Genome Wide Association Study (GWAS) for Southern Corn Rust (SCR) Disease Resistance in Maize

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ABSTRACT

Southern corn rust (SCR), caused by *Puccinia polysora* Undrew, is one of the most important maize diseases threatening maize production. Growing resistant varieties is the most practical and cost-effective approach to controlling the disease. Identification of resistance genes would help in the development of high-yielding resistant maize hybrids. Genome-wide association studies (GWAS) can efficiently reveal genomic loci associated with the desired phenotypic traits. In this study, the phenotypes of 262 maize recombinant inbreds against two isolates of SCR disease, namely, Nakhon Pathom and Chiang Mai, were investigated. Using 434,871 single nucleotide polymorphism (SNP) markers obtained from the maize SNP 600K genotyping array, GWAS was performed with the Fixed and random model Circulating Probability Unification (FarmCPU) model. The results showed that 19 SNPs distributed on chromosomes 1, 2, 3, 4, 5, 9 and 10 were significantly associated with resistance to SCR disease. As a result, 19 quantitative trait loci (QTL)s and 36 candidate genes were identified. In addition, the three major QTLs/SNP loci which included AX-90915192 on chromosome 4, AX-91151225 on chromosome 9 and AX-91648757 on chromosome 5, could distinguish the disease-resistant from disease-susceptible lines. These identified SNPs and genes provide useful information for cloning genes and understanding disease resistance mechanisms to SCR, and can be used in marker-assisted breeding programs to develop SCR resistant maize.

Keywords: Genome-wide association study (GWAS); *Puccinia polysora*; Southern corn rust (SCR); single nucleotide polymorphism (SNP) genotyping array

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INTRODUCTION

Maize rust caused by *Puccinia* spp., is considered the most persistent and destructive maize disease. In Thailand, the rust disease in different regions, as identified by its morphology and molecular markers was southern corn rust (SCR) caused by *P. polysora* Undrew, (Unartngam et al., 2011). The SCR is an obligate parasite that damages the entire corn plant. Symptoms first appear on the leaves and spread to the entire plant at maturity, resulting in leaf necrosis and complete destruction of photosynthetic areas until the plant eventually dies (Cammack, 1958). Yield losses caused by SCR can be as high as 45-50% with destruction being greatest in late season corn planting (Raid et al., 1988).

The use of resistant varieties is the most practical and cost-effective approach to control yield loss from SCR disease. To obtain a genotype with a high degree of resistance to SCR for breeding program, knowledge of the gene activity involved in the expression of the resistance response in the plant materials is a prerequisite. The main obstacle in using the conventional selection process to identify genotype with SCR resistance is the difficulty in selecting the right genes expressed in their morphological or agronomic traits for selection (Ashkani et al., 2015). Using molecular markers associated with SCR resistance could be a more convenient and time-saving approach for the selection of resistance genotypes. The mapping of quantitative trait loci (QTL) is the method that allows the discovery, localization, and characterization of genetic factors that contribute to polygenically inherited variation (Young, 1996). Researchers investigated the inheritance of resistance to SCR (Lv et al., 2021; Wanlayaporn et al., 2013) and suggested that the resistance mechanism is complex and polygenic.

Genome-wide association studies (GWAS) is an efficient approach to discover genomic loci associated with phenotypic traits (Ma et al., 2019). They involved high-resolution genome coverage using single nucleotide polymorphism markers (SNPs), and are the most recent approach for identifying natural variation and mapping chromosomal regions associated with traits of interest in plants, including maize (Elshire et al., 2011). GWAS utilizes different sets of inbred lines and provides a way to accurately locate QTLs for quantitative traits and identify candidate genes (Olukolu et al., 2016). Genetic studies of rust disease resistance in maize using GWAS were reported from the USA, Brazil and China (Olukolu et al., 2016; de Souza Camacho et al., 2019; Zhou et al., 2018). However, the maize germplasm of Thailand and Myanmar has less genetic information about SCR resistance for breeding programs

to develop resistant varieties. Therefore, the objective of this study was to identify the genomic regions and candidate genes associated with SCR disease resistance of maize by using SNPs markers in tropically adapted maize germplasm in Thailand.

MATERIALS AND METHODS

Phenotypic evaluation for SCR disease

In this study, 262 varieties and different recombinant inbred lines (RILs) of Thailand and Myanmar were evaluated. Of the 262 RILs, 188 lines were developed by the Department of Plant Science and Agricultural Resources of Khon Kaen University, 41 by Chai Nat Field Crop Research Center of the Department of Agriculture, Ministry of Agriculture and Cooperatives, 13 by Rajamangala University of Technology Lanna Lampang, 19 by Myanmar Agricultural Research Department, and Hybrix-3. Of 262 varieties, 71 were sweet corn, 168 were waxy corn and 23 were field corn. In these studies, Hybrix-3 was used as the susceptible and Ki-60 as the resistant controls for SCR.

Experiments were conducted using a randomized complete block design (RCBD) with three replicates. Disease screening was conducted 14 days after planting of the maize lines using two inoculum sources from Nakhon Pathom and Chiang Mai. For inoculation, the rust infected leaves were collected from the infected fields and the uredospores were obtained by washing. Then, the uredospores were filtrated with Whatman filter paper No. 1. The filter paper containing the ureodospores was cut into 3-5 mm pieces. After that, the paper pieces were placed on two leaves of each plant. One night after inoculation, they were removed from the plants.

Twenty-one days after inoculation, the severity of the disease was recorded by using a score (0-3); where 0=no symptom, 1=pustules or uredospores 1-25% of leaf area, small pustules that did not erupt, and no further development of pustules, 2=pustules or uredospores 25-50% of leaf area, <1 times development of pustules and 3=pustules or uredospores >50% of leaf area, erupted pustules, further development of pustules, and spread across leaves. Then, percent disease index (PDI) for SCR disease was calculated. Subsequently, the phenotypes of the tested lines were evaluated as a function of PDI as 0: Highly resistant (HR), 1-10: Resistant (R), 11-25: Moderately resistant (MR), 26-50: Moderately susceptible (MS), 51-75: Susceptible (S) and 76-100: Highly susceptible (HS) (Unartngam, 2019).

Statistical analyses

The phenotypic data from all trials were analyzed using the R program (R Core Team, 2018). Comparison of means was performed at the 5% level of least significant

difference (LSD). Estimates of variance components σ^2G (genotypic variance), σ^2ge (G x E) (interaction variance), and σ^2e (error variance) of the tested varieties were computed and the broad-sense heritability (h^2) was calculated using the following formula (Hallauer and Miranda, 1981).

$$h2 = \sigma^2G/(\sigma^2G+(\sigma^2ge/e)+(\sigma^2e/re))$$

Maize array genotyping and SNP calling

Genomic DNA was extracted using the magnetic bead method (Xin and Chen, 2012). Five plants per sample were pooled and used for DNA extraction. The pooled panel of 262 maize inbred lines was genotyped using the Axiom™ maize 600 K genotyping array with 616,201 variants (Thermo Fisher Scientific). To determine the quality of the data, quality control (Q.C.) was performed with a call rate of 90% (517,293 SNPs) (Axiom genomic suit). Subsequently, SNP markers with more than 10% missing data, 20% heterozygosity, less than 5% minor allele frequency (MAF) were excluded from the data set to obtain only bi-allelic sites (434,871 SNPs). These SNPs were used for GWAS analysis.

PCA and linkage disequilibrium analysis

Principal component analysis (PCA) was also performed to determine the relationship between samples using the software TASSEL (Bradbury et al., 2007), removing SNPs with minor allele frequencies (MAFs) of 5% and limiting the number of components to three. Linkage disequilibrium (LD) of all SNP pairs on each chromosome was determined using the PopLDdecay (https://doi.org/10.1093/bioinformatics/bty875), with the following parameters: MAF>5%; Hardy–Weinberg *P*-value cut-off, 0; and percentage of genotyped lines >0.75. In addition, SNPs with high LD were pruned using the in-depth-pairwise function implemented in PLINK (SNP window size: 50, shifted SNPS per step: 10, r2 thresholds: 0.1) (Niu et al., 2019) resulting in 160,991 SNP markers. This set of SNPs was used for population structure analysis using STRUCTURE (Evanno et al., 2005).

Genome-wide association analysis and candidate genes association analysis

A genome-wide association study (GWAS) was then performed using GAPIT (Genomic Association and Prediction Integrated Tool) (Lipka et al., 2012) in R program (R Core Team, 2018). The *P* values of testing markers and the associated markers were unified at each interaction. The threshold for a significant association was set based on the Bonferroni correction level of *P*-value ($P < 1/n$; n = total markers used). To determine the amount of variance explained by each significant SNP, an analysis of variance was performed for each significant

SNP and the ratio between mean square of each SNP by error mean square was used to represent the variance percentage explained (Rossi et al., 2020). Single regression analysis in R program (R Core Team, 2018) was performed.

The available reference genome sequence of maize (B73) was used to identify candidate genes. SNP probe sequences of ~150 bp on Axiom® Maize 600K Genotyping Array (Thermo Fisher Scientific) were used as queries in a BLAST algorithm-based search against the reference genome sequence in MaizeGDB (http://www.maizegdb.org/gbrowse). The 200 bp source sequences of each significant SNP was used for BLAST against the ZmB73_RefGen_v4 genome sequence in MaizeGDB (Portwood et al., 2019). Within the local LD block of significant SNPs, the annotated genes that are likely involved in disease resistance were identified as the putative candidate genes.

RESULTS AND DISCUSSION

Phenotypic evaluation for SCR disease

Resistance to southern corn rust was evaluated using two isolates from Nakhon Pathom and Chiang Mai. The tested lines differed significantly in their phenotypic response depending on their percent disease indices (PDI) ($p<0.01$) for both isolates. According to the results, the isolate from Nakhon Pathom showed a stronger response to the tested lines than the isolate from Chiang Mai. However, Pearson correlation analysis showed a correlation between these isolates ($p\leq0.05$), and the performance of the tested lines was not too different from these isolates (Table 1). It can be assumed that the pathogenicity of these isolates was not different and the tested varieties showed similar responses against these two isolates of SCR disease. The broad-sense heritability (h^2) was very low at 11.68%. This could be due to the fact that different isolates of the disease were used in these trials. The estimated heritability was low when the experiments were conducted in different environments (Brito et al., 2001). The low heritability implies that a great effort must be made in a breeding program to accumulate a large number of resistance alleles in the germplasm. However, because the probability of selecting superior genotypes (i.e. inbreds) is low, marker assisted selection (MAS) offers a strategy that could increase selection gain.

PDI from SCR against the two isolates ranged from 0-86.67% and 0-53.33% for Nakhon Pathom and Chiang Mai, respectively. The histogram for both isolates showed that most of the tested lines fell into the resistance class (Figure 1). For the isolate from Nakhon Pathom, 76 lines showed a highly resistant reaction, 54 were resistant, 53 were moderately resistant, 53 were

moderately susceptible, 21 were susceptible and 5 were highly susceptible. There was no highly susceptible line for the isolate from Chiang Mai. A total of 125 lines showed highly resistant reactions, 26 showed resistant, 70 showed moderately resistant, 39 showed moderately susceptible and only 2 lines showed susceptible reactions. The Ki-60, resistance check, showed high resistance to the isolate from Nakhon Pathom and moderately resistant to the isolate from Chiang Mai. Against the isolate from Nakhon Pathom, the commercial maize line Hybrix-3 showed moderate resistance, while it showed high resistance against the isolate from Chiang Mai (Figure 1). The population panel used in this study included tropical maize cultivars of waxy corn, sweet corn, and field corn, and association mapping was used to investigate the genetics of maize resistance to the disease SCR. The phenotypic responses of most RILs were resistance to the two isolates of SCR disease. These resistant lines can be used as the resistant source for the breeding program to develop SCR resistant varieties.

Table 1 Phenotypic variation for percent disease index (PDI) of SCR disease in 262 recombinant inbred lines of maize based on two inoculum sources (NP and CM)

Trait	Isolate	MEAN	MIN	MAX	SD	$LSD_{(0.05)}$	CV%	*P*
SCR	NP	19.17**	0	86.66	21.36	17.72	104.39	0.32**
	CM	10.41**	0	53.33	12.74	21.09	214.09	

SCR: Southern corn rust, NP: Nakhon Pathom, CM: Chiang Mai, MEAN: average, MIN: minimum, MAX: maximum, SD: standard deviation, *P*: Pearson correlation, **highly significant difference, CV: coefficient of variation, LSD: Least significant difference

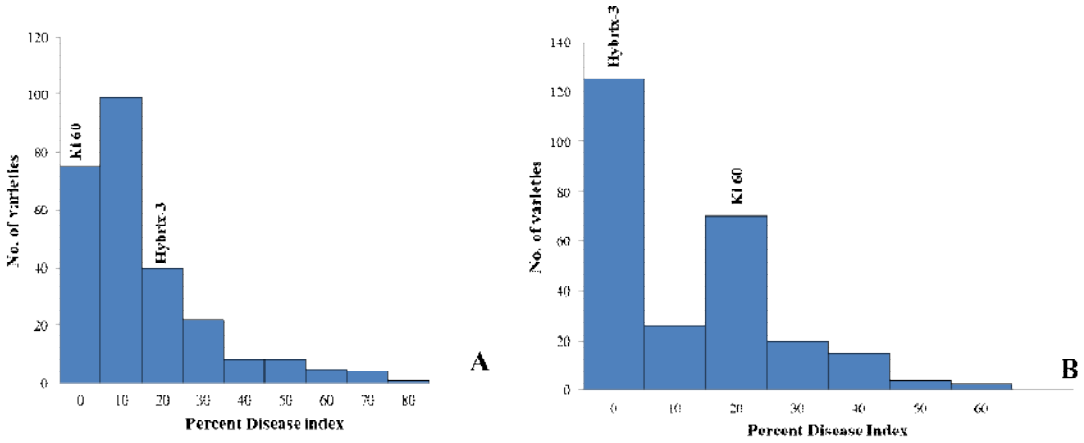

Figure 1 Frequency distribution of phenotypic variation in resistance to SCR. Frequency distribution of resistance to isolates from Nakhon Pathom (A) and Chiang Mai (B)

PCA and LD decay analysis

The genotype obtained from the high-density maize SNP array was analysed in a set of 262 maize lines using principal component analysis (PCA). Based on the genotyping of 434,871 SNPs from 262 maize lines after filtering with the parameters mentioned in materials and methods, PCA (total PCs=3) of all the genotype data was performed using the software PLINK. PC1 and PC2 explained most of the variation (16.6 and 12.1%, respectively) and were selected for visualization. Significant clusters were observed for corn type, sweet corn, waxy corn, and field corn. The average heterozygosity was 11.48 and ranged from 2.27 to 28.41. For linkage disequilibrium (LD) decay analysis, all 434,871 filtered SNPs were used as input data for calculating the genome-wide LD in the associated panel. The total LD decay in the genome of the 262 maize associated panel was 206 Kb at a cut-off r^2 = 0.1, so a 200 kb region flanking the left and right sides of an SNP was defined as a QTL (Figure 2). At a cut-off of r^2 = 0.2, the mean length of LD decay rapidly decreased to 48 kb.

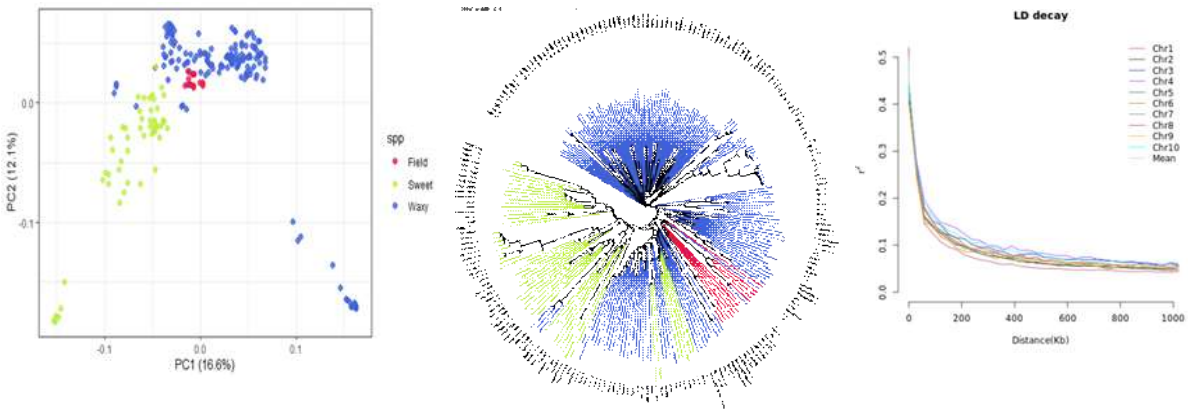

Figure 2 Principle component analysis of 262 recombinant inbred lines (RILs) of maize based on 434,871 SNP markers (A); Clustering of 262 RILs based on 434,871 SNP markers (B); LD, r^2 values versus physical distance (Mb) between all SNP pairs on the same chromosome (C). The total LD decay in the genome of 262 maize-associated panels using 434,871 SNP markers was 206 Kb at a cut-off r^2 = 0.1. At a cut-off of r^2 = 0.2, the mean length of LD decay rapidly decreased to 48 kb

GWAS analysis for marker trait associations

The responses of recombinant inbred lines against SCR disease were investigated and the QTLs associated with resistance to this disease were revealed by GWAS. GWAS utilizes different sets of inbred lines and provides a way to accurately locate QTLs for

quantitative traits and identify candidate genes (Olukolu et al., 2016). Association mapping was performed using Fixed and random model Circulating Probability Unification (FarmCPU) method incorporating population structure (PCA) and relatedness (kinship) within the tested panel, using 434,871 SNPs with rare alleles (MAF < 5%). Based on the results presented in Mahantan plots and quantile-quantile (QQ) plots (Figure 3), the significant SNPs were observed at a Bonferroni correction of $P \leq 2.29\times10^{6}$ ($P < 1/n$; n = total markers used).

In this study, the phenotypic data of two isolates, (Nakhon Pathom and Chiang Mai) of SCR disease were also used for GWAS with 434871 SNP markers. A total of 19 SNPs, 10 significant SNPs for Nakhon Pathom isolate and 9 significant SNPs for the Chiang Mai isolate, were identified with the SCR disease resistance (Table 2). The highest explained phenotypic variance (PVE) of these SNPs was 12%. For the isolate from Nakhon Pathom, the significant SNPs markers were identified on chromosome 1, 2, 3, 4, 5, 9 and 10. The most significant marker, S1_211766821, was

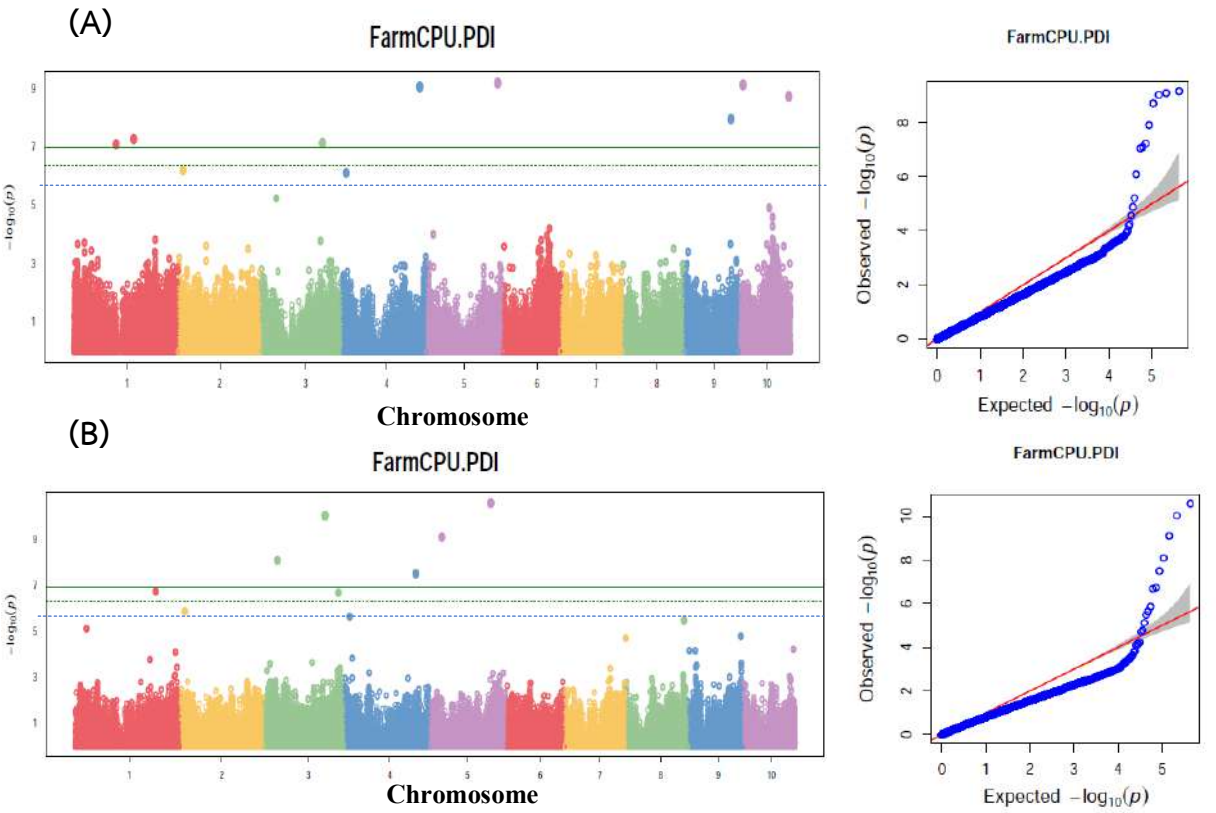

Figure 3 Manhattan plots and QQ plots from SNP-based GWAS for percent disease index (PDI) of rust disease, isolate Nakhon Pathom (A) and isolate Chiang Mai (B). The GWAS identified the most significant SNP markers that reached $p \leq 2.29\times 10-6$ (Bonferroni threshold=5.6) are listed using the FarmCPU model. The X axis indicates the physically mapped chromosomes. The Y axis indicates the significance calculated by -log10 (P)

found on chromosome 5 and had *P*-value of $6.78x10^{-10}$ and a MAF of 0.02. For the Chiang Mai isolate, a total of nine significant SNP markers were identified on chromosome 1, 2, 3, 4 and 5. They included one SNP each on chromosomes 1 and 2, 2 SNPs each on chromosome 4 and 5 and 3 SNP markers on chromosome 3. The most significant associated SNP, S5_179463254, was found on chromosome 5; this SNP had a *P*-value of $2.68x10^{-11}$ and an MAF of 0.08.

For SCR disease, 19 highly significant associated SNPs were detected on chromosomes 1, 2, 3, 4, 5, 9, and 10 for isolates from Nakhon Pathom and Chiang Mai. This result suggests that resistance to SCR disease is quantitatively inherited and controlled by many genes. Quantitative trait loci (QTL) conferring partial resistance to SCR were found on chromosomes 3 and 4 (Holland

Table 2 SNP markers associated with the trait of SCR disease resistant and their chromosomal location in 262 maize RILs by SNP-based GWAS

Trait	SNP Position(V4)	SNP Name	Chr	P-value	Allele	MAF	R^2
NP	S5_211766821	AX-90974807	5	$6.78x10^{-10}$	C/T	0.02	1.20
	S10_13757681	AX-91809638	10	$7.86x10^{-10}$	T/C	0.10	12.0
	S4_227597917	AX-90915192	4	$9.22x10^{-10}$	C/T	0.35	3.80
	S10_145635671	AX-91451802	10	$1.93x10^{-9}$	C/T	0.05	2.80
	S9_138444899	AX-91151225	9	$1.22x10^{-8}$	C/T	0.30	0.50
	S1_174752484	AX-91426201	1	$5.78x10^{-8}$	T/C	0.16	0.01
	S3_178254806	AX-91411063	3	$7.81x10^{-8}$	A/G	0.23	10.3
	S1_124668841	AX-90682835	1	$8.68x10^{-8}$	G/A	0.27	0.10
	S2_13599625	AX-90577822	2	$6.55x10^{-7}$	A/G	0.16	2.00
	S4_13128859	AX-90858848	4	$8.02x10^{-7}$	C/T	0.04	1.40
CM	S5_179463254	AX-90966401	5	$2.68x10^{-11}$	G/C	0.08	0.30
	S3_178600050	AX-90630900	3	$9.39x10^{-11}$	G/A	0.19	0.20
	S5_37538103	AX-91648757	5	$8.02x10^{-10}$	G/A	0.20	0.01
	S3_38803403	AX-91847440	3	$8.16x10^{-9}$	A/G	0.38	0.00
	S4_208062434	AX-91634643	4	$3.18x10^{-8}$	C/-	0.17	8.40
	S1_233445080	AX-90710167	1	$1.87x10^{-7}$	C/A	0.49	1.10
	S3_218037573	AX-91593556	3	$2.09x10^{-7}$	C/T	0.40	0.20
	S2_11930489	AX-91437579	2	$1.39x10^{-6}$	T/C	0.14	1.40
	S4_14738387	AX-90859313	4	$2.30x10^{-6}$	G/A	0.46	0.70

NP: Nakhon Pathom, CM: Chiang Mai, SNP: Single nucleotide polymorphism,
Chr: Chromosome, MAF: Minor allele frequency, R^2: Phenotypic variance explained

et al., 1998); 3, 4 and 9 (Jiang et al., 1999); 9 (Brunelli et al., 2002); 4, 8, 9 and 10 (Jines et al., 2007); 6 (Brewbaker et al., 2011); 1, 2, 5, 6, 9 and 10 (Wanlayaporn et al., 2013); 6, 9 and 10 (Lv et al., 2021) by biparental QTL mapping and on chromosomes 4, 8, 9 and 10 (de Souza Camacho et al., 2019); 4, 8 and 10 (Zhou et al., 2018) by GWAS analysis. Lv et al. (2021) identified 3 QTLs expressing resistance to SCR on chromosomes 6, 9, and 10 with an explained phenotypic variation of 43-78% PVE using 138 of recombinant inbred lines derived from CML496 (SCR-resistant) and Lx9801 (SCR-susceptible) and 9.4K SNP genotyping array marker platform. The significant SNPs in this study were found on 7 chromosomes as in the previous different studies; however, the positions of these SNP were different. So, all SNP found in this study may be the novel ones.

Zhou et al. (2018) performed GWAS using the SNP3K bead chip for maize and identified 7 QTLs conferring SCR resistance on chromosomes 4, 8, and 10 with PVE% ranging from 4.72-6.71%. Eight SNPs significantly associated with SCR resistance were observed on the seven chromosomes, 4, 5, 6, 7, 8, 9, and 10 by GWAS (de Souza Camacho et al., 2019). In this study, the phenotypic explanatory value of the significant SNPs for the disease SCR was also very low. The highest PVE% was 12% for the disease SCR. This result suggests that MAS alone is not recommended for routine use (Knapp, 1998). The best scheme should be the combination of MAS with conventional selection methods (ie: repeated selection) (Brito et al., 2001).

Candidate genes analysis

Version 4 of the B73 inbred line (RefGen_v4) available on MaizeGDB, was used to identify a candidate gene. A 200 kb radius around each SNP was searched for related genes involved in resistance to SCR. As a result, 19 QTLs were identified and 37 candidate genes encoding proteins related to the plant protection mechanism were selected for the rust isolates from Nakhon Pathom and Chiang Mai. However, the candidate genes linked to plant protection mechanism could not be identified in QTL_1.2_ AX-90682835 and the QTL_3.2_AX-91847440. And then, the SNP markers on chromosome 3, AX-91411063 and AX-90630900, were located on the same gene, Zm00001d04270, therefore 36 candidate genes were identified.

On chromosome 1, the significant SNPs were located in 3 candidate genes annotating F-box domain containing protein expressed, Patatin-like protein 6, LysM domain containing protein. The significant SNPs on chromosome 2 were located in 6 genes encoding binding partner of ACD11 1, myb domain protein 58, NDR1/HIN1-like 8, S-norcoclaurine synthase, MYB-related-

transcription factor 36 and 60S ribosomal protein L7-2. The associated SNPs on chromosome 3 were located in 9 genes, ethylene-responsive transcription factor ABR1, G-box-binding factor 4, histone H3, E3 ubiquitin-protein ligase PUB23, BTB/POZ domain-containing protein, GDSL esterase/ lipase, phospholipase A1-II gamma and alpha/beta-hydrolases superfamily protein. The significant SNPs on chromosome 4 were in 7 genes containing F-box/kelch-repeat protein, AGP16, chitinase 2 and disease resistance protein RPM1. On chromosome 5, the associated SNPs were located in 6 candidate genes encoding peroxidase 65, Rho-related protein from plants 1, protease Do-like 9, probable protein kinase, E3 ubiquitin-protein ligase ATL6 and myosin-15. The SNPs on chromosome 9 were located in the gene encoding a TPR (Tetratricopeptide repeat) superfamily protein associated with abiotic stresses. TPR proteins, which mediate protein–protein interactions, assemble as multiprotein complexes to defend against external stresses (Cerveny et al., 2013). On chromosome 10, the detected SNPs were located in 3 genes encoding soluble epoxide hydrolase, auxin response factor 2 and alpha/beta-hydrolases superfamily protein. Alpha/beta-Hydrolases superfamily protein, ABHs support a variety of unique catalytic functions for defense and hormone regulation. ABH esterase regulates the response of salicylic acid in plants, which is a key hormone to plant immune responses (Mindrebo et. al*.,* 2016). Subsequently, single candidate gene regions were found to contain significantly associated SNPs in the two isolates of SCR. The number of significant SNPs on candidate genes ranged from 19 to 128, and the phenotypic variance explained by these SNPs (R^2%) ranged from 1.51% and 9.96%.

To verify whether these loci are associated with disease resistance of SCR, allelic variation of significant SNP loci studies in GWAS was analysed by examining the genotypes of five resistant lines (Bio 18-50, Bio 18-53, Bio 18-56, Bio 18-62 and Bio 18-64) and five susceptible lines (13A-2, C.13-1 White-3, DRW-12, 13A-5 and DRW-29) against Nakhon Pathom isolate and five resistant lines (Bio 18-56, Bio 18-59, Bio 18-64, 2A-3 and 2A-4-1) and five susceptible lines (DRW-6, 12A-2, 13A-1, DRW-29 and DRY-9) against Chiang Mai isolate. For disease resistance SCR, only 3 QTLs (two for Nakhon Pathom isolate and one for Chiang Mai isolate) showed a difference in allelic composition when comparing the most resistant and susceptible cultivars to SCR disease. On chromosome 4, a QTL_4.1 AX-90915192 (physical position: 227597917) (C/T, *P*-value=9.22×10^{-10}), was identified. It can be observed that the average disease index of allele with C was 12.82%, which was

significantly lower than that of the allele with T (28.69%) ($p \leq 0.05$, Figure 4 (a)). Another QTL_9.1_AX-91151225 (physical position: 138444899) (C/T, *P*-value=1.22×10^{-8}) was found on chromosome 9. In this QTL, the average percent disease index of the allele with C (11.34%) was significantly lower than that of allele with T (29.93%) ($p \leq 0.05$, Figure 4 (b)). The third QTL_5.2_AX-91648757 (physical position: 14738387) (G/A, *P*-value = 8.02×10^{-10}) was observed on chromosome 5, and the average disease index for allele with G was 7.19%, while that of allele with A was 21.99% and significantly different from each other ($p \leq 0.05$, Figure 4 (c)).

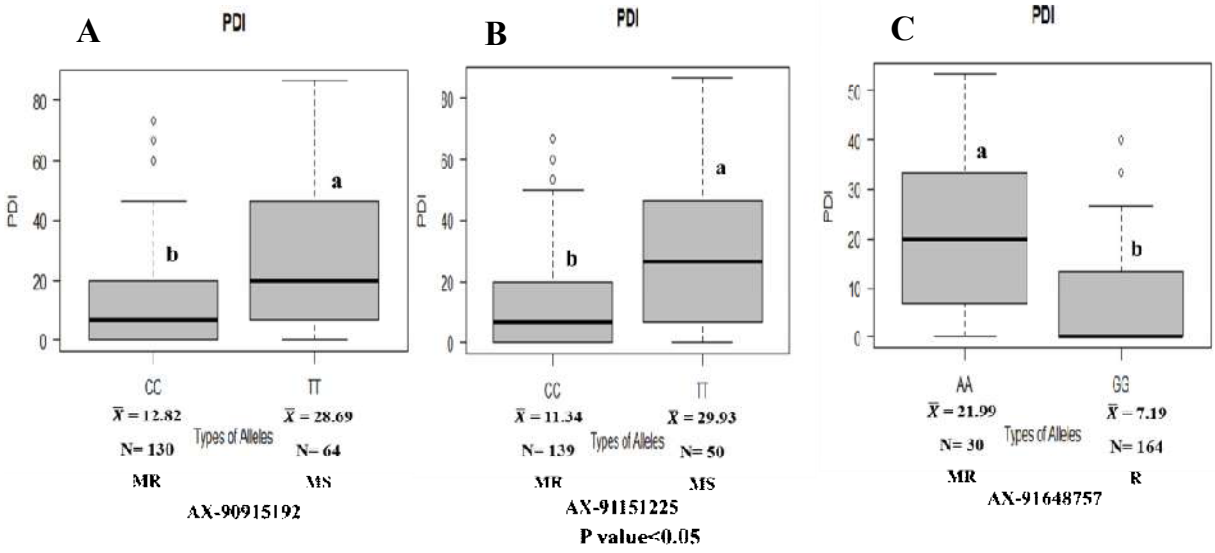

Figure 4 Allele effects of significant SNPs for the resistance trait SCR in waxy maize, (A) QTL_4.1_227597917_AX-90915192 (C/T), (B) QTL_9.1_ 138444899_AX-91151225 (T/C) and (C) QTL_5.2_14738387_AX-91648757 (G/A)

When comparing the genotypes of the five most resistant and susceptible lines to the disease SCR, three QTLs/SNP markers for waxy maize were identified in this study. The SNP locus, AX-90915192 on chromosome 4, has two alleles: CC, TT. In the resistant varieties, the CC accounts for 80% (number/ total) and the TT accounts for about 20%. In the susceptible materials, the TT accounts for 100%. The second SNP locus, AX-91151225 on the chromosome 9, has three alleles: CC, TT and CT. In the resistant varieties, the CC accounts for 80% and the TT for about 20%. In susceptible varieties, the TT is responsible for 60% and the CT for 40%. Therefore, the CC is responsible for much of the disease resistant materials at both SNPs. Another SNP locus, AX-91648757 on the chromosome 5, has three alleles: GG, AA and GA. The resistant materials contain 80% GG and 20% AA, whereas the susceptible materials contain 20% GG, 60% AA and

20% GA. Therefore, the GG allele accounts for a greater proportion of the disease-resistant materials. This information provides a scientific basis for a more detailed clarification of the mechanism of disease resistance SCR.

CONCLUSION

The genomic regions and candidate genes associated with SCR disease resistance in maize were investigated using SNPs markers. In this study, the resistant and susceptible RILs of maize against SCR disease were identified in the phenotypic evaluation. A GWAS was performed on a panel of 262 maize inbred lines using 434,871 SNP markers. Using FarmCPU model, a total of 19 SNP loci significantly associated with the disease SCR were identified. These SNP loci were located on chromosomes 1, 2, 3, 4, 5, 9 and 10, and a total of 19 QTLs and 36 candidate genes was detected at the SNP loci. These candidate genes are associated with biotic or abiotic stress. Analyses of allelic variation were also performed for the SNP loci selected by the GWAS and the results showed the three SNP loci, AX-90915192 on chromosome 4, AX-91151225 on chromosome 9 and AX-91648757 on chromosome 5 from top five resistant lines and that of susceptible ones of waxy corn. The candidate genes identified in this study could help future research to find resistance mechanism for SCR disease and facilitate in marker-assisted breeding programs to develop SCR resistant maize.

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