# *Supplementary material*

## Dissecting the Genetic Architecture of Seed Protein and Oil Content in Soybean from the Yangtze and Huaihe River Valleys Using Multi-Locus Genome-Wide Association Studies

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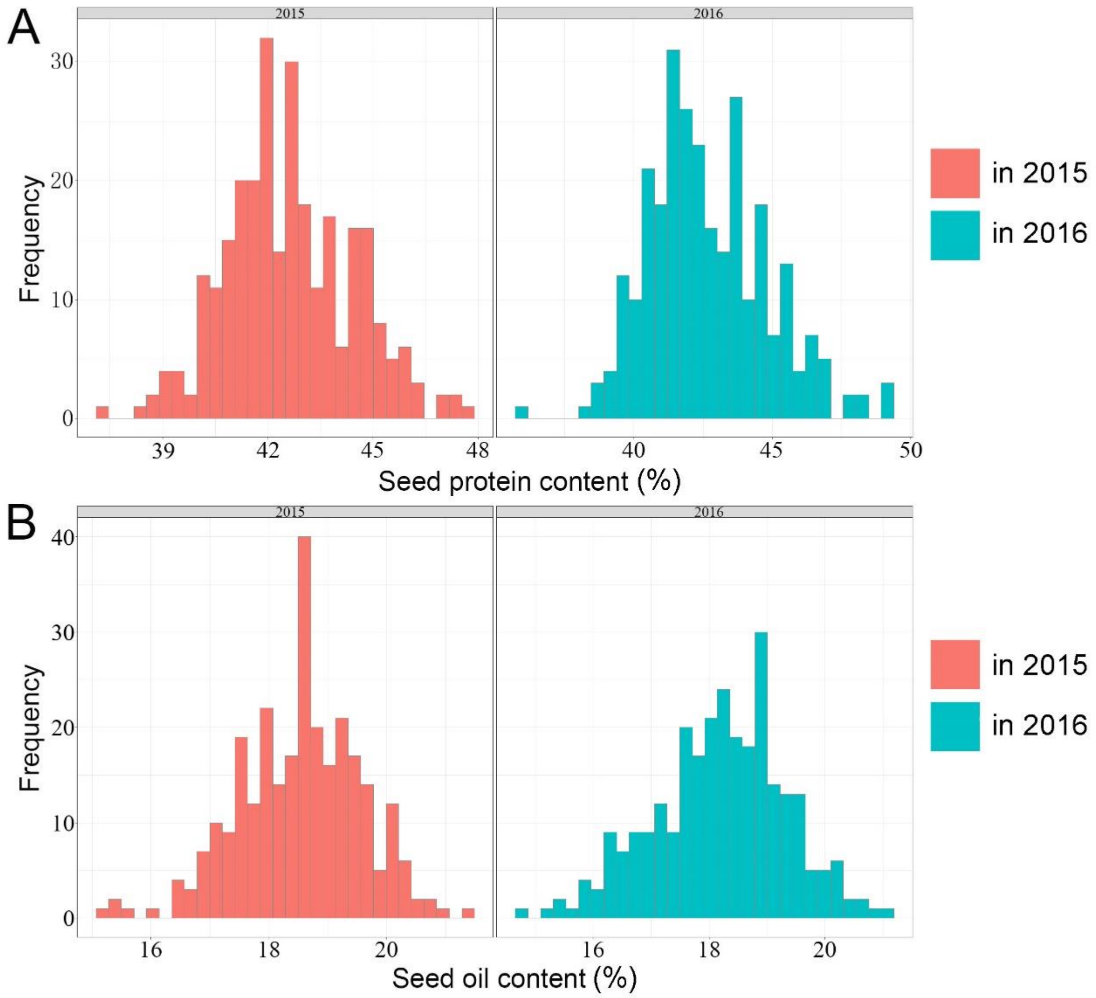
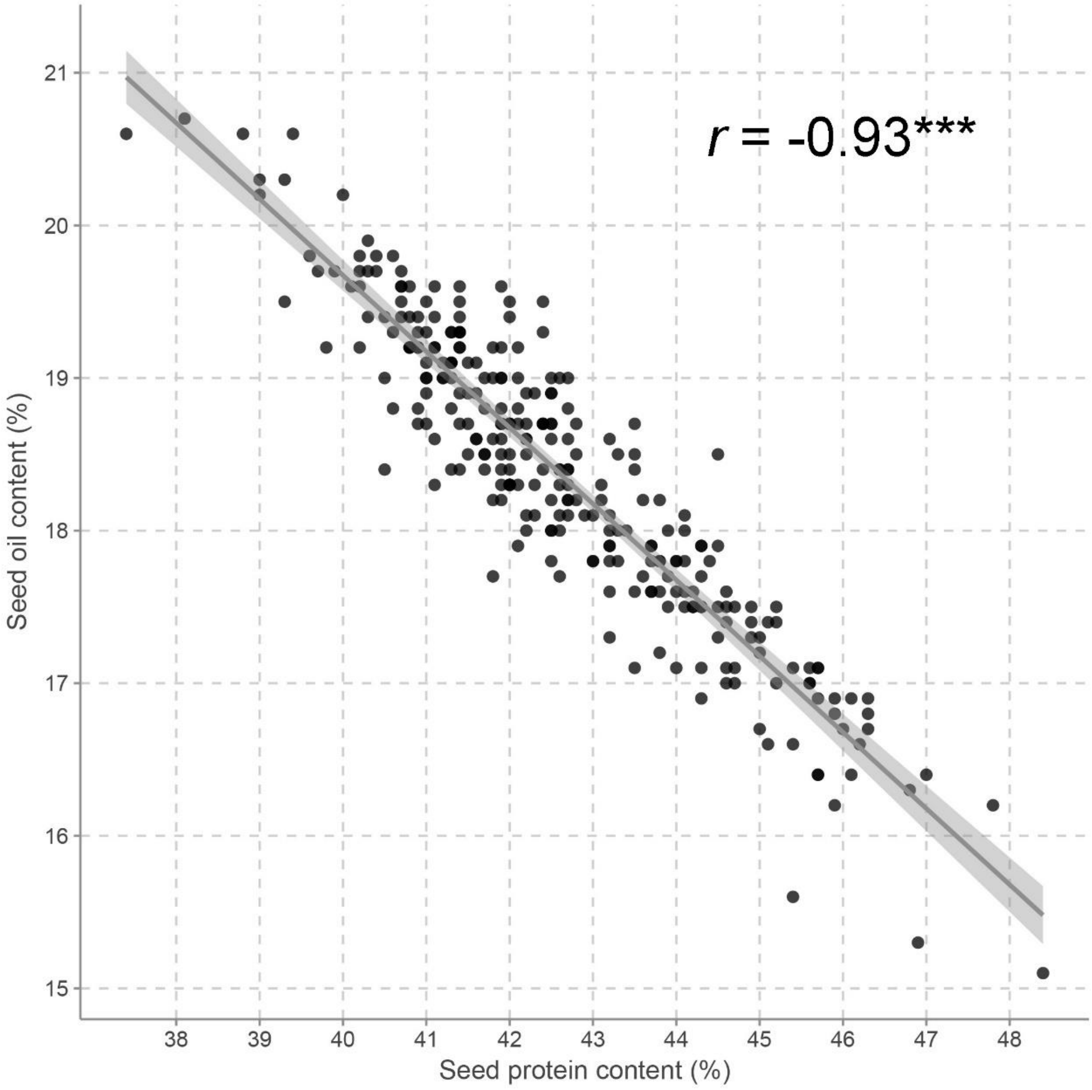
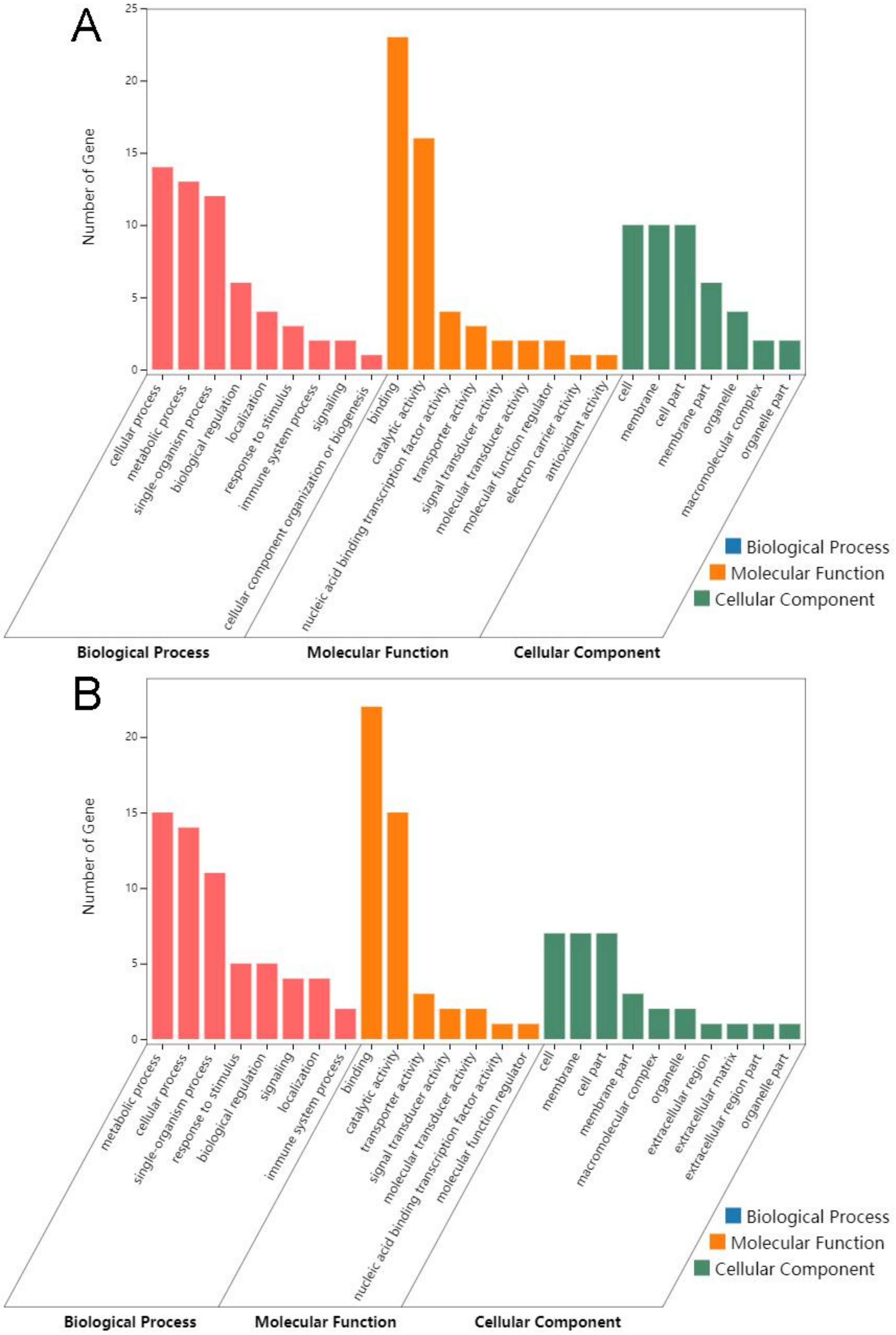


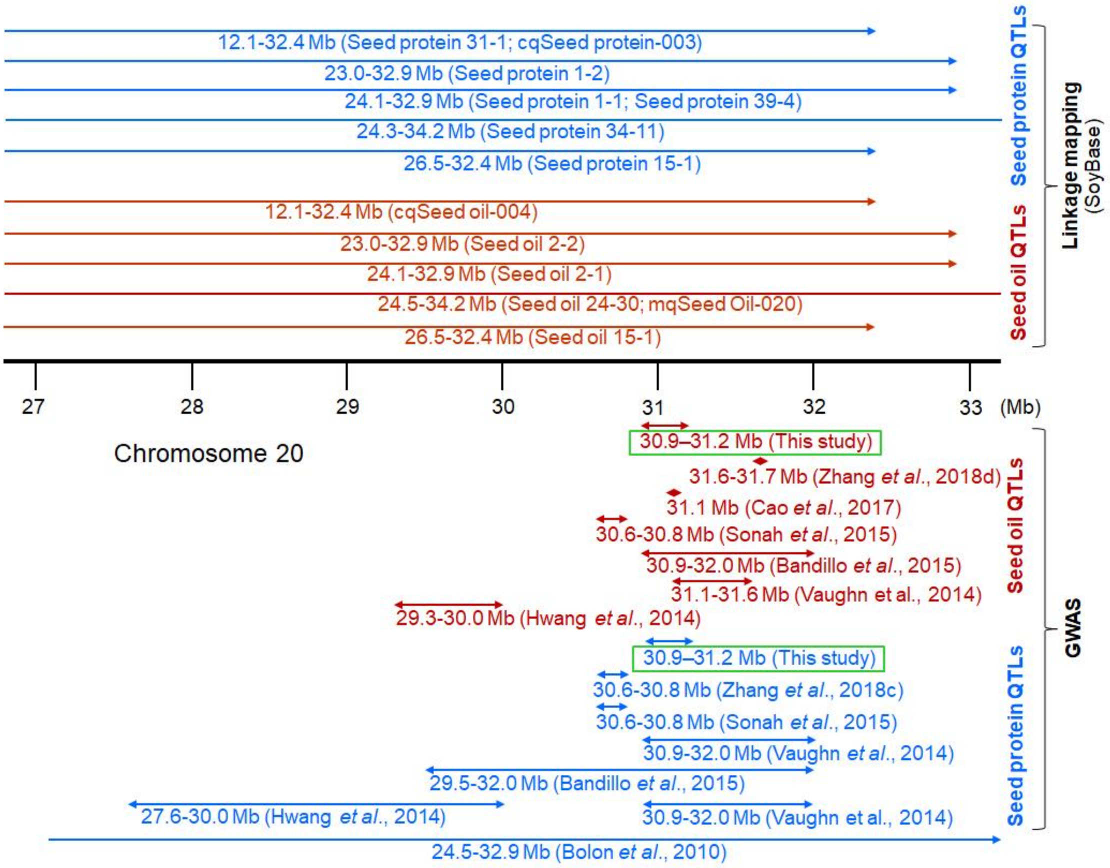
**Supplementary Figure 1** Phenotypic distribution of seed protein (A) and oil (B) content in the soybean association panel



**Supplementary Figure 2** Scatter plot for seed protein and oil content in the soybean association panel



**Supplementary Figure 3** Gene ontology classification of annotated genes for seed protein (A) and oil (B) content in the soybean association panel.



**Supplementary Figure 4** The candidate region of the major pleiotropic seed protein and oil content QTLs on Chromosome 20. Previously identified QTLs of seed protein and oil content through linkage mapping were obtained from SoyBase (http://soyba se.org) and are indicated above the horizontal axis. Previously identified QTLs of seed protein and oil content through GWAS are indicated below the horizontal axis. The seed protein and oil content QTLs are shown in blue and red, respectively. This study identified a major pleiotropic seed protein and oil content QTL in the 30.9-31.2 Mb region on chromosom 20 identified by RTM-GWAS procedure.

**Supplementary Table 1** The candidate genes annotated from the QTLs associated with the seed protein content detected in the soybean association panel

| QTL | $R^2$ (%) | Gene | Chromosome | Start | End | Functional annotation [a] |
|---|---|---|---|---|---|---|
| *qProt-2-1* | 1.0 | *Glyma02g31440* | Gm02 | 34348334 | 34355479 | NAD(P)-linked oxidoreductase superfamily protein |
| *qProt-3-1* | 0.6 | *Glyma03g40780* | Gm03 | 46431667 | 46441697 | Ankyrin repeat family protein |
| *qProt-4-1* | 2.4 | *Glyma04g02850* | Gm04 | 2059088 | 2063081 | Basic-leucine zipper (bZIP) transcription factor family protein |
| | | *Glyma04g02880* | Gm04 | 2082594 | 2084666 | Glyoxal oxidase-related protein |
| | | *Glyma04g02910* | Gm04 | 2103499 | 2103663 | – |
| | | *Glyma04g02980* | Gm04 | 2155604 | 2159914 | K-box region and MADS-box transcription factor family protein |
| | | *Glyma04g03180* | Gm04 | 2320692 | 2324583 | AAA-type ATPase family protein |
| | | *Glyma04g03230* | Gm04 | 2366242 | 2370703 | Sec14p-like phosphatidylinositol transfer family protein |
| *qProt-4-2* | 0.4 | *Glyma04g10451* | Gm04 | 8670881 | 8701308 | CRT (chloroquine-resistance transporter)-like transporter 2 |
| | | *Glyma04g10481* | Gm04 | 8701971 | 8719497 | Ubiquitin-protein ligase 1 |
| | | *Glyma04g10510* | Gm04 | 8725073 | 8727209 | Target SNARE coiled-coil domain protein |
| *qProt-6-1* | 1.1 | *Glyma06g40780* | Gm06 | 43967782 | 43972339 | Disease resistance protein (TIR-NBS-LRR class) family |
| | | *Glyma06g40820* | Gm06 | 44001679 | 44004225 | Disease resistance protein (TIR-NBS class) |
| *qProt-7-1* | 4.7 | *Glyma07g10621* | Gm07 | 8869326 | 8879181 | Protein kinase superfamily protein |
| *qProt-8-1* | 8.0 | *Glyma08g21340* | Gm08 | 16195831 | 16198280 | GDSL-like Lipase/Acylhydrolase superfamily protein |
| | | *Glyma08g21450* | Gm08 | 16289768 | 16295899 | Hexokinase 2 |
| | | *Glyma08g21530* | Gm08 | 16348536 | 16354299 | NADP-malic enzyme 4 |
| *qProt-8-2* | 1.2 | *Glyma08g42100* | Gm08 | 41999064 | 42001005 | RNI-like superfamily protein |
| | | *Glyma08g42110* | Gm08 | 42002502 | 42039978 | Glucan synthase-like 7 |
| | | *Glyma08g42170* | Gm08 | 42127192 | 42132559 | Protein kinase superfamily protein |
| | | *Glyma08g42230* | Gm08 | 42188239 | 42197436 | MEI2-like protein 5 |
| | | *Glyma08g42240* | Gm08 | 42224157 | 42231045 | MAP kinase 20 |
| *qProt-9-1* | 0.7 | *Glyma09g24910* | Gm09 | 30904477 | 30909668 | Hydroxyproline-rich glycoprotein family protein |
| | | *Glyma09g24956* | Gm09 | 30999671 | 31007110 | Divalent ion symporter |
| *qProt-10-1* | 1.0 | *Glyma10g01180* | Gm10 | 878255 | 880592 | Plant invertase/pectin methylesterase inhibitor superfamily |
| | | *Glyma10g01241* | Gm10 | 906726 | 913111 | Unknown protein |
| | | *Glyma10g01440* | Gm10 | 1070032 | 1070748 | – |
| | | *Glyma10g01450* | Gm10 | 1069805 | 1073166 | WRKY DNA-binding protein 23 |
| *qProt-10-2* | 3.6 | *Glyma10g29000* | Gm10 | 37895786 | 37900936 | Importin alpha isoform 2 |
| | | *Glyma10g29020* | Gm10 | 37907198 | 37912351 | Syntaxin of plants 43 |
| | | *Glyma10g29180* | Gm10 | 38076511 | 38080168 | Unknown function |
| | | *Glyma10g29190* | Gm10 | 38081913 | 38084017 | Dormancy/auxin associated family protein |
| | | *Glyma10g29280* | Gm10 | 38162014 | 38169053 | Respiratory burst oxidase homolog B |
| *qProt-10-3* | 1.5 | *Glyma10g29831* | Gm10 | 38598943 | 38610302 | Mitochondrial inner membrane translocase complex, subunit Tim44-related protein |
| | | *Glyma10g29851* | Gm10 | 38616053 | 38627075 | ROP binding protein kinases 2 |

| QTL | $R^2$ (%) | Gene | Chromosome | Start | End | Functional annotation [a] |
|---|---|---|---|---|---|---|
| | | *Glyma10g29861* | Gm10 | 38619549 | 38620910 | Protein of unknown function (DUF3049) |
| | | *Glyma10g29900* | Gm10 | 38652112 | 38654153 | Prefoldin chaperone subunit family protein |
| | | *Glyma10g29970* | Gm10 | 38676542 | 38684098 | Dehydroquinate dehydratase, putative / shikimate dehydrogenase, putative |
| | | *Glyma10g30040* | Gm10 | 38744503 | 38746299 | – |
| | | *Glyma10g30070* | Gm10 | 38768258 | 38776250 | Protein kinase superfamily protein |
| *qProt-15-2* | 1.3 | *Glyma15g28100* | Gm15 | 30878306 | 30937620 | Guanyl-nucleotide exchange factors; GTPase binding; GTP binding |
| *qProt-17-1* | 2.0 | *Glyma17g08880* | Gm17 | 6573195 | 6574595 | Nonsense-mediated mRNA decay NMD3 family protein |
| | | *Glyma17g08890* | Gm17 | 6576987 | 6586049 | AGAMOUS-like 8 |
| *qProt-19-1* | 0.6 | *Glyma19g29730* | Gm19 | 37457990 | 37461737 | Exostosin family protein |
| | | *Glyma19g29760* | Gm19 | 37499170 | 37501892 | F-box family protein |
| *qProt-19-2* | 0.9 | *Glyma19g31120* | Gm19 | 38858770 | 38898403 | Glutamate synthase 1 |
| *qProt-20-3* | 16.0 | *Glyma20g21693* | Gm20 | 31187632 | 31192534 | Subtilase family protein |
| | | *Glyma20g21726* | Gm20 | 31247519 | 31249743 | Aldehyde dehydrogenase 12A1 |
| *qProt-20-4* | 1.6 | *Glyma20g34880* | Gm20 | 43205506 | 43209164 | Differentiation and greening-like 1 |
| | | *Glyma20g34910* | Gm20 | 43226352 | 43228993 | Embryo sac development arrest 6 |
| | | *Glyma20g34975* | Gm20 | 43294976 | 43298657 | ROP-interactive CRIB motif-containing protein 1 |
| | | *Glyma20g35020* | Gm20 | 43315854 | 43330441 | COP1-interacting protein-related |
| | | *Glyma20g35030* | Gm20 | 43321080 | 43321497 | – |
| | | *Glyma20g35090* | Gm20 | 43366905 | 43377339 | Calcium-binding EF-hand family protein |
| | | *Glyma20g35170* | Gm20 | 43452291 | 43452785 | Unknown protein |
| **Total (18)** | **48.7** | **55** | | | | |

[a] Candidate gene annotation was obtained from SoyBase (http://www.soybase.org)

Note: Physical positions and genes are based on soybean reference genome Glyma. Wm82.a1.v1.1

**Supplementary Table 2** The candidate genes annotated from the QTLs associated with the seed oil content detected in the soybean association panel

| QTL | $R^2$ (%) | Gene | Chromosome | Start | End | Functional annotation [a] |
|---|---|---|---|---|---|---|
| *qOil-1-1* | 1.9 | Glyma01g30670 | Gm01 | 41495109 | 41496829 | PEBP (phosphatidylethanolamine-binding protein) family protein |
| | | Glyma01g30910 | Gm01 | 41681660 | 41690953 | BCL-2-associated athanogene 4 |
| | | Glyma01g30982 | Gm01 | 41712880 | 41714252 | – |
| *qOil-3-1* | 1.2 | Glyma03g04340 | Gm03 | 4465554 | 4469715 | Protein kinase superfamily protein |
| | | Glyma03g04420 | Gm03 | 4551604 | 4558159 | MATE efflux family protein |
| | | Glyma03g04470 | Gm03 | 4623994 | 4625733 | Ovate family protein 1 |
| *qOil-3-2* | 1.7 | Glyma03g09870 | Gm03 | 11876472 | 11888309 | protein kinase 1B |
| | | Glyma03g10002 | Gm03 | 11918753 | 11926518 | Peroxisomal membrane 22 kDa (Mpv17/PMP22) family protein |
| *qOil-4-2* | 0.6 | Glyma04g34745 | Gm04 | 40970963 | 40973184 | FAR1-related sequence 5 |
| | | Glyma04g34790 | Gm04 | 41008255 | 41014132 | ARM repeat superfamily protein |
| *qOil-4-3* | 0.4 | Glyma04g42167 | Gm04 | 47951206 | 47959456 | ACT domain repeat 3 |
| | | Glyma04g42174 | Gm04 | 47969886 | 47975364 | Protein kinase protein with adenine nucleotide alpha hydrolases-like domain |
| | | Glyma04g42240 | Gm04 | 48012311 | 48014973 | – |
| | | Glyma04g42246 | Gm04 | 48019840 | 48027339 | Target of Myb protein 1 |
| *qOil-6-1* | 0.6 | Glyma06g40780 | Gm06 | 43967782 | 43972339 | Disease resistance protein (TIR-NBS-LRR class) family |
| | | Glyma06g40820 | Gm06 | 44001679 | 44004225 | Disease resistance protein (TIR-NBS class) |
| *qOil-8-2* | 5.2 | Glyma08g21340 | Gm08 | 16195831 | 16198280 | GDSL-like Lipase/Acylhydrolase superfamily protein |
| | | Glyma08g21450 | Gm08 | 16289768 | 16295899 | Hexokinase 2 |
| | | Glyma08g21530 | Gm08 | 16348536 | 16354299 | NADP-malic enzyme 4 |
| *qOil-8-3* | 1.6 | Glyma08g23494 | Gm08 | 17922006 | 17924880 | Unknown protein |
| | | Glyma08g23640 | Gm08 | 18014906 | 18017420 | Unknown protein |
| | | Glyma08g23660 | Gm08 | 18031482 | 18035658 | Galactose oxidase/kelch repeat superfamily protein |
| | | Glyma08g23760 | Gm08 | 18103043 | 18123371 | Autoinhibited Ca(2+)-ATPase 9 |
| *qOil-10-1* | 1.0 | Glyma10g06660 | Gm10 | 5413833 | 5414572 | – |
| | | Glyma10g06670 | Gm10 | 5414057 | 5421473 | Protein phosphatase 2A regulatory B subunit family protein |
| | | Glyma10g06800 | Gm10 | 5502093 | 5516322 | Phosphatidyl inositol monophosphate 5 kinase |
| | | Glyma10g06820 | Gm10 | 5553816 | 5569768 | Glutamate-ammonia ligases;catalytics;glutamate-ammonia ligases |
| *qOil-10-3* | 2.6 | Glyma10g29831 | Gm10 | 38598943 | 38610302 | Mitochondrial inner membrane translocase complex, subunit Tim44-related protein |
| | | Glyma10g29851 | Gm10 | 38616053 | 38627075 | ROP binding protein kinases 2 |
| | | Glyma10g29861 | Gm10 | 38619549 | 38620910 | Protein of unknown function (DUF3049) |
| | | Glyma10g29900 | Gm10 | 38652112 | 38654153 | Prefoldin chaperone subunit family protein |
| | | Glyma10g29970 | Gm10 | 38676542 | 38684098 | Dehydroquinate dehydratase, putative / shikimate dehydrogenase, putative |
| | | Glyma10g30040 | Gm10 | 38744503 | 38746299 | – |

| QTL | $R^2$ (%) | Gene | Chromosome | Start | End | Functional annotation [a] |
|---|---|---|---|---|---|---|
| | | Glyma10g30070 | Gm10 | 38768258 | 38776250 | Protein kinase superfamily protein |
| *qOil-10-4* | 4.6 | Glyma10g38820 | Gm10 | 46597891 | 46602238 | Unknown protein |
| | | Glyma10g38890 | Gm10 | 46638029 | 46641386 | NAD(P)-linked oxidoreductase superfamily protein |
| | | Glyma10g38930 | Gm10 | 46662019 | 46664981 | Heat shock transcription factor A6B |
| | | Glyma10g38970 | Gm10 | 46684505 | 46690367 | TIFY domain/Divergent CCT motif family protein |
| *qOil-13-1* | 1.3 | Glyma13g27480 | Gm13 | 30660060 | 30670615 | Starch synthase 3 |
| | | Glyma13g27570 | Gm13 | 30770943 | 30775413 | RNA-binding (RRM/RBD/RNP motifs) family protein |
| | | Glyma13g27610 | Gm13 | 30790326 | 30791295 | Pentatricopeptide repeat 336 |
| *qOil-15-1* | 0.6 | Glyma15g14700 | Gm15 | 11189089 | 11197781 | Chaperone DnaJ-domain superfamily protein |
| *qOil-16-1* | 2.7 | Glyma16g27980 | Gm16 | 31945624 | 31952671 | WD-40 repeat family protein / notchless protein, putative |
| | | Glyma16g28080 | Gm16 | 31997661 | 32006814 | Cellulose synthase 6 |
| *qOil-17-1* | 1.8 | Glyma17g07341 | Gm17 | 5355165 | 5357969 | UDP-glucosyl transferase 78D2 |
| *qOil-17-2* | 1.1 | Glyma17g11950 | Gm17 | 8995174 | 9001876 | Tetratricopeptide repeat (TPR)-like superfamily protein |
| | | Glyma17g11961 | Gm17 | 9006506 | 9013109 | – |
| | | Glyma17g12040 | Gm17 | 9078459 | 9079741 | Calcium-binding EF-hand family protein |
| | | Glyma17g12120 | Gm17 | 9160275 | 9165307 | Zinc finger (ubiquitin-hydrolase) domain-containing protein |
| *qOil-20-1* | 15.1 | Glyma20g21693 | Gm20 | 31187632 | 31192534 | Subtilase family protein |
| | | Glyma20g21726 | Gm20 | 31247519 | 31249743 | Aldehyde dehydrogenase 12A1 |
| **Total (17)** | **44.2** | **51** | | | | |

[a] Candidate gene annotation was obtained from SoyBase (http://www.soybase.org)

Note: Physical positions and genes are based on soybean reference genome Glyma. Wm82.a1.v1.1

**Supplementary Table 3** The detected SNPs associated with seed protein content using mrMLM procedure in the soybean association panel

| SNP | Chromosome | Position | Effect | -log$_{10}$ *P* | R$^2$ (%) | R$^2$ (%) under RTM-GWAS procedure |
|---|---|---|---|---|---|---|
| Gm06_40739891 | 6 | 40739891 | -0.34 | 7.2 | 5.0 | - |
| Gm07_15027859 | 7 | 15027859 | -0.45 | 5.4 | 8.0 | - |
| Gm07_30989289 | 7 | 30989289 | -0.44 | 3.9 | 2.4 | - |
| Gm14_16005146 | 14 | 16005146 | 0.45 | 3.8 | 2.7 | - |
| Gm14_32054255 | 14 | 32054255 | 0.43 | 5.6 | 5.6 | - |
| **Total (5)** | | | | | **23.6** | |

**Supplementary Table 4** The detected SNPs associated with seed oil content using mrMLM procedure in the soybean association panel

| SNP | Chromosome | Position | Effect | -log$_{10}$ $P$ | $R^2$ (%) | $R^2$ (%) under RTM-GWAS procedure |
|---|---|---|---|---|---|---|
| Gm05_41503945 | 5 | 41503945 | 0.31 | 4.6 | 5.1 | - |
| Gm06_39998760 | 6 | 39998760 | -0.37 | 7.5 | 9.9 | - |
| Gm06_40810272 | 6 | 40810272 | 0.15 | 5.6 | 3.7 | - |
| **Gm06_43202237** | 6 | 43202237 | -0.22 | 4.7 | 2.0 | 0.6 |
| Gm08_2468904 | 8 | 2468904 | -0.15 | 5.0 | 3.7 | - |
| Gm11_38464734 | 11 | 38464734 | -0.21 | 5.0 | 3.8 | - |
| Gm14_28259042 | 14 | 28259042 | 0.24 | 5.2 | 5.8 | - |
| Gm15_16119053 | 15 | 16119053 | 0.16 | 4.3 | 4.1 | - |
| Gm20_8373052 | 20 | 8373052 | 0.27 | 4.4 | 2.9 | - |
| **Gm20_31299487** | 20 | 31299487 | 0.41 | 9.2 | 7.3 | 15.1 |
| Total (10) | | | | | 48.4 | |

[a] SNPs in bold font type are also detected by RTM-GWAS procedure