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應用集群分離 RNA 測序分析研究臺灣大豆「高雄 11 號」之白粉病抗病基因座

Application of Bulk Segregant RNA-Seq Analysis on the Powdery Mildew Resistance Loci in Soybean Cultivar 'KH11' of Taiwan

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口試委員會審定書

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本論文係黃承濬（學號 R10645004）在國立臺灣大學植物醫學碩士學位學程完成之碩士學位論文，於民國 112 年 10 月 2 日承下列考試委員審查通過及口試及格，特此證明。

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Abstract

Powdery mildew (PM) is one of the important soybean (*Glycine max*) diseases worldwide, and the implementation of soybean resistance has shown to be effective in managing PM. To date, there is only one PM resistance QTL, the *Rmd* locus identified on soybean chromosome (Chr) 16 based on studying the varieties from the United States and China. Whether additional PM resistance existed in soybean varieties from other geographic origins remains to be answered. This project focuses on the PM-resistant variety 'Kaohsiung 11' ('KH11') of Taiwan, and an F_3 population was generated by crossing 'KH11' with the PM-susceptible variety 'Hualien 1' ('HL1'). Bulk segregant RNA-Seq (BSR-Seq) was applied to capture the gene expression and genotypic variation between the resistant bulk (R bulk) and the susceptible bulk (S bulk), as well as the parents. The Δ(SNP-index) and G' value analyses identified significant SNPs and Indels on Chr06, Chr15, and Chr16 associated with PM resistance. To validate the differential expression of genes (DEGs), consensus 1687 DEGs identified by both HISAT2 and Kallisto methods were considered as candidate genes associated with PM resistance. The candidate genes on Chr06, Chr15, and Chr16 were selected based on the Log_2 fold change value and the relative position with the significant SNPs, and primers that can be used in the allele-specific polymerase chain reaction (AS-PCR) were designed. While the AS-PCR primers designed for Chr06 or Chr15 cannot distinguish the resistant and susceptible genotypes, AS-PCR primers on Chr16 were successful with an 82% accuracy rate, similar to the simple sequence repeat (SSR) marker Satt431 from previous studies. These results not only confirm the resistant source in the Taiwanese soybean cultivar 'KH11' is predominantly govern by the *Rmd* locus on Chr16, but also suggest the ASPs on Chr16

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designed in this study can be used as a molecular marker for breeding soybean PM resistance derived from 'KH11'.

Key words: Allele-specific PCR, Bulk-segregant RNA-Seq, Differentially expressed gene, Soybean, Powdery mildew, Resistance.

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中文摘要

大豆白粉病是重要的大豆病害之一，而使用抗病品種已被證實可以有效降低大豆白粉病所造成的危害。以往其他國家針對大豆白粉病抗病的研究多導向在大豆 16 號染色體上的 *Rmd* 基因座，而其他地區的大豆品種是否有其他抗性基因仍有待調查。本研究利用群聚分離分析法定位台灣大豆品種「高雄 11 號」上之抗病基因座。利用比較抗病親本「高雄 11 號」和感病親本「花蓮 1 號」、以及抗病子代和感病子代的 RNA 序列，找出與抗白粉病相關的基因座可能位於第 6、15 及 16 條染色體上。而使用 HISAT2 和 Kallisto 兩種方法分析抗感病相關差異表現基因，總共發現 1687 個差異表現基因在兩種方法間的分析上都被認為與白粉病抗性高度相關。另根據連鎖不平衡區間分析，在第 6、15 及 16 條染色體上選擇單核苷酸多態性以設計等位基因專一性引子對。結果發現第 6 及 15 條染色體的等位基因專一性引子對無法區分抗感病基因型，唯有第 16 條染色體上的單核苷酸多態性可區分抗感病基因型，且確率達為 82%，與前人文獻提及的 SSR 引子對 Satt431 相近。研究結果發現台灣大豆品種「高雄 11 號」上抗白粉病抗性主要由第 16 號染色體所調控。本篇研究亦設計了可以預測「高雄 11 號」抗病表現型之等位基因專一性引子對，期許可以應用於「高雄 11 號」作為親本之大豆抗病品種的育成。

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Chapter 1. Introduction

1.1 The Evolution and Pathogenicity of Powdery Mildew (PM) in Plants

PM is a plant disease caused by various obligate biotrophic fungal pathogens in the order Erysiphales, and PM can be found on more than 9000 plant species (Takamatsu 2013). Although different fungi have been identified as PM pathogens, the infection process remains similar across PM fungi. The primary infection occurs through the dissemination of ascospores and conidia, which are produced by cleistothecia and conidiophores, respectively. These inocula are spread by wind and they require water for germination. Once plants are severely infected, leaves would be covered by conidia and mycelia, leading to the appearance of white and powdery-like symptoms that named this disease (Chen 2003).

To identify the causal fungal species from different plants, researchers used to rely on the morphology of asci appendages, cleistothecia, and conidiophores. More recently, molecular identification using ITS sequence has become the predominant evidence (Alvaro 2008). These approaches have increased the discovery of a growing number of PM fungi, which have been suggested to have a long evolutionary history with plants dating back to the late Cretaceous period. The molecular clock of 28S rDNA supported that PM fungi were initially associated with gymnosperms and later splited to angiosperms.

PM fungi were suggested to adapt to herbaceous plants, resulting in the evolution of a new clade (Takamatsu 2013). Among the herbaceous plants, several important crops such as cucumber, grapevines, soybean, and wheat were reported to suffer from severe yield losses (Huang and Röder 2004; Imre 2013; Kunova et al. 2021; Kusch and Panstruga 2017; McTaggart et al. 2012; Pietrusinska and Tratwal 2020). Therefore,

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studies to develop effective PM-controlling methods or breeding for PM-resistant cultivars are desired (Heffer et al. 2006).

1.2 Fungicide Management for PM on Different Plants

Fungicides targeting sterol biosynthesis, amino acid synthesis, protein synthesis, or nucleic acid metabolism could be applied for PM management. However, studies have pointed to the emergence of fungicide-resistant isolates. The most used fungicides in soybean fields in Australia and the US are prothioconazole and tebuconazole (Dunn and Gaynor 2020). These fungicides belong to the triazole group with the mode of action as demethylation inhibitors (DMIs), which block the biosynthesis of fungal sterols (Petit et al. 2012; Swoboda and Pedersen 2009). The blocking target of these fungicides is the C14-demethylase (Erg11/Cyp51), and numerous studies had reported field-developed mutations on this gene to enhance DMI resistance in PM fungi. The barley PM fungus *Blumeria graminis* f. sp. *hordei*, the grapevine PM fungus *Erysiphe necator*, and the wheat PM fungus *Blumeria graminis* f. sp. *tritici* were all reported to have DMI-resistant isolates (Parker et al. 2014). In a severe case reported in 2013, only 18% of the PM disease on wheat seeds was controlled using DMI fungicide (Vielba-Fernández et al. 2020). Accordingly, the concerns of field-developed fungicide resistance should be noticed and alternative management strategies need to be considered.

1.3 PM Resistance Genes or Loci on Different Plants

Another strategy for PM management relies on PM-resistant varieties. PM-resistant genes can be generalized into the dominant resistance gene (*R* gene) and the recessive mildew resistance locus O (*mlo*). For the former, *R* gene-associated resistance is one common strategy to control PM (Jørgensen and Wolfe 1994), and *R* genes can effectively protect the host from pathogen invasion by recognizing pathogen effectors to trigger plant

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immunity (Jones and Dangl 2006). In wheat, over 100 *R* genes or *R* alleles were shown to be associated with PM resistance (Sun et al. 2022). For example, *Pm* genes such as *Pm4b* and *Pm4c* exhibit high PM resistance and they were used commonly in the wheat varieties of China and the US for years (Li et al. 2022; Sun et al. 2022).

Another type of resistant gene is the *mlo*. In some crops such as barley and pea, PM can be prevented by the *loss-of-function* mutations on the susceptible MLO locus, which in terms to enhance PM resistance. Different from the most *R* gene, *mlo* is a none race-specific resistance locus. PM infection on plants with *mlo* genotypes will be interrupted by the formation of papillae in the epidermal cell wall. The *mlo* resistance has been found to protect different plants against the vast majority of PM and it has also provided a durable resistance for decades (Czembor and Czembor 2021; Kusch and Panstruga 2017).

1.4 PM of Soybean (*Glycine max*) in Taiwan

Microsphaera diffusa is a known pathogen causing PM not only on soybean, but also on papaya and other legume species (McTaggart et al. 2012; Seress et al. 2021). The fungus may also survive on volunteer legumes and re-infecting soybean in the subsequent planting seasons (Dunn and Gaynor 2020). There are disease reports from Brazil, China, and the US to show yield losses over 30% caused by soybean PM (Kusch and Panstruga 2017; Zhou et al. 2022). In Taiwan, soybean production has been grappling with PM for two decades since the first report in 2003 (Chen 2003). Soybean production in Taiwan focuses on harvesting the R6 stage pods (edamame) and seeds. According to the annual report of the Council of Agriculture (CoA), soybean production had increased from 104 to 419 metric tons in 2011 to 2022. While soybean fields are gradually expanded in Taiwan, the production of edamame remains the primary focus because it is significantly profitable as an export crop with an annual export volume about 35,000 metric tons and economic value about NTD 2 billion. In contrast, exports of soybean seeds are minimal

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while imports reached as high as 2,746 metric tons in 2022. According to the annual CoA report, the expenditure on soybean imports exceeds 40 billion annually. Therefore, self-sufficiency in soybean seeds has become a major policy in Taiwan to expand soybean cultivation.

While Kaohsiung and Pingtung remain the mono-culture regions for edamame cultivation, soybean is recommended to be rotated with rice in Taoyuan and Tainan to reduce drought impacts (Lin 2021). Soybean planted in spring (March to June) can reduce water consumption, while rice can be planted in fall (July to November) after the precipitation brought in by typhoons. Although the rotation strategy addresses drought conditions, soybean cultivation in spring faces a higher risk of PM due to the cooler temperature. Therefore, disease management for soybean PM in cooler regions during spring such as Taoyuan becomes a new challenge.

Common PM management method in Taiwan relies on the application of fungicides. The fungicides recommended by the plant protection information system were fluxapyroxad+ pyraclostrobin, hexaconazole, and tridemorph. These fungicides belong to the methoxycarbamates, morpholines, pyridinylethylbenzamides, and triazoles group, respectively, and fungicides within these groups mainly inhibits the respiration pathway and the biosynthesis of sterol in the membrane (Vielba-Fernández et al. 2020). Although PM could be managed by these fungicides, the concerns of field-developed fungicide resistance should be noticed. Therefore, alternative management strategies such as the implementation of disease resistance in soybean varieties need to be considered for sustainable agriculture.

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1.5 PM Resistance in Soybean

At present, the soybean PM resistance genes are all directed to the *Rmd* locus on Chr16. There are three allelic types, *Rmd-c*, *Rmd*, and *rmd*, which exhibit the full life resistance, adult plant resistance, and susceptibility, respectively (Jiang et al. 2019; Kang and Mian 2010; Ramalingam et al. 2020). Many studies have focused on the US varieties such as 'Williams', 'Williams 82' and 'CNS', and the results found that the *Rmd* locus is linked to the upstream *Rps2* and downstream *Rj2* loci, which affect the occurrence of Phytophthora blight and root nodule formation (Ramalingam et al. 2020). It has been suggested that there are 17 PM-resistance genes at the *Rmd* locus, including Glyma.16G213700 to Glyma.16G215400 (except for Glyma.16G214400 which putatively encodes an exocyst subunit exo70 family protein). Instead, all the others were annotated as the toll-interleukin receptor (TIR)-nucleotide-binding site (NBS)-leucine-rich repeat (LRR) domains (TIR-NBS-LRR) proteins. Nine of them (Glyma.16G213900, Glyma.16G214200, Glyma.16G214300, Glyma.16G214500, Glyma.16G214800, Glyma.16G214900, Glyma.16G215100, Glyma.16G215200, and Glyma.16G215300) were related to PM resistance based on the differences in expression level between resistance and susceptible cultivars in this article (Jiang et al. 2019). Among these 9 genes, Glyma.16G214300 and Glyma.16G214500 were the two that received intensive interest because of the significant change in expression levels by PM (Zhou et al. 2022).

The *Rmd* locus was first identified using the single sequence repeat (SSR) marker including Satt431, Sat_366, and Sat_393, and these markers can be used for selecting progenies with the *Rmd* locus. (Jiang et al. 2019). As soybean takes an important place in export for countries like Brazil and the US, PM-resistant cultivars such as 'CNS', PI 243540 have been used as donor parents in resistance breeding (Kang and Mian 2010). However, current studies are mainly based on the US varieties, and there are no resistant loci or molecular markers for breeding PM resistance in the soybean varieties of Taiwan.

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Accordingly, studies on PM resistance in the soybean varieties of Taiwan would provide advanced understanding to compare the genetic diversity of PM resistance.

1.6 Strategies on Genetic Mapping for Disease Resistance

While marker-assisted selection (MAS) before planting can shorten the labor and time, a prerequisite to achieve this goal lies in the discovery of the phenotype-associated markers and quantitative trait loci (QTL). QTL are genomic regions associated with a phenotype, and interval mapping is a method for finding QTL associated between phenotypes and genotypes (Takagi et al. 2013). In order to obtain genotypes such as single nucleotide polymorphisms (SNPs), DNA or RNA sequencing can be applied. Normally, larger sample size can increase the credibility of the results, but it comes with cost on labor and time for preparing the crossing population.

One alternative method for minimizing the sample size and sequencing cost is the bulk-segregant analysis (BSA) (Wu et al. 2019). BSA was proposed to sample offspring with extreme phenotypes, especially for qualitative phenotypes such as rust resistance. For qualitative phenotypes, there will be two extreme ends such as resistance and susceptibility. Since BSA only takes the two most extreme populations, it is therefore cost-effective and easier to study the offspring populations. BSA can magnify the differences between the two phenotypes, meanwhile avoid redundant information for ambiguous or intermediate phenotypes. In addition to BSA, a modified approach named BSR-Seq, which utilizes RNA sequences instead of DNA sequences, can be performed to provide insights of gene expressions simultaneously (Mansfeld and Grumet 2018).

In many studies, including waterlogging tolerance in maize, PM and stem rust resistance in melon, as well as leaf rust, stem rust, and PM resistance in wheat, the QTLs associated with these traits were identified by BSA or BSR-Seq (Zou et al. 2016; Edae and Rouse 2019; Du et al. 2017; Zhu et al. 2020; Cao et al. 2021; Wu et al. 2018; Pandey

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et al. 2017; Takagi et al. 2013). For example, BSR-Seq was applied on maize to detect SNPs associated with waterlogging tolerance. In this case, RNA samples from a total of 20 inbred lines including sensitive and tolerant plants were used for BSA, and 128 SNPs were found to be associated with waterlogging tolerance. Integrating the information from DEG analysis and BSR-Seq, 18 SNPs and 18 genes were identified with potentials to be designed as markers for further use (Du et al. 2017). Another example using BSR-Seq to identify the SNPs linked to the PM resistance gene *Pm4b* in wheat. A total of 50 homozygous plants in each bulk were used for BSR-Seq. There were 283,866 SNPs and Indels found between the R bulks and S bulks, and 4 SNP-based markers flanking the *pm4b* gene were developed, and these markers have been used in the MAS (Wu et al. 2018). These examples showed that BSR-Seq is a useful tool for detecting SNPs, Indels, and DEGs simultaneously.

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1.7 PM-Resistant Soybean Variety 'Kaohsiung 11 (KH11)' of Taiwan

This study aims to study PM resistance locus on soybean in Taiwan by applying BSR-Seq on progenies of PM-resistant and PM-susceptible cultivars. The parents selected for this purpose were 'KH11' and 'HL1'. 'KH11' was developed by Kaohsiung District Agricultural Research and Extension Station in 2012 through the hybridization of 'Hsiang Chi' (香姬) × 'KVS1329'. It was first bred for edamame production with higher yields, and it showed higher resistance to PM, root rot, downy mildew, and anthracnose. The disadvantage of this cultivar was the appearance of purple spots while encountering late harvesting and low temperature (Chou 2015). These disadvantages make it not suitable for soybean production in Taoyuan. On the other hand, 'HL1' was developed by Hualien District Agricultural Research and Extension Station in 1979 by the hybridization of 'E32' × 'Wakajima'. The main characteristics were the resistance to lodging, the high protein content, and the high photosynthesis rate in low-light environments which was more suitable than 'KH11' for soybean production in Taoyuan in spring (Chen 2015). But 'HL1' is susceptible to PM, therefore, the application of BSR-Seq to study PM-resistance QTL in 'KH11' may facilitate the breeding of a new cultivar.

1.8 Principle of the Allele-Specific Polymerase Chain Reaction (AS-PCR)

AS-PCR is a method for direct distinguishing different genotypes in the PCR-amplified DNA sequences. This method is performed with normal thermal cycler protocol and contains two fluorescent-labeled universal probes (UPs), two non-labeled allele-specific primers (ASPs), and a non-labeled common reverse primer. The two ASPs differed in the 5’ UP-associated tails are recognized by the respective UPs based on the complement sequences and then excite two different fluorescences (e.g. HEX and FAM). This method has been used in resistant cultivar detection in different plants, such as maize, potato, and tea plants. (Chen et al. 2022; Cao et al. 2021; Fan et al. 2022; Kante et al.

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2021; Worthington et al. 2021). For example, the application of AS-PCR on potatoes differentiated the *Potato virus Y* (PVY) resistance genotypes (Kante et al. 2021). In this study, researchers amplified the sequence linking to the PVY resistance gene (*Ryadg* gene) and found the SNPs between resistant and susceptible cultivars. Based on the SNPs, nine AS-PCR markers were designed and two of them had 100 % concordance with the M6P2 high-resolution melting assay, which was developed by a previous study for PVY resistance gene identification. This example showed the feasibility of using AS-PCR marker for SNP and genotype differentiation.

1.9 Research Objectives

In facing unpredictable drought stress at Taoyuan, or even throughout Taiwan, in spring, the replacement of rice with soybean production can be helpful. The conversion in the rotation mode avoided the drought stress but increased the PM occurrence (Lin 2021). Previous literature have supported the use of PM-resistant soybean cultivars can effectively decrease the PM severity, however, there is no mapping studies regarding the PM-resistant soybean cultivars of Taiwan. To fill in this knowledge gap, the primary task of this study aimed to identify QTL associated with PM resistance of 'KH11' by applying BSR-Seq. Meanwhile, candidate resistance genes will be identified within the QTL considering their expression patterns. The identification of SNPs, Indels, and DEGs linked to PM resistance would also facilitate the design of ASP that can be used in MAS for breeding resistant soybean cultivars in Taiwan, improving the management of soybean PM, and promoting self-sufficiency in soybean seed production.

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Chapter 2. Materials and Methods

2.1 Plant Materials

The F_2 and F_3 populations were developed by crossing the PM-resistant variety 'KH11' and the PM-susceptible variety 'HL1'. F_2 populations were planted on August 1 2021, and the F_3 populations were planted on March 14 2022. All plant materials were placed in the greenhouse at Taoyuan District Agricultural Research and Extension Station. The cultivation method involved mechanical land preparation, ridge cultivation, double-row planting, and manual single-seed sowing. The row spacing was set at 50 cm x 10 cm, and the susceptible parent 'HL1' was evenly distributed as spreader rows. Standard agricultural practices were applied, while emamectin benzoate and clothianidin were applied for whitefly and *Spodoptera litura* management. No disease management was applied. The fertilizer application rate was N:P_2O_5:K_2O, at 20:60:60 kg/ha. P_2O_5 and K_2O fertilizers were used as the basal application, while N fertilizer was applied at half the dosage 20 days and 40 days after sowing. The water supply was facilitated through furrow irrigation three times in total: once after sowing, once at the onset of flowering (R1 stage), and once during pod filling (R5 stage). The total amount of water used for irrigation ranged from 660 to 990 m^3 ha^{-1}.

2.2 F_3 Population Phenotyping, RNA Preparation, and Sequencing

The phenotypic analysis for F_3 populations was carried out in fields on May 3 2022, while the PM symptoms on F_3 populations occurred naturally about 2 months after sowing. To determine the disease severity of the F_2 individual, putative phenotypes were traced back by the phenotypes of F_3 populations (Table 1). For F_2 lines with fully susceptible or fully resistant F_3 progenies, these F_2 and its derived F_3 were regarded as homozygous lines. And these F_3 samples were separated into R bulks and S bulks. A total

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of 10 F_3 samples were collected for each bulk, and there were three R bulks and three S bulks included in this study.

The leaf samples were collected in fields and fast-cleaned with water to remove any external dust. Subsequently, the cleaned leaf samples were excised from the plant and immediately frozen using dry ice to avoid RNA degradation. The leaf samples were brought back to lab for RNA extraction, and the frozen samples were ground into fine powders using liquid nitrogen and combined with 1 mL Invitrogen™ TRIzol™ reagent (ThermoFisher Scientific, Waltham, MA, USA) in 1.5 ml tubes. The TRIzol™ solution was then centrifuged at 24,000g for 1 minute, and the resulting supernatant was collected into new 1.5 ml tubes. The supernatant went through a two-step clean-up using 200 µl chloroform followed by centrifugation at 24,000g for 10 minutes in a refrigerated centrifuge set at 4°C. This step was repeated to ensure proper separation, followed by adding an equal volume of isopropanol to the supernatants and mixing thoroughly. The mixture was set on ice for 10 minutes before undergoing another round of centrifugation at 24,000g in a refrigerated centrifuge set at 4°C. The resulting pellet from the previous step was washed twice with 75% alcohol and then resolved in 25 µl ddH_2O. Ten samples with the same concentration of each bulk were pooled and treated with 8M lithium chloride (Merck KGaA, Darmstadt, Germany). Each 60 µl sample with 15,000 ng RNA was mixed with 30 µl 8M lithium chloride and precipitated at -20^{o}C for 30 minutes before the centrifugation at 14,000g for 15 minutes. Removed the supernatant and resuspended the pellet with 1 ml 75% alcohol and centrifugated at 14,000g for 15 minutes. This step was repeated for proper cleaning. The pallet was then resolved in 100 µl ddH_2O.

A total of 12 RNA-Seq samples for R bulk, S bulk, 'KH11', and 'HL1' (3 replicates for each) were prepared. The qualities of these 12 RNA-Seq samples were assessed through electrophoretic analysis and Nanodrop One (ThermoFisher Scientific).

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Subsequently, the quality of the RNA samples was checked by QSep 100 (Bioptic, New Taipei City, Taiwan), and the concentration of samples was ranging between 146.5 to 237.6 ng/μl. All samples were sequenced using the Illumina Novaseq 6000 platform with pair-end sequencing of 150 bp length (Biotools, New Taipei City, Taiwan).

2.3 Bulk Segregation Analysis (BSA)

After getting the sequence data, the quality assessment of the sequencing reads was performed using FastQC. The sequences were aligned to the reference genome of the soybean cultivar 'Williams 82' genome assembly a2.v1 using BWA software v0.7.17 With default settings. After alignment, the resulting Sequence Alignment Map (SAM) files underwent several normalization steps using SAMtools v1.13 (Li et al. 2009). The files were converted into Binary Alignment Map (BAM) format and sorted by the read names. To ensure accurate information without incorrect duplicate sequences produced in the Illumina sequencing step, the sorted files were processed using SAMtools function ‘fixmate’ to fill mate coordinates. Based on the mate score tags, duplicate sequences were identified and removed using SAMtools function ‘markdup’. The final output files from SAMtools were processed by Picard v2.26.0, which added read groups using ‘AddOrReplaceReadGroups’ before running GATK for variant calling.

For variant calling, GATK v3.8.1.0 function ‘UnifiedGenotyper’ was employed with default settings (Bathke and Lühken 2021). The resulting variants were further filtered using GATK function ‘FilterVariants’ to exclude those with quality scores less than 20. The output files were then processed by GATK function ‘VariantsToTable’ to extract essential information for subsequent analysis in R environment v4.3.0. The necessary information from the VCF file included chromosome ID, SNP position, required genotype field, allele depth, depth, reference allele, alternative allele, phred-scaled likelihood, and genotype quality.

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After the extraction of the necessary information in the VCF file, the R package 'QTLseqr' v0.7.5.2 (Mansfeld and Grumet 2018) was utilized for BSA with default settings, except for specifying a bulk size of 30 and designating F_2 as the population structure. The SNPs on Chr01 to Chr20 in the R bulk and S bulk were compared by calculating the Δ(SNP-index) and the outlier regions were excluded based on Hampel's rule. The thresholds for QTL output were set to 95% confidence intervals and the *q*-value at 0.01.

2.4 Differentially Expressed Genes (DEG) Analysis

For the analysis of DEGs, the alignment was performed in two different ways, one is the workflow including HISAT2 v2.2.1 (Pertea et al. 2016), SAMtools, and Subread v2.0.0 with DESeq2 v1.38.1 (Love et al. 2014), and the other is the workflow including Kallisto v0.46.2 with Sleuth version0.30 (Kim et al. 2019; Li et al. 2009; Liao et al. 2014; Pimental et al.).

For the HISAT2 pipeline, an index for alignment was constructed using the genome and annotation files of the soybean cultivar 'Williams 82' a2.v1. The functions of 'hisat2_extract_splice_sites' and 'hisat2_extract_exons' were utilized to extract splice sites and exons from the annotation files. The splicing sites and exons were then built with the reference genome using the function 'hisat2_build' into an index for further analysis. The RNA-seq files were aligned to the generated index using 'hisat2' with the default settings. Following the alignment, the files from HISAT2 underwent several normalization steps using SAMtools v1.13 (Li et al. 2009). Subsequently, the function 'featureCounts' from Subread was employed to calculate the number of reads aligned to each gene in the genome annotation using default settings. The final output files were text files containing information such as gene ID, chromosome, position, and read counts. All the individual sample files were combined into one file, and only the gene ID and read

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count information were selected for further analysis using the ‘DESeq2’ package in R environment. The read counts obtained from ‘featureCounts’ were transformed into a 'dds matrix' using the ‘DESeqDataSetFromMatrix’ function. The 'dds matrix' was filtered based on the condition that the total counts in a row were equal to or greater than 6 counts. The comparison between the R bulk and S bulk was adjusted with a significance threshold of adjusted *p*- value at 0.05.

For the Kallisto pipeline, the annotation and transcript file of 'Williams 82' a2.v1 reference genome were used for making an index using Kallisto function ‘index’. The 12 RNA-Seq data were then aligned to the index with Kallisto function ‘quant’ with the default settings. The DEGs between R bulk and S bulk were later found based on Wald test with q-value ≤ 0.05 setting with the ‘Sleuth’ package v0.30.1 (Pimental et al.). In order to increase the credibility of the results, the read counts from Kallisto were then compared with the read counts from HISAT2 using Pearson’s correlation.

2.5 Linkage disequilibrium (LD) analysis using the SoySNP50K

LD analysis was applied to identify genomic regions harboring candidate genes, and the SoySNP50K dataset with genotypes of 20,087 cultivars was downloaded from Soybase (Song et al. 2013). The regions chosen for LD mapping were based on the QTL regions on Chr06 and Chr15. As for Chr16, a comprehensive range from position 29,537,679 to 37,675,335 bp on Chr16 was selected to encompass QTL regions discovered in this study as well as in the previous literatures of soybean PM resistance (Jiang et al. 2019). The LD analysis was applied with TASSEL software v5.0 (Bradbury et al. 2007). Within these regions, the default function of 'full matrix' was applied, which means the association between every two SNPs was calculated. The analysis accounted for ‘heterozygous calls’ was set as the default setting ‘Set to missing’. Subsequently, the

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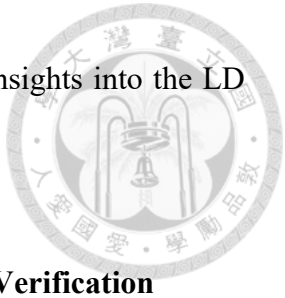
LD map based on the filtered genotypes was generated, providing insights into the LD blcoks within the QTL regions.



2.6 F_2 Population Collection, AS-PCR Primer (ASP) Design and Verification

On October 29 2021, the collection of F_2 population was applied. Leaf samples were cleaned with water to remove external dusts before collection. The leaf samples were frozen with dry ice and grind at 4,000 rpm for 1 minute by the homogenizer Prep-CB24 (Medclub, Taoyuan, Taiwan). The following DNA extraction protocol was completed with genomic DNA mint kit (plant) and followed the protocol (Geneaid, New Taipei City, Taiwan). A total of 249 samples were prepared for further verification of the AS-PCR markers and SSR markers.

For the allele-specific primer (ASP) design, one indel on Chr06, one SNP on Chr15 and one SNP on Chr16 were selected, which included the ASP_Gm06 (TTG/T), ASP_Gm15 (T/A), and ASP_Gm16 (T/C), respectively. In addition, three ASPs on Chr16, ASP_Gm16_front (G/A), ASP_Gm16_mid (C/T) and ASP_Gm16_back (A/G) were designed to verify the left and right border of LD block in the range from position 30,438,017 to 37,653,674 bp on Chr16. Previous studies had shown the increase of specificity by setting a single mismatch near the end of the ASPs (Liu et al. 2012), therefore, besides the SNPs at the end of the forward non-labeled ASPs, a single allele mismatch at the 3rd, 4th, or 5th bp from the 3' end was introduced to improve the specificity (Table 2).

The specificity of the primers with or without the mismatch was further verified with 'KH11', 'HL1', and heterozygous sample, in which the heterozygous sample was the DNA mixture of 'KH11' and 'HL1' samples. The ASPs with the highest specificity were chosen for downstream AS-PCR assay. Non-labeled primers and the UPs were synthesized using oligonucleotide purification cartridge and polyacrylamide gel

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electrophoresis for purification, respectively (Genomics, New Taipei City, Taiwan). To perform the AS-PCR assay, the reaction solution consisted of 1 μl ASPs mix (1 μM/μl), 7.05 μl ddH_2O, 1 μl DNA (10~50 ng/μl), 0.45 μl FAM & HEX UPs (4 μM/μl), 0.5 μl reverse primer (15 μM/μl), and 10 μl TOOLS Easy 2xProbe qPCR Mix (Biotools). The AS-PCR reaction was amplified using the CFX96 Real-Time PCR Systems (BIO-RAD, Hercules, CA, USA). The qPCR conditions were optimized for each primer set and tested using 'KH11' and 'HL1' (Figure 1).

To evaluate the accuracy and applicability of the ASPs, we conducted marker tests using the F_2 populations with the putative phenotypes traced back by the phenotypes of F_3 populations. The phenotypes of F_2 populations were the percentage of diseased plants within a group of F_3 populations derived from an F_2 (Table 1). The phenotypes were integrated with the results of the ASP test. The Kruskal-Wallis test and the Dunn's test were performed to determine significance at 0.05.

In addition, 14 SSR markers in the Chr16 region (Gm16: 29,537,679 to 37,675,335 bp), including BARCSOYSSR_16_0878, BARCSOYSSR_16_1291, BARCSOYSSR_16_1294, Sat_366, Sat_393, Sat_395, Satt215, Satt431, Satt547, Satt622, and Satt712 were selected to analyze the LB blocks and the AS-PCR results. The SSR marker sequences were shown (Table 3) and the PCR conditions for SSR markers were optimized with the DNA of 'KH11' and 'HL1' (Figure 1). The PCR solution consisted of 7 μl ddH_2O, 1 μl DNA (10 ng/μl), 1 μl forward primer (10 μM/μl), 10 μl PowerAmp 2X PCRmix-Green (Bioman, New Taipei City, Taiwan), and 1 μl reverse primer (10 μM/μl).

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Chapter 3. Results

3.1 PM Resistance Evaluation of the F_3 Population

PM symptoms on the susceptible parent 'HL1' and F_3 populations were observed on May 3rd 2022, approximately two months after planting on March 4th 2022. A total of 1,151 F_3 plants derived from 249 F_2 plants were evaluated in this study (Figure 2). The phenotypic analysis revealed that out of the 249 F_2 plants, F_3 progenies derived from 153 F_2 were observed to be fully resistant to PM and from 42 F_2 were fully susceptible (Table 1). These F_2 plants were regarded as homozygous resistant lineages and homozygous susceptible lineages, and the rest 54 F_2 plants were regarded as heterozygous lineages.

3.2 BSR-Seq Analysis of the F_3 Population

Twelve RNA libraries, with three biological replicates in each of the 'KH11', 'HL1', R bulk, and S bulk were subjected to BSR-Seq using Illumina sequencing platform. A total of 169,690,907, 187,508,668, 167,002,064, and 210,303,096 raw reads for 'KH11', 'HL1', R bulk, and S bulk library were generated, respectively. The GC content ranged from 40% to 43% and the average quality score stayed in 35 to 36. Mapping rates 99.83%, 99.23%, 99.80%, and 99.44% of each were mapped to the reference genome for 'KH11', 'HL1', R bulk, and S bulk, respectively. The mapping rate of twelve RNA-Seq data aligned to the reference genome of 'Williams 82' ranged from 85.49 % to 87.41 % (Table 8). To understand the homogeneity between replicates and the heterogeneity between different groups, principal component analysis (PCA) was performed. PC1 and PC2 explained 52.3% and 12.6% of the variation, respectively (Figure 3A). The PCA results demonstrated that the three replicates of the same samples ('KH11', 'HL1', R bulk, and S bulk) were grouped together, supporting the credibility of samples.

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By analyzing the Δ(SNP-index), which measures the allele frequency differences between resistant and susceptible bulks, 528 SNPs and 83 Indels passed the 95% confidence threshold, and 299 SNPs and 33 Indels passed the more stringent 99% confidence threshold (Figure 4). The SNPs and Indels that passed the 99% confidence threshold were primarily distributed on the Chr01, Chr06, Chr12, Chr13, Chr15, Chr16, Chr17, and Chr18, indicating their potential association with PM resistance. Additionally, for the analysis of G' value, which assesses the genetic differentiation between resistant and susceptible bulks, identified 4,105 SNPs and 629 Indels passing the filter (*q*-value<0.01). Notably, the most significant SNPs and Indels were concentrated on the Chr06, Chr15, and Chr16 (Figure 4), suggesting these chromosomes may harbor crucial genetic variants associated with PM resistance.

Through a comprehensive analysis incorporating Δ(SNP-index) and G' value, a total of 528 candidate SNPs and 83 indels were identified as differentially distributed between the resistant and susceptible bulks (Table 4). Three chromosomes, Chr06, Chr15, and Chr16 beacme the focus of research due to their higher G' values. These chromosomes collectively contained 44, 104, and 70 SNPs and 8, 14, and 10 Indels, respectively (Table 5, Table 6, and Table 7).

3.3 Differentially expressed gene (DEG) & Linkage disequilibrium (LD) Analysis

A total of 36,423 transcripts were identified using HISAT2, while 15,810 and 2,119 DEGs were found between the comparison of the two parents and the two bulks of progenies (*p*-value<0.05). Those 1907 DEGs overlapped between the comparison of the two parents and the two bulks were focused (Figure 5). As for Kallisto analysis pipeline, the alignment rate of the 12 RNA-Seq datasets ranged from 75.5% to 79.8% (Table 8). The PCA results explained 43.5% and 12.2% for PC1 and PC2 of the variation (Figure 3B), respectively. A total of 36,929 transcripts were detected by Kallisto, and 12,809 and

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7,085 DEGs were identified within the comparison of the two parents and the two bulks of progenies (Wald test, q-value ≤ 0.05). 4,269 DEGs overlapped between the two groups were focused and compared to the results from DESeq2 (Figure 5).

The correlation analysis of read counts showed a high correlation between each sample from Kallisto and HISAT2, with R^2 values ranging from 0.85 to 0.95 (Figure 6). Additionally, the correlation analysis of $\log_2$ fold change ($\log_2$ FC) values between the 36,929 mapped transcripts from Kallisto and the 36,423 mapped transcripts from HISAT2 showed an R^2 value of 0.69 (Figure 7). Focusing on the 1,687 DEGs identified by both Sleuth and DEGseq2 (Figure 8), a R^2 value of 0.98 was shown (Figure 7), indicating the correlation of the significant DEGs found between the two analysis methods was better than the expression levels of transcripts.

Within the 1687 DEGs, 97, 71, and 88 DEGs were found on the Chr06, Chr15, and Chr16, respectively. The DEGs within the QTL region on the Chr06, Chr15, and Chr16 were focused (Table 9). On the other hand, 34 of the 1687 DEGs were annotated as disease-resistance genes, in which 16 belonged to the TIR-NBS-LRR protein family. Two of the 16 DEGs, Glyma.16G213700 and Glyma.16G215100 were up-regulated in resistance cultivars, and the others were down-regulated. In addition, within the 1687 DEGs, four were annotated as seven transmembrane MLO family proteins, which was mentioned to decrease the resistance against PM while up-regulated in previous studies (Czembor and Czembor 2021; Kusch and Panstruga 2017, Santos et al. 2020). Three of the four MLO family DEGs, Glyma.02G063900, Glyma.06G286800, and Glyma.16G145600 were down-regulated.

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3.4 Selection of SNPs or Indels for ASP Design

Based on the LD analysis applied for the region selected, SNPs or Indels within the same LD group as the candidate DEGs were chosen for ASP design. In Chr06, the QTL region displayed two LD groups (Figure 9), where the first group spanned from 12.6 to 13.0 Mbp while the second group spanned from 13.0 to 13.9 Mbp. The majority of the SNPs and Indels discovered belonged to the second group. Within the second group, Glyma.06G162400 was the DEG with the highest absolute $\log_2$ FC value, and the Indel closest to it was selected for marker design.

According to the LD analysis in Chr15, all SNPs from SoySNP50K within the QTL region exhibited a strong linkage (Figure 10), indicating that the genes and the SNPs within this region were likely associated with each other. Additionally, within the QTL region, Glyma.15G170500 with the highest absolute $\log_2$ FC value was found as candidate DEG, and the SNP closest to it was chosen for marker design.

In Chr16, an LD analysis was performed in the region encompassing the QTL region and the *Rmd* locus. Within the region, the LD analysis showed R^2 less than 0.3, indicating a lack of linkage between most pairs of SNPs from SoySNP50K in the QTL region and the *Rmd* locus (Figure 11), showing no additional information for SNP selection. Hence, within the QTL region, the SNP closest to the DEG with the highest $\log_2$ FC value, meaning Glyma.16G195600, was chosen for marker design. As the LD analysis using SoySNP50K showed insufficient information for understanding the association between different regions on Chr16, the LD analysis was simply carried out with SSR markers and another two ASP, ASP_Gm16_mid and ASP_Gm16_back.

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3.5 Verification of ASPs & SSR Markers with F_2 Population

With the SNPs or Indels selected for ASPs, the ASPs were designed and later verified with the F_2 populations (Table 2; Figure 10). The Kruskal-Wallis test and the Dunn's test showed that the phenotypic separation for F_2 samples with allele1 genotype ('KH11') of Chr06 was not able to be differentiated from heterozygous and allele2 genotypes ('HL1'), meaning the indels on Chr06 were not available for PM-resistance phenotype prediction (Figure 12A).

As for ASP_Gm15, the results showed the difference between phenotypes of samples with allele1 genotype ('KH11') and allele2 genotype ('HL1'), but no difference between the phenotypes of samples with allele2 and heterozygous genotypes, meaning the ASP_Gm15 was not available for PM-resistance phenotype prediction (Figure 12B).

For ASP_Gm16, the Dunn's test separated allele1 ('KH11'), allele2 ('HL1'), and heterozygous samples into different phenotypes, showing the association between the phenotypes and the genotypes (Figure 12C). As PM resistance was a qualitative trait, the samples with heterozygous and allele1 genotypes were predicted to be resistant phenotypes, and 82 % of the predicted phenotypes matched the phenotypes. This means that the location selected for ASP_Gm16 can be used to predict phenotypic PM resistance.

In addition, BARCSOYSSR_16_0878, BARCSOYSSR_16_1291, BARCSOYSSR_16_1294, Sat_093, Sat_366, Sat_393, Sat_395, Satt215, Satt547, Satt622, and Satt712 are not polymorphic to distinguish genotypes between 'KH11' and 'HL1' (Figure 13). Only, Satt431 was polymorphic between 'KH11' and 'HL1' and could differentiate between allele1, allele2, and heterozygous F_2 population samples (Figure 13). The genotypes were shown in Table 1. For Satt431, the Dunn's test showed a significant difference between resistant, susceptible, and heterozygous samples, and an 87 % accuracy rate comparing to the phenotypes was shown after the conversion of heterozygous and allele1 genotypes into resistant phenotypes (Figure 12D).

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3.6 The LD Analysis of the Selected Region on Chr16 by ASPs

As mentioned, the LD analysis using SoySNP50K on Chr16 showed that most pairs of SNPs were not associated. To confirm the association of SNPs on Chr16, an alternative analysis to improve the LD analysis should be done. Because most of the SSR markers within the region on Chr16 interested were not polymorphic between 'KH11' and 'HL1', they were not usable for LD analysis for a wider region on Chr16. Three ASPs were designed for LD verification. They were located before and after the QTL region (Chr16: 34,328,014- 36,704,575). The ASP located after the QTL region was located on Chr16: 37,653,674 (ASP_Gm16_back), which covered the QTL region and the 9 TIR-NBS-LRR disease-resistance genes with ASP_Gm16. This marker was selected to verify if the region from our QTL region to Chr16: 37,653,674 were all linked. The other two ASPs were located on Chr16: 30,438,017 (ASP_Gm16_front) and Chr16: 32,279,648 (ASP_Gm16_mid). ASP_Gm16_front was located before the *mlo* gene Glyma.16G145600 (Chr16: 30,652,747- 30,659,192) and ASP_Gm16_mid was located between the *mlo* gene and the QTL region. This helped us know if the *mlo* gene was linked to the QTL region.

Comparing the results of ASP_Gm16_front, ASP_Gm16_mid and ASP_Gm16_back with ASP_Gm16 (Table 1), the chi-square test of all markers showed p-value <0.01, and the chi-square statistic were 59.875, 136.06 and 155.15, respectively. This means that the recombination between ASP_Gm16_front, ASP_Gm16_mid, and ASP_Gm16_back didn't segregate in the F_2 population.

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Chapter 4. Discussion

Rice production in Taoyuan suffered from drought stress in the first season in recent years, the rotation mode was converted from rice to soybean production. Under certain circumstance, soybean production was facing an increase in the occurrence of PM due to the cooler climate in spring. Previous literature has supported the use of PM-resistant soybean cultivars can effectively decrease the severity of PM, however, there is no mapping studies regarding the PM-resistant soybean cultivars of Taiwan. Hence, the primary task of this study aimed to identify QTL associated with PM resistance of 'KH11' by applying BSR-Seq, and the results may facilitate the breeding of PM-resistant soybean cultivars.

BSR-Seq analysis was applied to identify PM-associated DEGs and QTLs in 'KH11'. The results revealed significant loci on Chr06, Chr15, and Chr16, with corresponding DEGs found within these chromosomes. Three candidate DEGs, Glyma.06G162400, Glyma.15G170500, and Glyma.16G195600 were further investigated. The annotation of Glyma.06G162400 has not been available, but the other two DEGs, Glyma.15G170500, and Glyma.16G195600 had been investigated in previous studies. Glyma.15G170500 was annotated as a basic helix-loop-helix (bHLH) DNA-binding family protein, and a previous study showed its function associated not only with plant growth and abiotic stresses including drought, salinity, cold, and iron deficiency (Guo et al. 2021), but also disease resistance against *Pseudomonas syringae* pv. *tomato* (Pst) on tomato (Zhang et al. 2022). In tomato, the expression of bHLH protein suppressed the expression of the defense gene Arabinogalactan protein 1 (Agp1) and increased the susceptibility to Pst. On the contrary, the down-regulation of bHLH protein enabled the expression of Arabinogalactan protein 1 (Agp1) in tomato and recovered the Pst resistance. As for Glyma.16G195600 which encodes the cytochrome P450 family protein, it has been

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associated with the biosynthesis of secondary metabolites, antioxidants, and phytohormones in plants. Additionally, it protected plants from drought, salt, insect, and disease infestations (Pandian et al. 2020). In soybean, GmCYP82A3, a gene included in the cytochrome P450 family was found to confer disease resistance. With the overexpression of GmCYP82A3, a significant decrease in the lesion size caused by *Phytophthora parasitica* was shown (Yan et al. 2016). These studies showed the probability of the association of Glyma.15G170500 and Glyma.16G195600 with disease resistance. However, the genotypes assessed with the marker sets on Chr06 and Chr15 did not match the phenotypes in F_2 populations. This means that the QTL regions on Chr06 and Chr15 were not PM-associated regions or the associations were minor. The uncertainty of QTL regions in Chr06 and Chr15 may be due to the small bulk size, which could significantly decrease the Δ(SNP-index) and affect the results. Conversely, significant differences were observed among the three genotypes using marker sets on Chr16, confirming the association of the Chr16 with PM resistance.

Previous studies on PM resistance indicated qualitative resistance with upregulated PM resistance-associated genes on Chr16 of the resistant cultivars (Jiang et al. 2019). However, in our study, only two DEGs belonging to disease-resistance genes were up-regulated in the resistance bulk, while the others were down-regulated. The up-regulated disease-resistance genes were Glyma.16G213700 and Glyma.16G215100 in 'KH11' and R bulks. The difference between the up-regulated genes in our study and previous studies may be due to different cultivars, or the difference in the sampling time. Previous studies conducted expression level tests at 0, 3, 12, 24, 48, and 72 hours post-inoculation, whereas this study did not verify expression levels at a specific post-inoculation time. A time course expression experiment together with comparisons among different resistant

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soybean varieties with *Rmd* locus would be required to understand the mechanisms affecting the expression levels of the candidate DEGs.

In addition, the LD analysis using ASP_Gm16_front, ASP_Gm16_back and ASP_Gm16 showed that the whole region (Chr16: 30,438,017-37,653,674) was linked after the hybridization of 'KH11' and 'HL1'. And the results of the LD analysis using ASP_Gm16_front, ASP_Gm16_mid and ASP_Gm16 also showed that the region (Chr16: 30,438,017- 34,328,014) did not segregate in our crossing population, meaning that the association between PM-resistance and the *mlo* gene located on 30 Mbp could not be excluded yet. Compared to the results of DEGs, these imply that the PM resistance-associated locus predominantly resides on Chr16 in the Taiwanese soybean cultivar, 'KH11', but the regulation by which resistance genes varied between cultivars such as 'KH11', 'BRSMG68', and 'PI 243540'. Future studies on the expressions of PM resistance-associated genes at different time points would be needed to uncover the resistance mechanisms among cultivars.

Overall, the results of this study highlighted the predominant association of PM resistance in the Taiwanese soybean cultivar, 'KH11' with the *Rmd* locus on Chr16. The development of a reliable ASP on Chr16 holds a potential to assist molecular breeding of PM-resistant soybean cultivars in Taiwan using 'KH11' as the PM-resistance parent.

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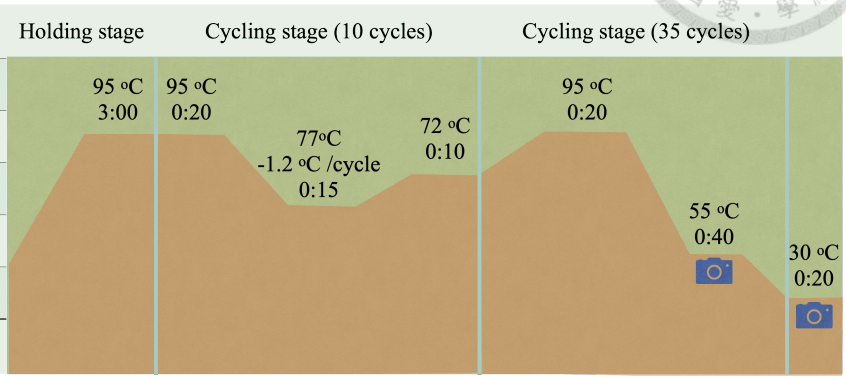
Figures

A

qPCR mix	10
F_1+F_2 (1 μM/ μL)	1
R (15 μM/ μL)	0.5
VIC+HEX (4 μM/μL)	0.45
DNA (25 ng/ μL)	1
H_2O	7.05
Total	20



B

PCR mix	10
F (10 μM/ μL)	1
R (10 μM/ μL)	1
DNA (20 ng/ μL)	1
H_2O	7
Total	20

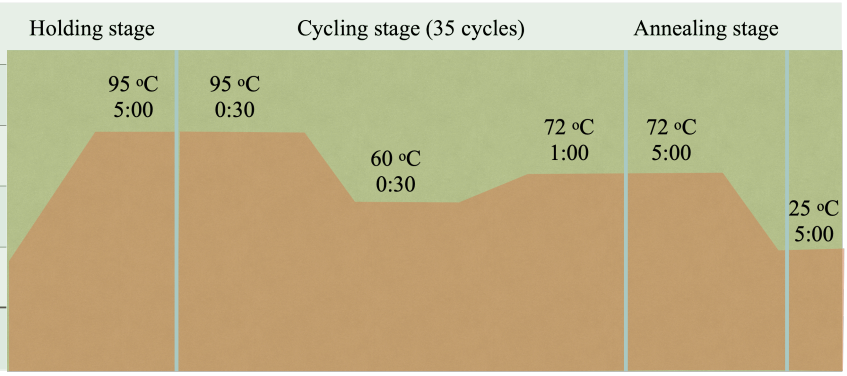


Figure 1. The PCR conditions for the ASPs and SSR markers. A. The PCR condition for ASPs markers, the camera represented the time for fluorescent detection, and the determination of genotype was based on the detection at 30°C.; B. The PCR condition for SSR markers.

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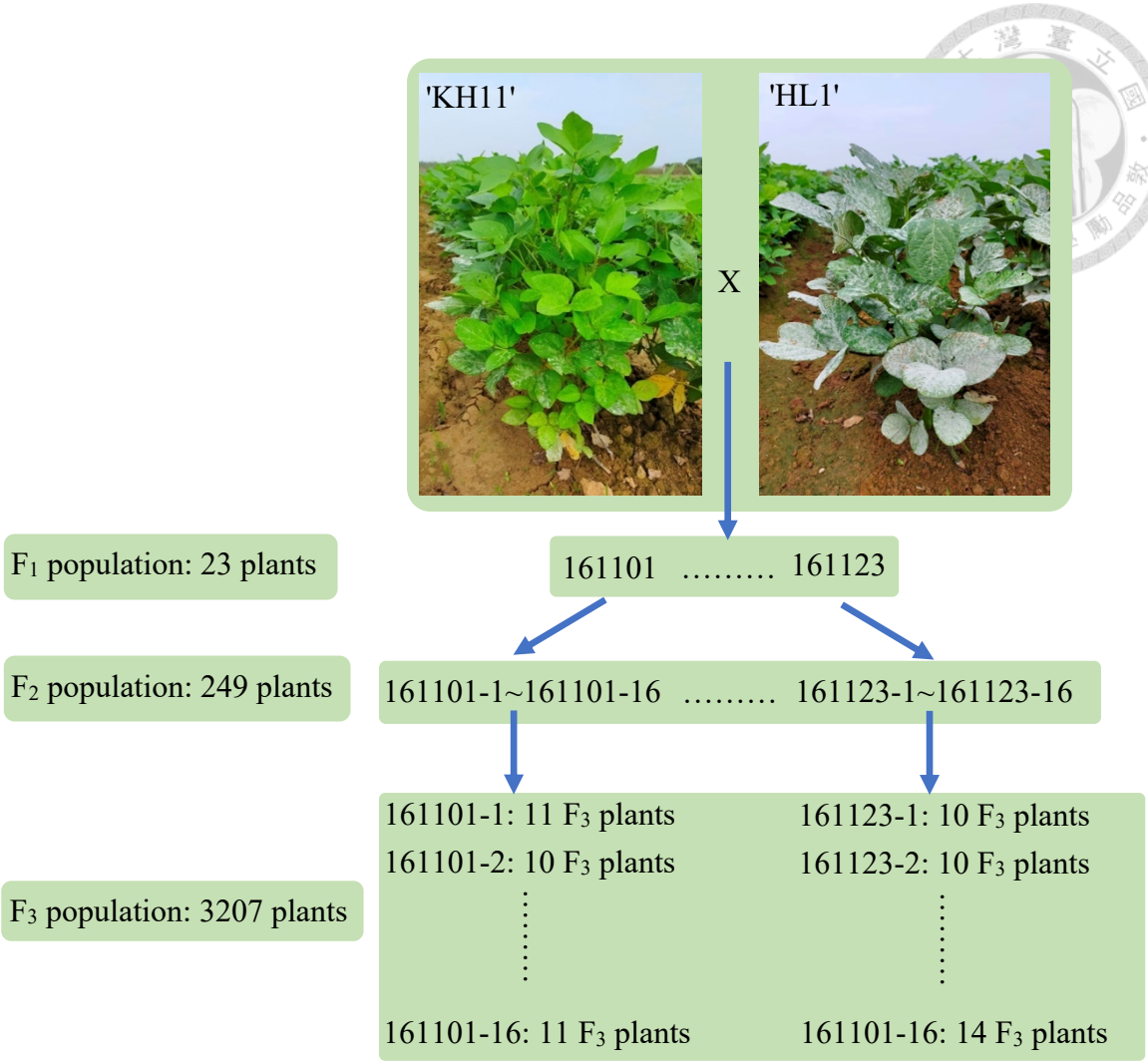

Figure 2. Progenies of the hybridization of 'KH11' and 'HL1'. The progenies were the hybridization of 'KH11' and 'HL1'. The pictures of the parents showed the differences between 'KH11' and 'HL1' against PM. For the progenies, 23, 249, and 3207 plants of F_1, F_2, and F_3 populations were generated.

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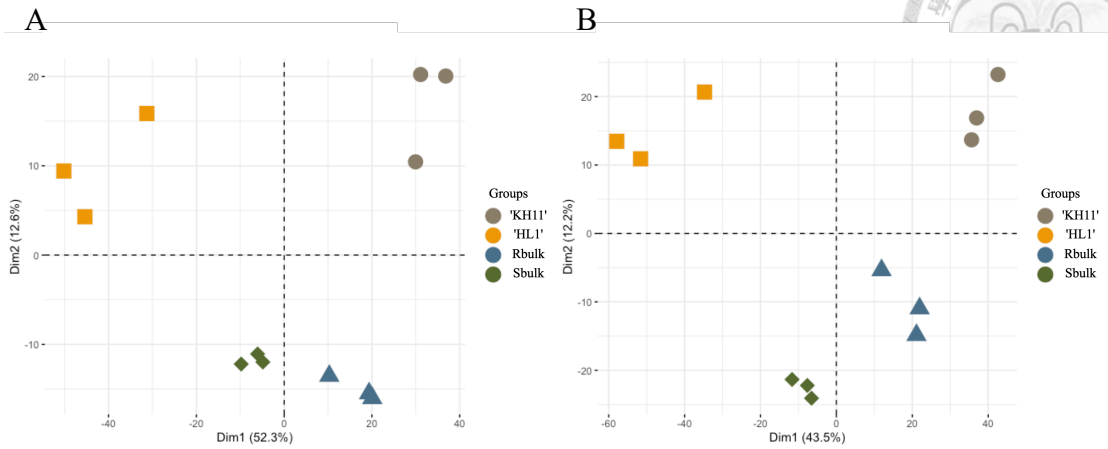

Figure 3. PCA analysis for RNA-Seq data. The PCA analysis for the RNA-Seq data aligned with HISAT2 and Kallisto showed the distribution of the twelve RNA samples. Twelve samples were separated into four groups in both analysis, which exactly corresponded to R bulk, S bulk, 'KH11', and 'HL1'. A. The PCA analysis for read counts from HISAT2, the PC1 and PC2 explained 52.3 % and 12.6 % of the variation.; B. The PCA analysis for read counts from Kallisto, the PC1 and PC2 explained 43.5 % and 12.2 % of the variation.

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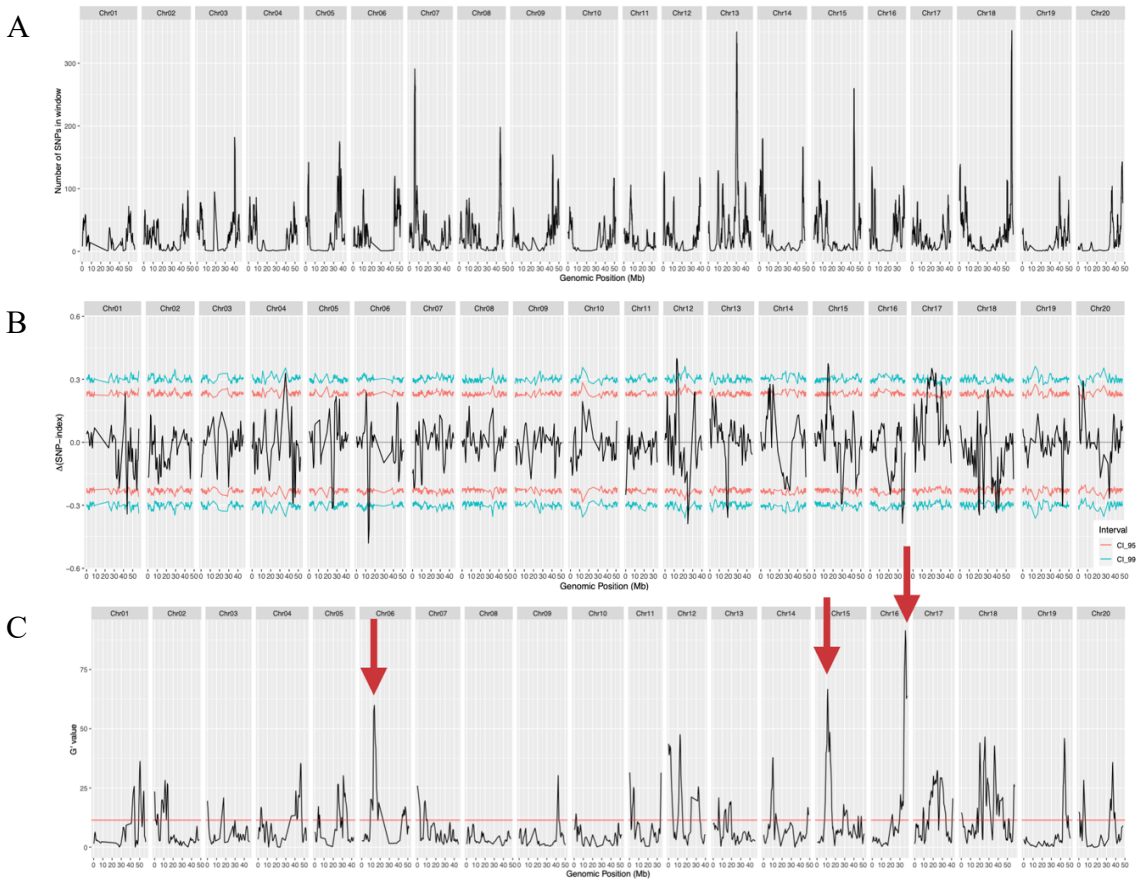

Figure 4. Quantitative trait locus (QTL) analysis plots. A. Numbers of SNPs per Mb in each chromosome; B. Smoothed Δ(SNP-index) plot, with the 95 (red) and 99 (blue) percent confidence interval thresholds.; C. Plot of G' values with the threshold of alpha = 0.01, the red arrow marked up the region with highest G' value.

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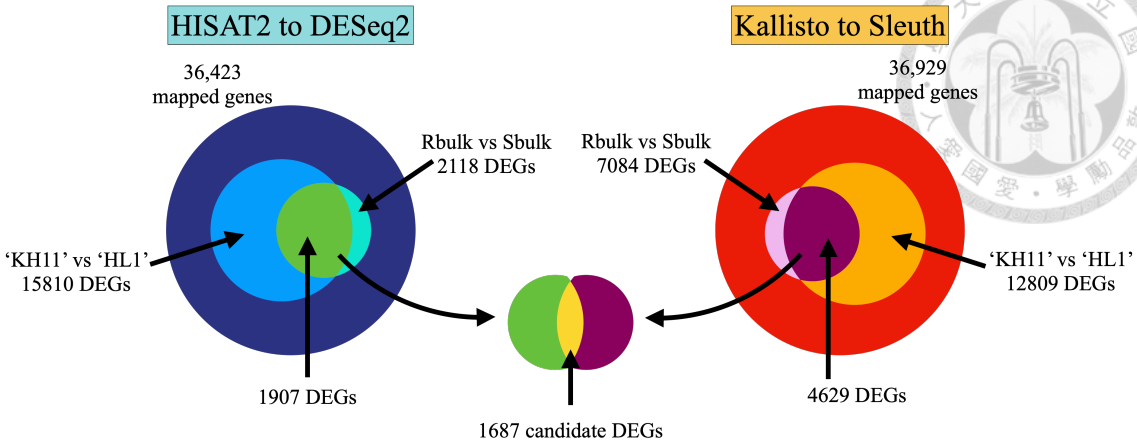

Figure 5. Venn diagram of the DEGs. The Venn diagram indicated the genes found in each step in HISAT2 to DESeq2 and Kallisto to Sleuth. In each, the total mapped genes, DEGs between parents, DEGs between progenies, and the DEGs overlapped between the aforementioned two groups were shown. And the final 1,687 candidate DEGs were shown to be overlapped between the result of two methods.

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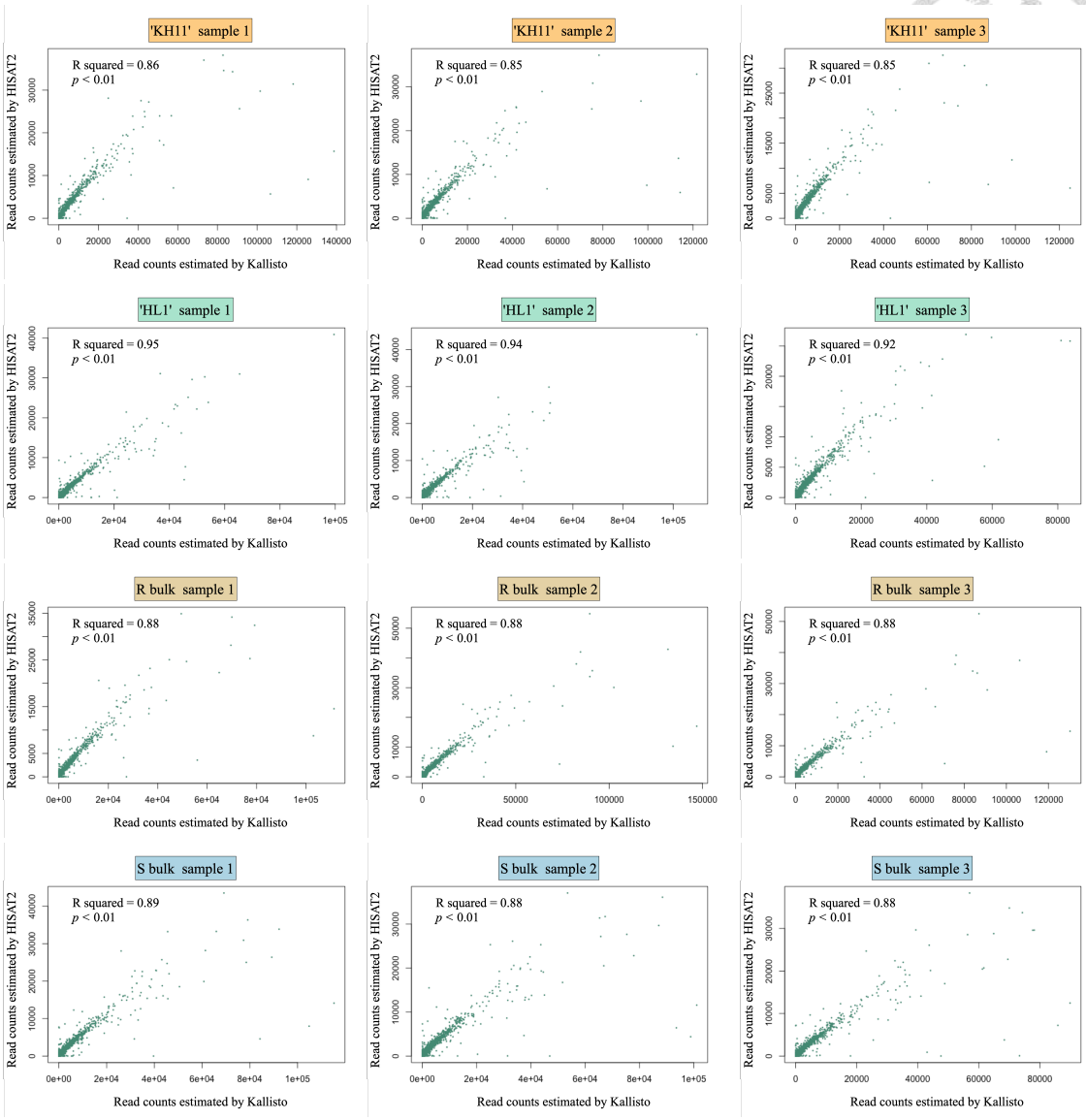

Figure 6. Correlation analysis of the read counts estimated by HISAT2 and Kallisto.

The X-axis represented the read counts estimated by Kallisto, and the Y-axis represented the read counts estimated by HISAT2. The R^2 of the 12 samples was between 0.88 to 0.95.

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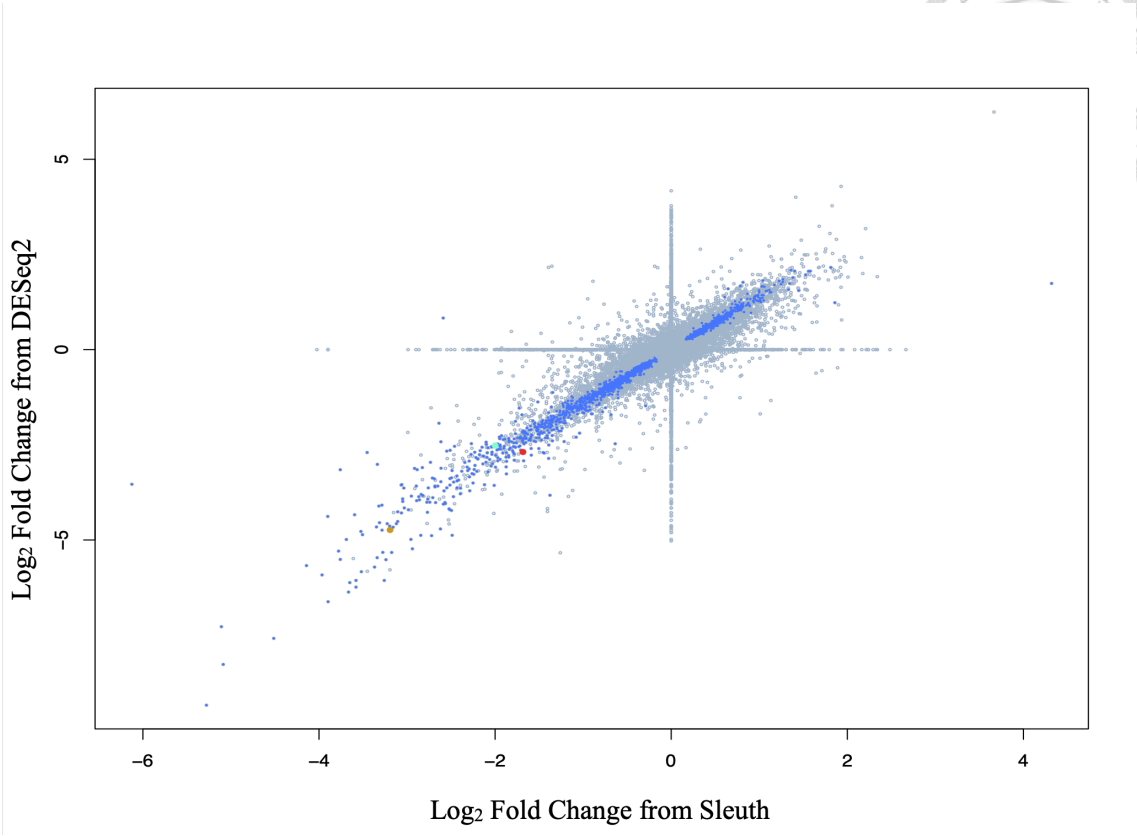

Figure 7. Correlation analysis of log_2 fold change (log_2 FC) estimated by DESeq2 and Sleuth. The X-axis represented the log_2 FC of the genes estimated by Sleuth, and the Y-axis represented the log_2 FC of the genes estimated by DESeq2. Gray and blue points represent all mapped genes and 1687 DEGs overlapped in the two methods, respectively. And within the blue dots, the red, light blue, and brown dots represented Glyma.06G162400, Glyma.15G170500, and Glyma.16G195600, respectively.

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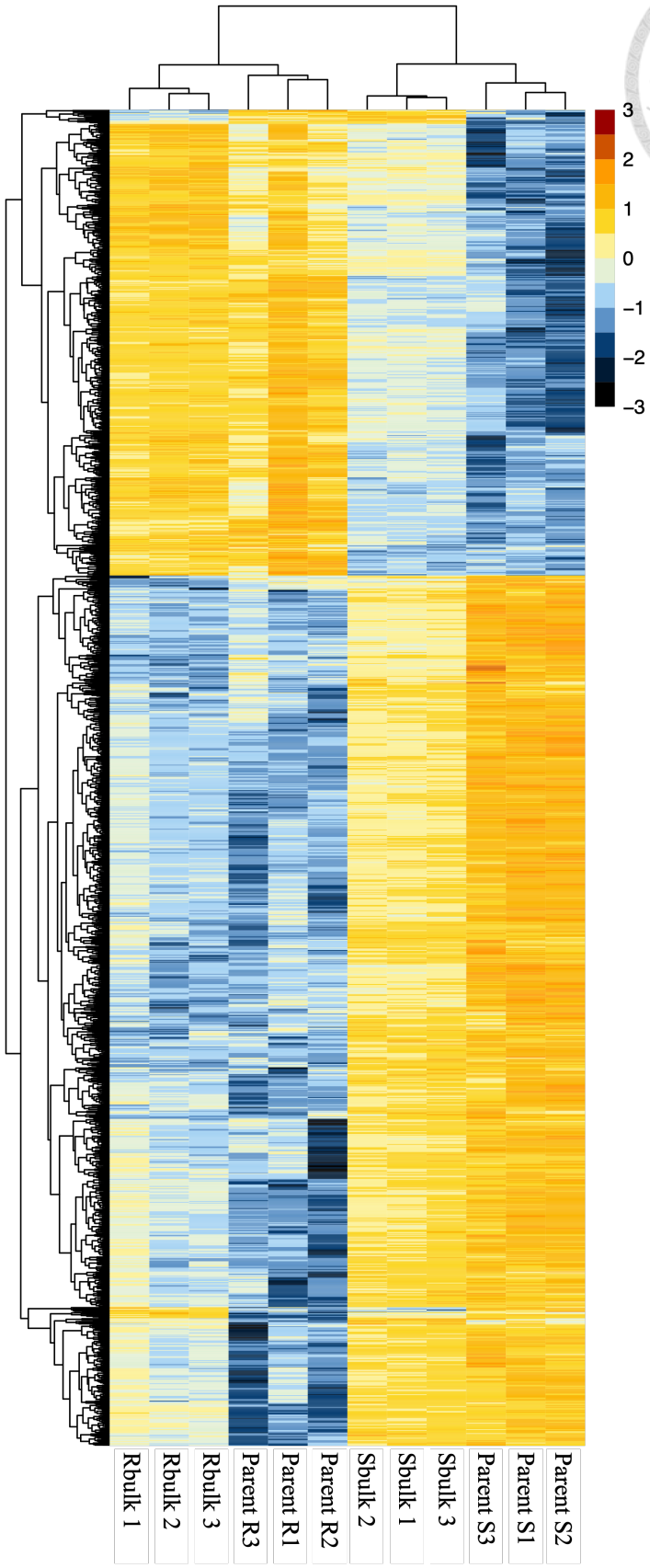

Figure 8. Heatmap of the 1687 DEGs. The expression level was presented with the $\log_2$ read counts.

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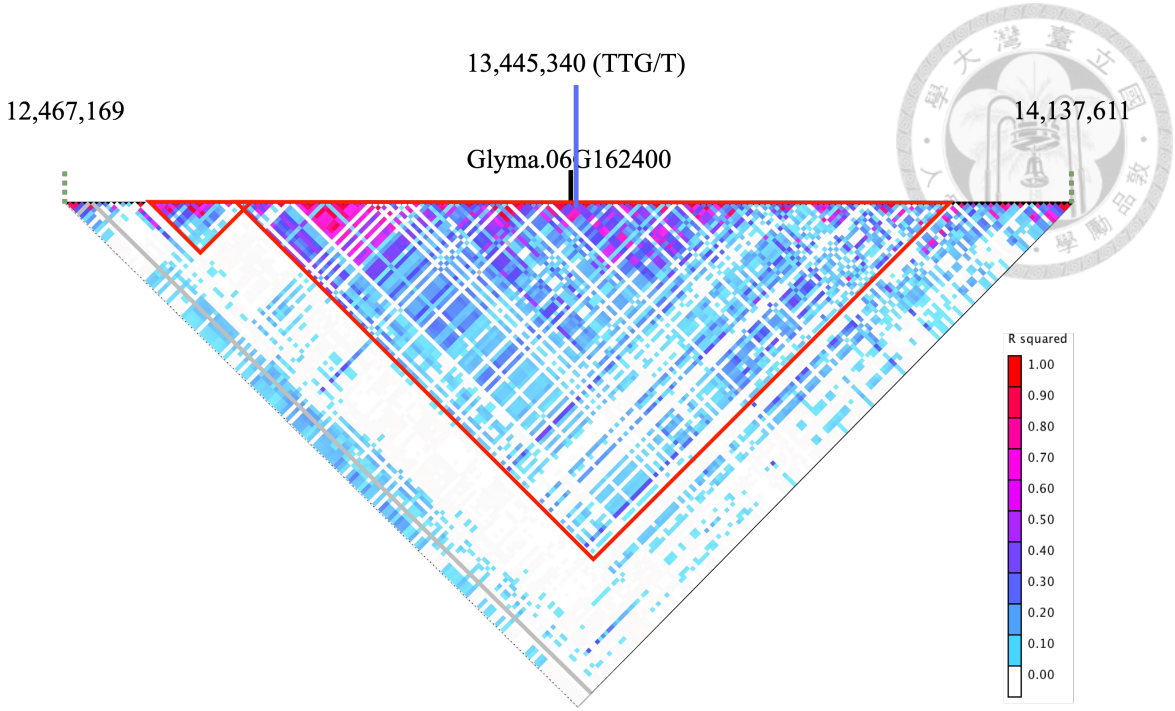

Figure 9. LD map of QTL regions in Chr06. The two green dot lines on the far left and right indicate the QTL region on Chr06. The black straight lines corresponded to the locations of candidate genes within this region. The regions marked by the red lines indicate the presence of the same LD groups. In the middle, the blue line represents the location of the SNP selected for the ASP design.

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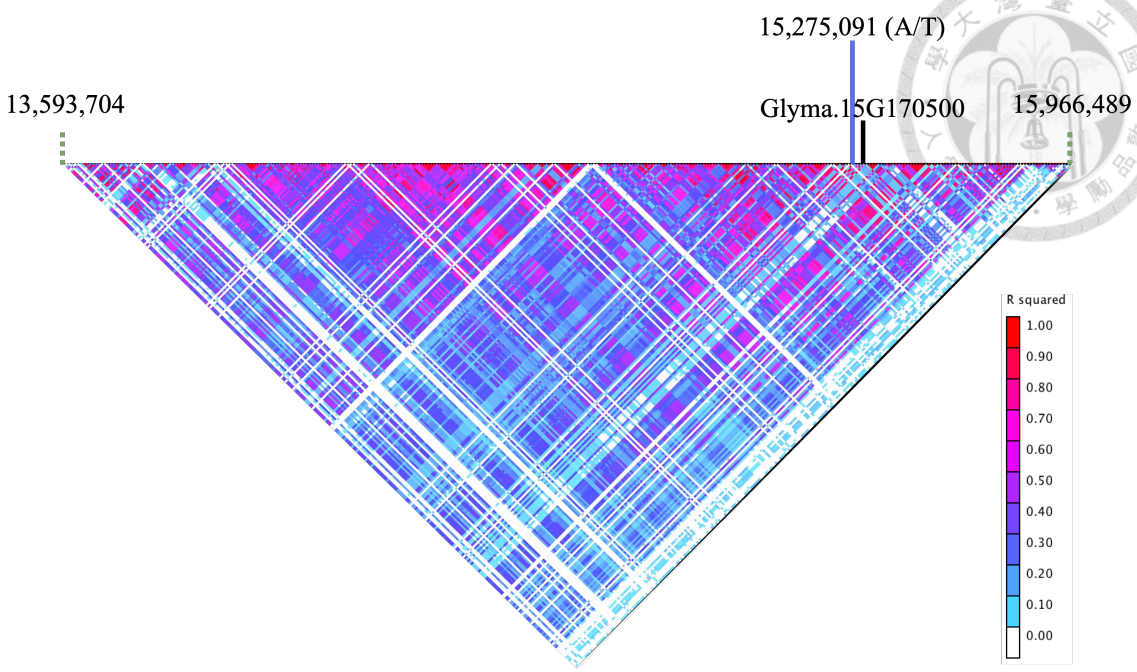

Figure 10. LD map of QTL regions in Chr15. The two green dot lines on the far left and right indicate the QTL region on Chr15. Every two SNPs within this region seemed to be linked. The black straight line corresponded to the locations of candidate genes within this region. Near the candidate gene, Glyma.15G170500, the blue line represented the location of the SNP selected for the ASP design.

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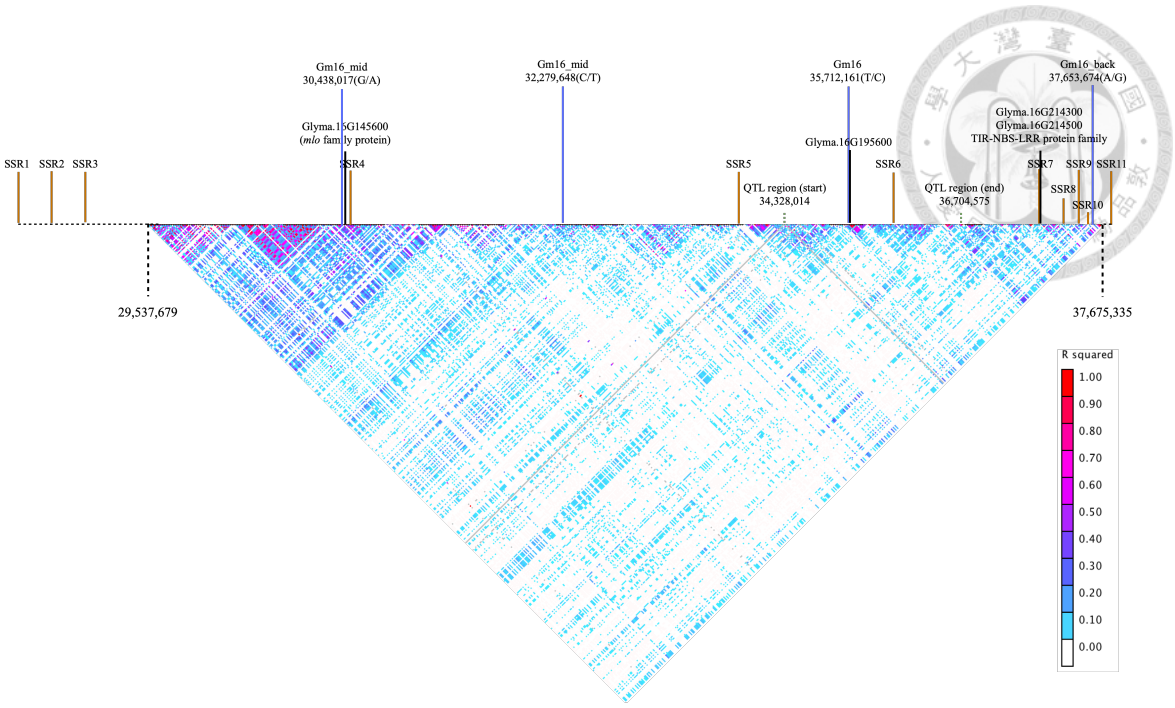

Figure 11. LD map of QTL regions in Chr16. The two black dot lines and green dot lines indicate the region for LD analysis and the QTL region on Chr16. The black straight lines corresponded to the locations of the genes interested, and the three blue straight lines represented the SNPs for ASP_Gm16, ASP_Gm16_front, ASP_Gm16_mid, and ASP_Gm16_back. In addition, the brown lines represented the relative position of the 11 SSR markers on Chr16, and SSR1 to SSR11 represented Satt622, BARCSOYSSR_16_0878, Satt215, Sat_366, Satt547, Satt431, BARCSOYSSR_16_1291, BARCSOYSSR_16_1294, Sat_395, Sat_393, and Satt712, respectively.

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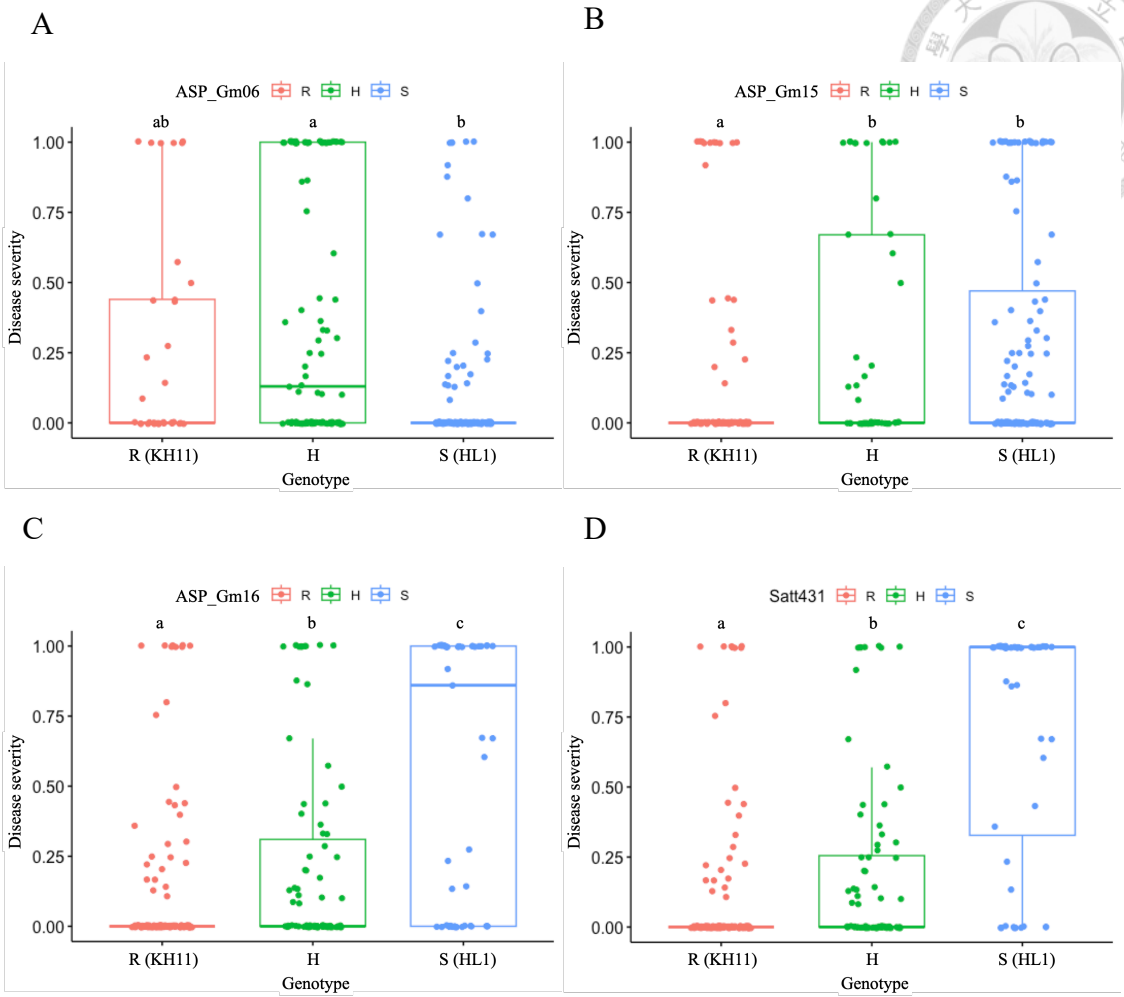

Figure 12. Mean seperation of ASPs & a SSR marker. The accuracy test was performed by the genotype and disease severity with F_2 population with the Dunn's test. R, H, and S correspond to the genotype allele1, heterozygous, and allele2, respectively. And the a, b, ab, and c correspond to the differentiation by Dunn's test. A. The Dunn's test of ASP_Gm06.; B. The Dunn's test of ASP_Gm15. C. The Dunn's test of ASP_Gm16.; D. The Dunn's test of SSR marker, Satt431.

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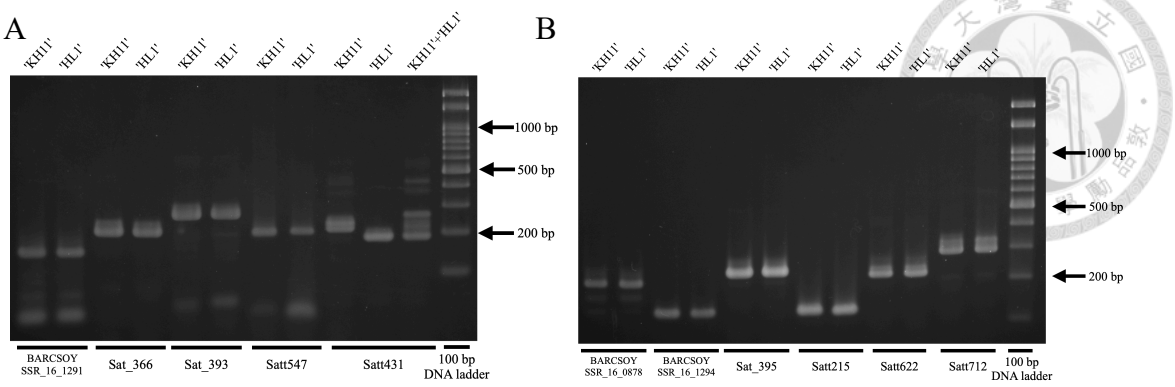

Figure 13. Differentiation of 'KH11' and 'HL1' with SSR markers. SSR markers, used to differentiate 'KH11' and 'HL1' serving as resistant and susceptible cultivars. The sequences flanked by BARCSOYSSR_16_0878, BARCSOYSSR_16_1291, BARCSOYSSR_16_1294, Sat_366, Sat_393, Sat_395, Satt215, Satt547, Satt622, and Satt712 showed no polymorphism between 'KH11' and 'HL1'. Only Satt431 was polymorphic between 'KH11', 'HL1', and heterozygous samples.

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Tables

Table 1. Phenotypes and the genotypes of 249 F_2 population

F_2 sample	disease incidence#	ASP_Gm06	ASP_Gm15	ASP_Gm16	Satt431	ASP_Gm16_mid	ASP_Gm16_back	ASP_Gm16_front
161101-1	0.00 (0/8)	R*	R	S*	R	S	R	S
161101-2	0.00 (0/11)	S	R	H*	R	H	R	H
161101-3	0.00 (0/10)	S	R	H	R	H	R	H
161101-4	0.00 (0/8)	S	R	S	R	S	R	S
161101-5	0.00 (0/8)	S	R	H	R	H	R	H
161101-6	0.00 (0/7)	S	R	H	R	H	R	H
161101-7	0.00 (0/10)	S	R	H	R	H	R	H
161101-8	0.00 (0/10)	S	R	H	R	H	R	H
161101-9	0.00 (0/10)	S	R	H	R	H	R	H
161101-10	0.00 (0/12)	S	R	H	R	H	R	H
161101-11	0.00 (0/8)	S	R	H	R	R	R	H
161101-12	0.00 (0/14)	S	R	H	R	H	R	H
161101-13	0.00 (0/11)	S	R	H	R	H	R	H
161101-14	0.00 (0/13)	S	R	H	R	H	R	H
161101-15	0.00 (0/10)	S	R	R	R	R	R	H
161101-16	0.00 (0/11)	S	R	S	R	S	R	S
161102-1	0.22 (2/9)	S	S	R	R	S	H	S
161102-2	0.36 (5/14)	H	S	R	S	R	S	S
161102-3	0.25 (2/8)	S	S	R	H	H	H	S
161102-4	0.00 (0/11)	H	S	R	R	H	R	S
161102-5	0.00 (0/5)	S	S	R	S	R	S	R
161102-6	0.00 (0/7)	R	S	R	H	R	H	R
161102-7	0.25 (3/12)	H	S	R	R	S	R	S
161102-8	0.00 (0/13)	H	S	R	R	S	R	S
161102-9	1.00 (13/13)	H	S	R	H	H	R	S
161102-10	0.43 (3/7)	R	S	R	S	H	H	H
161102-11	1.00 (9/9)	S	S	R	R	S	H	S
161102-12	0.29 (4/14)	H	S	R	H	H	H	R
161102-13	0.00 (0/8)	S	S	R	R	R	R	S
161102-14	0.00 (0/13)	R	S	R	S	R	S	R
161102-15	1.00 (13/13)	H	S	R	H	H	H	H
161102-16	0.30 (3/10)	H	S	R	H	H	H	H
161103-1	1.00 (8/8)	R	R	S	S	H	S	S
161103-2	1.00 (8/8)	H	H	S	S	H	S	S
161103-3	1.00 (6/6)	H	H	S	S	H	S	S
161103-4	1.00 (1/1)	R	R	S	S	S	S	S
161103-5	1.00 (9/9)	H	R	S	S	S	S	S
161103-6	1.00 (6/6)	H	R	S	S	S	S	S
161103-7	1.00 (12/12)	R	R	H	H	H	H	S
161103-8	1.00 (8/8)	H	H	S	S	H	S	S
161103-9	1.00 (9/9)	H	H	S	S	H	S	S
161103-10	1.00 (5/5)	H	R	S	S	H	S	S
161103-11	1.00 (8/8)	H	H	S	S	H	S	S
161103-12	0.00 (0/1)	R	S	S	S	S	S	S
161103-13	1.00 (12/12)	H	R	S	S	S	S	S
161103-14	1.00 (11/11)	H	H	S	S	S	S	S
161104-1	0.00 (0/1)	H	R	R	R	R	R	R
161104-2	0.00 (0/6)	H	R	R	R	R	R	R
161104-3	0.00 (0/6)	R	R	R	R	R	R	R
161104-4	0.00 (0/7)	R	R	R	R	R	R	R
161104-5	0.00 (0/3)	R	R	R	R	R	R	R
161105-1	0.00 (0/8)	S	S	R	R	H	R	S
161105-2	0.00 (0/7)	S	S	R	R	H	R	R
161105-3	0.00 (0/13)	S	R	R	R	S	R	S
161105-4	0.00 (0/9)	S	R	R	R	H	R	H
161105-5	0.00 (0/11)	S	S	R	R	R	R	R
161105-6	0.00 (0/12)	S	S	R	R	R	R	R
161106-1	0.00 (0/14)	S	H	H	H	S	S	S
161106-2	0.23 (3/13)	S	R	R	R	S	R	S
161106-3	0.00 (0/9)	R	H	R	R	S	R	S
161106-5	0.00 (0/13)	H	H	H	R	S	R	S
161106-6	0.00 (0/9)	S	H	H	H	S	H	S
161106-7	1.00 (14/14)	H	R	H	H	S	H	S
161106-8	0.08 (1/13)	S	H	H	H	S	H	S
161106-9	0.00 (0/1)	H	S	H	H	S	H	S
161106-10	0.25 (3/13)	H	S	H	H	S	H	S
161106-11	0.67 (4/6)	S	H	S	S	S	S	S

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161106-12	0.00 (0/15)	S	R	R	R	S	R	S
161106-13	0.40 (4/10)	S	S	R	R	S	R	S
161107-1	0.00 (0/10)	R	R	H	H	H	H	R
161107-2	0.57 (4/1)	R	S	H	H	H	H	R
161107-3	0.36 (4/11)	H	S	H	H	H	H	H
161107-4	0.44 (4/9)	R	R	H	H	R	H	H
161107-5	0.00 (0/5)	H	R	R	R	R	R	H
161107-6	0.00 (0/4)	H	R	R	R	R	R	H
161107-7	1.00 (8/8)	H	H	S	S	S	S	S
161107-8	0.17 (2/12)	H	H	R	R	R	S	R
161107-9	0.44 (7/16)	H	R	R	R	R	S	R
161107-10	0.44 (4/9)	R	R	H	H	H	S	R
161107-11	0.13 (2/15)	H	H	H	H	H	S	R
161107-13	0.33 (5/15)	R	H	S	S	S	S	H
161107-14	0.00 (0/10)	H	R	H	H	H	H	H
161107-15	0.00 (0/12)	R	H	H	H	H	H	R
161107-16	1.00 (17/17)	H	R	H	H	H	H	H
161108-1	1.00 (11/11)	R	R	S	S	S	S	S
161108-2	1.00 (12/12)	H	S	S	S	S	S	S
161108-3	1.00 (8/8)	H	S	S	S	S	S	S
161108-4	1.00 (8/8)	H	H	S	S	S	S	S
161108-5	1.00 (16/16)	H	S	S	S	S	S	S
161108-6	1.00 (14/14)	H	S	S	S	S	S	S
161108-7	1.00 (17/17)	H	S	S	S	S	S	S
161110-1	0.00 (0/13)	H	S	S	S	S	S	S
161110-2	0.00 (0/7)	S	H	R	R	R	R	R
161110-3	0.00 (0/14)	R	S	R	R	R	R	H
161110-4	0.00 (0/11)	S	S	R	R	R	R	H
161110-5	0.00 (0/10)	S	S	R	R	R	R	H
161110-6	0.00 (0/10)	H	R	R	R	R	R	H
161110-7	0.00 (0/3)	S	S	R	R	R	R	H
161110-8	0.00 (0/11)	S	S	R	R	R	R	H
161110-9	0.00 (0/6)	H	H	R	R	R	R	H
161110-10	0.00 (0/1)	S	H	R	R	R	R	S
161110-11	0.00 (0/5)	H	H	R	R	R	R	H
161110-12	0.00 (0/10)	H	H	R	R	R	R	H
161110-13	0.00 (0/10)	S	R	R	R	R	R	H
161110-14	0.00 (0/14)	H	H	R	R	R	R	R
161110-15	0.00 (0/7)	S	H	R	R	R	R	R
161110-16	0.00 (0/14)	S	R	R	R	R	R	R
161110-17	0.00 (0/4)	H	H	R	R	R	R	R
161110-18	0.00 (0/11)	H	H	R	R	R	R	R
161110-19	0.00 (0/10)	H	H	R	R	R	R	H
161110-20	0.00 (0/7)	H	H	R	R	R	R	H
161110-21	0.00 (0/8)	H	H	R	R	R	R	H
161110-22	0.00 (0/15)	S	S	R	R	R	R	S
161110-23	0.00 (0/8)	S	H	R	R	R	R	S
161110-24	0.00 (0/10)	H	H	R	R	R	R	H
161110-25	0.00 (0/12)	H	H	R	R	R	R	S
161110-26	0.00 (0/11)	H	H	R	R	R	R	R
161111-19	0.00 (0/17)	H	R	R	R	R	R	H
161111-20	0.00 (0/13)	S	S	H	H	R	R	R
161111-21	1.00 (10/10)	H	S	H	H	R	H	R
161111-22	0.86 (6/7)	H	S	S	S	R	S	R
161111-23	1.00 (13/13)	H	S	S	S	R	S	H
161111-26	1.00 (9/9)	S	S	H	H	R	S	R
161111-27	1.00 (13/13)	H	S	H	S	R	S	R
161111-28	0.10 (1/10)	H	S	H	S	R	S	R
161111-29	0.00 (0/13)	H	S	H	H	R	H	R
161112-1	0.00 (0/21)	H	S	H	H	R	H	H
161112-2	0.00 (0/13)	H	R	H	H	S	H	S
161112-3	1.00 (8/8)	R	S	S	S	H	S	H
161112-4	1.00 (6/6)	H	H	R	R	R	R	R
161112-5	0.00 (0/14)	H	S	H	H	H	S	H
161112-6	1.00 (11/11)	H	H	S	S	R	S	H
161112-7	0.33 (5/15)	S	H	R	R	H	R	R
161112-8	0.00 (0/8)	H	S	H	R	H	R	H
161112-9	0.00 (0/7)	R	R	S	S	H	H	H
161112-10	0.00 (0/8)	R	R	H	H	H	H	H
161112-11	0.00 (0/7)	H	R	R	R	H	R	H
161112-12	0.50 (4/8)	R	H	H	H	H	H	H
161112-13	0.00 (0/7)	R	H	H	H	H	H	H
161113-1	1.00 (12/12)	R	H	H	H	H	H	H
161113-2	0.86 (12/14)	S	S	H	S	H	S	H
161113-3	1.00 (12/12)	H	S	H	S	H	S	H

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161113-4	1.00 (11/11)	H	S	H	S	H	S	H
161113-5	1.00 (9/9)	H	R	S	S	S	S	S
161113-6	0.88 (7/8)	R	S	H	S	S	S	S
161114-1	0.92 (11/12)	S	S	H	S	H	S	H
161114-2	0.00 (0/10)	S	R	S	H	S	H	S
161114-3	0.17 (1/6)	S	R	H	R	H	H	H
161114-4	0.50 (1/2)	S	S	R	R	R	H	H
161114-5	0.60 (3/5)	S	S	R	R	R	R	H
161114-6	0.20 (1/5)	H	H	S	S	H	S	S
161114-7	0.00 (0/7)	S	H	R	R	R	R	H
161114-8	0.13 (1/8)	S	H	S	S	H	S	H
161114-9	0.67 (4/6)	S	H	H	H	H	H	H
161114-10	0.20 (1/5)	S	H	H	H	H	S	R
161114-11	0.80 (8/10)	S	R	H	H	S	H	S
161114-12	0.00 (0/10)	S	H	R	R	R	R	S
161115-1	0.00 (0/10)	S	S	H	H	S	H	S
161115-5	0.00 (0/10)	R	S	R	R	H	R	S
161115-6	0.00 (0/12)	H	S	R	R	R	R	S
161115-7	0.00 (0/5)	H	S	R	R	R	R	H
161115-8	0.10 (1/10)	H	S	H	H	H	R	S
161115-9	0.00 (0/10)	H	S	H	H	H	R	H
161115-10	0.00 (0/4)	H	S	R	R	R	R	R
161115-11	0.00 (0/2)	R	S	S	S	S	R	S
161115-12	0.00 (0/7)	H	S	R	R	R	R	R
161115-13	0.00 (0/6)	H	S	H	H	H	R	H
161115-14	0.00 (0/11)	H	S	S	S	S	R	S
161115-15	0.00 (0/14)	H	S	H	H	H	R	H
161116-1	0.00 (0/7)	S	S	H	H	H	R	H
161116-2	0.00 (0/14)	S	R	R	R	R	R	R
161116-3	0.00 (0/10)	S	R	R	R	H	R	H
161117-1	0.00 (0/4)	S	R	R	R	H	R	H
161117-2	0.00 (0/6)	S	S	H	H	R	H	S
161117-3	0.17 (1/6)	R	S	H	H	H	H	S
161117-4	0.20 (2/10)	S	S	H	R	H	R	S
161117-5	0.00 (0/10)	H	S	H	H	H	H	S
161117-6	0.44 (4/9)	R	S	H	H	H	H	S
161117-7	0.11 (1/9)	H	S	R	R	R	H	S
161117-8	1.00 (4/4)	H	S	R	R	R	R	S
161117-9	0.13 (1/8)	H	S	R	R	R	R	S
161117-10	0.00 (0/1)	H	S	S	S	H	S	S
161117-11	0.00 (0/11)	S	S	H	H	R	H	S
161117-12	0.27 (3/11)	H	S	S	H	S	H	S
161117-13	0.00 (0/12)	R	S	S	H	S	H	S
161117-14	0.11 (1/9)	S	S	S	S	S	R	S
161117-15	0.00 (0/9)	H	S	H	H	S	H	S
161117-16	0.00 (0/7)	S	S	H	H	H	H	S
161117-17	0.25 (3/12)	H	S	H	H	H	H	S
161117-18	1.00 (8/8)	S	S	H	H	H	H	S
161117-19	0.00 (0/12)	S	S	H	H	H	H	S
161117-20	0.67 (4/6)	H	S	S	H	S	S	S
161119-1	0.00 (0/14)	S	S	S	S	S	S	S
161119-2	0.00 (0/6)	S	R	R	H	R	R	R
161119-3	0.00 (0/9)	S	R	R	H	R	R	R
161119-4	0.00 (0/6)	S	R	R	R	R	R	R
161119-5	0.00 (0/7)	S	R	R	R	R	R	R
161119-6	0.00 (0/14)	S	R	R	H	R	R	R
161119-7	0.00 (0/9)	S	R	R	R	R	R	R
161119-8	0.00 (0/5)	S	R	R	H	R	R	H
161119-9	0.00 (0/12)	S	R	R	R	R	R	R
161119-10	0.00 (0/8)	S	R	R	H	R	R	H
161119-11	0.00 (0/10)	S	R	R	H	R	R	R
161119-12	0.00 (0/24)	S	R	R	H	R	R	R
161119-13	0.00 (0/10)	S	R	R	H	R	R	R
161119-14	0.00 (0/9)	S	R	R	R	R	R	H
161119-15	0.00 (0/17)	S	R	R	R	R	R	R
161119-16	0.00 (0/11)	S	R	R	R	R	R	R
161119-17	0.00 (0/13)	S	R	R	R	R	R	R
161120-1	0.00 (0/4)	S	R	R	R	R	R	R
161120-2	0.00 (0/8)	S	S	R	R	R	R	R
161120-3	0.00 (0/8)	S	S	R	R	R	R	R
161120-4	0.00 (0/11)	S	S	R	H	R	R	R
161120-5	0.00 (0/3)	S	S	R	R	R	R	R
161120-6	0.00 (0/7)	S	S	R	R	R	R	R
161120-7	0.13 (1/8)	S	S	R	H	R	R	H
161120-8	0.00 (0/7)	S	S	R	R	R	R	R

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161120-9	0.00 (0/9)	S	S	R	R	R	R	R
161120-10	0.00 (0/4)	S	R	R	R	R	R	R
161120-11	0.00 (0/2)	S	S	R	R	R	R	R
161120-12	0.00 (0/11)	S	S	R	R	R	R	R
161121-1	0.00 (0/8)	S	S	R	R	R	R	R
161121-2	0.00 (0/3)	S	R	R	R	R	R	S
161121-3	0.00 (0/10)	S	R	H	R	H	R	S
161121-4	0.00 (0/10)	S	R	R	R	R	R	S
161121-5	0.00 (0/11)	S	R	H	R	S	R	S
161121-6	0.14 (2/14)	S	R	H	R	H	R	S
161121-7	0.29 (4/14)	S	R	R	R	R	R	S
161121-8	0.00 (0/10)	S	R	H	R	S	R	S
161121-9	0.00 (0/6)	S	R	H	R	S	R	S
161121-10	0.00 (0/6)	S	R	S	R	S	R	S
161121-11	0.00 (0/15)	S	R	H	R	S	R	S
161121-12	0.00 (0/10)	S	R	R	R	R	R	S
161121-13	0.00 (0/8)	S	R	S	R	H	R	S
161123-1	0.00 (0/7)	S	R	S	R	S	R	S
161123-2	0.14 (1/7)	H	S	H	H	S	H	S
161123-3	1.00 (8/8)	S	S	H	H	S	H	S
161123-4	1.00 (7/7)	R	S	R	R	S	R	S
161123-5	0.00 (0/7)	H	S	R	R	S	R	S
161123-6	0.14 (3/22)	R	S	H	H	S	S	S
161123-7	0.00 (0/11)	R	S	S	H	S	S	S
161123-8	0.09 (1/11)	H	S	H	H	S	H	S
161123-9	0.00 (0/9)	R	S	H	H	S	H	S
161123-10	0.40 (4/10)	H	S	S	H	S	S	H
161123-11	1.00 (6/6)	H	S	H	H	S	H	S
161123-12	0.75 (6/8)	H	S	R	R	S	R	S
161123-13	0.00 (0/11)	H	S	R	R	S	H	S
161123-16	0.00 (0/10)	H	S	H	H	S	H	S

[#]Disease incidence: diseased F_3 plants of one F_2/ F_3 plants of one F_2

[*]R, H, and S correspond to the genotype allele1, heterozygous, and allele2, respectively

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Table 2. Primer sequences of ASPs and universal probes (UPs)

Primer name	SNP position & SNP type	Primer sequence	Product size
UP-FAM		FAM-AGCGATGCGTTCGAGCATCGC(*T)GAAGGTGACCAAGTTCATGCT	
UP-HEX		HEX-AGGACGCTGAGATGCGTCC(*T)GAAGGTCGGAGTCAACGGATT	
ASP_Gm06_F1	Chr06: 13,445,340 (TTG, T)	GAAGGTGACCAAGTTCATGCTTGGAAAGGTAGCTAGGC*cTTG	117
ASP_Gm06_F2		GAAGGTCGGAGTCAACGGATTTGGAAAGGTAGCTAGGCATTA	
ASP_Gm06_R		GGAGACTCGAGTGTTTGAGC	
ASP_Gm15_F1	Chr15: 15,275,091 (T, A)	GAAGGTGACCAAGTTCATGCTTCTTTGCCCACTTGT*gGGCT	100
ASP_Gm15_F2		GAAGGTCGGAGTCAACGGATTTCTTTGCCCACTTGTTG*tCA	
ASP_Gm15_R		CACTGCAGTTGAATTTATTAC	
ASP_Gm16_F1	Chr16: 35,712,161 (T, C)	GAAGGTGACCAAGTTCATGCTGGTTTGTTTTCGGCA*gCTTT	110
ASP_Gm16_F2		GAAGGTCGGAGTCAACGGATTGGTTTGTTTTCGGCATC*aTC	
ASP_Gm16_R		AGTTTGGGATATTTCCTCCC	
ASP_Gm16_front_F1	Chr16: 30,438,017 (G, A)	GAAGGTGACCAAGTTCATGCTCGTTTTGTCCCTGAcAAG	98
ASP_Gm16_front_F2		GAAGGTCGGAGTCAACGGATTCGTTTTGTCCCTGAcAAA	
ASP_Gm16_front_R		CTGCTTTGCTATTGATCAGG	
ASP_Gm16_mid_F1	Chr16: 32,279,648 (C, T)	GAAGGTGACCAAGTTCATGCTGTGGATGATGAGTCAGTTcAC	115
ASP_Gm16_mid_F2		GAAGGTCGGAGTCAACGGATTGTGGATGATGAGTCAGTTcAT	
ASP_Gm16_mid_R		CTGTGTCATCCTTTGGAATGCC	
ASP_Gm16_back_F1	Chr16: 37,653,674 (A, G)	GAAGGTGACCAAGTTCATGCTATTCCCTCTGTTCCCACgCA	115
ASP_Gm16_back_F2		GAAGGTCGGAGTCAACGGATTATTCCCTCTGTTCCCACgCG	
ASP_Gm16_back_R		GTCGTTTATGACGGAGATGTCGG	

FAM & HEX: represented the fluorescent probe
(*T): represented the location of the quencher (BHQ1)
*x: represented the mismatch
X: represented the UP-associated tail

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Table 3. Primer sequences of SSR markers

SSR marker	Primer sequence	Reference
BARCSOYSSR_16_0878_F	TCTGCAGTGGTCTCATCGAC	Anne et. al
BARCSOYSSR_16_0878_R	TGGTGTGTTCCTTGGGATTT	
BARCSOYSSR_16_1291_F	GATGCGGGTTAAAATCAGGA	Anne et. al
BARCSOYSSR_16_1291_R	GGTTATGCTTTCTTTTCCGC	
BARCSOYSSR_16_1294_F	CCCTCTTCATTTCCCTTGGT	Anne et. al
BARCSOYSSR_16_1294_R	CCTCCCAATATCTTTGGGATAA	
Sat_366_F	GCGGACATGGTACATCTATATTACGAGTATT	Song et al.
Sat_366_R	GCGGCACAAGAACAGAGGAAACTATT	
Sat_393_F	GCGGTCCTGCATGTTAATGTTGATT	Song et al.
Sat_393_R	GCGGGTCCCTACAATGTGAGTGG	
Satt395_F	GCGTCTTAGTCGTGCTGACACTACTC	Cregan et al.
Satt395_R	GCGAGTTTGTTTAGCTCAATCTTTCACTCA	
Satt215_F	CCCATTCAATTGAGATCCAAAATTAC	Cregan et al.
Satt215_R	GCGCCTTCTTCTGCTAAATCA	
Satt431_F	GCGTGGCACCCTTGATAAATAA	Cregan et al.
Satt431_R	GCGCACGAAAGTTTTTCTGTAACA	
Satt547_F	GCGCTATCCGATCCATATGTG	Cregan et al.
Satt547_R	TGATTTCGCTAGGTAAAATCA	
Satt622_F	GCGGTGTAGGTAATAATTTTAATTCTCAT	Song et al.
Satt622_R	GCGGTGTAGGTTTCACACTTCATTCAC	
Satt712_F	GCGAATATAGCCAAATTTAGGTTGAATGACA	Song et al.
Satt712_R	GCGACCACCCATCACCTCCACCTCAAACAAC	

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Table 4. Quantitative trait locus (QTL) on each chromosome

Chromosome	start	end	length	nSNPs
Chr01	42,022,380	42,022,384	4	2
Chr01	44,039,080	44,039,080	0	1
Chr04	35,995,923	36,085,835	89,912	2
Chr04	44,006,761	44,592,458	585,697	14
Chr04	46,484,895	46,788,048	303,153	3
Chr05	25,031,503	26,483,572	1,452,069	37
Chr06	10,494,052	10,494,052	0	1
Chr06	12,467,169	14,137,611	1,670,442	51
Chr11	143,592	455,675	312,083	15
Chr12	12,572,720	14,538,682	1,965,962	23
Chr12	24,784,017	24,784,017	0	1
Chr12	31,863,197	32,426,001	562,804	8
Chr13	18,126,034	19,313,959	1,187,925	11
Chr14	8,185,612	8,657,103	471,491	7
Chr14	9,716,858	10,178,559	461,701	16
Chr14	13,184,157	14,017,630	833,473	26
Chr15	13,662,988	15,966,489	2,303,501	116
Chr15	28,958,496	29,881,792	923,296	2
Chr16	21,775,132	21,775,132	0	1
Chr16	34,328,014	36,718,314	2,390,300	79
Chr17	15,480,307	24,792,714	9,312,407	85
Chr17	30,686,217	31,414,190	727,973	24
Chr18	20,122,341	20,981,201	858,860	3
Chr18	25,362,528	25,654,977	292,449	4
Chr18	29,657,906	30,561,365	903,459	2
Chr18	34,518,559	34,849,312	330,753	7
Chr18	38,910,252	40,217,786	1,307,534	19
Chr18	41,733,839	42,095,313	361,474	14
Chr19	42,855,894	43,134,090	278,196	23
Chr20	71,417	835,174	763,757	8
Chr20	5,256,787	5,256,787	0	1
Chr20	33,702,942	33,702,999	57	5

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Table 5. SNPs and Indels in Chr06

	Position (bp)	Reference allele	Alternative allele	G′ value	*p*-value	*q*-value
1	10,494,052	C	CT	20.09	2.15E-05	2.14E-04
2	12,467,169	G	A	30.61	2.33E-07	5.19E-06
3	12,479,153	T	TA	31.24	1.83E-07	4.18E-06
4	12,772,969	CT	C	46.70	1.03E-09	8.02E-08
5	12,841,611	A	T	50.32	3.60E-10	3.05E-08
6	12,990,670	C	A	57.15	5.63E-11	5.06E-09
7	12,990,700	T	G	57.15	5.63E-11	5.06E-09
8	13,194,784	A	T	57.95	4.58E-11	4.19E-09
9	13,196,862	T	A	57.95	4.57E-11	4.19E-09
10	13,249,048	G	A	58.16	4.33E-11	4.01E-09
11	13,249,210	A	G	58.16	4.33E-11	4.01E-09
12	13,280,124	T	TC	58.28	4.20E-11	3.92E-09
13	13,292,483	C	T	58.33	4.15E-11	3.89E-09
14	13,309,964	A	C	58.40	4.07E-11	3.84E-09
15	13,310,531	A	C	58.40	4.07E-11	3.84E-09
16	13,310,698	G	A	58.40	4.07E-11	3.84E-09
17	13,313,030	C	T	58.41	4.06E-11	3.84E-09
18	13,314,280	T	C	58.41	4.06E-11	3.84E-09
19	13,316,902	G	A	58.42	4.05E-11	3.84E-09
20	13,317,122	C	A	58.43	4.05E-11	3.84E-09
21	13,317,174	C	T	58.43	4.04E-11	3.84E-09
22	13,317,995	C	G	58.43	4.04E-11	3.84E-09
23	13,321,803	A	C	58.44	4.03E-11	3.84E-09
24	13,331,468	T	A	58.48	3.99E-11	3.84E-09
25	13,337,047	A	C	58.50	3.96E-11	3.84E-09
26	13,353,697	T	A	58.57	3.90E-11	3.84E-09
27	13,362,386	G	A	58.60	3.87E-11	3.84E-09
28	13,362,488	A	G	58.60	3.86E-11	3.84E-09
29	13,394,883	A	G	58.73	3.74E-11	3.80E-09
30	13,445,340	T	TTG	58.93	3.56E-11	3.65E-09
31	13,447,999	T	TA	58.94	3.55E-11	3.65E-09
32	13,448,039	AT	A	58.94	3.55E-11	3.65E-09
33	13,448,141	G	T	58.94	3.55E-11	3.65E-09
34	13,448,149	C	T	58.94	3.55E-11	3.65E-09
35	13,455,418	C	A	58.97	3.52E-11	3.65E-09
36	13,455,439	A	C	58.97	3.52E-11	3.65E-09
37	13,455,468	C	T	58.97	3.52E-11	3.65E-09
38	13,455,754	G	A	58.97	3.52E-11	3.65E-09
39	13,455,922	C	T	58.97	3.52E-11	3.65E-09
40	13,457,732	G	A	58.98	3.51E-11	3.65E-09
41	13,457,865	C	G	58.98	3.51E-11	3.65E-09
42	13,466,136	C	T	59.01	3.48E-11	3.65E-09
43	13,471,300	T	A	59.03	3.47E-11	3.65E-09
44	13,486,266	T	A	59.09	3.41E-11	3.65E-09
45	13,499,250	A	T	59.14	3.37E-11	3.65E-09
46	13,782,174	C	G	59.91	2.77E-11	3.12E-09
47	13,832,233	A	C	59.01	3.48E-11	3.65E-09
48	13,906,143	A	G	57.68	4.90E-11	4.45E-09
49	13,906,246	G	C	57.68	4.91E-11	4.45E-09
50	13,997,351	G	GT	56.03	7.54E-11	6.75E-09
51	14,057,955	C	A	54.94	1.01E-10	8.98E-09
52	14,137,611	A	T	53.51	1.48E-10	1.30E-08

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Table 6. SNPs and Indels in Chr15

	Position (bp)	Reference allele	Alternative allele	G′ value	*p*-value	*q*-value
1	13,662,988	A	G	47.29	8.63E-10	7.02E-08
2	13,671,589	TA	T	47.38	8.41E-10	6.87E-08
3	13,737,536	G	T	48.07	6.86E-10	5.70E-08
4	13,776,924	C	T	48.48	6.09E-10	5.15E-08
5	13,850,034	G	T	47.38	8.41E-10	6.87E-08
6	13,879,954	C	T	46.93	9.60E-10	7.64E-08
7	13,879,956	A	T	46.93	9.60E-10	7.64E-08
8	13,880,883	A	C	46.91	9.64E-10	7.64E-08
9	13,881,120	A	G	46.91	9.65E-10	7.64E-08
10	13,882,421	A	G	46.89	9.71E-10	7.64E-08
11	13,972,273	G	A	45.54	1.46E-09	1.04E-07
12	13,979,623	C	G	45.43	1.51E-09	1.06E-07
13	14,011,231	G	T	44.95	1.74E-09	1.17E-07
14	14,037,164	T	C	44.56	1.96E-09	1.31E-07
15	14,043,799	TGAAGAAGAAGAA	T,TGAAGAAGAA	44.46	2.02E-09	1.32E-07
16	14,043,853	G	A	44.46	2.02E-09	1.32E-07
17	14,043,886	C	A	44.46	2.02E-09	1.32E-07
18	14,043,961	TTCGA	T	44.46	2.02E-09	1.32E-07
19	14,048,098	T	C	44.40	2.06E-09	1.34E-07
20	14,060,840	A	AAAAAAAAAATTAAG,AAAAAAAAAATTAAG	44.20	2.19E-09	1.39E-07
21	14,086,956	A	G	43.81	2.47E-09	1.51E-07
22	14,099,040	A	G	43.63	2.61E-09	1.55E-07
23	14,099,045	T	A	43.63	2.61E-09	1.55E-07
24	14,106,494	A	AT	43.52	2.70E-09	1.59E-07
25	14,106,530	T	TA	43.52	2.70E-09	1.59E-07
26	14,281,900	G	A	40.88	6.25E-09	3.07E-07
27	14,282,272	A	G	40.87	6.26E-09	3.07E-07
28	14,336,487	T	C	40.05	8.16E-09	3.51E-07
29	14,336,521	G	A	40.05	8.16E-09	3.51E-07
30	14,336,593	A	G	40.05	8.17E-09	3.51E-07
31	14,340,826	G	T	39.99	8.34E-09	3.52E-07
32	14,340,872	G	A	39.99	8.34E-09	3.52E-07
33	14,340,881	A	C	39.99	8.34E-09	3.52E-07
34	14,340,884	G	A	39.99	8.34E-09	3.52E-07
35	14,340,894	C	A	39.99	8.34E-09	3.52E-07
36	14,362,945	T	C	39.66	9.30E-09	3.56E-07
37	14,363,338	G	C	39.65	9.32E-09	3.56E-07
38	14,364,593	C	T	39.63	9.38E-09	3.56E-07
39	14,364,596	T	C	39.63	9.38E-09	3.56E-07
40	14,365,955	C	T	39.61	9.44E-09	3.56E-07
41	14,366,138	G	A	39.61	9.45E-09	3.56E-07
42	14,375,919	G	T	39.46	9.92E-09	3.56E-07
43	14,375,933	C	A	39.46	9.92E-09	3.56E-07
44	14,381,232	A	G	39.38	1.02E-08	3.56E-07
45	14,381,264	A	G	39.38	1.02E-08	3.56E-07
46	14,405,147	A	T	39.02	1.15E-08	3.88E-07
47	14,405,207	T	A	39.02	1.15E-08	3.88E-07
48	14,405,338	C	T	39.02	1.15E-08	3.88E-07
49	14,405,482	C	T	39.02	1.15E-08	3.88E-07
50	14,410,839	C	CAAA,CAAAAA	38.93	1.18E-08	3.97E-07
51	14,410,936	T	C	38.93	1.18E-08	3.97E-07
52	14,435,865	CAGCACGCCCAGG	C	38.56	1.34E-08	4.48E-07
53	14,438,684	C	G	38.52	1.36E-08	4.54E-07
54	14,446,111	A	G	38.40	1.41E-08	4.69E-07
55	14,446,340	A	G	38.40	1.41E-08	4.69E-07
56	14,446,494	A	G	38.40	1.41E-08	4.69E-07
57	14,464,067	G	A	38.13	1.55E-08	5.11E-07
58	14,464,143	G	A	38.13	1.55E-08	5.11E-07
59	14,464,247	C	A	38.13	1.55E-08	5.11E-07

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60	14,471,632	A	C	38.02	1.61E-08	5.26E-07
61	14,471,637	C	G	38.02	1.61E-08	5.26E-07
62	14,471,851	A	C	38.02	1.61E-08	5.26E-07
63	14,471,891	T	C	38.02	1.61E-08	5.26E-07
64	14,471,893	T	G	38.02	1.61E-08	5.26E-07
65	14,471,992	T	C	38.01	1.61E-08	5.26E-07
66	14,472,114	C	T	38.01	1.61E-08	5.26E-07
67	14,472,436	C	T	38.01	1.61E-08	5.26E-07
68	14,489,369	G	T	37.75	1.76E-08	5.71E-07
69	14,490,881	G	A	37.73	1.77E-08	5.75E-07
70	14,536,439	T	C	37.04	2.24E-08	7.02E-07
71	14,541,393	G	A	36.97	2.30E-08	7.19E-07
72	14,543,523	G	GA	36.94	2.32E-08	7.26E-07
73	14,551,414	A	G	36.82	2.42E-08	7.55E-07
74	14,625,059	G	A	36.12	3.09E-08	9.40E-07
75	14,625,090	C	A	36.12	3.09E-08	9.40E-07
76	14,625,095	T	C	36.12	3.09E-08	9.40E-07
77	14,625,136	G	A	36.12	3.09E-08	9.40E-07
78	14,625,160	C	T	36.12	3.09E-08	9.40E-07
79	14,625,175	T	C	36.12	3.09E-08	9.40E-07
80	14,667,405	GTGGGCA	G	35.80	3.45E-08	1.04E-06
81	14,671,869	A	C	35.77	3.49E-08	1.04E-06
82	14,684,518	C	T	35.68	3.60E-08	1.06E-06
83	14,781,141	G	C	34.96	4.64E-08	1.32E-06
84	14,820,434	T	C	34.67	5.15E-08	1.45E-06
85	15,000,088	G	GAAC	33.34	8.32E-08	2.17E-06
86	15,000,303	G	T	33.34	8.33E-08	2.17E-06
87	15,000,310	G	A	33.34	8.33E-08	2.17E-06
88	15,011,862	T	G	33.26	8.59E-08	2.24E-06
89	15,024,156	TTG	T	33.17	8.89E-08	2.30E-06
90	15,134,393	T	C	32.35	1.20E-07	2.98E-06
91	15,135,487	C	G	32.34	1.21E-07	2.99E-06
92	15,175,528	T	G	32.05	1.35E-07	3.26E-06
93	15,175,593	G	A	32.05	1.35E-07	3.26E-06
94	15,177,817	A	G	32.03	1.35E-07	3.27E-06
95	15,223,884	GTATTTT	G	31.69	1.54E-07	3.67E-06
96	15,236,641	G	T	31.60	1.60E-07	3.79E-06
97	15,236,666	G	T	31.60	1.60E-07	3.79E-06
98	15,270,937	G	A	31.34	1.76E-07	4.09E-06
99	15,271,234	T	C	31.34	1.76E-07	4.09E-06
100	15,271,302	T	C	31.34	1.76E-07	4.09E-06
101	15,273,141	T	A	31.33	1.77E-07	4.09E-06
102	15,273,156	T	C	31.33	1.77E-07	4.09E-06
103	15,273,317	T	A	31.32	1.77E-07	4.09E-06
104	15,274,681	C	G	31.31	1.78E-07	4.09E-06
105	15,274,905	A	T	31.31	1.78E-07	4.09E-06
106	15,275,091	A	T	31.31	1.78E-07	4.09E-06
107	15,513,033	C	CGAGA	27.05	9.61E-07	1.68E-05
108	15,556,974	T	G	25.97	1.51E-06	2.36E-05
109	15,642,145	G	A	23.89	3.72E-06	5.01E-05
110	15,642,278	A	C	23.88	3.73E-06	5.01E-05
111	15,675,119	C	T	23.08	5.34E-06	6.69E-05
112	15,701,815	C	G	22.42	7.19E-06	8.66E-05
113	15,783,276	A	G	20.42	1.84E-05	1.90E-04
114	15,783,288	T	C	20.42	1.84E-05	1.90E-04
115	15,800,877	T	A	19.99	2.26E-05	2.23E-04
116	15,966,489	G	T	15.93	1.81E-04	1.10E-03
117	28,958,496	C	G	18.07	5.88E-05	4.47E-04
118	29,881,792	C	T	13.14	8.70E-04	4.08E-03

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Table 7. SNPs and Indels in Chr16

	Position	Reference allele	Alternative allele	G′ value	*p*-value	q-value
1	21,775,132	A	C	13.84	5.80E-04	2.89E-03
2	34,328,014	C	A	46.97	9.47E-10	7.64E-08
3	34,330,701	G	C	47.15	8.98E-10	7.27E-08
4	34,349,153	C	T	48.39	6.25E-10	5.24E-08
5	34,349,160	G	GTGCTTGATCT	48.39	6.25E-10	5.24E-08
6	34,955,172	G	C	75.89	6.74E-13	1.76E-10
7	35,204,428	G	A	78.94	3.54E-13	1.01E-10
8	35,204,644	G	C	78.94	3.53E-13	1.01E-10
9	35,204,690	A	G	78.95	3.53E-13	1.01E-10
10	35,204,700	C	T	78.95	3.53E-13	1.01E-10
11	35,204,848	T	C	78.95	3.53E-13	1.01E-10
12	35,204,888	A	G	78.95	3.53E-13	1.01E-10
13	35,204,896	TCG	T	78.95	3.53E-13	1.01E-10
14	35,626,209	G	C	86.45	7.73E-14	2.79E-11
15	35,633,631	ATC	A	86.60	7.52E-14	2.77E-11
16	35,633,639	C	G	86.60	7.52E-14	2.77E-11
17	35,636,027	T	A	86.65	7.45E-14	2.77E-11
18	35,636,166	A	G	86.65	7.44E-14	2.77E-11
19	35,636,427	A	C	86.65	7.43E-14	2.77E-11
20	35,636,449	G	T	86.65	7.43E-14	2.77E-11
21	35,644,275	C	G	86.81	7.22E-14	2.77E-11
22	35,672,693	A	T	87.36	6.47E-14	2.77E-11
23	35,672,715	C	T	87.36	6.47E-14	2.77E-11
24	35,672,828	A	ACT	87.36	6.47E-14	2.77E-11
25	35,673,211	C	T	87.37	6.46E-14	2.77E-11
26	35,673,266	G	C	87.37	6.46E-14	2.77E-11
27	35,673,363	G	A	87.37	6.46E-14	2.77E-11
28	35,673,586	C	A	87.38	6.45E-14	2.77E-11
29	35,673,610	C	T	87.38	6.45E-14	2.77E-11
30	35,682,762	G	T	87.56	6.23E-14	2.77E-11
31	35,682,817	G	C	87.56	6.23E-14	2.77E-11
32	35,703,960	G	C	87.97	5.75E-14	2.77E-11
33	35,711,480	TGTGCG	T	88.12	5.58E-14	2.77E-11
34	35,712,161	T	C	88.13	5.57E-14	2.77E-11
35	35,879,522	T	G	91.40	2.99E-14	2.77E-11
36	35,913,263	C	T	91.40	2.99E-14	2.77E-11
37	35,913,625	A	C	91.40	2.99E-14	2.77E-11
38	35,917,816	T	A	91.36	3.01E-14	2.77E-11
39	35,917,973	C	T	91.36	3.01E-14	2.77E-11
40	35,917,987	C	T	91.36	3.01E-14	2.77E-11
41	35,945,203	T	A	91.10	3.16E-14	2.77E-11
42	36,030,953	G	A	90.29	3.69E-14	2.77E-11
43	36,030,957	T	G	90.29	3.69E-14	2.77E-11
44	36,030,961	T	A	90.29	3.69E-14	2.77E-11
45	36,030,968	A	C	90.29	3.69E-14	2.77E-11
46	36,030,984	A	T	90.29	3.69E-14	2.77E-11
47	36,030,991	C	T	90.29	3.69E-14	2.77E-11
48	36,031,607	G	A	90.28	3.69E-14	2.77E-11
49	36,031,662	T	G	90.28	3.69E-14	2.77E-11
50	36,031,666	G	A	90.28	3.69E-14	2.77E-11
51	36,031,706	G	A	90.28	3.70E-14	2.77E-11
52	36,031,826	T	A	90.28	3.70E-14	2.77E-11
53	36,031,865	ATAGTGG	A,ATGG	90.28	3.70E-14	2.77E-11
54	36,031,894	A	C	90.28	3.70E-14	2.77E-11
55	36,032,535	T	C	90.27	3.70E-14	2.77E-11
56	36,032,606	C	T	90.27	3.70E-14	2.77E-11
57	36,067,690	A	C	89.94	3.94E-14	2.77E-11

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58	36,067,691	A	G	89.94	3.94E-14	2.77E-11
59	36,074,020	T	C	89.88	3.99E-14	2.77E-11
60	36,074,021	G	A	89.88	3.99E-14	2.77E-11
61	36,074,561	C	T	89.87	3.99E-14	2.77E-11
62	36,320,218	A	T	87.54	6.25E-14	2.77E-11
63	36,384,423	A	T	86.93	7.04E-14	2.77E-11
64	36,384,475	A	C	86.93	7.04E-14	2.77E-11
65	36,385,269	T	G	86.93	7.05E-14	2.77E-11
66	36,402,893	T	C	86.76	7.28E-14	2.77E-11
67	36,416,266	C	CAA	86.63	7.46E-14	2.77E-11
68	36,624,428	ATGTCTC	A	80.20	2.72E-13	9.65E-11
69	36,626,757	G	A	80.11	2.77E-13	9.67E-11
70	36,631,856	A	T	79.91	2.89E-13	9.74E-11
71	36,631,887	A	G	79.91	2.89E-13	9.74E-11
72	36,645,461	A	AGGGTT	79.37	3.24E-13	1.01E-10
73	36,650,365	C	CA	79.17	3.37E-13	1.01E-10
74	36,655,555	T	G	78.96	3.52E-13	1.01E-10
75	36,703,953	T	G	77.04	5.28E-13	1.42E-10
76	36,704,353	A	G	77.02	5.30E-13	1.42E-10
77	36,704,370	A	G	77.02	5.30E-13	1.42E-10
78	36,704,527	T	C	77.01	5.31E-13	1.42E-10
79	36,704,575	T	C	77.01	5.31E-13	1.42E-10
80	36,718,314	T	A	76.46	5.97E-13	1.57E-10

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Table 8. Sequence information and mapping rate of 12 RNA-Seq data with HISAT2 and Kallisto

Sample	Total sequences	HISAT2	Kallisto
R bulk sample 1	19,437,965	86.27%	79.8%
R bulk sample 2	23,592,353	86.52%	79.4%
R bulk sample 3	21,675,835	87.14%	79.6%
S bulk sample 1	27,728,769	86.08%	77.8%
S bulk sample 2	27,779,334	85.72%	76.9%
S bulk sample 3	26,945,146	85.71%	75.5%
'KH11' sample 1	24,253,695	86.68%	79.1%
'KH11' sample 2	24,161,669	85.70%	76.6%
'KH11' sample 3	24,606,510	85.49%	77.7%
'HL1' sample 1	21,812,899	85.58%	79.5%
'HL1' sample 2	22,869,448	87.41%	79.1%
'HL1' sample 3	21,565,699	85.92%	78.1%

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Table 9. Information of DEGs on Chr06, Chr15, and Chr16

GeneID	Annotation	Log_2FC*	Padj#
Glyma.06G003100	Arabidopsis phospholipase-like protein (PEARLI 4) family	-1.92	1.53E-05
Glyma.06G014500	Photosystem II reaction center PsbP family protein	0.38	2.71E-02
Glyma.06G015400	Ribophorin I	-0.29	2.30E-02
Glyma.06G015900	glyceraldehyde-3-phosphate dehydrogenase B subunit	0.75	6.90E-04
Glyma.06G018300	Pentatricopeptide repeat (PPR-like) superfamily protein	0.51	3.26E-05
Glyma.06G019400	FtsH extracellular protease family	0.58	5.30E-05
Glyma.06G020700	sterol methyltransferase 2	-0.39	3.85E-02
Glyma.06G024000	Protein of unknown function (DUF668)	-0.86	1.78E-03
Glyma.06G024100	tubulin beta-1 chain	0.60	4.36E-02
Glyma.06G032200	CBS domain-containing protein with a domain of unknown function (DUF21)	-0.57	6.30E-03
Glyma.06G034700	calmodulin-like 41	-0.99	5.82E-03
Glyma.06G045500	adenine phosphoribosyl transferase 1	-0.28	1.37E-02
Glyma.06G053000	Putative lysine decarboxylase family protein	0.49	7.62E-03
Glyma.06G053300	Protein of unknown function (DUF3511)	-1.03	2.14E-03
Glyma.06G061000	proton gradient regulation 3	0.38	3.97E-02
Glyma.06G065000	cellulose synthase family protein	1.55	2.81E-02
Glyma.06G066300	Cysteinyl-tRNA synthetase, class Ia family protein	0.37	2.68E-02
Glyma.06G069100	DnaJ/Hsp40 cysteine-rich domain superfamily protein	0.38	4.87E-02
Glyma.06G073600	Mog1/PsbP/DUF1795-like photosystem II reaction center PsbP family protein	0.40	1.67E-02
Glyma.06G077400	WRKY DNA-binding protein 11	-0.80	2.19E-02
Glyma.06G078600	AIG2-like (avirulence induced gene) family protein	0.38	4.10E-02
Glyma.06G082000	Sulfite exporter TauE/SafE family protein	-0.96	2.02E-02
Glyma.06G090800	Leucine-rich receptor-like protein kinase family protein	-0.78	1.56E-02
Glyma.06G096100	MSCS-like 2	0.47	1.26E-02
Glyma.06G100200	RNA-binding (RRM/RBD/RNP motifs) family protein	0.60	7.25E-04
Glyma.06G100900		-2.39	6.91E-04
Glyma.06G106100	sulfate transporter 1;3	-0.64	9.57E-03
Glyma.06G113200	Chlorophyll A-B binding family protein	1.01	3.16E-03
Glyma.06G114800	PDI-like 1-2	-0.43	2.17E-02
Glyma.06G119300	wall associated kinase 3	-0.94	7.58E-04
Glyma.06G119500		1.17	2.20E-02
Glyma.06G122400	Mog1/PsbP/DUF1795-like photosystem II reaction center PsbP family protein	0.61	1.76E-02
Glyma.06G125600	WRKY DNA-binding protein 30	-1.60	7.77E-05
Glyma.06G129300	IQ-domain 21	0.94	1.52E-02
Glyma.06G129400	Glycogen/starch synthases, ADP-glucose type	0.38	3.89E-02
Glyma.06G135200	CTC-interacting domain 11	-0.28	4.27E-02
Glyma.06G141600		-0.83	3.49E-02
Glyma.06G142000	WRKY DNA-binding protein 70	-1.29	1.34E-03
Glyma.06G143000	Chalcone-flavanone isomerase family protein	-1.13	8.79E-06
Glyma.06G143900		1.80	4.36E-02
Glyma.06G145700	ABC2 homolog 13	0.51	1.84E-03
Glyma.06G146900	general control non-repressible 5	0.70	3.14E-02
Glyma.06G154700	embryo defective 2170	-1.64	1.59E-04
Glyma.06G156800		0.53	2.92E-02
Glyma.06G158400		1.78	1.17E-02
Glyma.06G158800	Eukaryotic aspartyl protease family protein	1.54	1.85E-02
Glyma.06G160200	Sec14p-like phosphatidylinositol transfer family protein	0.62	2.71E-02
Glyma.06G161100	Protein prenylyltransferase superfamily protein	0.58	1.46E-03
Glyma.06G162200	Clp ATPase	-0.54	1.48E-02
Glyma.06G162400		-2.69	7.10E-06
Glyma.06G166200	Protein kinase superfamily protein	-0.86	1.15E-03
Glyma.06G166700	DHHC-type zinc finger family protein	-0.79	1.52E-05
Glyma.06G167500	SAUR-like auxin-responsive protein family	-3.97	2.57E-05
Glyma.06G169300	thylakoid lumenal 17.9 kDa protein, chloroplast	0.63	5.68E-03
Glyma.06G169400	Major facilitator superfamily protein	0.68	9.68E-04
Glyma.06G171400	RNA-metabolising metallo-beta-lactamase family protein	0.42	1.32E-03
Glyma.06G172400	Nucleic acid-binding, OB-fold-like protein	0.40	2.37E-04
Glyma.06G175400	31-kDa RNA binding protein	0.55	1.47E-03
Glyma.06G186400	ADP-glucose pyrophosphorylase family protein	0.48	2.17E-02
Glyma.06G187200	alpha/beta-Hydrolases superfamily protein	-0.88	1.92E-03

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Glyma.06G187300	alpha/beta-Hydrolases superfamily protein	-1.38	2.01E-04
Glyma.06G187400	alpha/beta-Hydrolases superfamily protein	-0.71	9.43E-05
Glyma.06G188100	BIG PETAL P	0.56	5.30E-03
Glyma.06G194900	light-harvesting chlorophyll-protein complex I subunit A4	0.91	4.05E-03
Glyma.06G214700	SOUL heme-binding family protein	0.74	7.84E-03
Glyma.06G217400	DNA/RNA polymerases superfamily protein	0.50	4.90E-04
Glyma.06G220700	blue-copper-binding protein	-2.87	3.62E-06
Glyma.06G227200	SecY protein transport family protein	-0.56	3.17E-02
Glyma.06G245200	thioredoxin M-type 4	0.99	1.77E-04
Glyma.06G249300	Cyclophilin-like peptidyl-prolyl cis-trans isomerase family protein	0.46	5.39E-03
Glyma.06G249400	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein	-1.86	1.79E-02
Glyma.06G255900	receptor kinase 3	-0.57	8.60E-05
Glyma.06G258000	Calcium-binding EF-hand family protein	-1.28	1.08E-03
Glyma.06G261100	S-locus lectin protein kinase family protein	-2.74	8.07E-06
Glyma.06G261500	disease resistance protein (TIR-NBS-LRR class), putative	-1.23	3.87E-02
Glyma.06G261900	receptor kinase 3	-0.98	2.15E-02
Glyma.06G262000	S-locus lectin protein kinase family protein	-2.47	7.80E-03
Glyma.06G265500	GRAS family transcription factor	-1.71	7.47E-03
Glyma.06G265700	NAD(P)-binding Rossmann-fold superfamily protein	1.06	4.03E-04
Glyma.06G268100	disease resistance protein (TIR-NBS-LRR class), putative	-1.48	4.89E-03
Glyma.06G277600	ATP synthase protein I -related	0.35	6.84E-03
Glyma.06G283400	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein	0.82	3.68E-02
Glyma.06G286800	Seven transmembrane MLO family protein	-2.10	3.96E-02
Glyma.06G295400		-1.72	3.55E-07
Glyma.06G299900	myb domain protein 14	-2.09	1.64E-03
Glyma.06G302300	xyloglucan endotransglucosylase/hydrolase 9	1.40	3.00E-02
Glyma.06G303600	RING-box 1	-0.35	4.65E-02
Glyma.06G305000	glutamate receptor 3.4	-0.51	2.57E-05
Glyma.06G306200		-0.89	4.06E-02
Glyma.06G307700	WRKY family transcription factor	-0.82	4.91E-02
Glyma.06G308800	strictosidine synthase-like 3	-0.69	1.39E-03
Glyma.06G309000	proline-rich family protein	0.94	9.49E-06
Glyma.06G310100	alpha/beta-Hydrolases superfamily protein	-0.90	4.42E-02
Glyma.06G315100	aminophospholipid ATPase 1	-1.53	3.41E-05
Glyma.06G317800	DERLIN-1	-1.17	8.07E-04
Glyma.06G320000	Eukaryotic protein of unknown function (DUF842)	-2.13	3.99E-03
Glyma.06G321600	FAD-binding Berberine family protein	-2.54	2.21E-02
Glyma.15G007200	Protein kinase superfamily protein	-0.57	4.71E-05
Glyma.15G011500	nucleotide transporter 1	0.82	1.94E-05
Glyma.15G011900	pleiotropic drug resistance 12	-1.50	7.10E-06
Glyma.15G012000	pleiotropic drug resistance 12	-2.64	1.41E-03
Glyma.15G016800	PLAC8 family protein	-2.32	2.32E-08
Glyma.15G018800	AAA-type ATPase family protein	0.38	2.81E-02
Glyma.15G021400	Leucine-rich repeat transmembrane protein kinase protein	-0.75	2.15E-03
Glyma.15G028400		-1.34	2.75E-02
Glyma.15G034100	lysophosphatidyl acyltransferase 2	0.69	1.04E-02
Glyma.15G045200	LOB domain-containing protein 11	-2.20	3.68E-04
Glyma.15G051600	BRI1-associated receptor kinase	-0.43	3.32E-03
Glyma.15G053700	Protein phosphatase 2C family protein	-0.69	2.20E-05
Glyma.15G054600	EXORDIUM like 2	-1.10	3.94E-02
Glyma.15G061100	methionine sulfoxide reductase B 2	0.31	2.71E-02
Glyma.15G062500	basic pathogenesis-related protein 1	-1.35	3.97E-03
Glyma.15G064900	S-locus lectin protein kinase family protein	-1.60	4.64E-03
Glyma.15G065100	receptor kinase 3	-2.47	1.21E-03
Glyma.15G069100	secretory carrier 3	-0.71	3.66E-04
Glyma.15G079100	ethylene responsive element binding factor 2	-1.01	4.20E-09
Glyma.15G080200	P-loop containing nucleoside triphosphate hydrolases superfamily protein	0.40	2.01E-02
Glyma.15G086500	Eukaryotic protein of unknown function (DUF872)	-0.39	5.85E-03
Glyma.15G087200	Aldolase-type TIM barrel family protein	0.45	6.03E-03
Glyma.15G088000	heat shock protein 70B	-0.81	1.14E-02
Glyma.15G110300	WRKY family transcription factor	-0.91	2.17E-02
Glyma.15G115600	Auxin-responsive family protein	-1.27	1.88E-03

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Glyma.15G122200	Copper transport protein family	-2.56	9.29E-03
Glyma.15G124700	ferric reduction oxidase 7	1.04	1.34E-03
Glyma.15G126800	Transducin/WD40 repeat-like superfamily protein	0.61	1.88E-02
Glyma.15G127200	NPR1-like protein 3	-0.60	2.81E-02
Glyma.15G127500	Pectin lyase-like superfamily protein	0.84	1.23E-03
Glyma.15G131400		-4.27	7.25E-03
Glyma.15G132500	fatty acid hydroxylase 1	0.41	2.79E-02
Glyma.15G133600	Glycosyl hydrolases family 31 protein	-0.81	1.18E-04
Glyma.15G148700	Cysteine proteinases superfamily protein	-0.72	2.11E-03
Glyma.15G154500	Mitochondrial transcription termination factor family protein	1.23	1.32E-03
Glyma.15G158200	VQ motif-containing protein	-2.58	1.66E-05
Glyma.15G160400	Ankyrin repeat family protein	-0.58	4.08E-04
Glyma.15G160900	Disease resistance protein (TIR-NBS-LRR class) family	-1.48	4.27E-03
Glyma.15G161000	HISTIDINE TRIAD NUCLEOTIDE-BINDING 2	0.55	9.91E-03
Glyma.15G161100		1.43	2.07E-08
Glyma.15G161600	lectin protein kinase family protein	-1.71	6.93E-06
Glyma.15G162600	H(+)-ATPase 2	-1.31	2.71E-02
Glyma.15G168500	NB-ARC domain-containing disease resistance protein	-0.93	3.99E-04
Glyma.15G168700	Glutaredoxin family protein	-0.36	2.81E-02
Glyma.15G169500	beta-galactosidase 12	0.97	3.79E-02
Glyma.15G170500	basic helix-loop-helix (bHLH) DNA-binding family protein	-2.52	9.50E-06
Glyma.15G171000	calmodulin-binding family protein	-0.82	4.58E-02
Glyma.15G172800	Ribosomal protein L18ae family	-1.32	2.74E-04
Glyma.15G177900	Protein kinase superfamily protein	-2.06	5.27E-03
Glyma.15G185000	Ribosomal L18p/L5e family protein	0.37	2.92E-02
Glyma.15G197200	Mitochondrial transcription termination factor family protein	0.89	3.24E-04
Glyma.15G201100	flavin-dependent monooxygenase 1	1.55	3.27E-03
Glyma.15G202300	Cyclophilin-like peptidyl-prolyl cis-trans isomerase family protein	0.67	7.54E-06
Glyma.15G205900	temperature-induced lipocalin	0.64	1.38E-04
Glyma.15G211500	Kunitz family trypsin and protease inhibitor protein	-4.51	2.85E-05
Glyma.15G212400	LEM3 (ligand-effect modulator 3) family protein / CDC50 family protein	-0.59	5.65E-04
Glyma.15G215600	Leucine-rich repeat protein kinase family protein	1.67	2.74E-02
Glyma.15G216400	ribonuclease Ps	0.38	2.04E-02
Glyma.15G232200	sigma factor binding protein 1	-3.12	7.43E-04
Glyma.15G242900	Chalcone-flavanone isomerase family protein	0.92	5.92E-03
Glyma.15G243100	solanesyl diphosphate synthase 1	1.18	8.02E-03
Glyma.15G249800	VQ motif-containing protein	-2.65	7.32E-04
Glyma.15G250200	receptor-like protein kinase 1	2.06	2.23E-02
Glyma.15G251800	glutathione S-transferase TAU 19	-1.30	1.70E-02
Glyma.15G253500	Protein kinase superfamily protein	-0.99	6.21E-03
Glyma.15G254900	Pentatricopeptide repeat (PPR) superfamily protein	0.48	1.24E-02
Glyma.15G256300		-0.83	8.37E-05
Glyma.15G258800	SAUR-like auxin-responsive protein family	-0.94	5.07E-03
Glyma.15G265600	P-loop containing nucleoside triphosphate hydrolases superfamily protein	0.59	1.91E-03
Glyma.15G268200		-4.01	3.68E-05
Glyma.15G268400	cofactor of nitrate reductase and xanthine dehydrogenase 2	0.69	5.52E-03
Glyma.16G008600	Cytochrome P450 superfamily protein	-0.86	1.06E-02
Glyma.16G010200	Tetratricopeptide repeat (TPR)-like superfamily protein	0.49	1.17E-02
Glyma.16G014700	RING/U-box superfamily protein	-3.13	2.71E-02
Glyma.16G015900	Protein of unknown function (DUF1295)	-0.86	2.15E-02
Glyma.16G016100	photosystem I light harvesting complex gene 2	0.93	1.56E-03
Glyma.16G019900	D-arabinono-1,4-lactone oxidase family protein	-2.90	4.11E-03
Glyma.16G021300	cytochrome B5 isoform E	-0.71	3.68E-03
Glyma.16G023000	myb domain protein 111	-1.86	6.45E-05
Glyma.16G024100	embryo defective 1703	0.34	1.07E-02
Glyma.16G026900	Auxin-responsive GH3 family protein	-0.83	9.42E-05
Glyma.16G034900	receptor-like protein kinase 4	-0.49	4.01E-02
Glyma.16G035200		-0.61	3.71E-02
Glyma.16G037100	Peptidase M50 family protein	0.45	9.32E-03
Glyma.16G041600	evolutionarily conserved C-terminal region 2	-0.26	4.83E-02
Glyma.16G044900	glyceraldehyde 3-phosphate dehydrogenase A subunit 2	0.65	3.44E-02
Glyma.16G046000	DEAD/DEAH box helicase, putative	0.59	9.66E-06
Glyma.16G050700	GRAM domain family protein	-1.18	5.60E-03

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Glyma.16G054400	WRKY DNA-binding protein 75	-2.71	6.26E-05
Glyma.16G055900	Peroxidase superfamily protein	-4.63	5.78E-06
Glyma.16G056900	Peptidase family M48 family protein	0.70	3.28E-03
Glyma.16G060700	RmlC-like cupins superfamily protein	-6.24	7.53E-04
Glyma.16G060800	RmlC-like cupins superfamily protein	-5.92	2.50E-04
Glyma.16G061800	glutamate receptor 2.5	-1.44	1.07E-02
Glyma.16G062600	lysine histidine transporter 1	-1.21	3.84E-02
Glyma.16G063200	glucose-6-phosphate dehydrogenase 6	-0.99	3.01E-03
Glyma.16G069200	Protein kinase superfamily protein	-0.47	3.00E-02
Glyma.16G075800	Pectinacetylesterase family protein	0.71	1.55E-02
Glyma.16G076300	Long-chain fatty alcohol dehydrogenase family protein	0.91	3.87E-02
Glyma.16G076400	Protein of unknown function (DUF1218)	-1.51	1.78E-02
Glyma.16G078900	Protein kinase family protein with leucine-rich repeat domain	-1.52	6.33E-05
Glyma.16G079200	BAK1-interacting receptor-like kinase 1	-0.56	2.21E-02
Glyma.16G080800	Yippee family putative zinc-binding protein	-0.90	3.45E-03
Glyma.16G090700	RPM1 interacting protein 4	-0.46	2.36E-03
Glyma.16G096600	phototropin 2	0.55	2.54E-03
Glyma.16G102000	C2H2 type zinc finger transcription factor family	-1.19	2.56E-02
Glyma.16G103900	pinoresinol reductase 1	-1.72	5.20E-09
Glyma.16G106500	putrescine-binding periplasmic protein-related	0.57	2.49E-03
Glyma.16G132500	alpha/beta-Hydrolases superfamily protein	-2.11	5.60E-05
Glyma.16G137200	disease resistance protein (TIR-NBS-LRR class), putative	-1.14	1.82E-02
Glyma.16G138000	alpha/beta-Hydrolases superfamily protein	0.88	1.96E-03
Glyma.16G141200	Protein phosphatase 2C family protein	0.65	1.35E-02
Glyma.16G145200	methyl esterase 5	-2.76	8.26E-04
Glyma.16G145600	Seven transmembrane MLO family protein	-3.10	3.14E-04
Glyma.16G146700	phosphate transporter 3;1	-1.98	3.70E-03
Glyma.16G147900	Protein of unknown function (DUF3754)	0.51	2.80E-02
Glyma.16G151500	NAC domain containing protein 47	-1.92	2.95E-02
Glyma.16G155700	glutathione-disulfide reductase	-0.27	1.82E-02
Glyma.16G156300	5\'-3\' exonuclease family protein	0.34	3.56E-02
Glyma.16G159100	Disease resistance protein (TIR-NBS-LRR class) family	-1.03	2.12E-02
Glyma.16G159300	Disease resistance protein (TIR-NBS-LRR class) family	-1.55	1.77E-03
Glyma.16G162400	Pyridoxal phosphate (PLP)-dependent transferases superfamily protein	-9.34	4.17E-07
Glyma.16G166600		-1.06	2.89E-02
Glyma.16G167200	tubby like protein 5	-0.43	2.67E-02
Glyma.16G170000		-3.82	1.28E-02
Glyma.16G173500	SLAC1 homologue 3	1.29	4.14E-03
Glyma.16G173900	disease resistance family protein / LRR family protein	-1.60	1.10E-05
Glyma.16G174000	receptor like protein 1	-1.74	4.83E-07
Glyma.16G174600	disease resistance family protein / LRR family protein	-1.37	2.08E-03
Glyma.16G174700	disease resistance family protein / LRR family protein	-1.30	2.40E-03
Glyma.16G175100	disease resistance family protein / LRR family protein	-1.16	4.76E-03
Glyma.16G176200	phosphatase-related	-0.32	4.05E-03
Glyma.16G176600	disease resistance family protein / LRR family protein	-1.51	1.87E-02
Glyma.16G176900	disease resistance family protein / LRR family protein	-1.20	1.77E-02
Glyma.16G189300	Copper transport protein family	-1.10	1.31E-02
Glyma.16G194100	membrane-associated progesterone binding protein 3	0.64	1.56E-03
Glyma.16G195600	cytochrome P450, family 71, subfamily A, polypeptide 26	-4.74	2.62E-08
Glyma.16G199200	GroES-like zinc-binding alcohol dehydrogenase family protein	0.49	2.96E-02
Glyma.16G199800		-1.90	4.87E-06
Glyma.16G199900	peptidoglycan-binding LysM domain-containing protein	-2.36	7.18E-04
Glyma.16G201500	Protein kinase superfamily protein	-1.02	8.52E-07
Glyma.16G202000	Embryo-specific protein 3, (ATS3)	-2.70	2.32E-03
Glyma.16G204100	Double Clp-N motif protein	0.34	2.19E-02
Glyma.16G205100	Leucine-rich repeat protein kinase family protein	1.81	8.19E-07
Glyma.16G205500	Arabidopsis thaliana protein of unknown function (DUF794)	0.52	2.78E-02
Glyma.16G208300	carboxyesterase 18	1.05	1.72E-03
Glyma.16G208800	SWIB/MDM2 domain; Plus-3;GYF	-0.88	2.03E-02
Glyma.16G212600	GRAM domain-containing protein / ABA-responsive protein-related	-0.94	1.21E-05
Glyma.16G212900	YELLOW STRIPE like 7	-1.11	2.55E-09
Glyma.16G213700	Disease resistance protein (TIR-NBS-LRR class) family	1.35	2.77E-08
Glyma.16G213800	Disease resistance protein (TIR-NBS-LRR class) family	-1.19	3.44E-04
Glyma.16G213900	Disease resistance protein (TIR-NBS-LRR class) family	-1.93	1.25E-07

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Glyma.16G214000	disease resistance protein (TIR-NBS-LRR class), putative	-3.01	1.78E-15
Glyma.16G214100	Disease resistance protein (TIR-NBS-LRR class) family	-1.54	7.35E-09
Glyma.16G214500	Disease resistance protein (TIR-NBS-LRR class) family	-3.54	1.78E-09
Glyma.16G215100	disease resistance protein (TIR-NBS-LRR class), putative	1.74	1.57E-15
Glyma.16G218000	cyclic nucleotide-binding transporter 1	-0.31	1.20E-02
Glyma.16G219800	WRKY DNA-binding protein 70	-2.49	5.82E-04
Glyma.16G220900	HAD superfamily, subfamily IIIB acid phosphatase	-2.48	4.88E-03

*: Log_2 Fold Change: The log_2 FC values uses the susceptible variety as the denominator to calculate the expression level.
#: Adjusted *p*-value

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