

# **Analysis of High-Resolution QTL Markers Associated with Rice Yields Using Data for Two Consecutive Years in Different Environmental Conditions**

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## Abstract

Previously we reported the identification of seven quantitative trait loci (OTLs) associated with the rice yield measuring five parameters including panicles per plant (PPP), spikelets per panicle (SPP), seed set percentage (SSP), 1000-grain weight (TGW) and yield in 2012. Here we report the analysis of QTLs using the same trait parameters data of the mapping population in 2013 for detecting highly conserved QTL markers. A total of 6 QTLs were identified from chromosomes 1, 7, 8, 10, 11, and 12, which were contrasted with our previous results (chromosomes 1, 2, 4, 5, 6, 8, and 11). In this comparison, three QTLs from chromosome 1, 8, and 11 were only found to be associated with the components of yield over two consecutive years indicating high sensitivity of QTL markers to the environment. Of those three QTLs, SPP-associated marker RM12285 was found to be dominantly expressed by real-time PCR (qPCR). In addition, compared to our previous report the numbers of mapping population and markers were significantly increased for higher resolution markers from 70 to 120, and from 143 to 217, respectively. We also found that the parameter SPP was dominantly correlated with the rice yield. Furthermore, the double haploid (DH) population facilitated to analyze the epistatic effects for yield and yield components in rice. Taken together, combining multiple mapping population data over years possibly enables narrowing down to the highly conserved QTL markers against diverse environmental fluctuation caused by such as drought and high temperature. Thus, these data would be critically exploited to improve for the crop breeding strategy.

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## **Keywords**

#### QTL, Rice Yield Component, Epistatic Interaction, qPCR

#### 1. Introduction

One of the most demanded crops is rice being favored by more than half of the world population. The three major types in rice are *indica*, *joponica*, and *javanicas* showing their own specific phenotypes particularly depending on different environmental conditions. Almost 70% of populations in Korea and most Asian countries rely on their daily nourishment from rice. Even though the development of new varieties by crop breeding program has been operated successfully, the significant increment of world population hardly attenuates the problem of famine, particularly in developing countries. For this reason, it is still a top priority to increase the yield of rice. The advent of molecular markers assisted technology and molecular linkage genetic maps have made it possible to characterize the performance of individual QTLs. These techniques have been widely applied to identify QTLs controlling yield and its related traits on various crops, such as maize [1]-[3], tomato [4]-[6], wheat [7] [8]. The most important rice traits such as grain yield and quality have been focused to utilize QTL analysis [9]-[11]. Diverse rice genetic backgrounds in different environment allow the identification of a number of QTLs [12]-[16] providing breeders information to improve breeding strategies via marker-assisted selection (MAS). Classical quantitative genetic analysis enabled to find out the effect of epistasis. Using morphological markers the existence of digenic epistatic interactions in barley has been described [17] [18]. Recent studies of QTL mapping revealed that epistatic interactions among multiple loci played an important role in complex quantitative traits such as yield and yield components. To detect epistatic effects for yield and related traits, such as the F2, F2:3 chromosome segment substitution lines (CSSL), several mapping populations have been developed. To detect additive effects within a single locus and additive effects between loci, the double haploid (DH) population with homozygous genotypes derived from F1 plants via anther culture can be utilized. The control of quantitative traits is achieved by the polygenes that are characteristics of small effects and sensitive to the environment. The interaction of QTL environment (QE) is a critical component for plant evolution and breeding strategy. The yield and its related traits-associated QE have been extensively investigated in many crops [18]-[20]. In the previous studies, a number of QTLs detected in one or several different environments were found to have significant QE effects, indicating that QE interactions commonly occur on quantitative traits in various environments.

Further to our previous report that investigated the QTLs associated with yield in a single year-produced mapping population, in this study we collected a second consecutive year-produced data to improve selective efficiency via detection on QTLs controlling yield and yield components in different environments. In addition, we analyzed the effect of epistatic effects and QE interactions using the DH population in rice. Therefore, more conserved QTL markers with higher credibility under diverse environmental conditions might significantly elevate the efficiency of breeding strategy for the development of new crop varieties.

#### 2. Materials and Methods

#### **2.1. Plant Materials and Field Experiments**

A population of 120 doubled haploid (DH) lines derived from a cross between "Cheongcheong" (*Oryza sativa* L. ssp. Indica) and "Nagdong" (*Oryza sativa* L. ssp. Japonica) was used in this study, which was conducted on the experimental farm of Kyungpook National University. The germinated seeds were sown in a seedling bed, and seedlings were transplanted into three-row plots after 30 days. The population was evaluated in a randomized complete block design with two replications of 2012 and 2013. Seventeen plants were cultivated in each row, with 30 cm between rows and 15 cm between plants within each row. The parents, "Cheongcheong" and "Nagdong", were cultivated in ten plots as controls. All of the panicles from each plant were harvested and kept in different envelopes to avoid seed shattering. Panicles per plant (PPP) were identified as the mean number of-panicles per plant. Spikelets per panicle (SPP) were counted as the average number of spikelets per panicle. Seed set percentage (SSP) was evaluated using the number of filled grains per plant divided by the number of

spikelets per plant. The 1000-grain weight (TGW) was measured as the average weight of 1000 filled grains. Yield was evaluated as the weight of all filled grains of the plant, which was converted to kg per 10a (kg/10a). Mean values of the two replications were calculated for each trait and used in data analysis.

#### 2.2. Genetic Linkage Map and QTLs Analysis

A genetic map consisting of 222 DNA makers was constructed using MAPMAKER/EXP Version 3.0 [21]. Distances between markers were presented in centiMorgans (cM) using the Kosambi function with an average interval of 10.6 cM. The completed genetic map used by the Plant Molecular Breeding Laboratory at the School of Applied Biosciences in Kyungpook National University. To identify the putative QTLs, composite interval mapping was employed using WinQTL Cartographer 2.5 [22] [23]. AnLOD value of 2.5 was used as the significant threshold for claiming a QTL at P < 0.05. QTLMapper 1.6 was used to detect the main effect QTLs, epistatic interactions and their environmental interactions with a threshold of P  $\leq$  0.005 [24]. The QTL locations identified in the current research were compared with previously reported QTLs affecting rice yield and related traits using agenomic database (http://gramene.org). The methods were similar to those described [14].

#### 2.3. Real-Time PCR (qPCR)

The qPCR was conducted to compare phenotype and genotype with transcriptional expression based on relative expression. Microsatellite markers using codon region of OsAct 1 (accession no. Os3g0718100) as a reference gene was employed to confirm amplification with specific primers

(5'-AGGCTCCTCTCAACCCCAAGGCCAATCG-3', 5'-AGGTAATCAGTGAGATCACGCCCAGC-3')

and melting curves of the DH population of rice from a cross of "Cheongcheong" and "Nagdong". Briefly a 20  $\mu$ l mixture containing 3  $\mu$ l of 30 ng/ $\mu$ l template cDNA and DNA, 10  $\mu$ l of QuantiSpeed SYBR NO-ROX (Phile-Korea, Cat. No. QS105-05), 1  $\mu$ l of 20 pM of each primer and 5  $\mu$ l of nuclease-free water (Qiagen, Cat. No. 129114) was analyzed byqPCR.qPCR was carried out using the following program: 95°C for 2 min, followed by 45 cycles of 95°C for 5 s, 60°C for 10 s and 72°C for 10 s.

#### 3. Results

The yield and components were summarized for parents and 120 DH lines in 2012 and 2013 (Table 1). Phenotypic transgressive and continuous variations were observed for most traits. 2012 "Nagdong" had higher values for spikelets per panicle, seed set percentage and grain yield, while 2012 "Cheongcheong" had higher panicles per plant and 1000-grain weight. 2013 "Nagdong" had higher values for seed set percentage. 2013 "Cheongcheong" had higher panicles per plant, spikelets per panicle, and grain yield. Although the parents have higher grain yield, some extremely low values for grain yield were observed due to the low seed set percentage in the DH population.

This phenomenon was probably attributable to the origin of the progeny from sub-specific (indica-japonica) crosses. Significant correlations (P < 0.05, P < 0.01) were calculated among yield and related traits in 2012 and 2013 (**Table 1**). Spikelets per panicle, seed set percentage and 1000-grain weight showed strong positive correlations with yield. The 1000-grain weight had a strong positive correlation with seed set percentage. In addition, negative correlations existed among panicles per plant, spikelets per panicle and 1000-grain weight (**Table 2**).

In the DH population, the frequency distributions of yield and the yield components PPP, SPP and TGW had a normal distribution. On the other hand, SSP and YIELD have learned to right.Putative QTLs and significant QTLs detected for yield and yield components are shown in Table 2.

In 2012 a total of 12 QTLs were identified on seven chromosomes (all except for chromosomes 1, 7, 9, 10, 12), while in 2013 a total of 12 QTLs were identified on six chromosomes (all except for 2, 3, 4, 5, 6, 9). Two of the QTLs were detected in both years on chromosomes 8 and 11, respectively.

One of the QTLs identified at qPPP 8 between markers 72-RM22499 in 2012. Another QTL was detected at qPPP11 between markers RM26002-26162 in 2013 Four QTLs were identified on chromosomes 2, 3, 5 and 6 in 2012 and chromosome 7 in 2013. SPP QTLs identified qSPP2 between RM6-RM213, qSPP3 between RM15749-RM3525, qSPP5 between RM18130-RM3381, and qSPP6 between RM20196-RM20355 in 2012. In 2013 qSPP7 only identified between RM8261 and RM420. Alleles from "Cheongcheong" showed increased effects at qSPP2 andqSPP6, while the "Nagdong" allele had an increased effect at qSPP3, qSPP5 and qSPP7. A total of four QTLs were resolved for seed set percentage over two years. One of the QTLs, qSSP9, was identi-

Voors	Troite -	Parei	nts	- DU population
Tears	mans	Cheongcheong	Nagdong	DH population
	PPP	$13.9\pm1.9^{\rm a}$	$11.9\pm0.8$	$13.3\pm3.1$
	SPP	$94.9\pm4.8$	$118.1\pm24.3$	$96.9 \pm 44.5$
2012	SSP	$71.3 \pm 2.5$	$78.0\pm5.2$	$58.2\pm20.5$
	TGW	$25.9 \pm 1.2$	$24.3\pm0.7$	$23.9\pm3.9$
	YIELD	$734.9\pm82.8$	$740.6 \pm 196.1$	$409.2\pm247.5$
	PPP	$13.9 \pm 2.6$	$12.9\pm1.6$	$15.2\pm4.2$
	SPP	$130.2\pm20.6$	$125.9 \pm 14.5$	$116.5\pm41.5$
2013	SSP	$88.9\pm4.5$	$89.9\pm2.3$	$86.4\pm39.2$
	TGW	$29.8\pm4.4$	$22.6\pm4.0$	$24.2\pm4.8$
	YIELD	$878.6 \pm 134.1$	$864.4 \pm 112.3$	$864.1\pm725.7$

Table 1. Analysis of yield and yield components between parents and DH population in 2012 and 2013.

<sup>a</sup>Mean ± standard deviation.

Table 2. Phenoty	vpic correlations a	mong vield and	vield com	ponents in a 120	) DH population.
			J		

Year	Traits	PPP	SPP	SSP	TGW	YIELD
2012	PPP	1.000	-0.058	0.106	-0.139	0.256**
	SPP			0.490**	0.004	$0.783^{**}$
	SSP				-0.034	$0.681^{**}$
	TGW					$0.194^{*}$
	YIELD					1.000
2013	PPP	1.000	-0.059	$-2.09^{*}$	-0.113	0.119
	SPP			0.835**	$-2.00^{*}$	0.837**
	SSP				-0.111	$0.846^{**}$
	TGW					$0.194^{*}$
	YIELD					1.000

\*\*Significant at P = 0.01.

fied in both years. The "Cheongcheong" allele had an increased effect at qSSP8andqSSP8-1, while the "Nagdong" allele showed an increased effect at qSSP1. Four and six QTLs were detected in 2012 and 2013, respectively. One of the QTLs, qTGW8, was identified during both years at chromosome 8. The "Nagdong" alleles contributed to heavy 1000-grain weight at these loci, while the increasing effect caused by the "Cheongcheong" allele was only identified at qTGW2 and qTGW8-5 in 2012 and qTGW1-1 andqTGW1-2 in 2013. Two QTLs, qYIELD4 and qYIELD11, were detected on chromosomes 4 and 11 in 2012. Another two QTLs, qYIELD1 and qYIELD12, were detected on chromosomes 1 and 12 in 2013. The "Cheongcheong" alleles showed increased yield at qYIELD1, qYIELD4, qYIELD11, qYIELD1 and qYIELD12 (Table 3, Figure 1).

A total of 25 digenic interactions were detected for yield and yield components in this study (**Table 3**). The highest LOD was qYIELD11 6.18 between RM3428 and RM27123 in 2012, and qTGW11 7.05 between RM27123 and RM27161 in 2013. In addition, the main effect QTLs made greater contributions to the phenotype variations for spikelets per panicle and 1000-grain weight. Selection was the high coincidence at relationship between 18 selected CNDH lines and the QTL at parent's genotype in 2013.

Hypostasis and epistasis selected 18CNDH lines related about strong and weak phenotype, respectively.We selected yield hypostasis related four markers RM3428, RM26771, RM26748, and RM26801 for coincidence between phenotype and genotype in 2012 (Table 4). PPP hypostasis related RM26002 (Table 5), SSP hypostasis related two markers RM12285 and RM3482 (Table 6), and TGW epistasis related RM3512 (Table 7).

Amplification efficiencies were calculated from the slopes of standard curves generated by the qPCR data analysis program. It is qPCR result with CNDH lines and OsAct1 using RM12285, this could confirm that is been shown revelation aspect of specification gene connected with phenotype, and reveals variously (**Table 8**, **Figure 2**). Relation with revelation of specification gene by Phenotype and marker agreed a little (T value was 1.240<sup>ns</sup>). Also, was construed that there are been a lot of SSPs as specific gene expression is larger because re-

e 3. QTLs	detected for yield a	nd yield components in a 1	20 double-	haploid po	opulation in 2012 an	d 2013.
Year	QTL traits	Interval markers <sup>a</sup>	LOD	R2 <sup>b</sup>	Additive effect <sup>c</sup>	Increasing allele <sup>d</sup>
	qPPP8	RM72-RM22499	3.23	0.38	0.95	Cheongcheong
	qSPP2	RM6-RM213	3.10	0.39	14.50	Cheongcheong
	qSPP3	RM15749-RM3525	4.71	0.36	-16.28	Nagdong
	qSPP5	RM18130-RM3381	3.80	0.31	-13.86	Nagdong
	qSPP6	RM20196-RM20355	4.00	0.36	15.11	Cheongcheong
	qSSP8	RM23036-RM22701	2.84	0.29	6.62	Cheongcheong
2012	qSSP8-1	RM72-RM22861	2.72	0.25	6.15	Cheongcheong
	qTGW2	RM3512-RM213	2.77	0.29	0.76	Cheongcheong
	qTGW8-3	RM1148-RM22334	2.91	0.36	-1.89	Nagdong
	qTGW8-4	RM22197-RM23314	5.80	0.46	-8.99	Nagdong
	qTGW8-5	RM23314-RM1235	3.74	0.30	1.42	Cheongcheong
	qYIELD4	RM16429-RM1205	3.83	0.38	82.39	Cheongcheong
	qYIELD11	RM3428-RM27123	6.18	0.42	127.30	Cheongcheong
	qPPP11	RM26002-RM26162	2.47	0.25	2.47	Cheongcheong
	qSPP7	RM8261-RM420	2.61	0.39	-0.39	Nagdong
	qSSP1	RM12285-RM3482	5.15	-0.39	-0.39	Nagdong
	qSSP8	RM404-RM22499	2.77	0.39	0.41	Cheongcheong
	qTGWP7	RM1134-RM420	2.62	0.38	-5.47	Nagdong
2012	qTGW1-1	RM10458-RM11194	2.50	0.32	27.8	Cheongcheong
2013	qTGW1-2	RM12285-RM212	5.63	0.31	4.86	Cheongcheong
	qTGW8	RM23178-RM149	3.22	0.35	-7.05	Nagdong
	qTGW10	RM25890	5.07	0.40	-5.84	Nagdong
	qTGW11	RM27123-RM27161	7.05	0.40	-0.39	Nagdong
	qYIELD1	RM11285-RM212	5.75	0.38	311.80	Cheongcheong
	qYIELD12	RM8216-RM1159	3.55	0.37	322.76	Cheongcheong

<sup>a</sup>Interval markers are those within the significance threshold on each border of the QTL range; <sup>b</sup>The proportion of evaluated phenotype variation attributable to a particular QTL was estimated by the coefficient of determination ( $\mathbb{R}^2$ ); <sup>c</sup>Positive values of the additive effect indicate that alleles from "Cheongcheong" are in the direction of increasing the traits; <sup>d</sup>Increase allele is the source of the allele causing an increase in the measured trait.

		•											•		U							
									Y	TEL	D (hy	post	asis)									Index of
Chr.	Maker		I	Parents	5													CNE	H lin	es		(%)
		$\mathbf{C}^{\mathrm{a}}$	$N^{b}$	112	33	46	116	101	100	84	92	78	91	90	81	82	77	105	95	4	83	
	RM3428	0	Х	-	0	0	0	Х	0	0	0	0	0	0	0	0	0	0	0	0	0	88.9
11	RM26771	0	Х	0	0	0	0	Х	0	0	0	0	0	0	0	0	0	0	0	0	0	94.4
11	RM26748	0	Х	0	0	Х	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	88.9
	RM26801	0	Х	0	0	Х	0	Х	0	Х	0	0	0	0	0	0	0	Х	Х	0	0	72.2

Table 4. Relationship between 18 selected CNDH lines and the QTLS at parent's genotype in 2012.

<sup>a</sup>Cheongcheong indicates maternal line; <sup>b</sup>Nagdong indicates fetal line and each sample's correspondence with chromosome markers. This table shows the highest index of concordance (over 70%).

Table 5. I	Relationship	between 18	selected CNDH lines	and the RM26002	of aPPP11	at parent's genotype in 2	013
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								Р	PP (hy	posta	sis)									Index of
	Parents CNDH lines															(%)				
$\mathbf{C}^{\mathrm{a}}$	$N^{b}$	49	11	70	86	88	20	27	96	99	85	93	120	52	63	82	97	55	87	
0	Х	0	0	Х	0	Х	0	Х	0	0	0	0	0	0	Х	0	0	Х	0	72.2

 $^{a}$ Cheongcheong indicates maternal line;  $^{b}$ Nagdong indicates fetal line and each sample's correspondence with chromosome markers. This tab le shows the highest index of concordance (over 70%).

#### Table 6. Relationship between 18 selected CNDH lines and the QTLS at parent's genotype in 2013.

										SS	SP (h	ypost	asis)									Index of
Chr.	Maker		Р	arent	s													Cl	NDH 1	ines		(%)
		$\mathbf{C}^{\mathrm{a}}$	$\mathbf{N}^{\mathrm{b}}$	84	100	92	115	30	56	25	90	12	50	68	11	14	105	8	119	29	31	
1	RM12285	0	Х	0	0	0	Х	0	-	0	0	0	0	0	0	0	0	Х	Х	0	0	77.8
1	RM3482	0	Х	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Х	0	Х	0	88.9

<sup>a</sup>Cheongcheong indicates maternal line; <sup>b</sup>Nagdong indicates fetal line and each sample's correspondence with chromosome markers. This table shows the highest index of concordance (over 70%).

#### Table 7. Relationship between 18 selected CNDH lines and the QTLS at parent's genotype in 2012.

										Т	GW	(epist	asis)									Index of
Chr.	Maker		Pa	rents														CN	DH	lines		(%)
		$C^{a}$	$N^{b}$	96	63	98	99	89	41	45	27	9	40	87	78	50	119	82	11	56	94	
2	RM3512	0	Х	0	Х	0	Х	Х	0	0	0	Х	0	0	0	0	0	0	0	Х	0	72.2

<sup>a</sup>Cheongcheong indicates maternal line; <sup>b</sup>Nagdong indicates fetal line and each sample's correspondence with chromosome markers. This table shows the highest index of concordance (over 70%).

				1 .	•		1 .	1 4
<b>ODIO</b>		OTIO1	nchin	hotwoon ov	nroccion ·	rotio n	honotuno	andganotung
		מוומו		DELWEELLEX	DICSSIUIL	LALIO. D	HEHUIVDE.	
	~	Letter Or				races, p		and genocy per

			Item		Parents											CND	H lines			
	$\mathbf{C}^{\mathrm{a}}$	$\mathbf{N}^{\mathrm{b}}$	92	115	30	56	25	90	12	50	68	11	14	105	8	119	29	31	84	100
Е	1.0	0.0	17.0	7.3	118.9	3.0	37.3	53.2	9.4	5.4	692.6	0.0	50.9	15.8	148.6	1.0	18.3	34.8	19.6	9.8
Р	129	129	134	217	64	129	129	33	88	139	173	123	151	86	70	75	62	83	59	119
G	0	Х	0	0	0	Х	0	-	0	0	0	0	0	0	0	0	Х	Х	0	0

qPCR data shown the efficiency ratio of each sample. E is the expression ratio based on qPCR. P is phenotype at spikelets per panicle in 2013. G is relationship between 18 selected CNDH lines and the RM12285 marker of qSPP1 at parent's genotype.

gression line appears by Y = 0.814X - 23.260 (Figure 3).

#### 4. Discussion

In this study, only 3 of 12 QTLs affecting yield and yield components were identified over two years, indicating that individual QTLs are sensitive to the environment. Using populations derived from a combination between japonica and indica, 16 HD QTLs were consecutively identified [25]. An interval was identified on chromosome 1 between markers RZ730 and RZ801, where OTLs were associated with panicle number, plant height, maximum tiller number and flag leaf length [19]. This is the first report of the qPPP1-1 flanking marker 1008 and RM575. A OTL for spikelets per panicle flanked by markers RM237 and RM128 on chromosome 1 and 4 was reported. In this study, qSPP2 was between RM6 and RM213 which were the same regions [14]. A QTL associated seed set percentage was found with a peak marker RM23 on chromosome 1 [26]. The same chromosome region between markers RM35 and RG81A for the 35.3 percentage ripened grain was reported [27]. A QTL flanked by markers 1019 and RM488 in the same region was detected for two years [28]. In this study, we also detected a QTL flanked by markers 404 and RM22861 in the same region for two years. QTLs of 1000-grain weight, qTGW3 and qTGW5, were the most important genes for grain length and grain width in rice, respectively [29]. In this study, qTGW8 was detected both years. The qYIELD4 flanked by C2807 and RM252 on chromosome 4 was reported [30]. This QTL was on same loci detected in studies for yield [9] [31]. In the present study, the qYIELD4 flanked by markers 16429 and 1205 was identified in the same region. Investigation of phenotypic correlations between five traits indicated that SPP and SSP showed the highest correlation. The "Cheongcheong" alleles showed an increased number of panicles per plant. A specification gene expressed the highest amplification by RM12285of qSPP's using qPCR. CNDH lines were compared estimate ratio and genotype, phenotype. As a result of the spikelets per panicle is many tillering type so this crop suitable early season cultivar at mountain region.

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Figure 1. QTLs for yield and yield components were identified in 120 double-haploid lines derived from a "Cheongcheong/Nagdong" cross. The approximate positions of centromeres are represented within 12 chromosomes by the black bars. Diagonal symbols indicate that the QTLs were identified in 2012; clear symbols indicate that the QTLs were detected in 2013. The epistatic loci are shown on the left sides of chromosomes.



Figure 2. Quantitative analysis of RM12285 with OsAct 1 in the DH population. (a) Auburn indicates OsAct 1 and blue indicates RM12285. (b) The relative expression of the DH population of rice from a cross of "Cheongcheong" and "Nagdong".





## **5. Conclusion and Recommendations**

We focused on analysis of high-resolution QTL markers associated with rice yields using data for two consecutive years in different environmental conditions. Three QTLs from chromosome 1, 8, and 11 were only found to be associated with the components of yield over two consecutive years indicating high sensitivity of QTL markers to the environment as a total of 6 chromosomes in each single year. Of those three QTLs, SPP-associated marker RM12285 was found to be dominantly expressed by qPCR. With multiple data profiling over years it would facilitate to narrow down to closely associated with yield components, thus we are further planning for fine mapping by collecting more data of QTLs over next few years under diverse environmental conditions.

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