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META-QTL ANALYSIS ASSOCIATED WITH BACTERIAL STALK ROT RESISTANCE IN MAIZE (*Zea mays* L.) SEGREGATING POPULATIONS

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SUMMARY

Bacterial stalk rot (BSR) caused by *Dickeya zeae* is one of the important diseases of maize that significantly affects maize yield performance. Resistance to *D. zeae* is influenced by high humidity and temperature. Affected tissues are described as soft, mushy, and emitting a foul odor. Yield losses can reach approximately 98.8% of the grower's potential. Quantitative trait locus (QTL) mapping experiments using seven biparental populations were conducted at Syngenta Philippines, Inc., from 2014–2020 to locate consistent QTL and markers involved in BSR resistance. The QTL detected in NMM033, NMM073, NMM089, NMM090, NMM091, NMX003, and NMX001 populations were used to estimate the numbers and positions of consensus QTL with BioMercator V4.2.3 software. Meta-analysis for BSR resistance was conducted by considering all QTL for BSR resistance traits identified in 2014–2020. Among the 49 distinct markers on chromosomes (chrs) 1 to 10, eight most significant loci were detected, *i.e.,* $MSRQTL_{1\text{-}1}$, $MSRQTL_{2\text{-}1}$, $MSRQTL_{3\text{-}1}$, $MSRQTL_{3\text{-}2}$, $MSRQTL_{5\text{-}1}$, $MSRQTL_{5\text{-}2}$, $MSRQTL_{6\text{-}1}$, and $MSRQTL_{10\text{-}1}$. Meta-QTL were identified in chrs 1, 5, and 10 in four populations; in chr 2 in three populations; and in chrs 3 and 6 in two populations evaluated in this study. The regions identified in chrs 1, 2, 3, 5, 6, and 10 with high QTL colocalization across biparental populations were considered as important QTL for BSR resistance traits. Further implementation through fine-mapping is recommended for marker development. The impact of this discovery would strengthen downstream applications in marker-assisted backcrossing and is not only limited to maize BSR resistance but also to other native traits of different crops.

Keywords: *Zea mays* L., bacterial stalk rot, Meta-QTL analysis

Key findings: Through meta-QTL analysis, we focused on the chromosomal regions for BSR-resistance traits identified in chrs 1, 2, 3, 5, 6, and 10 with high QTL colocalization across biparental populations. Meta-QTL analysis provides a preliminary step in identifying important regions across different studies before downstream applications, such as fine-mapping and marker development.

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INTRODUCTION

Maize (*Zea mays* L.) is the third most important crop after wheat and rice in the world (Shiferaw *et al.*, 2011). Maize production is constrained by several abiotic and biotic factors. Among these biotic factors, bacterial stalk rot (BSR) caused by *Dickeya zeae* has economic importance given that it reduces crop yield by up to 98.8% (Kumar *et al.*, 2017). BSR occurs at all stages of crop growth. It causes top rot, stalk rot, and ear rot depending on the tissue or organ affected. In general, affected tissues are described as soft, mushy, and emitting a foul odor. Infected plants show wilted leaves and may either lodge or remain standing depending on the stage of the crop (Thind and Payak, 1985). The pathogen infects the plant through natural openings, such as stomata and hydathodes. Infection also occurs through wounds in the leaf whorl, stalks, and roots caused by insects and injuries induced by strong winds and/or mechanical means. Host plant resistance remains the most economical approach to managing BSR. Resistance sources in line and hybrid development have been identified by various researchers (Ebron *et al.*, 1987; Sah and Arny, 1990; Subekti and Salazar, 2007). The classification of available inbreds into distinct heterotic groups is critical for the development of superior hybrids, synthetics, and breeding populations with disease resistance (Akinwale *et al.*, 2014).

Through the years, quantitative trait locus (QTL) mapping has consistently been a reliable tool in identifying genomic regions involved in the genetic variation of traits, including BSR resistance. In the past decades, several QTL associated with various maize traits (i.e., days to pollen shed, silking date, plant height, leaf number, plant phosphorous content, fibrous root number, plant weight, and seedling root) in different mapping populations and under different growth conditions (Chardon *et al.*, 2004; Chen *et al.*, 2008; Song *et al.*, 2016) have been reported. However, the significance of QTL mapping results is influenced by numerous factors, which include experimental design, population type and size, population structure, genetic marker density, and statistical methods (Guo *et al.*, 2018). Meta-analysis is an effective approach for combining the QTL results from independent studies and refining QTL positions on consensus maps (Chen *et al.*, 2017). The end goal of meta-QTL analysis is to improve the predictive inference of "true" QTL locations for the trait of interest. One of the key factors in selecting QTL data to be included in a meta-analysis is reliance on published reports that are statistically significant. However, most of the time, such reliance leads to a "file drawer problem" or publication bias (Wu and Hu 2012). Importantly, meta-analysis should not be only limited to the utilization of published data but should also be instead used on independent studies being done in private companies.

Between QTL and meta-QTL, an individual QTL is a locus that, in a given environment, segregates between two or more parents and thus shows a correlation between allelic and phenotypic variation for a given trait (Miles *et al.*, 2008). An individual QTL has different attributes, such as a confidence interval (CI), LOD value, R^2 value, and genetic effects. A meta-QTL indicates the most likely consensus position of a group of individual colocalizing QTL. It has a peak position, a CI, a weight but no LOD value, no R^2 value, and no allelic effect. It can be seen as the *in silico* validation of genomic regions because they are detected multiple times (Goffinet and Gerber, 2000).

Several meta-QTL studies on various crops have been reported. However, only limited QTL concerning various maize BSR traits have been reported. A 2021 study by Shariatipour *et al*. showed the utility of meta-QTL analysis in narrowing the CI of QTL identified in several independent studies on zinc and iron in wheat. Khowaja *et al.* (2009) established that in rice, the *sd1* semidwarfing gene coincides with the plant height meta-QTL, and the drought avoidance meta-QTL is not likely associated with the *sd1* gene. The authors concluded that meta-analysis is valuable for providing an improved capability to dissect the complex genetic structure of traits and gives relatively confined target regions for the identification of positional candidate genes.

To the authors' knowledge, the publication by Canama and Hautea (2010) is the only paper reporting QTL mapping for BSR resistance. In the current study, which was conducted in Syngenta Philippines, Inc., from 2014 to 2020, QTL associated with BSR

resistance traits in maize were detected in seven biparental populations, and meta-QTL were identified through a meta-analysis method based on the consensus reference map created by Syngenta. The objectives of the present meta-QTL analysis are to (1) summarize the QTL related to BSR resistance traits that were detected between 2014 to 2020, (2) refine the positions of the detected QTL, and (3) identify of a set of promising candidate genes associated with BSR traits

MATERIALS AND METHODS

Plant materials and field experiments

Seven biparental populations were screened for BSR resistance and utilized for meta-analysis from 2014 to 2020. In total, 2122 plant materials (i.e., 246–360 entries per population) consisting of F_3, F_4, and B_1F_4 generations were evaluated, *see* Table 1.

Table 1. Corn biparental populations screened for BSR resistance from 2014–2020.

Population	Gene-ration Code	Number of Segregating Lines	Rep	Susceptible Parent	BSR Score (%)	Donor Parent	BSR Score (%)	Year	Location
NMM033	F3	360	2	08MZF-DJCS	81	07MZF-BLTH	17	2014	General Santos City
NMM073	F3	300	2	12MZF-FL48	77	07MZF-BLTH	37	2016	General Santos City
NMM089	F3	305	2	13MZF-SJQ4	59	07MZF-BLTH	30	2017	General Santos City
NMM090	F3	310	2	13MZF-SJQ3	97	07MZF-BLTH	28	2017	General Santos City
NMM091	F3	320	2	13MZF-FL8V	60	07MZF-BLTH	22	2017	General Santos City
NMX003	B_1F_4	246	3	06MZF-D129	67	10MZF-L4K2	16	2020	Alabel
NMX001	F4	281	3	10MZF-MPNW	80	10MZF-L4K2	20	2020	Alabel

Five populations from heterotic pool 1 (hp1) and two populations from heterotic pool 2 (hp2) were screened in General Santos City, Philippines, for BSR resistance. Artificial inoculation was performed by using the stab method (Pascual and Salazar, 2001) to ensure homogeneous BSR infection. Field experiments were set up by using randomized complete block design with two replications for hp1 populations and three replications for hp2 populations. Following the selection of parents as described by Yang *et al.* (2004), the maize inbred lines 07MZF-BLTH and 10MZF-L4K2 with high resistance were selected. The inbred lines 08MZF-DJCS, 12MZF-FL48, 13MZF-SJQ4, 13MZF-SJQ3, 13MZF-FL8V, 06MZF-D129, and 10MZF-MPNW were susceptible to BSR. Test entries, including resistant and susceptible checks, were planted in the experiment. Two seeds were sown per hill in a 5 m row with a 20 cm planting distance. Thinning was performed 15–20 days after planting (DAP). A total of 26 healthy plants were retained in the plot. The final stand count was recorded before inoculation. All cultural practices used for growing maize, except bactericide application, were applied. The degrees of freedom in the analysis of variance (ANOVA) of segregating populations were partitioned into replication, entries, and error terms. All plant materials used in this study are proprietary to Syngenta Philippines, Inc.

Artificial inoculation of BSR and phenotyping of segregating lines

Phenotyping experiments for BSR were conducted in Syngenta RnD Station, General Santos City, Philippines, and in Alabel, Saranggani Province, Philippines. Test entries, including resistant and susceptible checks, were planted in the BSR nursery.

Inoculum (Pascual and Salazar, 2001) was standardized to approximately 107–108 cfu/mL by using a spectrophotometer (1.0 absorbance at 600 nm O.D.). Inoculation was done at 45–50 DAP via the stab method. A 1 cm needle (attached to a handle) was dipped in the inoculum and stabbed into the stalk's second internode from the base of the plant. Irrigation was provided to favor disease development. Data collection for BSR rating (BACSR) and BSR number (BACSN) were recorded at 5, 10, and 15 days after inoculation (DAI). BSR percentage (BACSP) was calculated by dividing the BACSN by the final stand count. Evaluation of resistance (BACSP) was done at 15 DAI. Rating scales for BSR (BACSR) are provided in Table 2 by following the scale used by Syngenta for the stab inoculation method. Note that artificial inoculation was performed on all plants to ensure the homogenous infection of the plants by the pathogen.

Table 2. Evaluation and recording of BSR disease reactions.

Scale	Reaction	Description
1	Highly resistant	0%–5% wilted plants
3	Resistant	6%–10% wilted plants
5	Moderately resistant	11%–25% wilted plants
7	Susceptible	26%–50% wilted plants
9	Highly susceptible	51%–100% wilted plants

Table 3. QTL used in the meta-analysis.

Population	Population type	Number of polymorphic markers	Trait	Number of QTL detected
NMM033	F_3	234	BACSP, BACSN	7
NMM073	F_3	252	BACSP, BACSN	10
NMM089	F_3	278	BACSP, BACSN	6
NMM090	F_3	336	BACSP, BACSN	5
NMM091	F_3	352	BACSP, BACSN	7
NMX003	B_1F_4	2235	BACSP, BACSR, BACSN	10
NMX001	F_4	2821	BACSP, BACSR, BACSN	4
Legend	BACSP	BSR percentage		
	BACSR	BSR Rating		
	BACSN	BSR number		

Statistical analyses of phenotypic data

Data analysis was run on the R Studio Syngenta Server by using the "lmer" package in R (Bates *et al*. 2015). Quantitative genetic parameters were estimated on the basis of the progenies. All variables were set as random to compute heritability estimates. Variance components were determined via the restricted maximum likelihood method assuming a random model (Liu *et al.*, 2011). Broad-sense heritability on an entry-mean basis was calculated as the ratio of genotypic-to-phenotypic variance.

$$H^2 = \sigma^2_G/[\sigma^2_G + \sigma^2_e],$$

where σ^2_G refers to genotypic variance, and σ^2_e refers to residual variance.

In the case of biparental mapping, analysis was performed by setting entries as fixed to obtain the mean LS values.

Genotyping of material in biparental populations and linkage analysis

Each of the lines in hp1 populations and their parents were genotyped by using a set of TaqMan markers (Biosearch, USA). The average number of polymorphic markers used in these studies was approximately 290 for each of the five populations. Two hp2 populations and their parental lines were genotyped with a haplotype-based approach by using the 15K Axiom® Maize Genotyping Array designed by Syngenta (Thermo Fisher Scientific, 2018). Composite interval mapping was used to map QTL and estimate their genetic effects for each trait (Zeng, 1994). An empirical threshold was determined by using 1000 permutations (Churchill and Doerge, 1994) to identify the significance threshold for each trait. QTL positions were identified on the basis of the maximum likelihood odds ratio (LOD). QTL CIs were obtained by subtracting the start QTL position from the end QTL position.

Preparation of QTL data, consensus map, and QTL projection

A total of 49 QTL for three BSR resistance traits (*i.e.,* BACSP, BACSR, and BACSN) were detected in seven biparental populations, *see* Table 3. These studies covered different population sizes that ranged from 246 to 360 genotypes. The QTL information, including the flanking markers, phenotypic variation explained by each QTL, and CIs, was extracted from the 49 QTL for the three maize BSR resistance traits from Syngenta's historical data. BioMercator V4.2.3 (Sosnowski *et al*., 2012) was used to project the identified QTL in seven mapping populations onto the consensus map. The QTL projection was based on LOD scores (>2.5), phenotypic variation explained by each QTL (R^2), CIs, and QTL positions (cM). The CIs of 49 QTL were obtained on the basis

of on the genetic positions of the flanking markers on the consensus map. For the markers without genetic positions, the closest markers of the QTL flanking markers from the reference were used to project QTL on the consensus map. QTL that cannot be mapped onto the consensus map and/or mapped location outside the consensus map were removed (Sosnowski *et al.*, 2012).

Moreover, the LOD scores varied from 2.63 to 16.59. QTL were selected on the basis of significance threshold determination via multiple permutations (n. perm = 1000) and varied across populations. All QTL above the threshold values were included in the meta-analysis. As mentioned in the literature review, meta-analysis did not consider LOD and R^2 values except for genetic position and CI among QTL included in the analysis.

Meta-analysis with BioMercator V4.2.3

The analysis was performed with the two-stage clustering procedure reported by Veyrieras *et al*. in 2007, implementing two kinds of clustering algorithms. Gaussian and unbiased approximation originated from the asymptotic Gaussian distribution of the maximum-likelihood estimation of parameters applying the Expectation–Maximization algorithm. The main output of first-step ClustQTL is the optimal number of QTL locations in accordance with the model choice criteria. The Akaike Information Criterion (AIC) was used to select QTL models for each chromosome (chr). Significant models were selected with the lowest AIC value, indicating a better fit in predicting future values. The second step MQTLView provides the number of meta-QTL as suggested in the model. The final selection of meta-QTL has been determined if CI < 90 cM and QTL probability of membership > 30%.

The position and CI of each of the original QTL were projected on the consensus map. Quality control was applied to select only QTL with LOD greater than 2.5. BACSP, BACSR, and BACSN traits were combined into the single trait BAC_n to cover all related traits in BSR QTL locations that were assumed to be normally distributed around their true locations with variances that were obtained from the reported CI and R^2 values.

RESULTS

Response of the populations to BSR

The result of aggregated ANOVA among populations was significant for all traits, *see* Tables 4, 5, and 6. Similar to the study conducted by Li *et al*. (2011), this study included only populations with significant ANOVA in the meta-analysis. All traits differed greatly between the two parents. The inbred lines 07MZF-BLTH and 10MZF-L4K2 had lower BACSP, BACSR, and BACSN than the inbred lines 08MZF-DJCS, 12MZF-FL48, 13MZF-SJQ4, 13MZF-SJQ3, 13MZF-FL8V, 06MZF-D129, and 10MZF-MPNW, *see* Table 1. The resistant parental line checks and the susceptible parental line checks were consistent in expressing resistance and susceptible response across testing years, *see* Figure 1. The values of the histogram showed that BACSP was normally distributed in all populations, *see* Appendix Figure 1. Phenotypic correlations between replications were observed to be higher in three replicated trials (ranging from 52% to 66%) than in two replicated trials (ranging from 6% to 38%), *see* Table 7. Transgressive segregation was also observed for all traits.

Broad-sense heritability for all the traits ranged from 19.92% to 53.87%, *see* Tables 4, 5, and 6. Heritability for BACSP was observed with the highest average at 38.6% compared with that for BACSR (29.4%) and BACSN (33%). In general, heritability in the broad sense does not yield a figure that is predictive and is therefore helpful in QTL analysis. For example, if H^2 = 0.7, then the trait being measured is likely highly attributable to genetic control. Thus, QTL analysis can be performed. On the other hand, if H^2 = 0.3, the trait (e.g., diseases) being measured is likely to have low heritability, and QTL analysis may not be needed. In the practical sense, broad-sense heritability can serve as a guide for performing QTL analysis and depends on the context of the traits being measured. As mentioned by Rebetzke *et al*. (2008), decreasing the environmental variance (for example, increasing the number of replications or by using a more uniform environment) generally increases heritability.

Table 4. Significance of genotypic effects and heritability estimates for the segregating populations screened in BACSP by using RCB design.

Trial ID	d.f.	SS	MS	F value	Pr(>F)	H^2	Residual
NMM033	361	359318	995.34	2.3087	4.360E−16***	36.52%	431.10
NMM073	302	267675	886.34	1.933	4.174E−09***	31.55%	458.50
NMM089	307	236852	771.51	2.5251	2.200E−16***	35.25%	305.50
NMM090	312	292134	936.33	2.3141	1.720E−14***	34.14%	404.60
NMM091	322	315911	981.09	2.1966	2.890E−13***	31.69%	446.65
NMX003	218	361945	1660.3	7.0133	2.200E−16***	53.87%	236.73
NMX001	284	510050	1796	5.3023	2.200E−16***	47.33%	338.71

BACSP: BSR Percentage, d.f.: degrees of freedom, SS: Sum of squares, MS: Mean squares, F: Value variance between the means of two populations residual error, Pr(>F) results likely did not occur by chance, H^2: Heritability estimate, **: significant at 0.1%, ***: significant at 0.01%.

Table 5. Significance of genotypic effects and heritability estimates in the segregating populations screened in BACSR using RCB design.

Trial ID	d.f.	SS	MS	F value	Pr(>F)	H^2	Residual
NMM033	361	1548	4.288	1.8545	1.210E−09***	27.34%	2.31
NMM073	302	6.7309	302	1.5819	2.855E−05***	22.25%	4.26
NMM089	307	1795.6	5.849	1.5373	5.244E−05***	20.06%	3.81
NMM090	312	1436.2	4.6033	1.5471	3.600E−05***	19.92%	2.98
NMM091	322	2210.3	6.8431	1.7701	7.437E−08***	24.85%	3.87
NMX003	218	2376.1	10.899	5.6767	2.200E−16***	48.91%	1.92
NMX001	284	3860.9	13.595	4.2167	2.200E−16***	42.78%	3.22

BACSR: BSR Rating, d.f.: degrees of freedom, SS: Sum of squares, MS: Mean squares, F: Value variance between the means of two populations' residual error, Pr(>F) results likely did not happen by chance, H^2: Heritability estimate, **: significant at 0.1%, ***: significant at 0.01%.

Table 6. Significance of genotypic effects and heritability estimates in the segregating populations screened in BACSN using RCB design.

Trial ID	d.f.	SS	MS	F value	Pr(>F)	H^2	Residual
NMM033	361	15942	44.161	2.0039	1.000E−11***	31.01%	22.04
NMM073	302	12515	41.442	1.8288	6.354E−08***	29.16%	22.66
NMM089	307	8947.9	29.146	1.8161	3.905E−08***	25.71%	16.05
NMM090	312	12667	40.599	1.7735	9.800E−08***	25.19%	22.89
NMM091	322	13160	40.745	1.8468	8.696E−09***	26.78%	22.06
NMX003	218	29435	135.02	5.2983	2.200E−16***	46.90%	25.48
NMX001	284	42774	150.61	4.447	2.200E−16***	46.52%	33.76

BACSN = BSR number, d.f.: degrees of freedom, SS: Sum of squares, MS: Mean squares, F: Value variance between the means of two populations' residual error, Pr(>F) results likely did not happen by chance, H^2: Heritability estimate, **: significant at 0.1%, ***: significant at 0.01%.

Table 7. Correlation between replications in seven independent populations used for the meta-analysis.

Populations	Rep1/Rep2	Rep1/Rep3	Rep2/Rep3
NMM033	6%	-	-
NMM073	30%	-	-
NMM089	38%	-	-
NMM090	38%	-	-
NMM091	34%	-	-
NMX003	66%	62%	64%
NMX001	59%	52%	55%

Figure 1. a) Susceptible check, b) Resistant check plant responses to BSR.

Moreover, higher heritability was observed for three replicated trials (ranging from 42.78% to 53.87%) than for two replicated trials (ranging from 19.92% to 36.52%) for all of the evaluated BSR resistance traits. As reported by Casler (2014), increasing the number of replicates has a direct and positive effect on experimental precision, specifically in reducing the standard error (SE) of the experiments. Given the results of this study, three replicated trials had a higher precision (i.e., lower SE) in estimating background variances (noise) of treatment effects on all traits, except for BASCN in the NMX003 (Residual value at 25.48) and NMX001 (Residual value at 33.76) populations, *see* Table 4, 5, and 6.

QTL for BSR resistance traits

The reported QTL for three BSR resistance traits were distributed randomly in 10 chrs, with the total number of QTL per chr ranging from 1 (chr 4) to 8 (chr 7), *see* Table 8. Chrs 1, 3, 5, 7, and 10 showed a relatively high number of QTL (6–8) associated with BSR resistance traits. The 49 QTL were classified on the basis of the percentage of the phenotypic variance explained by each QTL. A total of 39 (80%) of the 49 QTL displayed an R^2 value of less than 10%, whereas 10 QTL (20%) explained more than 10% of the phenotypic variance. Of these 49 QTL, most for individual traits were for BACSP and BACSN. The number of QTL identified per chr across the seven mapping populations were unevenly distributed, *see* Figure 2.

Interestingly, QTL for BACSR traits were also detected in the NMX003 and NMX001 biparental populations likely because of the highly dense SNPs used for these populations. As mentioned above, all values above the significance threshold were included in the meta-analysis. This study validated the 2010 report from Canama and Hautea on identifying major QTL located at 35.05 cM on chr 2 with R^2 = 25.9%. Across different mapping populations, population structures, marker types (SSR, AFLP, RGA vs SNPs), and genetic maps, common QTL on chr 2 were identified as major QTL conferring resistance to BSR (R^2 = 25.9% vs 19.46%). However, a high R^2 does not necessarily mean high CI.

TaqMan and Axiom markers are SNP markers with different platforms and number of markers involved. In general, few markers were used in mapping analysis on the NMM033, NMM073, NMM089, NMM090, and NMM091 populations, *see* Table 3. By contrast, approximately 15 000 markers were run through the Axiom® genotyping platform. The advent of SNP markers on the Axiom genotyping platform overlays a more precise genetic map that represents the genome of maize (Thermo Fisher Scientific, 2018). Finally, estimates of genetic effects were obtained from QTL analysis. In general, additive and dominant genetic effects were observed for BACSP, BACSR, and BACSN in 10 chrs.

Table 8. QTL identified in seven biparental populations consisting of three BSR resistance traits, *i.e.,* BACSP, BACSR, and BACSN.

Populations	QTL_ID	Trait	Year	Chr#	LOD	R^2	Position (cM)	Start (cM)	Stop (cM)	CI
NMM073	qbacsp1-16	BACSP	2016	1	3.55	5.07	538	408.52	565.59	157.07
NMM089	qbacsp1-17	BACSP	2017	1	2.83	3.11	29.6	11.64	390.66	379.02
NMX001	qbacsp1-20	BACSP	2020	1	8.56	11.54	417	401.72	419.25	17.52
NMM033	qbacsp2-14	BACSP	2014	2	12.06	12.83	110	64.99	369.51	304.53
NMM090	qbacsp2-17a	BACSP	2017	2	14.28	19.46	57.8	31.15	84.51	53.36
NMM091	qbacsp2-17b	BACSP	2017	2	2.99	3.64	22.4	6.38	468.42	462.05
NMX003	qbacsp3-19	BACSP	2019	3	13	21.64	405	379.55	407.23	27.68
NMX001	qbacsp3-20	BACSP	2020	3	8.19	10.41	433	432.8	441.36	8.56
NMM033	qbacsp5-14	BACSP	2014	5	4.81	4.27	415	371.39	424.06	52.67
NMM089	qbacsp5-17a	BACSP	2017	5	5.81	6.35	389.4	376.92	411.11	34.19
NMM091	qbacsp5-17b	BACSP	2017	5	4.9	5.81	37.9	18.95	59.98	41.04
NMM073	qbacsp6-16	BACSP	2016	6	2.63	2.76	303	208.14	330.64	122.5
NMM091	qbacsp6-17	BACSP	2017	6	3.98	4.12	323.1	311.05	334.86	23.81
NMM073	qbacsp7-16	BACSP	2016	7	2.76	3.93	315	17.4	331.59	314.18
NMM089	qbacsp7-17a	BACSP	2017	7	3.1	3.07	20.3	12.14	266.74	254.61
NMM091	qbacsp7-17b	BACSP	2017	7	5.18	5.29	255.2	29.24	293.79	264.56
NMX001	qbacsp7-20	BACSP	2020	7	4.92	5.02	356	352.1	359.78	7.68
NMM073	qbacsp8-16	BACSP	2016	8	3.44	3.84	153	28.78	331.72	302.94
NMM073	qbacsp9-16	BACSP	2016	9	2.95	4.65	272	212.81	293.75	80.93
NMM033	qbcasp10-14	BACSP	2014	10	4.91	3.57	273	257.39	288.94	31.55
NMM073	qbacsp10-16	BACSP	2016	10	3.32	3.78	293	4.96	298.54	293.58
NMM089	qbacsp10-17a	BACSP	2017	10	3.91	4.83	271.2	236.45	296.47	60.03
NMM090	qbacsp10-17b	BACSP	2017	10	5.66	8.41	251.4	187.84	265.1	77.26
NMX001	qbacsr1-20	BACSR	2020	1	9.78	13.43	403	401.72	419.25	17.52
NMX003	qbacsr3-19	BACSR	2019	3	13.4	21.76	397	379.55	407.23	27.68
NMX001	qbacsr3-20	BACSR	2020	3	7.75	8.86	433	429.22	437.42	8.2
NMX001	qbacsr5-20	BACSR	2020	5	3.77	3.3	447	1.8	446.6	444.8
NMX001	qbacsr7-20	BACSR	2020	7	3.76	4.12	356	251.2	361.82	110.62
NMM033	qbacsn1-14	BACSN	2014	1	3.29	4.34	36.1	4.08	544.7	540.62
NMX001	qbacsn1-20	BACSN	2020	1	9.2	14.09	402	400.11	419.25	19.14
NMM033	qbacsn2-14	BACSN	2014	2	8.5	1.67	112	64.99	369.51	304.53
NMM090	qbacsn2-17	BACSN	2017	2	9.72	12.53	57.8	31.15	84.51	53.36
NMX003	qbacsn3-19	BACSN	2019	3	16.59	24.98	394	385.6	404.8	19.2
NMX001	qbacsn3-20	BACSN	2020	3	6.85	9.79	433	412.19	436.1	23.91
NMM090	qbacsn4-17	BACSN	2017	4	3.51	3.21	62.2	41.03	342.07	301.05
NMM033	qbacsn5-14	BACSN	2014	5	3.67	4.1	419.1	371.39	427.69	56.3
NMM089	qbacsn5-17a	BACSN	2017	5	3.57	3.66	389.4	341.96	418.94	76.99
NMM091	qbacsn5-17b	BACSN	2017	5	5.28	6.13	43.2	18.95	59.98	41.04
NMM073	qbacsn6-16	BACSN	2016	6	2.86	2.9	304	208.14	327.67	119.54
NMM091	qbacsn6-17	BACSN	2017	6	3.36	4.14	322.7	254.26	334.86	80.61
NMM089	qbacsn7-17a	BACSN	2017	7	4.54	5.03	21.9	12.14	266.74	254.61
NMM091	qbacsn7-17b	BACSN	2017	7	6.06	6.83	257.2	158.02	286.6	128.58
NMX001	qbacsn7-20	BACSN	2020	7	3.98	4.75	355	260.93	361.82	100.88
NMM073	qbacsn8-16	BACSN	2016	8	3.76	3.95	139	28.78	320.78	292
NMM073	qbacsn9-16	BACSN	2016	9	3.07	3.79	276	212.81	293.75	80.93
NMX003	qbacsn9-19	BACSN	2019	9	4.57	6.37	54	46.79	261.1	214.31
NMM033	qbacsn10-14	BACSN	2014	10	4.49	4.72	283.2	257.39	292.23	34.84
NMM073	qbacsn10-16	BACSN	2016	10	3.79	5.23	291	252.29	296.47	44.19
NMM090	qbacsn10-17	BACSN	2017	10	6.18	6.62	251.4	187.84	265.1	77.26

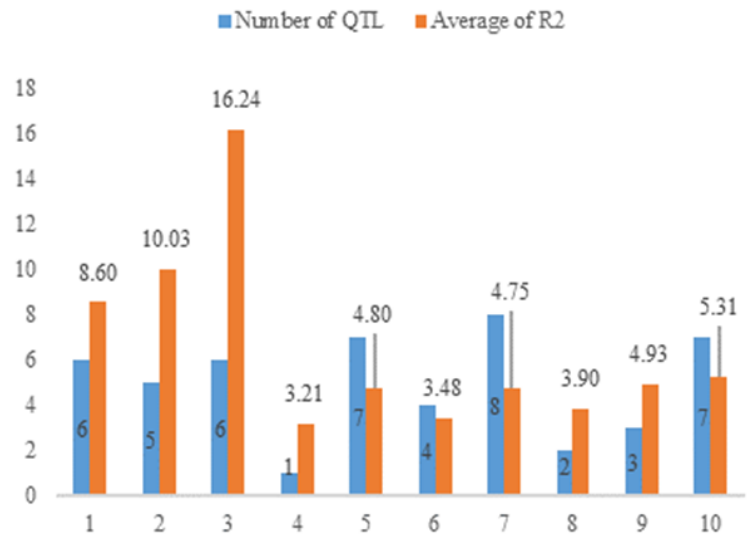

Figure 2. QTL distributed on each chr *(blue bar*) and average (R^2) phenotypic variance explained (*orange bar*).

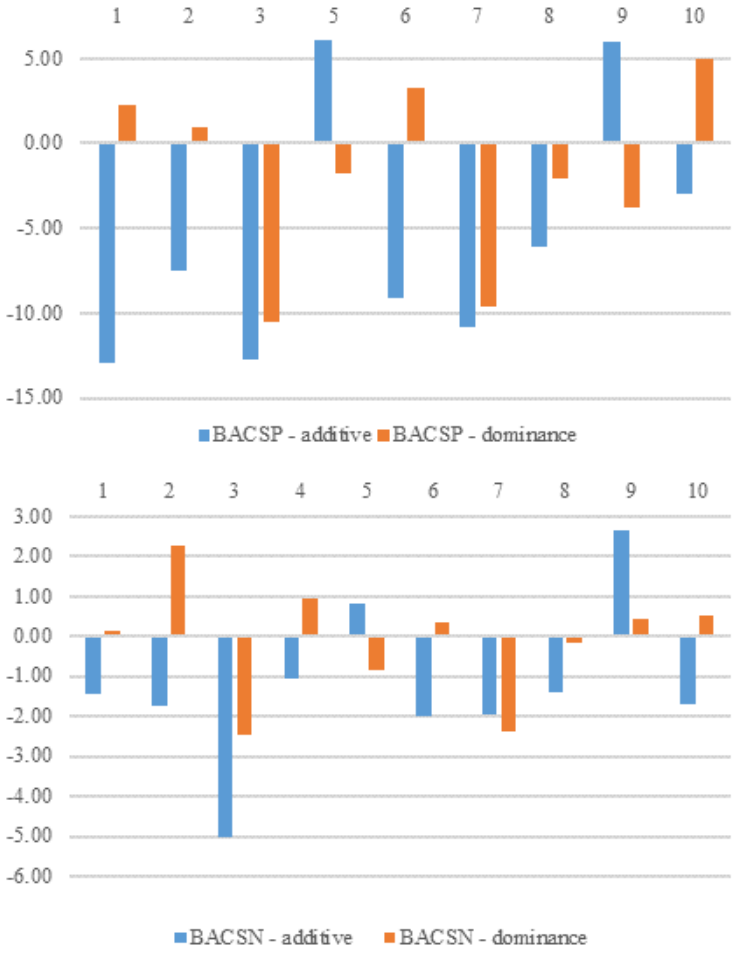

Figure 3. Estimates of additive and dominant effects across 10 chrs in maize.

Overall, genetic effects had a positive effect in reducing BSR infection, except in chr 5 and 9 in BACSP and chr 2 in BACSN. The estimate of QTL additive effect for BACSP varied from −12.93% (chr 1) to 6.32% (chr 5), whereas the dominance effect ranged from −10.55% (chr 3) to 5.01% (chr 10). Interestingly, chr 1 (−12.93), chr 3 (−12.71), and chr 7 (−10.85) had high additive effects, indicating a positive contribution in terms of BSR resistance. Moreover, a high dominance effect was observed for chr 3 (−10.55) and chr 7 (−9.59) that corresponded to a high additive effect. In the case of BACSN, a high additive effect was observed for chr 3 (−5.03), *see* Figure 3.

Meta-QTL analysis

The BSR resistance QTL was projected onto the consensus map via BioMercator V4.2.3 software. Meta-analysis was conducted on the 49 QTL identified for the three BSR resistance traits, and eight meta-QTL (*see* Figure 4) were selected on the basis of the lowest AIC value criteria and CI < 90 cM. The meta-QTL were unevenly distributed over all chrs. The number of meta-QTL ranged from 1 to 2 on chrs 1 to 10 but not on chrs 4 and 8. One meta-QTL was selected on chrs 1, 2, 6, and 10, and two meta-QTL were selected on chrs 3 and 5. The 95% CI of the selected meta-QTL ranged from 2.15 cM to 89.31 cM with an average of 29.14 cM.

The corresponding genetic intervals were 404.32–411.02 cM for the meta-QTL $MSRQTL_{1\text{-}1}$, 14.41–103.64 cM for $MSRQTL_{2\text{-}1}$, 391.02–404.08 cM for $MSRQTL_{3\text{-}1}$, 431.57–433.59 cM for $MSRQTL_{3\text{-}2}$, 26.38–54.10 cM for $MSRQTL_{5\text{-}1}$, 381.83–419.37 cM for $MSRQTL_{5\text{-}2}$, 306.48–337.65 cM for $MSRQTL_{6\text{-}1}$, and 265.93–287.01 cM for $MSRQTL_{10\text{-}1}$, *see* Table 9.

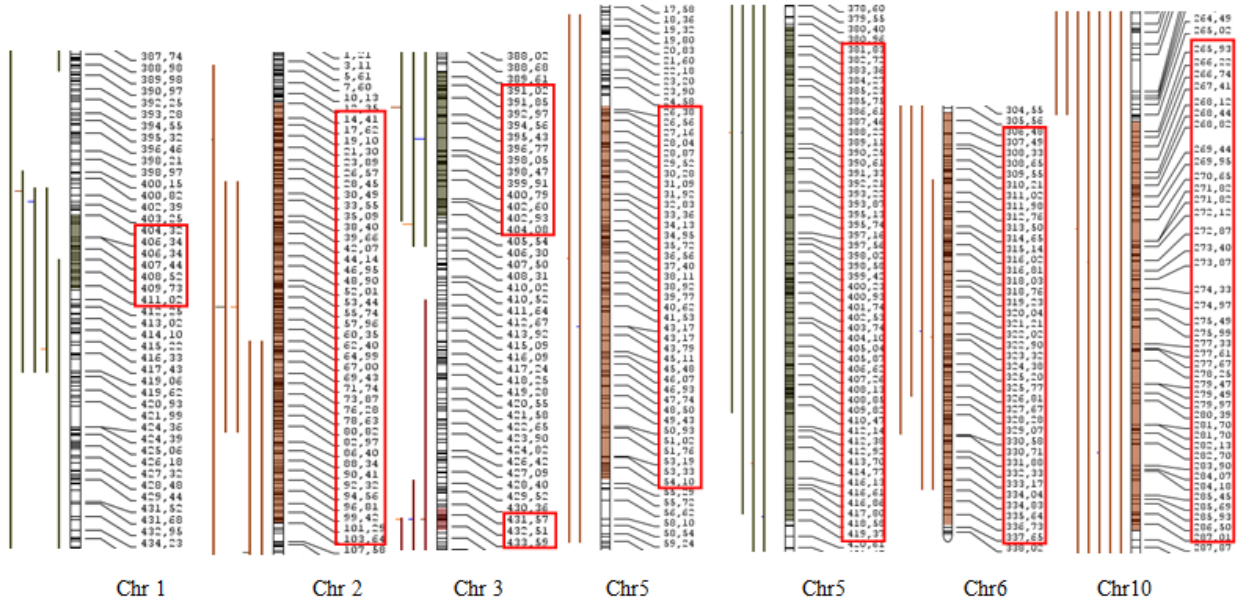

Figure 4. Meta-QTL position defined for BACSP, BACSR, and BACSN across biparental populations.

DISCUSSION

Applications of meta-QTL in plant breeding

The implementation of molecular applications is becoming an important aspect of most plant breeding programs, such as rice and corn breeding programs. The native trait conversion (specifical introgression) of BSR into lines needs robust, stable, and refined QTL that are identified in the target marker regions and validated across different genetic populations (Collard and Mackill, 2008). Meta-QTL analysis allows validation and focuses on QTL regions identified from independent populations with different marker platforms (SNP TaqMan vs SNP Axiom), genetic backgrounds, and

Table 9. Meta-analysis of QTL controlling BSR resistance traits in maize.

Meta-QTL	Chr	Position (cM)	Flanking regions	Weight	CI (95%)	Decision
$MSRQTL_{1-1}$	1	407.78	404.32−411.02	1	7.01	Select
$MSRQTL_{2-1}$	2	59.15	14.41−103.64	1	89.31	Select
$MSRQTL_{3-1}$	3	397.43	391.02−404.08	0.5	13.71	Select
$MSRQTL_{3-2}$	3	433	431.57−433.59	0.5	2.15	Select
	4	-		-	-	
$MSRQTL_{5-1}$	5	40.55	26.38−54.10	0.33	29.01	Select
$MSRQTL_{5-2}$	5	400.34	381.83−419.37	0.67	38.28	Select
$MSRQTL_{6-1}$	6	321.77	306.48−337.65	1	31.68	Select
	7	21.38		0.4	180.06	
	7	263.63		0.6	766.42	
	8	-		-	-	
	9	54		0.33	214.38	
	9	273.99		0.67	213.16	
$MSRQTL_{10-1}$	10	276.41	265.93−287.01	1	21.95	Select

environments (Sosnowski *et al*., 2012; Kumar and Nadarajah, 2020). Our results showed the position of consensus QTL for BSR resistance.

Comparing independent studies is difficult because the results of QTL detection are highly influenced by several interacting factors, such as genetic material (parents, populations, generations, and population structure), markers, and mapping methods (Austin *et al.*, 2000; Ho *et al.*, 2002; Mihaljevic *et al.*, 2004; Lan *et al.*, 2005; Li *et al.*, 2009). Moreover, utilizing these reported QTL for direct implementation in maize breeding programs is difficult. At the same time, integrating distinct genetic maps into a single consensus map and extracting information integral to fine mapping activities and marker development are promising. The impact of this discovery would strengthen downstream applications in marker-assisted backcrossing not only for maize BSR but also for other native traits of different crops.

Löffler *et al.* (2009) identified the parent of wheat (i.e., Chinese spring donor 'Sumai 3') with high consistency contributing to *Fusarium* head blight resistance through meta-QTL analysis. The authors recommended a) choosing parents for crossing to cover different resistance loci/QTL intervals, b) exploring new meta-QTL, and c) selecting markers from new meta-QTL for use in marker-assisted selection. Rong *et al.* (2007) suggested that for cotton, the compilation of different QTL mapping results yields a highly complete picture of the genetic control of a trait and may reveal patterns in the organization of trait variation. Furthermore, the authors recommended crossing closely related genotypes that differ by single-gene mutants that may yield profoundly different QTL landscapes, indicating that meta-analysis linked to synteny-based and expression-based information provides clues regarding specific genes for fiber variation and families involved in QTL networks (i.e., interacting genes).

Distribution of QTL and meta-QTL across the maize genome

The initial BSR resistance traits were projected unequally over different chrs in the maize genome. Across the genome, chr 4 had the lowest number of QTL, whereas chr 7 had the highest number of QTL detected (*see* Figure 2). Importantly, high (additive) phenotypic variance in each of the mapping populations could be attributed to QTL in chrs 1 (R^2 = 8.60), 2 (R^2 = 10.03), and 3 (R^2 = 16.24).

The distribution of meta-QTL across the genetic linkage map was similar to that of the individual QTL. Genetic maps from several independent studies were merged via homothetic projection (Arcade *et al.*, 2004). Given that different genetic maps share a sufficient number of common loci, these loci function as bridges between maps, and the projection of remaining loci is possible. Finally, a compiled map was obtained from multiple sources via iterative map compilation/projection.

Mining of candidate genes in meta-QTL

Several meta-QTL studies identifying consensus QTL for maize root-related traits have been reported. Guo *et al*. (2018) identified 45 maize homologs as candidate genes controlling maize root traits. In addition, three maize genes (GRMZM5G813206, GRMZM2G1 67220, and GRMZM2G467069)

identified in $MQTL_{8-5}$ were associated with lateral root and crown root development. Two meta-QTLs, i.e., $MQTL_{7-2}$ and $MQTL_{9-1}$, were involved in nitrogen and phosphorus stress responses. The identified meta-QTL could be used indirectly for the abiotic stress improvement of maize root traits. However, the meta-QTL on chrs 7 and 9 were not selected due to their high CI, and no meta-QTL was detected on chr 8.

In this study, the alignment of the genomic regions tagged by QTL was performed with the Maize Genetics and Genomics Database (Portwood *et al*., 2018). Two characterized proteins were found in 197 853–202 163 Mb. Interestingly, in chr 3, this region harbors the protein-coding gene Defective Kernel 1 (*DEK1*). Tran *et al*. (2017) reported that the *DEK1* protein is associated with a mechanically activated Ca^{2+} current in plants, suggesting that the perception of mechanical stress plays a critical role in plant development. The upregulation of the *DEK1* protein results in root inhibition by Gd^{3+} ions. In the practical sense, BSR infection induces mechanical stress in maize plants. Specifically, *D. zeae* weakens the stalk portion of the plants.

CONCLUSIONS

Studies conducted from 2014 to 2020 on different types and sizes of populations and density of genetic markers showed that the 49 identified QTL were unevenly distributed in 10 chrs. The regions identified in chr 1, 2, 3, 5, 6, and 10 across biparental populations with high QTL colocalization were considered as important QTL for BSR resistance traits with CIs of 2.15–89.31 cM. Furthermore, the 197 853 Mb – 202 163 Mb physical positions on chr 3 harboring the protein-coding gene *DEK1* provides insights into the correlation between mechanical stress and BSR resistance. Validation across different genetic backgrounds and population structures is recommended for fine mapping and marker development.

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Appendix

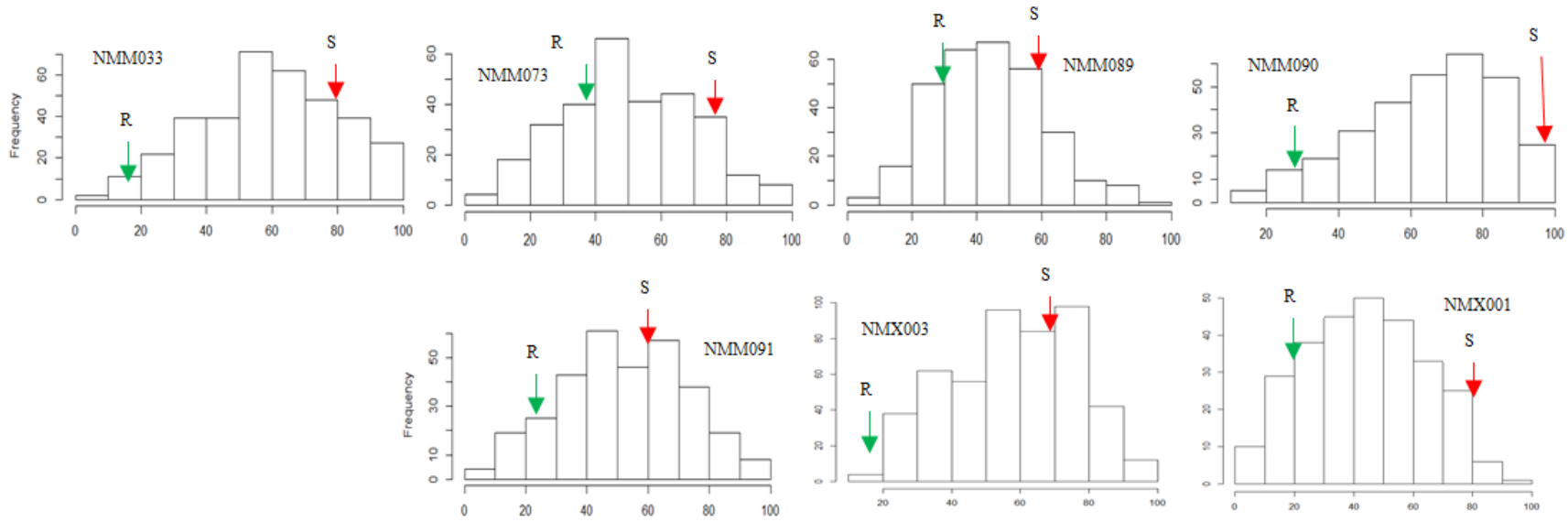

Figure 1. Distribution of BACSP in seven independent studies derived from donor parents 07MZF-BLTH and 10MZF-L4K2. The values of the resistant and susceptible parents are indicated by arrows.