

Table S1 Information about tested rice varieties used in the experiment.

| Variety | abbreviation | Female parent | Male parent | Type | Year | Area (10^4 mu) |
|-------------------------|--------------|---------------|-------------|------------|------|-------------------|
| Longliangyouhuazhan | LLYHZ | Longke638S | Huazhan | Super rice | 2015 | >1000 |
| Longliangyouhuanglizhan | LLYHLZ | Longke638S | Huanglizhan | — | 2016 | >100 |
| Longliangyou8612 | LLY8612 | Longke638S | Huahui8612 | — | 2019 | <100 |
| Longliangyou534 | LLY534 | Longke638S | R534 | — | 2016 | >400 |
| Longliangyou3189 | LLY3189 | Longke638S | R3189 | — | 2017 | <100 |
| Longliangyou1988 | LLY1988 | Longke638S | R1988 | Super rice | 2016 | >200 |
| Longliangyou149 | LLY149 | Longke638S | R149 | — | 2016 | <100 |
| Longliangyou1377 | LLY1377 | Longke638S | R1377 | Super rice | 2017 | >200 |
| Longliangyou1212 | LLY1212 | Longke638S | R1212 | Super rice | 2016 | >100 |
| Longliangyou1206 | LLY1206 | Longke638S | R1206 | — | 2016 | >100 |
| Longliangyou1125 | LLY1125 | Longke638S | R1125 | — | 2017 | <100 |
| Jingliangyouhuazhan | JLYHZ | Jing4155S | Huazhan | Super rice | 2015 | >1000 |
| Jingliangyouhuanglizhan | JLYHLZ | Jing4155S | Huanglizhan | — | 2018 | <100 |
| Jingliangyou8612 | JLY8612 | Jing4155S | Huahui8612 | — | 2018 | <100 |
| Jingliangyou3189 | JLY3189 | Jing4155S | R3189 | — | 2017 | <100 |
| Jingliangyou1988 | JLY1988 | Jing4155S | R1988 | — | 2018 | <100 |
| Jingliangyou1377 | JLY1377 | Jing4155S | R1377 | — | 2016 | >200 |
| Jingliangyou1212 | JLY1212 | Jing4155S | R1212 | Super rice | 2016 | >200 |
| Jingliangyou1206 | JLY1206 | Jing4155S | R1206 | — | 2017 | <100 |
| Jingliangyou1199 | JLY1199 | Jing4155S | R149 | — | 2018 | <100 |
| Jingliangyou1125 | JLY1125 | Jing4155S | R1125 | — | 2017 | <100 |
| Fengliangyou4 | FLY4 | Feng39s | Yandao4 | — | 2009 | — |

Data was from China Rice Data Center (<https://www.ricedata.cn/>). Super rice was designated by Ministry of Agriculture and Rural Affairs. FLY4 was used as the control variety since it was the control variety in variety certification. One hectare equals 15 Chinese Mu.

Table S2 Yield components of tested hybrid rice varieties in 2019 and 2020.

| Variety | Panicles | Spikelets per | Spikelets | Grain filling | Grain weight | Panicles | Spikelets per | Spikelets | Grain filling | Grain weight |
|------------|-----------------|---------------|-----------------|----------------|--------------|-----------------|---------------|-----------------|----------------|--------------|
| | m ⁻² | panicle | m ⁻² | percentage (%) | (mg) | m ⁻² | panicle | m ⁻² | percentage (%) | (mg) |
| 2019 | | | | | | | | | | 2020 |
| LLYHZ | 227.1 | 230.6 | 52321.1 | 76.6 | 20.4 | 222 | 240 | 53173 | 75.9 | 19.8 |
| LLY534 | 225.7 | 214.9 | 48562.2 | 80.3 | 19.9 | 210 | 233 | 49064 | 85.5 | 19.8 |
| LLY1206 | 209.7 | 220.3 | 46220.5 | 84.0 | 21.6 | 221 | 214 | 47151 | 83.6 | 21.1 |
| LLY8612 | 184.0 | 240.3 | 44026.3 | 80.7 | 22.1 | 191 | 263 | 50156 | 78.8 | 22.2 |
| LLY149 | 177.8 | 202.7 | 36019.1 | 82.2 | 25.3 | 201 | 220 | 44174 | 83.2 | 24.4 |
| LLY1377 | 208.6 | 230.1 | 47983.0 | 78.3 | 20.4 | 234 | 233 | 54466 | 71.9 | 20.3 |
| JLYHZ | 244.4 | 208.4 | 50854.9 | 82.5 | 18.3 | 231 | 211 | 48693 | 85.0 | 18.9 |
| JLY1206 | 248.6 | 179.9 | 44693.9 | 81.9 | 20.2 | 229 | 213 | 48526 | 84.0 | 19.6 |
| JLY1988 | 210.4 | 203.2 | 42758.5 | 87.8 | 20.7 | 222 | 216 | 47800 | 80.6 | 20.5 |
| LLY1988 | 217.4 | 202.4 | 43875.3 | 76.4 | 22.3 | 199 | 204 | 40677 | 77.5 | 22.8 |
| LLYHLZ | 225.0 | 226.9 | 51129.6 | 79.7 | 20.8 | 219 | 207 | 45224 | 75.7 | 20.9 |
| LLY3189 | 206.4 | 188.5 | 38976.0 | 80.5 | 25.2 | 187 | 205 | 38259 | 78.9 | 25.4 |
| JLYHLZ | 204.9 | 215.3 | 44020.9 | 82.9 | 19.4 | 238 | 249 | 59256 | 78.7 | 18.4 |
| JLY8612 | 204.2 | 218.4 | 44522.2 | 77.8 | 19.6 | 203 | 243 | 49351 | 72.7 | 20.2 |
| JLY1377 | 220.1 | 220.7 | 48543.1 | 81.2 | 19.4 | 230 | 235 | 53909 | 76.8 | 19.2 |
| JLY3189 | 204.9 | 190.6 | 39026.1 | 85.4 | 23.2 | 222 | 183 | 40488 | 82.3 | 24.0 |
| LLY1125 | 208.3 | 215.3 | 44804.0 | 79.6 | 18.8 | 211 | 267 | 56242 | 81.9 | 18.3 |
| JLY1212 | 206.3 | 204.6 | 42076.3 | 82.1 | 19.6 | 217 | 219 | 47380 | 86.3 | 19.6 |
| JLY1125 | 210.4 | 233.4 | 48996.2 | 81.7 | 17.8 | 223 | 242 | 53842 | 79.8 | 17.5 |
| JLY1199 | 220.8 | 168.8 | 37293.2 | 77.4 | 22.8 | 217 | 202 | 43749 | 78.6 | 22.6 |
| LLY1212 | 221.5 | 207.8 | 45864.3 | 77.0 | 20.7 | 222 | 198 | 43688 | 81.2 | 20.9 |
| FLY4 | 194.6 | 186.3 | 36232.4 | 79.5 | 24.9 | 201 | 202 | 40573 | 72.4 | 24.6 |
| LSD (0.05) | 21.8 | 24.5 | 6042.4 | 4.4 | 1.0 | 24 | 27 | 5412 | 7.4 | 0.5 |

Critical values based on LSD test (0.05) were used for comparison in each parameter among different varieties.

Table S3 Dry matter accumulation and harvest index of tested hybrid rice varieties in 2019 and 2020.

| Variety | TDW_PM | TDW_HD | TDW_GF | HI | TDW_PM | TDW_HD | TDW_GF | HI |
|------------|--------|--------|--------|------|--------|---------|--------|------|
| | 2019 | | | | 2020 | | | |
| LLYHZ | 1662.5 | 946.5 | 716.1 | 49.0 | 1607.5 | 1226.65 | 380.84 | 49.5 |
| LLY534 | 1633.0 | 1133.6 | 499.3 | 47.6 | 1612.7 | 1215.97 | 396.71 | 51.5 |
| LLY1206 | 1692.8 | 1021.0 | 671.8 | 49.6 | 1629.9 | 1164.94 | 464.97 | 50.9 |
| LLY8612 | 1648.7 | 975.8 | 672.8 | 47.7 | 1700.7 | 1210.40 | 490.31 | 51.5 |
| LLY149 | 1554.7 | 1065.4 | 489.2 | 48.1 | 1730.8 | 1248.42 | 482.42 | 51.8 |
| LLY1377 | 1617.5 | 1158.9 | 458.6 | 47.4 | 1807.0 | 1299.94 | 507.07 | 44.0 |
| JLYHZ | 1587.6 | 1051.4 | 536.2 | 48.6 | 1572.7 | 1162.86 | 409.86 | 49.6 |
| JLY1206 | 1585.2 | 978.5 | 606.7 | 46.7 | 1516.9 | 1157.81 | 359.11 | 52.5 |
| JLY1988 | 1617.6 | 957.9 | 659.7 | 48.2 | 1647.2 | 1127.92 | 519.31 | 47.9 |
| LLY1988 | 1661.7 | 1069.3 | 592.4 | 44.9 | 1545.6 | 1251.29 | 294.27 | 46.4 |
| LLYHLZ | 1843.0 | 1129.1 | 713.9 | 46.0 | 1654.0 | 1282.67 | 371.37 | 43.3 |
| LLY3189 | 1692.6 | 1020.6 | 672.1 | 46.7 | 1555.9 | 1252.06 | 303.85 | 49.4 |
| JLYHLZ | 1461.4 | 951.3 | 510.1 | 48.4 | 1671.5 | 1119.30 | 552.20 | 51.2 |
| JLY8612 | 1522.1 | 912.1 | 610.0 | 44.5 | 1521.1 | 1125.91 | 395.20 | 47.6 |
| JLY1377 | 1592.5 | 953.8 | 638.7 | 48.0 | 1614.1 | 1140.71 | 473.35 | 49.2 |
| JLY3189 | 1601.5 | 1070.2 | 531.3 | 47.9 | 1572.8 | 1229.01 | 343.77 | 50.8 |
| LLY1125 | 1402.5 | 935.0 | 467.5 | 47.8 | 1645.0 | 1152.94 | 492.05 | 51.3 |
| JLY1212 | 1434.5 | 1008.9 | 425.6 | 47.4 | 1537.8 | 1200.76 | 337.07 | 52.0 |
| JLY1125 | 1548.6 | 963.8 | 584.8 | 45.9 | 1538.0 | 1157.72 | 380.33 | 48.8 |
| JLY1199 | 1605.4 | 996.0 | 609.3 | 41.0 | 1609.2 | 1172.98 | 436.20 | 48.4 |
| LLY1212 | 1686.5 | 1104.6 | 581.9 | 43.3 | 1648.2 | 1230.58 | 417.61 | 45.0 |
| FLY4 | 1476.2 | 931.1 | 545.1 | 48.6 | 1558.0 | 1305.22 | 252.76 | 46.2 |
| LSD (0.05) | 202.9 | 88.2 | 201.8 | 3.7 | 147.23 | 97.43 | 152.47 | 3.06 |

Critical values based on LSD test (0.05) were used for comparison in each parameter among different varieties. TDW-Total dry weight, PM-physiological maturity, HD-heading stage, GF-grain filling period, HI-harvest index.

Table S4 Number of differentially expressed genes between LLYHZ, JLYHZ, and JLY1212 in spikelets at flowering stage in 2020

| Comparison | up | down |
|------------------|------|------|
| LLYHZ vs JLYHZ | 2124 | 1768 |
| LLYHZ vs JLY1212 | 1900 | 1775 |
| JLYHZ vs JLY1212 | 779 | 1044 |

Table S5 The overlapping genes differentially expressed between LLYHZ and JLYHZ and between LLYHZ and JLY1212 in panicle at flowering stage

| Location | Group | Gene name | Gene function |
|-------------------------|-------|-------------------------------|---|
| Growth and yield | | | |
| LOC_Os07g45570 | down | OsBLE2 | Aldolase; Root length. |
| LOC_Os03g48740 | down | OsIAAGLU OsIAGT1 | Dwarfism; Tillering; Auxin catabolism |
| LOC_Os02g49370 | down | OsHAP3E | Panicle flower; Dwarfism; Leaf angle; Floral organ formation |
| LOC_Os01g25484 | up | PSR1 OsNiR | Shoot regeneration; Nitrate assimilation |
| LOC_Os05g13900 | up | OsPRP RePRP1.1 | Root growth; Root cell elongation; ABA sensitivity |
| LOC_Os07g23660 | up | RePRP2.1 | Root; Root growth; Root cell elongation; ABA sensitivity |
| LOC_Os01g54270 | up | D10 OsCCD8 OsCCD8b | Root; Strigolactone biosynthesis; Crown root length |
| LOC_Os07g23640 | up | RePRP2.2 | Root; Root growth; Root cell elongation; ABA sensitivity |
| LOC_Os01g45550 | up | OsPIN3t | Culm leaf; Tiller angle; Crown root development; Root gravitropism; Drought tolerance |
| LOC_Os02g17780 | up | OsCPS OsCPS1 | Dwarfism; Gibberellin biosynthesis |
| LOC_Os09g37330 | up | SAUR39 | Dwarfism; Leaf angle; Root growth; Regulation of auxin level and transport |
| LOC_Os03g49880 | up | OsTB1 FC1 SCM3 | Culm leaf; Tiller growth |
| LOC_Os06g12210 | up | BU1 OsbHLH174 | Seed; Leaf angle; Grain size; Brassinosteroid sensitivity |
| LOC_Os07g38130 | up | OsFOR1 PGIP | Panicle flower; Regulation of floral organ number |
| LOC_Os05g39990 | up | NA | Flowering; Dwarfism; Cell wall extensibility; Flowering time |
| LOC_Os05g51360 | up | OsEBS | Panicle flower; Plant height; Leaf size; Grain number |
| LOC_Os05g50890 | up | OsJar1 OsGH3.5 OsGH3-5 | Sterility; Seed development |
| LOC_Os04g45330 | up | OsYABBY5 OsYAB3 TOB1 | Panicle flower; Spikelets morphology |
| LOC_Os02g44630 | up | OsPIP1;1 RWC1 | Germination dormancy; Seed germination |
| Grain quality | | | |
| LOC_Os06g04200 | down | Wx | Eating quality; Seed amylose content. |
| LOC_Os01g44220 | down | OsAPL2 osagpl2-3 OsAGPL2 GIF2 | Eating quality; Starch biosynthesis |
| LOC_Os05g33570 | down | FLO4 OsPPDKB ppdk OsC4PPDK | Eating quality; Seed protein and lipid content |
| LOC_Os08g09230 | down | OsSSIIIa Flo5 | Eating quality; Seed starch content |
| LOC_Os08g40930 | down | OsISA1 OsPHS8 | Eating quality; Seed starch content |

| | | | |
|-------------------------|------|--------------------------|---|
| LOC_Os06g12450 | down | ALK SSIIa | Eating quality; Gelatinization temperature; Gel consistency |
| LOC_Os03g55090 | down | Pho1 | Seed; Seed starch content; Grain maturation |
| LOC_Os03g61120 | down | OASA1 | Sterility; Trp level in grain; Fertility; Germination ability |
| LOC_Os05g03040 | up | RSR1 | Seed; Seed amylose content; Grain size |
| Stress tolerance | | | |
| LOC_Os06g16270 | down | OsHSBP2 | Heat shock tolerance |
| LOC_Os01g70310 | down | OsbHLH001 OsICE2 | Salinity tolerance; Salinity tolerance; Regulation of Na and K homeostasis |
| LOC_Os02g33770 | down | Osgtgamma-1; | Salinity tolerance; Salinity tolerance |
| LOC_Os01g64000 | down | NA | Sterility; Salinity tolerance. Fertility. |
| LOC_Os11g35500 | down | Xa21 | Bacterial blight resistance; Bacterial blight resistance |
| LOC_Os02g51110 | up | Lsi1 OsNIP2;1 OsLsi1 | Other soil stress tolerance; Si uptake; Pests resistance |
| LOC_Os06g36560 | up | OsMIOX | Drought tolerance |
| LOC_Os03g03370 | up | DSM2 | Other stress resistance; Drought and oxidative stress tolerance |
| LOC_Os09g25850 | up | OsGL1-1 WSL2 | Drought tolerance; Synthesis of leaf cuticular wax; Drought tolerance; Cuticle permeability |
| LOC_Os10g11980 | up | OsAT1 | Blast resistance; Spotted leaf. Resistance to Magnaporthe grisea and Xanthomonas oryzae pv oryzae |
| LOC_Os03g20550 | up | OsWRKY55 WRKY55a WRKY55b | Resistance to Magnaporthe grisea; Lateral root growth |
| LOC_Os05g31140 | up | OsEGL1 | Blast resistance; Resistance to Magnaporthe grisea; Lesion mimic |
| LOC_Os12g03040 | up | ONAC131 | Blast resistance; Resistance to Magnaporthe grisea |
| LOC_Os11g03300 | up | ONAC122 OsNAC10 | Blast resistance; Resistance to Magnaporthe grisea |
| LOC_Os08g35760 | up | OsGLP1 | Dwarf; Blast resistance; Semi-dwarf |

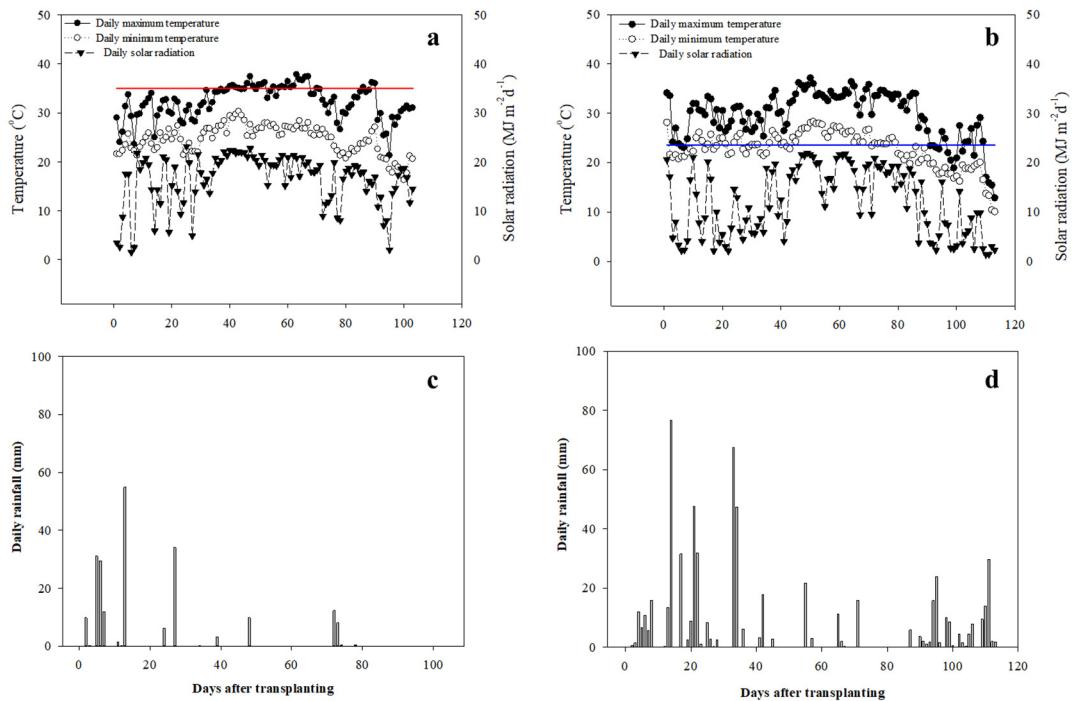


Figure S1 Climate conditions during rice growing period in 2019 and 2020

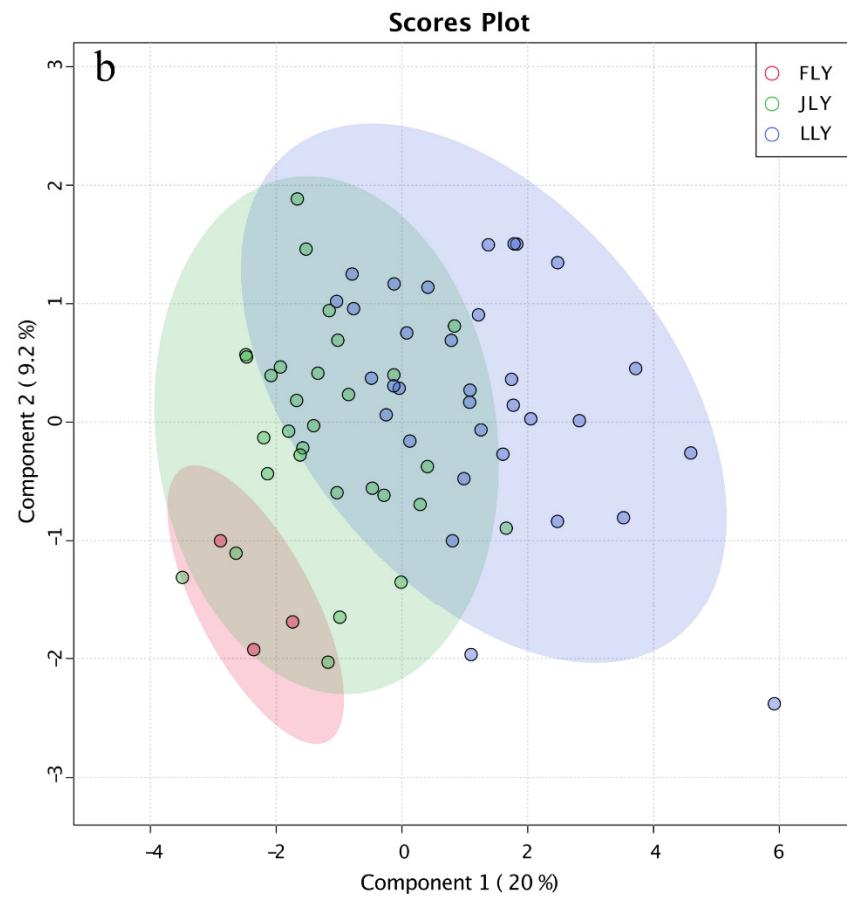
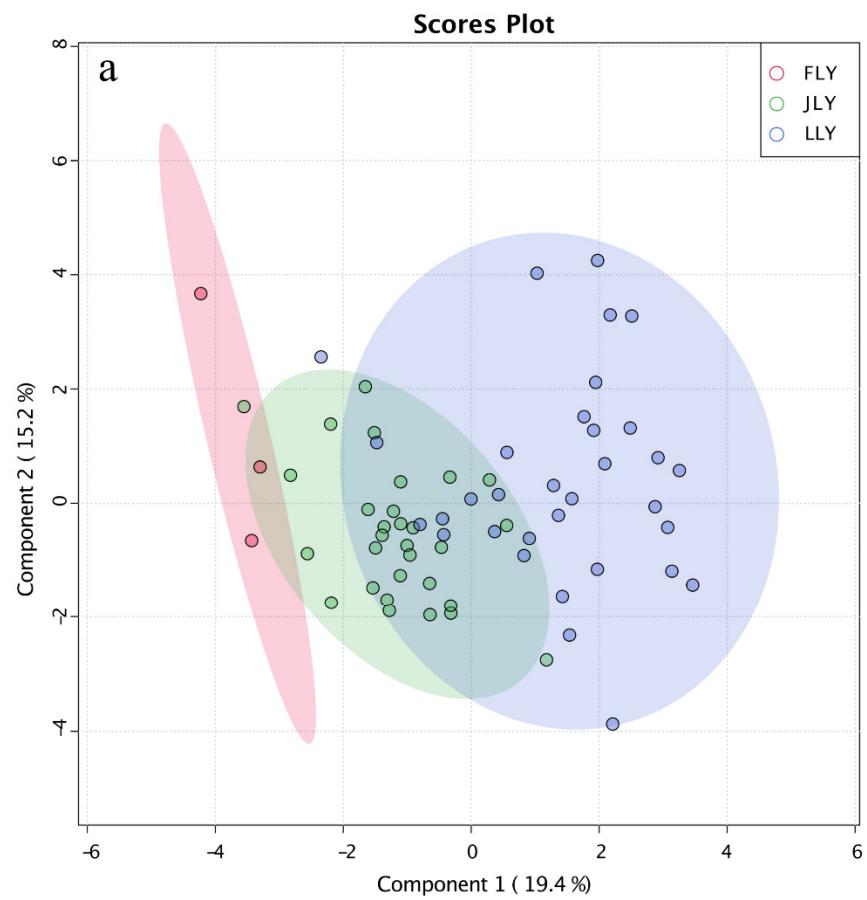


Figure S2 Scores plots of hybrid varieties with Longke638S as the female parent (LLY), those with Jingke4155S as the female parent (JLY) and the control variety (Fengliangyou4, FLY) based on traits related with grain yield, quality and nitrogen use efficiency in 2019 (a) and 2020 (b).

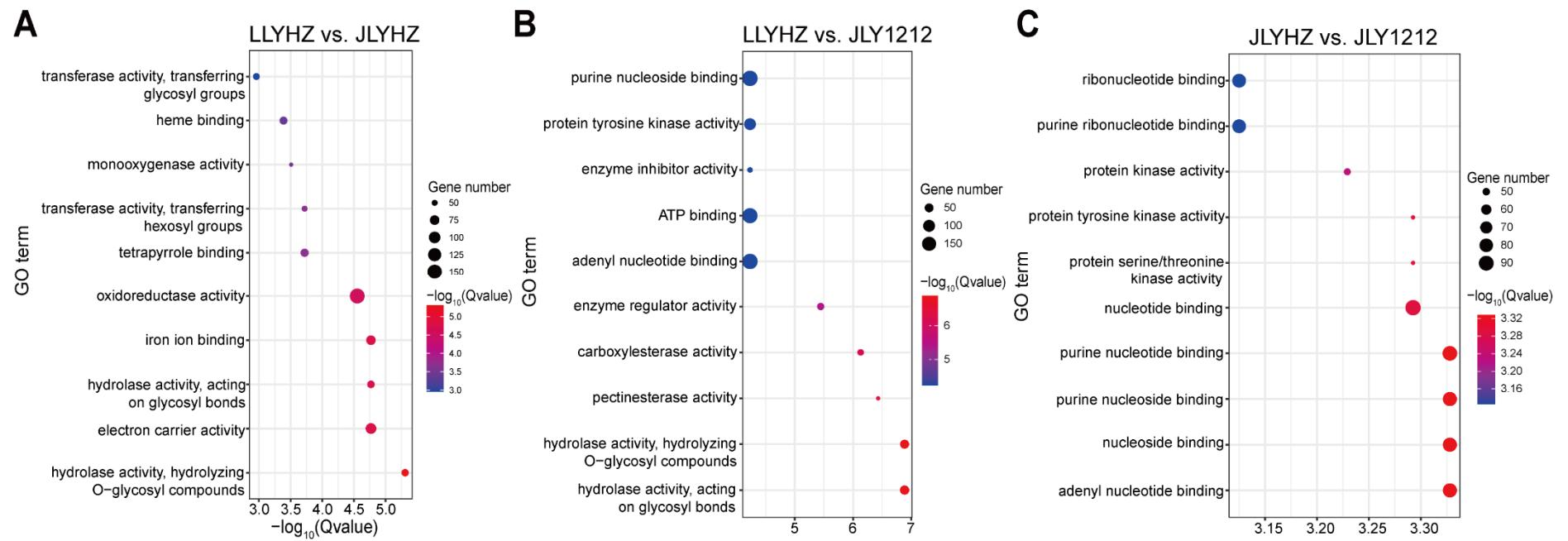


Figure S3 GO analysis of the molecular function of up-regulated genes in spikelets at flowering stage between LLYHZ and JLYHZ (A), between LLYHZ and JLY1212 (B), and between JLYHZ and JLY1212 (C).

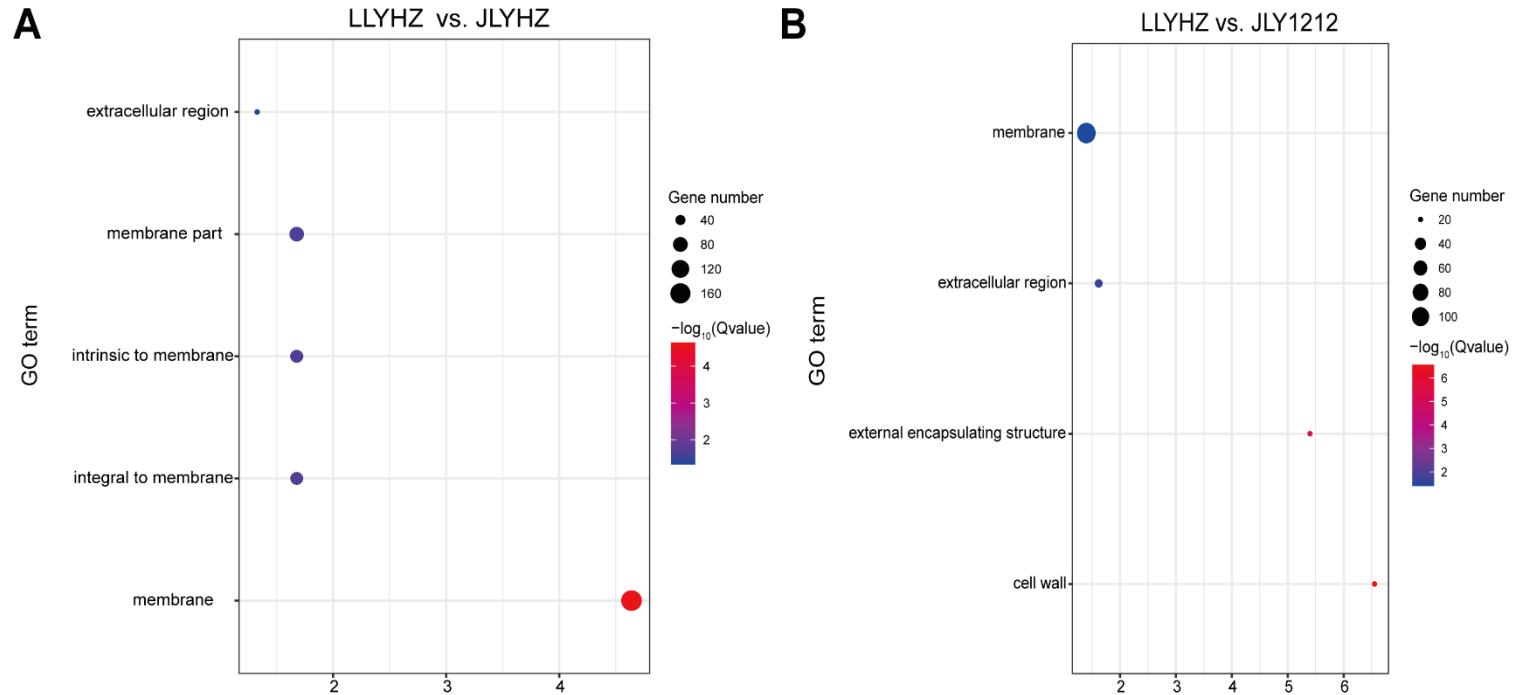


Figure S4 GO analysis of the cellular component of up-regulated genes in spikelets at flowering stage between LLYHZ and JLYHZ (A), and between LLYHZ and JLY1212 (B).