

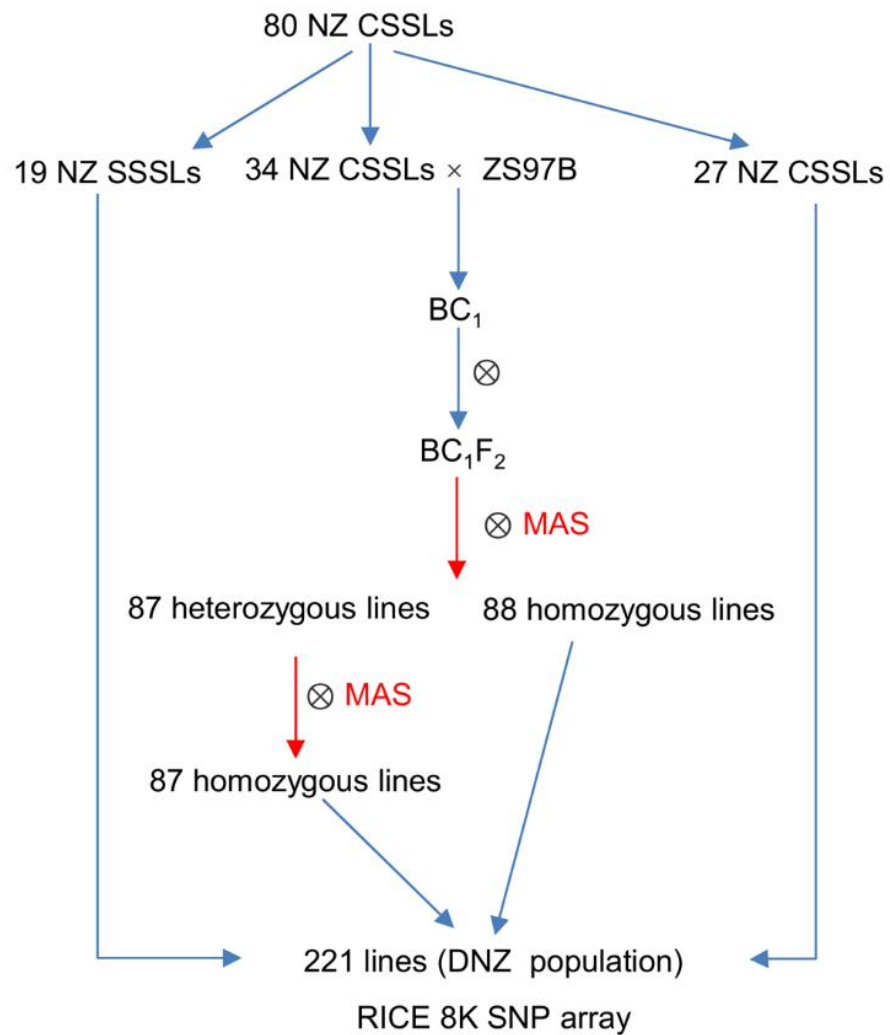
# **Identification of a novel QTL and candidate gene associated with grain size using chromosome segment substitution lines in rice**

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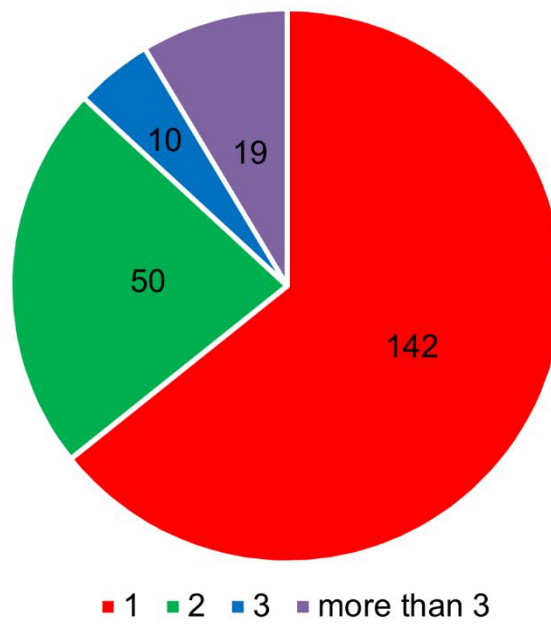
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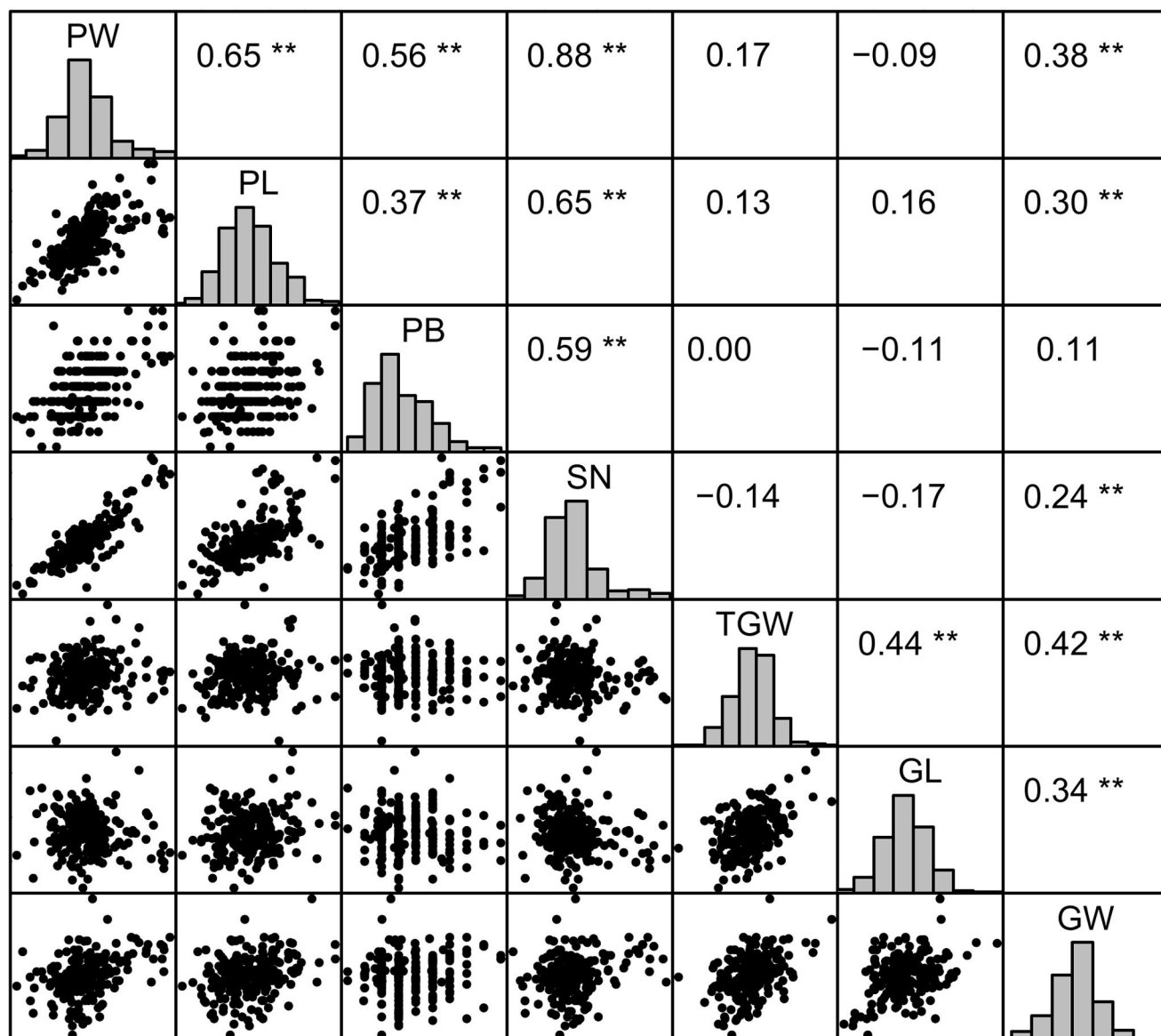
**Supplementary Figures S1-5 and Table S1-3, S6-7**



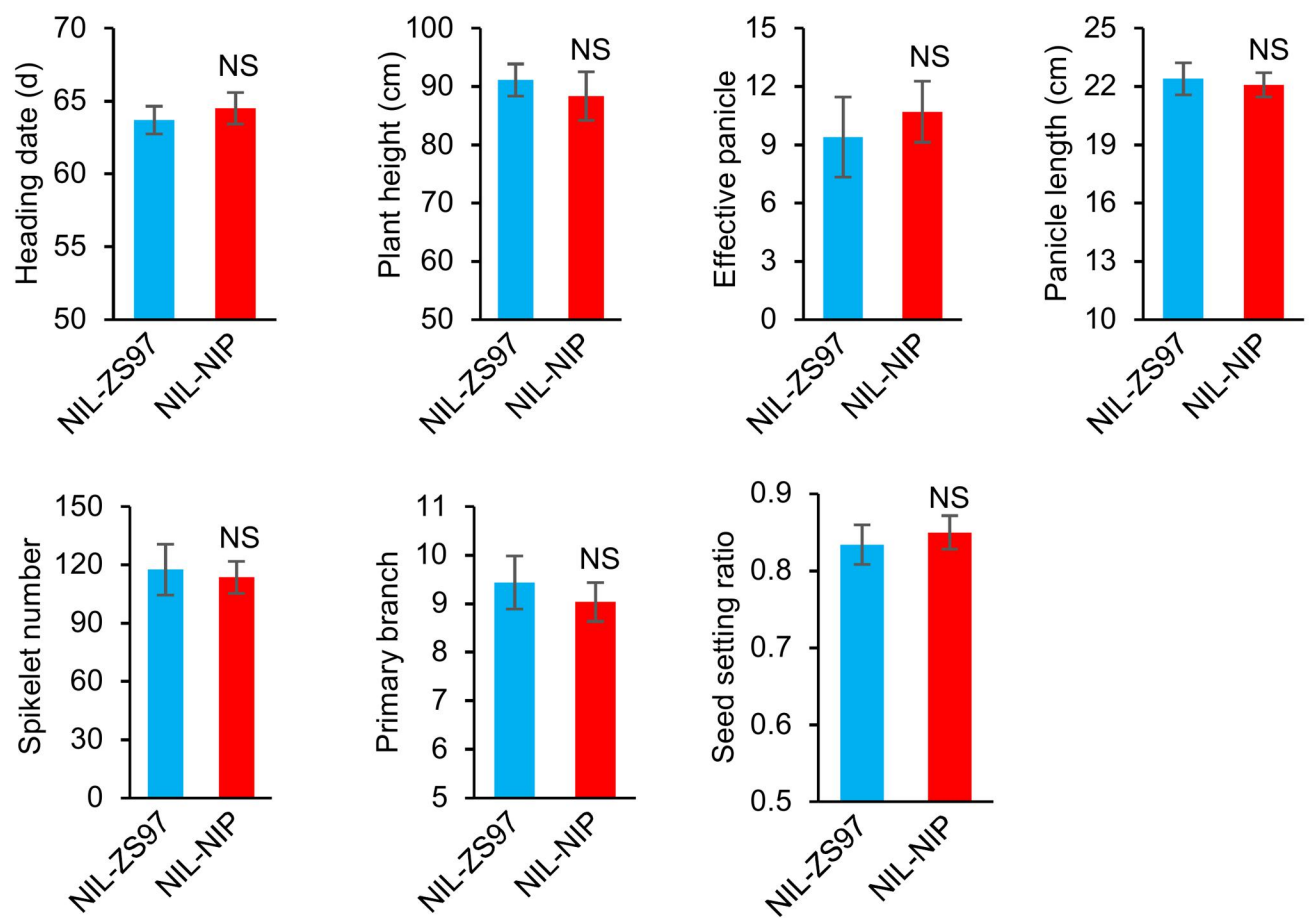
**Supplementary Figure S1.** Flowchart of developing chromosome segment substitution line (CSSL) population (DNZ). The red arrow indicates that the process of genotyping by marker-assisted selection approach (MAS). The black circle represents a self-cross.



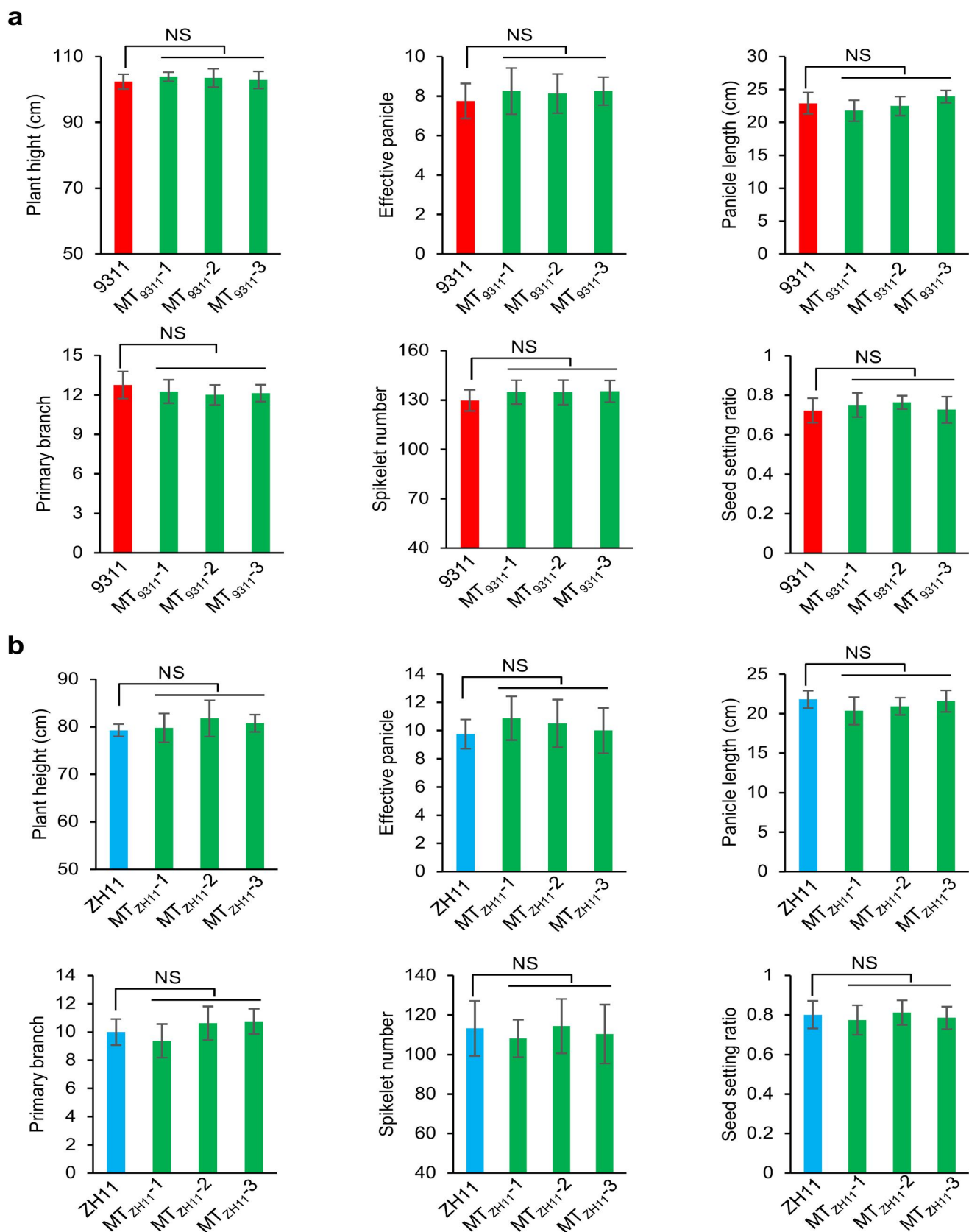
**Supplementary Figure S2.** Pie chart showing the number of chromosome segment substitution lines that are classed as four types in which 1, 2, 3, and more than 3 substitution segments are carried.



**Supplementary Figure S3.** Histogram of frequency distribution and correlation coefficient of seven yield-related traits in the CSSL population. GL, GW, TGW, SN, PB, PL and PW represent grain length, grain width, thousand grain weight, spikelet number, primary branch, panicle length and panicle weight, respectively. The upper panel contains correlation coefficients and the lower panel contains frequency distribution of the assayed parameters. The diagonal represents the histogram of the assayed traits. Double asterisks represent significance at  $P < 0.01$ .



**Supplementary Figure S4.** Comparison of the yield-related traits in NILs of *qGL11*. Data are given as the mean and SE ( $n = 10$ ). NS, no significance by Student's *t*-test.



**Supplementary Figure S5.** Comparison of the yield-related traits in the CRISPR/Cas9 induced mutants and corresponding wild type. (a) and (b) represent the mutations of *OsGH3.13* in 9311 and ZH11, respectively. Data are given as the mean and SE ( $n = 8$ ). NS, no significant difference from the wild type by Student's *t*-test.

**Supplementary Table S1.** Distribution of polymorphic SNPs between ZS97 and NIP across the whole genome

| Chr   | Number of SNPs | Distance between SNPs (bp) |           |         |
|-------|----------------|----------------------------|-----------|---------|
|       |                | Min                        | Max       | Median  |
| 1     | 451            | 21                         | 2,533,264 | 47,336  |
| 2     | 415            | 17                         | 1,122,836 | 47,535  |
| 3     | 401            | 12                         | 931,647   | 53,140  |
| 4     | 408            | 15                         | 1,660,306 | 33,728  |
| 5     | 354            | 15                         | 777,527   | 44,729  |
| 6     | 381            | 12                         | 1,136,278 | 39,963  |
| 7     | 367            | 12                         | 1,021,740 | 45,705  |
| 8     | 260            | 57                         | 1,250,806 | 59,528  |
| 9     | 160            | 152                        | 3,011,420 | 44,429  |
| 10    | 137            | 17                         | 1,119,873 | 131,061 |
| 11    | 158            | 29                         | 1,063,284 | 124,376 |
| 12    | 118            | 132                        | 1,277,886 | 173,441 |
| Total | 3610           | 12                         | 3,011,420 | 50,282  |

**Supplementary Table S2.** Distribution of substitution segments and bins on the rice chromosomes in the DNZ population

| Chr   | Number of segments | Median of segment (Mb) | Segment coverage length (Mb) | Coverage rate of segment (%) | Number of bins | Min of bin (kb) | Max of bin (Mb) | Median of bin (kb) |
|-------|--------------------|------------------------|------------------------------|------------------------------|----------------|-----------------|-----------------|--------------------|
| 1     | 51                 | 1.9                    | 39.1                         | 90                           | 50             | 22.4            | 8.2             | 406.7              |
| 2     | 46                 | 1.0                    | 35.9                         | 100                          | 46             | 12.3            | 4.2             | 408.1              |
| 3     | 61                 | 2.3                    | 35.8                         | 98                           | 53             | 25.5            | 5.8             | 478.5              |
| 4     | 49                 | 3.0                    | 34.2                         | 97                           | 56             | 1.8             | 6.5             | 290.0              |
| 5     | 40                 | 2.5                    | 29.9                         | 100                          | 42             | 29.3            | 3.9             | 481.9              |
| 6     | 22                 | 2.6                    | 24.8                         | 81                           | 30             | 2.0             | 6.0             | 399.3              |
| 7     | 35                 | 1.7                    | 29.7                         | 100                          | 35             | 44.6            | 4.0             | 457.2              |
| 8     | 21                 | 3.2                    | 28.4                         | 100                          | 26             | 69.2            | 7.2             | 620.9              |
| 9     | 27                 | 5.3                    | 23.0                         | 100                          | 20             | 34.5            | 6.5             | 532.8              |
| 10    | 12                 | 2.6                    | 23.5                         | 100                          | 15             | 166.6           | 3.6             | 1527.9             |
| 11    | 30                 | 3.9                    | 28.5                         | 100                          | 30             | 35.3            | 4.2             | 461.6              |
| 12    | 14                 | 4.9                    | 24.6                         | 89                           | 15             | 128.0           | 5.0             | 1277.9             |
| Total | 408                | 2.3                    | 357.4                        | 96                           | 418            | 1.8             | 8.2             | 472.7              |

**Supplementary Table S3.** Seven panicle and grain-related traits in the DNZ population

| Traits                    | NIP             | ZS97            | DNZ population   |             |      |
|---------------------------|-----------------|-----------------|------------------|-------------|------|
|                           | Mean $\pm$ SD   | Mean $\pm$ SD   | Mean $\pm$ SD    | Range       | CV   |
| Grain length (mm)         | 7.8 $\pm$ 0.08  | 8.3 $\pm$ 0.08  | 8.3 $\pm$ 0.23   | 7.7 ~ 9.2   | 2.8  |
| Grain width (mm)          | 3.2 $\pm$ 0.05  | 3.5 $\pm$ 0.05  | 3.4 $\pm$ 0.11   | 3.1 ~ 3.8   | 3.2  |
| Thousand-grain weight (g) | 21.3 $\pm$ 0.56 | 25.4 $\pm$ 0.89 | 24.8 $\pm$ 1.19  | 20.5 ~ 29.4 | 4.8  |
| Panicle length (cm)       | 17.9 $\pm$ 0.52 | 22.8 $\pm$ 0.78 | 22.8 $\pm$ 1.54  | 18.8 ~ 27.8 | 6.7  |
| Primary branch            | 10 $\pm$ 0.88   | 10 $\pm$ 0.88   | 10.6 $\pm$ 1.71  | 7 ~ 16      | 16.1 |
| Spikelet number           | 87 $\pm$ 6.2    | 120 $\pm$ 7.7   | 127.2 $\pm$ 24.7 | 69 ~ 216    | 19.4 |
| Panicle weight (g)        | 1.5 $\pm$ 0.17  | 3 $\pm$ 0.29    | 2.9 $\pm$ 0.63   | 1.3 ~ 4.9   | 21.5 |

Phenotype data of NIP and ZS97 are given as mean with SD ( $n = 10$ ), SD and CV stand for standard deviation and coefficient of variation, respectively.

**Supplementary Table S6. Primers used in the study**

| Primer          | Forward primer sequence (5'-3') | Purpose                 |
|-----------------|---------------------------------|-------------------------|
| M5F             | AGCATGATGTTTCATACGTGA           | mapping<br><i>qGL11</i> |
| M5R             | ATGGGATATTGCTTCACCAC            |                         |
| M9F             | AAGGTTGACAAGGACAGAAG            |                         |
| M9R             | TCGCAGGAATGGATAAAA              |                         |
| M8F             | ACAGTATTCCGTAGGCACGG            |                         |
| M8R             | GCTCCATGAGGGTGGTAGAG            |                         |
| M10F            | GTCAACGGTGTCAAACATTTTCG         |                         |
| M10R            | TTAGGAGTGGCCTGCTATGTATGC        |                         |
| M7F             | CGACACAGTTACAAAGCCACAGC         |                         |
| M7R             | TGGGAGGACGTTGATATGTCTCG         |                         |
| M11F            | GATGATCAGGAGGCAACAC             |                         |
| M11R            | CGTGATCACCTTACAATCCA            |                         |
| M12F            | CCCTCCATTTTCAGATTATACG          |                         |
| M12R            | TTGATACTTCATCCGTTTAAGG          |                         |
| M3F             | TAGCTACATTGCCGTGAGAG            |                         |
| M3R             | TGGTTATTTGCGATAGGACA            |                         |
| ID5L            | GGGTCAAAGGTCAGTGATGT            |                         |
| ID5R            | TGTACCTTGGCAGTTATGGGT           |                         |
| ID1F            | TCACTGTGCAGAATCGACCA            |                         |
| ID1R            | GGGATGTCTCTTGCAGCATG            |                         |
| ID3F            | TTTCTTCAGGTCGCAGTG              |                         |
| ID3R            | CAACAGGTCCAATTTTCG              |                         |
| ID2F            | CCCAGTGGTTATAGATGG              |                         |
| ID2R            | TTTAGCAAGTAGTGTCCC              |                         |
| M1F             | CACTTCAACTTCCTCCTCCTTTCC        |                         |
| M1R             | AGTGTCGGTCTTGTGGTTCTTGC         |                         |
| M2F             | AAGAGGAGGAGGAGAGAGAAGC          |                         |
| M2R             | CAGAGAGTTGTACACATTCTGAGC        |                         |
| M4F             | GCGGACACACCAGAGAATAAGC          |                         |
| M4R             | GTGCTGTCCTGTCCTTGAATCC          |                         |
| M6F             | TTCCCTCCTATGTAGCATTAGC          |                         |
| M6R             | ATGGTGGAGAGTACTTCATTCC          |                         |
| ID6F            | TCGATAGAAATCAACCCT              |                         |
| ID6R            | AGATATCATTAGCGGAGA              |                         |
| ID7F            | CAAGCGTCACTACTACAGAT            |                         |
| ID7R            | GCATTCGCCTAGTTCCAG              |                         |
| ID8F            | AGCCCAAGAACAAGAGGA              |                         |
| ID8R            | AGGTCCTGCCGAGATTTA              |                         |
| ID9F            | ATCGGTGGATGGATGGCT              |                         |
| ID9R            | GGGACCAGGAGGCTTCAC              |                         |
| ID10F           | TTGCTTGGTGAGATTGAC              |                         |
| ID10R           | GACATCCTAAACCCTCCC              |                         |
| Os11g0528200RTF | TGATTTCTGGCTATTATGCG            | Realtime PCR            |
| Os11g0528200RTR | GGACTACAAACCAAAGGACC            |                         |
| Os11g0528300RTF | GCATTGAAGGACCAAAGTA             |                         |
| Os11g0528300RTR | CTATAGGGGCAACAAAACA             |                         |
| Os11g0528400RTF | TACACTATCCGTGCTTGGT             |                         |
| Os11g0528400RTR | CATCTGGTGTGTCAGGCTTT            |                         |
| Os11g0528500RTF | GTTTCTTGTGCTGCGGTGAG            |                         |

|                 |                                                |                         |
|-----------------|------------------------------------------------|-------------------------|
| Os11g0528500RTR | GCGTGCAGAACGATGACG                             |                         |
| Os11g0528700RTF | CTTCTCCACCTTGATGAACTCG                         |                         |
| Os11g0528700RTR | CTCTACAACCTCCTCGTCCC                           |                         |
| UBQ-F           | AACCAGCTGAGGCCCAAGA                            |                         |
| UBQ-R           | ACGATTGATTTAACCAGTCCATGA                       |                         |
| U3F             | CCCCTTTCGCCAGGGGTACCGTAATTCATCCAGGTCTCCAAG     | Vector<br>construction  |
| U3R             | TACGAATTCGAGCTCGGTACCGCTGTGCCGTACGACGGTACG     |                         |
| OsGH3.13-U3R    | ACTACAAGAGCCGCCACTTCGTTTTAGAGCTAGAAATAGCAAGTTA |                         |
| OsGH3.13-U3F    | GAAGTGGCGGCTCTTGTAGTGCCACGGATCATCTGCACAATC     |                         |
| CAS9F           | GAGCGGATAACAATTTACACAG                         | Transgenic<br>detection |
| CAS9R           | TCTATGTTACTAGATCGGGAATTCA                      |                         |
| SCROsGH3.13     | GGGGCTCGTGTACCGCGTGT                           |                         |
| SCROsGH3.13     | CCGTCGTGATCTCCGGCTTCA                          |                         |

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**Supplementary Table S7.** Sequence comparison of the 3-kb promoter region and coding region of *OsGH3.13<sup>NIP</sup>* and *OsGH3.13<sup>ZS97</sup>*

| Position | Sequence of NIP (5'-3')      | Sequence of ZS97 (5'-3')     | Conserved motifs                |
|----------|------------------------------|------------------------------|---------------------------------|
| -38      | TGAGAACTAGG                  | TGAGAGCTAGG                  |                                 |
| -61      | AAGAA(CA) <sub>8</sub> CCAAA | AAGAA(CA) <sub>7</sub> CCAAA |                                 |
| -127     | TAGAGACCAAG                  | TAGAGGCCAAG                  | GCC (Ethylene-response element) |
| -179     | TTGTCCGACTA                  | TTGTCTGACTA                  |                                 |
| -185     | CTCTCTTTGTC                  | CTCTCTTTGTC                  | CTCC-motif                      |
| -387     | TCCTC - AAAAA                | TCCTCTAAAAA                  |                                 |
| -727     | AACATAAAGAG                  | AACATCAAGAG                  |                                 |
| -898     | AATACTGGAGT                  | AATACAGGAGT                  |                                 |
| -1327    | AACAG(A) <sub>9</sub> TACTG  | AACAG(A) <sub>10</sub> TACTG |                                 |
| -1671    | AGCTGCAGCAG                  | AGCTGAAGCAG                  |                                 |
| -1892    | TTACTTCTTTC                  | TTACTCCTTTC                  | AAGAA-motif                     |
| -1985    | CTATGCATAT                   | CTATGTCATAT                  |                                 |
| -2037    | CATATGTGTGTG                 | CATATGGTGTG                  |                                 |
| -2458    | TGCAGAATACA                  | TGCAGGATACA                  | GATACA (auxin response element) |
| -2709    | TTATAGCATT                   | TTATAACATT                   |                                 |
| -2760    | TCCCAACACTG                  | TCCCATCACTG                  |                                 |
| -2821    | GAACCGAATCT                  | GAACCAATCT                   | CAAT-box                        |
| 29       | CCC                          | CTC                          | Non-synonymous variation        |
| 260      | GTC                          | GCC                          | Non-synonymous variation        |
| 521      | TCA                          | TCG                          | Synonymous variation            |
| 745      | GGG                          | GGA                          | Synonymous variation            |
| 769      | ACG                          | ACT                          | Synonymous variation            |
| 943      | TCC                          | TCT                          | Synonymous variation            |
| 5424     | GAC                          | AAC                          | Non-synonymous variation        |

The position of the start codon (ATG) of *OsGH3.13* is recorded as 1. Natural variations in the 3-kb promoter region and coding region are shown in red font; subscript digit indicates the number of base repeats in parentheses. Conserved motifs are predicted based on the PlantCARE database, which contains natural variations are underlined.