±0.014876 |
| **Energy metabolism** |
| 14 | ATP synthase subunit beta | -0.0885587±0.6456 | 2.09357±0.0475617 | 0.340623±0.469927 |
| 18 | Enolase1 | -0.337305±0.35686 | 0.226159±0.800169 | 2.51494±0.0041992 |
| 30 | Enolase3 | 1.0928±0.0976647 | 2.02433±0.0021595 | -1.0712±0.152627 |
| 68 | Enolase2 | -0.395662±0.35512 | -3.44661±0.004078 | -0.108217±0.97390 |
| 15 | ATP synthase subunit beta | -0.337305±0.35686 | 3.46312±0.0009063 | 0.996649±0.224036 |
| **Transport** |
| 13 | luminal-binding protein | - | 0.200921±0.47323 | 2.14608±0.0087995 |
| 62 | ABC transporter ATP-binding protein | -0.962437±0.16824 | -0.771385±0.29258 | -1.94149±0.035698 |
| 42 | LysR family transcriptional regulator | 1.52985±0.0595039 | -2.03956±0.008029 | 0.603973±0.455842 |
| 24 | far upstream element-binding protein 1-like | -0.492631±0.28534 | 2.15622±0.013198 | 0.354565±0.563222 |
| 53 | double-stranded RNA-binding protein 2-like protein | -0.1307±0.834389 | 0.284186±0.525305 | -1.76012±0.02408 |
| **Amino-acid biosynthesis** |
| 41 | Ferredoxin-dependent glutamate synthase | 0.198844±0.321542 | -1.61137±0.022521 | -0.860556±0.16643 |
| 10 | tryptophan synthase subunit alpha | 0.723199±0.250455 | -0.170993±0.30477 | 3.00329±0.0009061 |
| 47 | glutamine synthetase cytosolic isozyme-like | -2.03597±0.135225 | 0.501266±0.249882 | 2.45687±0.004781 |
| **Oxidation reduction** |
| 36 | Thioredoxin, nucleoredoxin2 | -0.308916±0.47835 | 0.327108±0.612409 | 1.8707±0.0154 |
| 0 | Thioredoxin, nucleoredoxin3 | -0.0831856±0.9489 | 1.06453±0.101122 | 1.68506±0.025497 |
| **Molecular chaperones** |
| 58 | 20 kDa chaperonin, chloroplastic-like | - | 4.04639±0.0311327 | -0.581248±0.31519 |
| 17 | ruBisCO large subunit-binding protein subunit beta | -0.141635±0.28042 | 2.83277±0.0049858 | -0.44845±0.764828 |
| 32 | ruBisCO large subunit-binding protein subunit alpha | 0.897925±0.556501 | 1.46534±0.0445307 | 1.97924±0.034659 |
| **Stress response** |
| 9 | Stromal 70 kDa heat shock-related protein | 1.86752±0.0225207 | 0.719232±0.215478 | 0.331451±0.436583 |
| 26 | activator of 90 kDa heat shock protein ATPase homolog | 0.647824±0.44136 | -16.2451±0.004179 | 1.40954±0.0351479 |
| 37 | heat shock 70 kDa protein | -0.158448±0.87070 | -0.578875±0.60112 | 1.5627±0.0254 |
| 22 | Heat shock protein 70 family | -0.541101±0.22963 | 1.56534±0.0445307 | 0.808282±0.228996 |
| 33 | Heat shock protein 70 family | -0.237975±0.62450 | -0.925184±0.21466 | 4.79865±0.015497 |
| **Abscisic acid signaling pathway** |
| 16 | AraC family transcriptional regulator | -0.503466±0.73783 | -0.948694±0.14511 | 2.07127±0.0010800 |

Spot number indicated in Fig. 3; Seed bud (Period B, 5 d), visible cracks in seed coat; Seed germination (9 d, Period C), completely broken seed coat; Radical elongation (Period D, 13 d), color-changing embryo occurred. Data indicated Mean of relative protein abundance ± SE (standard error).