*Plant Genetic Resources*

***Supplementary material***

**Molecular characterization of QTL-allele system for drought tolerance at seedling stage and optimal genotype design using multi-locus multi-allele genome-wide association analysis in a half-sib population of soybean [*Glycine max* (L.) Merr.]**

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**Supplementary Table S1** ANOVA of two DT indicators in MTZ half-sib/NAM population

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| SOV | Df | SS | MS | *F*-value | *Pr>F* |
| **RRL** |  |  |  |  |  |
| Geno | 403 | 25.82 | 0.064 | 6.01 | <.0001 |
| Rep | 1 | 0.122 | 0.122 | 11.45 |  |
| Error | 343 | 3.65 | 0.0106 |  |  |
| Total | 747 | 29.61 |  |  |  |
| **RSL** |  |  |  |  |  |
| Geno | 403 | 10.75 | 0.026 | 7.63 | <.0001 |
| Rep | 1 | 0.0017 | 0.0017 | 0.49 |  |
| Error | 343 | 1.19 | 0.0035 |  |  |
| Total | 747 | 20.41 |  |  |  |

SOV: Source of variance, Df: degrees of freedom, SS: sum of square, MS: mean square, RRL: relative root length,

RSL: relative shoot length, Geno: genotype, Rep: replication.

**Supplementary Table S2** Details of QTLs/SNPLDBs for RRL indicator in MTZ half-sib/NAM population

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| QTL | SNPLDB | Position | QTL in SoyBase | QTL in previous RTM-GWAS literature | Reference |
| ***RRL2.1*** | **BLOCK\_2** | **1753138\_1938465** |  | Novel |  |
| ***RRL3.1*** | **BLOCK\_3** | **35661055\_35786897** |  | Novel |  |
| ***RRL3.2*** | **BLOCK\_3** | **40660640\_40701639** |  | Novel |  |
| *RRL4.1* | Gm04 | 41553879 |  | Novel |  |
| *RRL4.2* | Gm04 | 44742587 |  | Novel |  |
| ***RRL5.1*** | **BLOCK\_5** | **31321826\_31420764** |  | Novel |  |
| ***RRL5.2*** | **BLOCK\_5** | **31464538\_31660162** |  | Novel |  |
| ***RRL7.1*** | **BLOCK\_7** | **14696530\_14701703** |  | Novel |  |
| ***RRL8.1*** | **Gm08** | **42822124** |  | Novel |  |
| ***RRL10.1*** | **Gm10** | **7270006** |  | ***RPL10.1*** | Khan *et al.,* (2019) |
| ***RRL11.1*** | **BLOCK\_11** | **11504560\_11700307** |  | Novel |  |
| *RRL12.1* | BLOCK\_12 | 111357\_186286 |  | Novel |  |
| ***RRL15.1*** | **BLOCK\_15** | **29838173\_29838452** |  | *RPL15.1* | Khan *et al.,* (2019) |
| ***RRL15.2*** | **BLOCK\_15** | **47590909\_47672694** |  | Novel |  |
| ***RRL18.1*** | **BLOCK\_18** | **1901132\_2099117** |  | ***RPL18.1*** | Khan *et al.,* (2019) |

QTL: a QTL such as *RRL2.1* is designated in the following way: RRL stands for relative root length, 2 means chromosome number 2 and .1 means its order in that chromosome according to its physical position; QTL in bold face means the locus is a large-contribution major QTL (LC major QTL) with *R2* value more than 1%, while a QTL in normal case is considered as a small-contribution major QTL (SC major QTL). SNPLDB: the SNPLDB with only single SNP is designated as, for example, “Gm08” where Gm08 represents Chromosome 08, while the column of position indicates that locus’s physical position in bp; while the SNPLDB with multiple SNPs is designated as, for example, “BLOCK\_18” where 18 means chromosome 18, followed by its physical position in bp

**Supplementary Table S3** Details of QTLs/SNPLDBs for RSL indicator in MTZ half-sib/NAM population

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| QTL | SNPLDB | Position | QTL in SoyBase | QTL in previous RTM-GWAS literature | Reference |
| ***RSL1.1*** | **BLOCK\_1** | **18376976\_18575793** |  | Novel |  |
| *RSL1.2* | BLOCK\_1 | 18903261\_18921779 |  | Novel |  |
| ***RSL3.1*** | **Gm03** | **37902066** |  | *RPL3.1* | Khan *et al.,* (2019) |
| ***RSL4.1*** | **BLOCK\_4** | **44742805\_44935708** |  | Novel |  |
| *RSL6.1* | Gm06 | 9604346 |  | Novel |  |
| *RSL6.2* | Gm06 | 44183574 |  | Novel |  |
| ***RSL7.1*** | **Gm07** | **7162007** |  | Novel |  |
| ***RSL9.1*** | **BLOCK\_9** | **41829601\_41830331** |  | Novel |  |
| *RSL10.1* | Gm10 | 43637054 |  | Novel |  |
| *RSL10.2* | BLOCK\_10 | 43914696\_43923482 |  | Novel |  |
| ***RSL11.1*** | **BLOCK\_11** | **10796920\_10886936** | mqCanopy-wilt-010 | ***RPL11.1*** | Hwang *et al.,* (2016a) |
|  |  |  |  |  | Khan *et al.,* (2019) |
| ***RSL11.2*** | **BLOCK\_11** | **11281795\_11467146** |  | Novel |  |
| ***RSL13.1*** | **BLOCK\_13** | **43640771\_43640816** |  | *RRL13.6* | Khan *et al.,* (2018) |
| ***RSL14.1*** | **BLOCK\_14** | **44998759\_45188985** |  | Novel |  |
| ***RSL15.1*** | **BLOCK\_15** | **11112792\_11310309** |  | Novel |  |
| *RSL16.1* | BLOCK\_16 | 35357235\_35370483 |  | Novel |  |
| ***RSL17.1*** | **BLOCK\_17** | **2421707\_2422009** |  | Novel |  |
| ***RSL17.2*** | **BLOCK\_17** | **37620685\_37640402** |  | Novel |  |
| ***RSL18.1*** | **BLOCK\_18** | **58542397\_58738796** |  | Novel |  |
| ***RSL19.1*** | **Gm19** | **21779180** |  | Novel |  |
| ***RSL19.2*** | **Gm19** | **29384143** |  | Novel |  |
| *RSL19.3* | BLOCK\_19 | 44377195\_44558289 |  | ***RSL19.5*** | Khan *et al.,* (2018) |
| ***RSL19.4*** | **BLOCK\_19** | **48219290\_48305154** |  | Novel |  |
| ***RSL19.5*** | **BLOCK\_19** | **49643418\_49779822** |  | Novel |  |
| *RSL20.1* | BLOCK\_20 | 35319103\_35343249 |  | ***RPL20.1*** | Khan *et al.,* (2019) |

QTL: a QTL such as *RSL 4.1* is designated in the following way: RSL stands for Relative shoot length, 4 means chromosome number 4 and .1 means its order in that chromosome according to its physical position; QTL in bold face means the locus is a large-contribution major QTL (LC major QTL) with *R2* value more than 1%, while a QTL in normal case is considered as a small-contribution major QTL (SC major QTL). SNPLDB: the SNPLDB with only single SNP is designated as, for example, “Gm07” where Gm07 represents Chromosome 07, while the column of position indicates that locus’s physical position in bp; while the SNPLDB with multiple SNPs is designated as, for example, “BLOCK\_19” where 19 means chromosome 19, followed by its physical position in bp.

**Supplementary Table S4** Summary of the detected QTL-allele system that confers drought tolerance in MTZ half-sib/NAM population

|  |  |  |  |
| --- | --- | --- | --- |
| QTL‒allele | RRL | RSL | Total |
| **QTL** |  |  |  |
| Whole | 25.84 (15, 0.86~4.31) | 43.66 (25, 0.55~10.91) | 40 |
| LC-major QTL | 23.05 (12,1.23~4.31) | 38.28 (17, 1.03~10.91) | 29 |
| SC-major QTL | 2.79 (3, 0.86~0.98) | 5.38 (8, 0.55~0.85) | 11 |
| Unmapped minor QTL | 57.26 | 43.44 |  |
| Total contribution (*h*2) | 83.10 | 87.10 |  |
| **Allele** |  |  |  |
| Whole | 36(2.4, 2~3) | 57(2.3, 2~3) | 93 |
| Positive allele | 18(0.003~0.250) | 27 (0.002~0.088) | 45 |
| Negative allele | 18(-0.231~-0.0001) | 30(-0.12~-0.001) | 48 |

In QTL column, "Whole" is the total QTL; LC-major QTL, large-contribution major QTL with genetic contribution (*R2*) of more than 1.0%; SC-major QTL: small-contribution major QTL with *R2* less than 1.0%. In the columns of the two indicators for "QTL"; the number outside the parentheses is the total *R2* of the corresponding QTL; the first number in parentheses is the number of QTL; the second number is a range of *R2* for the individual QTL. The *R2* of the unmapped minor QTL is calculated from the Total contribution (*h2*) ‒ the contribution of whole detected QTL.

In allele column, in the columns of the two indicators for "Allele", the number outside the parentheses is the total alleles, the first number in parentheses for "Whole" is the average number of alleles per locus followed by a range of allele numbers per locus, and the number in parentheses for "Positive allele" and "Negative allele" is a range of corresponding allele effects.

In the Total column, the number is the total number of QTLs or alleles detected.

**Supplementary Table S5** The genetic constitution of RRL of the three parents in MTZ half-sib/NAM population

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| QTL | *R*2(%) | M | T | Z | Best |
| *RRL10.1* | 4.31 | 1(0.04) | 2(-0.04) | 2(-0.04) | 1 |
| *RRL5.2* | 2.52 | 1(-0.089) | 2(-0.162) | 3(0.25) | 3 |
| *RRL18.1* | 2.19 | 1(-0.00014) | 2(-0.065) | 3(0.066) | 3 |
| *RRL5.1* | 1.97 | 1(0.104) | 2(0.13) | 3(-0.23) | 2 |
| *RRL11.1* | 1.85 | 1(-0.035) | 2(0.0036) | 3(0.031) | 3 |
| *RRL15.2* | 1.71 | 1(-0.037) | 2(0.037) | 1(-0.037) | 2 |
| *RRL3.2* | 1.68 | 1(-0.027) | 2(0.027) | 2(0.027) | 2 |
| *RRL8.1* | 1.57 | 1(0.024) | 2(-0.024) | 2(-0.024) | 1 |
| *RRL2.1* | 1.45 | 1(0.017) | 2(-0.036) | 3(0.019) | 3 |
| *RRL7.1* | 1.33 | 1(0.052) | 1(0.052) | 3(-0.052) | 1 |
| *RRL3.1* | 1.24 | 1(-0.032) | 2(-0.011) | 3(0.043) | 3 |
| *RRL15.1* | 1.23 | 1(0.056) | 2(-0.056) | 2(-0.056) | 1 |
| *RRL4.2* | 0.98 | 1(-0.022) | 2(0.022) | 2(0.022) | 2 |
| *RRL12.1* | 0.95 | 1(-0.023) | 2(0.023) | 2(0.023) | 2 |
| *RRL4.1* | 0.86 | 1(0.030) | 2(-0.030) | 2(-0.030) | 1 |
| **Total** |  | **1.027** | **0.84** | **0.982** | **1.820** |

The QTLs (abbreviated as chromosome-order, such as 18.1 means the 1stQTL on 18th Chromosome) detected using RTM‒GWAS are arranged in a descending order according to their genetic contribution (*R*2).

The three parents are arranged as M8206 (abbreviated as M), TongShan (T) and ZhengYang (Z);the number (outside of parenthesis) represents the code of the detected allele, which is designated as 1 to 3;the value in parentheses is the corresponding allele effects.

The Column of “Best allele” indicates the code of the best allele among the alleles in the same row. Total: the total of all the allelic effects added with mean of each trait

**Supplementary Table S6** The genetic constitution of RSL of the three parents in MTZ half-sib/NAM population

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| QTL | *R*2(%) | M | T | Z | Best | QTL | *R*2(%) | M | T | Z | Best |
| *RSL11.2* | 10.91 | 1(0.044) | 2(0.076) | 3(-0.12) | 2 | *RSL18.1* | 1.20 | 1(-0.002) | 2(-0.031) | 3(0.034) | 3 |
| *RSL11.1* | 3.42 | 1(-0.067) | 2(-0.020) | 3(0.088) | 3 | *RSL19.4* | 1.13 | 1(0.027) | 2(-0.001) | 3(-0.025) | 1 |
| *RSL7.1* | 3.11 | 1(-0.028) | 2(0.028) | 1(-0.028) | 2 | *RSL19.1* | 1.04 | 1(-0.045) | 2(0.045) | 2(0.045) | 2 |
| *RSL4.1* | 2.13 | 1(-0.0094) | 2(0.026) | 3(-0.016) | 2 | *RSL3.1* | 1.03 | 1(-0.023) | 1(-0.023) | 2(0.023) | 2 |
| *RSL13.1* | 1.99 | 1(0.019) | 2(-0.019) | 1(0.019) | 1 | *RSL19.3* | 0.85 | 1(-0.016) | 2(0.016) | 1(-0.016) | 2 |
| *RSL9.1* | 1.98 | 1(-0.023) | 2(0.023) | 2(0.023) | 2 | *RSL10.2* | 0.80 | 1(0.031) | 2(-0.031) | 2(-0.031) | 1 |
| *RSL15.1* | 1.61 | 1(-0.019) | 2(0.017) | 3(0.0022) | 2 | *RSL6.2* | 0.67 | 1(-0.021) | 1(-0.021) | 2(0.021) | 2 |
| *RSL14.1* | 1.59 | 1(0.029) | 1(0.029) | 3(-0.029) | 1 | *RSL10.1* | 0.66 | 1(-0.037) | 2(0.037) | 2(0.037) | 2 |
| *RSL19.2* | 1.58 | 1(-0.025) | 2(0.025) | 2(0.025) | 2 | *RSL1.2* | 0.65 | 1(0.071) | 2(-0.071) | 2(-0.071) | 1 |
| *RSL17.2* | 1.46 | 1(-0.024) | 2(0.038) | 3(-0.013) | 2 | *RSL20.1* | 0.61 | 1(0.011) | 2(-0.011) | 2(-0.011) | 1 |
| *RSL19.5* | 1.41 | 1(-0.022) | 2(0.022) | 2(0.022) | 2 | *RSL16.1* | 0.59 | 1(0.011) | 2(-0.011) | 2(-0.011) | 1 |
| *RSL17.1* | 1.37 | 1(0.020) | 2(-0.020) | 2(-0.020) | 1 | *RSL6.1* | 0.55 | 1(0.011) | 2(-0.011) | 1(0.011) | 1 |
| *RSL1.1* | 1.32 | 1(-0.088) | 2(0.088) | 2(0.088) | 2 | **Total** |  | **0.374** | **0.75** | **0.597** | **1.299** |

The QTLs (abbreviated as chromosome-order, such as 18.1 means the 1st QTL on 18th Chromosome) detected using RTM‒GWAS are arranged in a descending order according to their genetic contribution (*R*2).

The three parents are arranged as M8206 (abbreviated as M), TongShan (T) and ZhengYang (Z); the number (outside of parenthesis) represents the code of the detected allele, which is designated as 1 to 3; the value in parentheses is the corresponding allele effects.

The Column of “Best allele” indicates the code of the best allele among the alleles in the same row. Total: the total of all the allelic effects added with mean of each trait

**Supplementary Table S7** Top ten superior single crosses for RRL and RSL indicators

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Code | Cross | RRL | RSL | WAV |
| 1 | T132 | 1.714 | 0.506 |  |
|  | Z061 | 1.480 | 0.753 |  |
| Progeny 95th percentile | | 1.772 | 0.767 | 1.257 |
| 2 | T097 | 1.403 | 0.776 |  |
|  | T132 | 1.714 | 0.506 |  |
| Progeny 95th percentile | | 1.712 | 0.789 | 1.239 |
| 3 | T063 | 1.377 | 0.740 |  |
|  | Z061 | 1.480 | 0.753 |  |
| Progeny 95th percentile | | 1.574 | 0.911 | 1.235 |
| 4 | T066 | 1.512 | 0.629 |  |
|  | Z061 | 1.480 | 0.753 |  |
| Progeny 95th percentile | | 1.633 | 0.854 | 1.234 |
| 5 | T174 | 1.365 | 0.664 |  |
|  | Z061 | 1.480 | 0.753 |  |
| Progeny 95th percentile | | 1.588 | 0.894 | 1.233 |
| 6 | T035 | 1.298 | 0.856 |  |
|  | Z012 | 1.268 | 0.527 |  |
| Progeny 95th percentile | | 1.631 | 0.845 | 1.229 |
| 7 | T097 | 1.403 | 0.776 |  |
|  | Z061 | 1.480 | 0.753 |  |
| Progeny 95th percentile | | 1.583 | 0.889 | 1.228 |
| 8 | T035 | 1.298 | 0.856 |  |
|  | Z061 | 1.480 | 0.753 |  |
| Progeny 95th percentile | | 1.503 | 0.964 | 1.227 |
| 9 | T259 | 1.084 | 0.987 |  |
|  | Z061 | 1.480 | 0.753 |  |
| Progeny 95th percentile | | 1.435 | 1.027 | 1.226 |
| 10 | T081 | 1.360 | 0.719 |  |
|  | Z061 | 1.480 | 0.753 |  |
| Progeny 95th percentile | | 1.599 | 0.853 | 1.218 |
| Best genotype predicted from three parents | | **1.820** | **1.299** | **1.553** |

Code: code of the cross, in RRL and RSL columns are the predicted genotypic 95th percentile under linkage model obtained from a simulation with 2,000 progenies per cross, In the WAV column is the weighted average value of the two indicators with their heritability values as weights, WAVs of the parents are 0.693, 0.794 and 0.785 for M8206, TongShan and ZhengYang, respectively, all the ten crosses will have their 95th percentile progenies to be transgressive

**Supplementary Table S8** Top ten superior single crosses for RRL, RSL, RPL and RPDW as set of DT indicators

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Code | Cross | RRL | RSL | RPL | RPDW | WAV |
| 1 | T035 | 1.298 | 0.856 | 1.096 | 0.713 |  |
|  | Z048 | 0.813 | 0.833 | 0.607 | 1.712 |  |
| Progeny 95th percentile | | 1.235 | 1.012 | 0.851 | 1.027 | 1.044 |
| 2 | T081 | 1.360 | 0.719 | 1.039 | 0.640 |  |
|  | Z048 | 0.813 | 0.833 | 0.607 | 1.712 |  |
| Progeny 95th percentile | | 1.201 | 0.991 | 0.823 | 1.041 | 1.028 |
| 3 | Z048 | 0.813 | 0.833 | 0.607 | 1.712 |  |
|  | Z061 | 1.480 | 0.753 | 0.847 | 0.795 |  |
| Progeny 95th percentile | | 1.312 | 0.979 | 0.727 | 0.906 | 0.997 |
| 4 | T279 | 1.027 | 0.420 | 0.691 | 0.297 |  |
|  | Z048 | 0.813 | 0.833 | 0.607 | 1.712 |  |
| Progeny 95th percentile | | 1.217 | 0.909 | 0.649 | 0.972 | 0.958 |
| 5 | T097 | 1.403 | 0.776 | 1.062 | 0.426 |  |
|  | Z048 | 0.813 | 0.833 | 0.607 | 1.712 |  |
| Progeny 95th percentile | | 1.240 | 0.969 | 0.243 | 0.874 | 0.876 |
| 6 | T049 | 1.195 | 0.742 | 1.012 | 0.706 |  |
|  | Z048 | 0.813 | 0.833 | 0.607 | 1.712 |  |
| Progeny 95th percentile | | 1.177 | 0.946 | 0.834 | 0.939 | 0.983 |
| 7 | T035 | 1.298 | 0.856 | 1.096 | 0.713 |  |
|  | Z065 | 1.253 | 0.713 | 0.709 | 1.173 |  |
| Progeny 95th percentile | | 1.401 | 0.922 | 0.902 | 0.651 | 0.965 |
| 8 | T081 | 1.360 | 0.719 | 1.039 | 0.640 |  |
|  | Z065 | 1.253 | 0.713 | 0.709 | 1.173 |  |
| Progeny 95th percentile | | 1.473 | 0.818 | 0.874 | 0.671 | 0.956 |
| 9 | T132 | 1.714 | 0.506 | 1.094 | 0.463 |  |
|  | Z048 | 0.813 | 0.833 | 0.607 | 1.712 |  |
| Progeny 95th percentile | | 1.363 | 0.831 | 0.850 | 0.919 | 0.997 |
| 10 | T066 | 1.512 | 0.629 | 1.081 | 0.742 |  |
|  | Z048 | 0.813 | 0.833 | 0.607 | 1.712 |  |
| Progeny 95th percentile | | 1.306 | 0.837 | 0.844 | 0.920 | 0.983 |
| Best genotype predicted from three parents | | **1.820** | **1.299** | **1.320** | **1.889** | **1.605** |

Code: code of the cross, in RRL, RSL, RPL and RPDW columns are the predicted genotypic 95th percentile under linkage model obtained from a simulation with 2,000 progenies per cross, In the WAV column is the weighted average value of the four indicators with their heritability values as weights, WAVs of the parents are 0.663, 0.738 and 0.636 for M8206, TongShan and ZhengYang, respectively, all the ten crosses will have their 95th percentile progenies to be transgressive

**Supplementary Table S9** Twenty six candidate genes harboring SNPs associated with RRL in MTZ half-sib/NAM population at *P*‒value <0.0001 by χ2-analysis

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| QTL | *R*2 (%) | Gene | Start(bp) | End(bp) | Putative functional protein description | **A** | **S** | **T** | **D** | **PM** | **TF** | **PK** | **U** | **O** |
| ***RRL2.1*** | 1.45 | *Glyma02g02450* | 1836948 | 1844648 | Unknown |  |  |  |  |  |  |  | √ |  |
|  |  | *Glyma02g02600* | 1932148 | 1936451 | PAC motif |  |  |  |  | √ |  |  |  |  |
|  |  | *Glyma02g02610* | 1937277 | 1938821 | Unknown |  |  |  |  |  |  |  | √ |  |
| ***RRL3.1*** | 1.24 | *Glyma03g27830* | 35604981 | 35613864 | [ATP-binding cassette (ABC) transporter](http://pantherdb.org/panther/category.do?categoryAcc=PC00003) |  |  | √ |  |  |  |  |  |  |
|  |  | *Glyma03g27890* | 35673291 | 35675971 | MYB family | √ | √ |  |  |  | √ |  |  |  |
| ***RRL3.2*** | 1.68 | *Glyma03g32890* | 40613121 | 40618246 | [Cation/h + antiporter](http://pantherdb.org/panther/family.do?clsAccession=PTHR32468) |  |  | √ |  |  |  |  |  |  |
|  |  | *Glyma03g32940* | 40656951 | 40662253 | Potassium channel |  | √ |  |  | √ |  |  |  |  |
|  |  | *Glyma03g33000* | 40689640 | 40693587 | Soluble inorganic pyrophosphatase |  |  | √ |  | √ |  |  |  |  |
| *RRL4.1* | 0.86 | *Glyma04g35210* | 41546880 | 41553823 | Cyclic nucleotide-gated ion channel |  |  | √ |  |  |  |  |  |  |
| ***RRL5.1*** | 1.97 | *Glyma05g25130* | 31281384 | 31283061 | Unknown |  |  |  |  |  |  |  | √ |  |
|  |  | *Glyma05g25180* | 31312861 | 31315433 | Bidirectional sugar transporter |  |  | √ |  |  |  |  |  |  |
|  |  | *Glyma05g25190* | 31319246 | 31322363 | U3 small nucleolar RNA-associated protein |  | √ |  |  |  | √ |  |  | √ |
| ***RRL5.2*** | 2.52 | *Glyma07g14830* | 14666162 | 14668465 | RNA recognition domain-containing protein |  |  |  |  |  |  |  |  |  |
|  |  | *Glyma07g14860* | 14701203 | 14707342 | Dehydratase |  |  |  | √ | √ |  |  |  |  |
|  |  | *Glyma07g14870* | 14709171 | 14713495 | Translation initiation factor |  |  |  |  | √ |  |  |  |  |
| ***RRL8.1*** | 1.57 | *Glyma08g42810* | 42774428 | 42788133 | Nucleolar pre-ribosomal-associated protein |  | √ |  |  |  | √ |  |  |  |
|  |  | *Glyma08g42820* | 42785107 | 42791663 | Unknown |  |  |  |  |  |  |  | √ |  |
| ***RRL11.1*** | 1.85 | *Glyma11g15810* | 11466243 | 11468866 | LOB domain |  | √ |  |  |  |  |  |  |  |
|  |  | *Glyma11g15841* | 11498539 | 11521484 | Translation elongation factor ef-1 alpha | √ | √ |  |  |  | √ |  |  |  |
| *RRL12.1* | 0.95 | *Glyma12g00350* | 108779 | 113486 | Threonylcarbamoyladenosine tRNA |  |  | √ |  |  |  |  |  |  |
| ***RRL15.2*** | 1.71 | *Glyma15g40570* | 47570429 | 47571953 | Unknown |  |  |  |  |  |  |  | √ |  |
| ***RRL18.1*** | 2.19 | *Glyma18g02900* | 1878560 | 1887232 | UTP-glucose-1-phosphate uridylyltransferase 3 |  |  | √ |  |  |  |  |  |  |
|  |  | *Glyma18g02960* | 1931777 | 1944321 | Clathrin heavy chain |  |  |  |  |  |  |  |  | √ |
|  |  | *Glyma18g03040* | 2013003 | 2016594 | Unknown |  |  |  |  |  |  |  | √ |  |
|  |  | *Glyma18g03180* | 2092774 | 2099488 | AARF domain containing kinase |  |  |  |  |  |  | √ |  |  |
|  |  | *Glyma18g03200* | 2117710 | 2122683 | Nuclear pore complex protein transporter |  |  | √ |  |  |  |  |  |  |

In Classification column, A, S, T, D, PM, TF, PK, UF and O, represent the nine biological categories of candidate genes, i.e. ABA responders, stress responders, transporters, development factors, protein metabolism, transcription factors, protein kinases, unknown function and other minor categories, respectively. The bold faced QTLs are LC major ones.

**Supplementary Table S10** Thirty nine candidate genes harboring SNPs associated with RSL in MTZ half-sib/NAM population at *P*‒value <0.0001 by χ2-analysis

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| QTL | R2 (%) | Gene | Start(bp) | End(bp) | Putative functional protein | A | S | T | D | PM | TF | PK | U | O |
| ***RSL3.1*** | 1.03 | *Glyma03g29860* | 37878377 | 37887807 | TBCC domain |  |  | √ |  |  |  |  |  |  |
|  |  | *Glyma03g29891* | 37900852 | 37906703 | Leucine rich repeat n-terminal domain |  | √ |  |  | √ |  |  |  |  |
| ***RSL4.1*** | 2.13 | *Glyma04g38480* | 44875756 | 44880786 | Unknown |  |  |  |  |  |  |  | √ |  |
|  |  | *Glyma04g38560* | 44933843 | 44935783 | NAC transcription factor | √ | √ |  |  |  | √ |  |  |  |
| ***RSL7.1*** | 3.11 | *Glyma07g08595* | 7162123 | 7162653 | NAC transcription factor | √ | √ |  |  |  | √ |  |  |  |
|  |  | *Glyma07g08601* | 7162766 | 7163062 | Unknown |  |  |  |  |  |  |  | √ |  |
| *RSL10.1* | 0.66 | *Glyma10g35410* | 43616040 | 43621443 | Synaptotagmin-3 |  |  |  |  |  |  |  |  | √ |
| *RSL10.2* | 0.8 | *Glyma10g35640* | 43900600 | 43905731 | Synaptotagmin-3 |  |  |  |  |  |  |  |  | √ |
| ***RSL11.1*** | 3.42 | *Glyma11g15140* | 10834124 | 10835095 | Zinc finger domain | √ | √ |  |  |  |  |  |  |  |
|  |  | *Glyma11g15180* | 10884136 | 10885737 | MYB4 | √ | √ |  |  |  | √ |  |  |  |
|  |  | *Glyma11g15075* | 10799344 | 10803663 | Unknown |  |  |  |  |  |  |  | √ |  |
| ***RSL11.2*** | 10.91 | *Glyma11g15580* | 11232271 | 11255186 | Stress response regulator |  | √ |  |  |  |  |  |  |  |
|  |  | *Glyma11g15810* | 11466243 | 11468866 | Two-component response regulator |  |  | √ |  |  | √ | √ |  |  |
|  |  | *Glyma11g15841* | 11498539 | 11521484 | LOB domain-containing protein 1 |  |  |  |  |  |  |  |  | √ |
| ***RSL15.1*** | 1.61 | *Glyma15g14700* | 11189089 | 11197781 | Translation elongation factor ef-1 alpha |  | √ |  |  | √ | √ |  |  |  |
| *RSL16.1* | 0.59 | *Glyma16g32230* | 35394552 | 35398580 | Unknown |  |  |  |  |  |  |  | √ |  |
|  |  | *Glyma16g32250* | 35408840 | 35416022 | Fbox protein pp2-a14 |  | √ |  |  |  |  |  |  |  |
|  |  | *Glyma16g32121* | 35305441 | 35308719 | Transcriptional regulator slk1 |  | √ |  |  | √ | √ |  |  |  |
|  |  | *Glyma16g32236* | 35400692 | 35405759 | protein root Primordium defective1 |  | √ |  | √ |  |  |  |  |  |
| ***RSL17.1*** | 1.37 | *Glyma17g03560* | 2370418 | 2379333 | Unknown |  |  |  |  |  |  |  | √ |  |
|  |  | *Glyma17g03570* | 2382766 | 2398213 | Dihydrolipoyl dehydrogenase |  |  |  |  |  |  |  |  | √ |
|  |  | *Glyma17g03660* | 2431405 | 2437778 | Serine/threonine-protein phosphatase | √ | √ |  |  |  |  | √ |  |  |
|  |  | *Glyma17g03680* | 2451441 | 2456345 | Nucleic acid-binding protein-related |  |  |  |  |  |  |  |  |  |
|  |  | *Glyma17g03700* | 2460791 | 2467923 | Protein high chlorophyll fluorescent |  | √ |  | √ |  |  |  |  |  |
| ***RSL18.1*** | 1.2 | *Glyma18g49110* | 58493633 | 58498394 | ATP-citrate synthase beta chain protein |  |  |  |  |  |  |  |  | √ |
|  |  | *Glyma18g49390* | 58767060 | 58768883 | Unknown |  |  |  |  |  |  |  | √ |  |
| *RSL19.3* | 0.85 | *Glyma19g37190* | 44396091 | 44405653 | Zinc finger domain | √ | √ |  |  |  |  |  |  |  |
|  |  | *Glyma19g37350* | 44530898 | 44531935 | Unknown |  | √ |  |  |  |  |  |  |  |
|  |  | *Glyma19g37380* | 44543693 | 44545886 | Homeobox-leucine zipper protein |  | √ |  |  | √ |  |  |  |  |
|  |  | *Glyma19g37108* | 44370650 | 44379782 | Udp-glycosyltransferase |  |  | √ |  |  |  |  |  |  |
|  |  | *Glyma19g37132* | 44377103 | 44377696 | Unknown |  |  |  |  |  |  |  | √ |  |
|  |  | *Glyma19g37395* | 44556756 | 44559936 | [Calcium-binding protein 39-related](http://pantherdb.org/panther/family.do?clsAccession=PTHR10182) |  | √ | √ |  |  |  |  |  |  |
| ***RSL19.4*** | 1.13 | *Glyma19g42100* | 48166591 | 48172962 | [Ubiquitin-protein ligase](http://pantherdb.org/panther/category.do?categoryAcc=PC00234) |  |  |  | √ |  |  |  |  |  |
|  |  | *Glyma19g42180* | 48208643 | 48212956 | ATP-dependent clp protease proteolytic |  |  |  |  |  |  |  |  | √ |
|  |  | *Glyma19g42240* | 48260948 | 48262037 | histone H2A |  |  |  | √ |  |  |  |  |  |
|  |  | *Glyma19g42280* | 48294125 | 48299090 | [Non-receptor serine/threonine Protein kinase](http://pantherdb.org/panther/category.do?categoryAcc=PC00167) |  | √ |  |  |  |  | √ |  |  |
|  |  | *Glyma19g42290* | 48319914 | 48327457 | [Kinase modulator](http://pantherdb.org/panther/category.do?categoryAcc=PC00140) |  | √ |  |  |  |  | √ |  |  |
| ***RSL19.5*** | 1.41 | *Glyma19g44080* | 49609097 | 49613123 | ABL interactor 2 | √ | √ |  |  |  |  |  |  |  |
| *RSL20.1* | 0.61 | *Glyma20g25670* | 35314786 | 35321939 | [Amino acid transporter](http://pantherdb.org/panther/category.do?categoryAcc=PC00046) |  |  | √ |  |  |  |  |  |  |

In Classification column, A, S, T, D, PM, TF, PK, UF and O, represent the nine biological categories of candidate genes, i.e. ABA responders, stress responders, transporters, development factors, protein metabolism, transcription factors, protein kinases, unknown function and other minor categories, respectively. The bold faced QTLs are LC major ones.

**Supplementary Table S11** Candidate genes related to DT reported in the literature

|  |  |  |  |
| --- | --- | --- | --- |
| QTL | Reported Gene | Functional protein | Reference |
| ***RRL3.1*** | *Glyma03g27830* | [*ATP-binding cassette (ABC) transporter*](http://pantherdb.org/panther/category.do?categoryAcc=PC00003) | Khan *et al.,* (2018) |
|  | *Glyma03g27890* | *MYB family* | Khan *et al.,* (2018) |
| ***RRL3.2*** | Glyma03g32900 | [*Cation/h + antiporter*](http://pantherdb.org/panther/family.do?clsAccession=PTHR32468) | Do *et al.,* (2016) |
| ***RRL5.1*** | *Glyma05g25180* | *Sugar transporter protein* | Patil *et al.,* (2015) |
| ***RRL18.1*** | *Glyma18g02900* | *UTP-glucose-1-phosphate uridylyltransferase* | Khan *et al.,* (2019) |
|  | *Glyma18g02960* | *Clathrin heavy chain* | Khan *et al.,* (2019) |
|  | *Glyma18g03040* | *Unknown* | Khan *et al.,* (2019) |
|  | *Glyma18g03180* | *AARF domain containing kinase* | Khan *et al.,* (2019) |
|  | *Glyma18g03200* | *Nuclear pore complex protein transporter* | Khan *et al.,* (2019) |
| ***RSL3.1*** | *Glyma03g29860* | *TBCC domain* | Khan *et al.,* (2019) |
|  | *Glyma03g29891* | *Leucine rich repeat n-terminal domain* | Khan *et al.,* (2019) |
| ***RSL4.1*** | *Glyma04g38560* | *NAC transcription factor* | Le *et al.,* (2012) |
| *RSL10.1* | *Glyma10g35410* | *Synaptotagmin-3* | Khan *et al.,* (2019) |
| ***RSL11.1*** | *Glyma11g15140* | *Zinc finger domain* | Khan *et al.,* (2019) |
|  | *Glyma11g15180* | *MYB4* | Khan *et al.,* (2019) |
|  | *Glyma11g15075* | *Unknown* | Khan *et al.,* (2019) |
| ***RSL11.2*** | *Glyma11g15580* | *Stress response regulator* | Marcolino-Gomes *et al.,* (2014) |
| *RSL19.3* | *Glyma19g37190* | *Zinc finger domain* | Khan *et al.,* (2018) |
|  | *Glyma19g37380* | *Homeobox-leucine zipper protein* | Chen *et al.,* (2014); Khan *et al.,* (2018) |
|  | *Glyma19g37108* | *Udp-glycosyltransferase* | Khan *et al.,* (2018) |
|  | *Glyma19g37395* | [*Calcium-binding protein 39-related*](http://pantherdb.org/panther/family.do?clsAccession=PTHR10182) | Khan *et al.,* (2018) |
| ***RSL19.5*** | *Glyma19g44080* | *ABL interactor 2* | Khan *et al.,* (2019) |
| *RSL20.1* | *Glyma20g25670* | [*Amino acid transporter*](http://pantherdb.org/panther/category.do?categoryAcc=PC00046) | Khan *et al.,* (2019) |

Bold faced are LC (major) QTLs with PHC equal to or more than 1%