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Analysis of the genetic basis of plant height-related traits in response to ethylene by QTL mapping in maize (*Zea mays* L.)

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Abstract

Ethylene (ET) is critical importance in the growth, development, and stress responses of plants. Plant hormonal stress responses have been extensively studied, however, the role of ET in plant growth, especially plant height (PH) remains unclear. Understanding the genetic control for PH in response to ET will provide insights into the regulation of maize development. To clarify the genetic basis of PH-related traits of maize in response to ET, we mapped QTLs for PH, ear height (EH), and internode length above the uppermost ear (ILAU) in two recombinant inbred line (RIL) populations of *Zea mays* after ET treatment and in an untreated control (CK) group. Sixty QTLs for the three traits were identified. Twenty-two QTLs were simultaneously detected under both ET treatment and untreated control, and five QTLs were detected at two geographic locations under ET treatment only. Individual QTL can be explained 3.87–17.71% of the phenotypic variance. One QTL (*q2PH9-1*, *q1PH9*, *q1EH9*/*q1ILAU9-1*, *q2ILAU9*, and *q2EH9*) for the measured traits (PH, EH, ILAU) was consistent across both populations. Two QTLs (*q2PH2-5*, *q2ILAU2-2*, *q1PH2-2*, and *q1ILAU2-2*; *q1PH8-1*, *q1EH8-1*, *q2PH8-1*) were identified for up to two traits in both locations and populations under both ET treatment and untreated control. These consistent and stable regions are important QTLs of potential hot spots for PH, ear height (EH), and internode length above the uppermost ear (ILAU) response to ET in maize; therefore, QTL fine-mapping and putative candidate genes validation should enable the cloning of PH, EH, and ILAU related genes to ET response. These results will be valuable for further fine-mapping and quantitative trait nucleotides (QTNs) determination, and elucidate the underlying molecular mechanisms of ET responses in maize.

Introduction

The gaseous endogenous plant hormone ethylene (ET) is important for plant growth and development [1–3]. By restricting cell elongation and regulating cell division, ET is most commonly associated with cell size regulation [4, 5]. In terms of development, ET is thought to be an 'aging'

hormone, due to the role it plays in accelerating such processes as abscission, senescence, and ripening [6–8]. Components of the ET signal transduction pathway in *Arabidopsis* have been identified through genetic approaches. The basic model of ET signal transduction works as follows: ET receptors (ETR1, ERS1, ETR2, ERS2 and EIN4) and receptors activating CTR1 in the absence of ET keeps downstream signaling components EIN2 and EIN3 inactive. Upon binding ET, the receptors no longer activate CTR1, while EIN2 activates the EIN3/EIL transcription factors, thus inducing a transcriptional cascade and the establishment of ET responses [3, 9, 10].

ET normally causes the inhibition of stem elongation [6, 11]. However, ET treatment also causes a stunted and thick inflorescence stem, which is also observed in the untreated *ctr1* mutant of *Arabidopsis* [12]. Rapid shoot growth in aquatic species is controlled by the levels of ET synthesis and action. Thus, the elongation response of deepwater rice, commonly known as 'supergrowth', is primarily dependent on the hypoxic induction of ACC synthase [12–14]. The resulting increase in ET modulates the balance between gibberellic acid (GA) and abscisic acid (ABA) and induces stem elongation [12, 15–16]. Plant height has been shown to decrease with decreasing internode length upon ET application in maize, barley, oats, and wheat [17–22]. In *Rumex* species, ET sensitivity was shown to be the key factor controlling submergence-induced shoot elongation [23]. These observations confirm that ET is indispensable for internode elongation in higher plants. Therefore, further studies ET-responsive gene of stem elongation related traits (e.g., PH) is important for reveal the molecular mechanisms of ET signal transduction cascade and interacting with other plant hormones. Multiple QTLs for PH and ear height have been detected using different populations in maize [24–28], and genetic analysis of ear to plant heights (EPR, ear height /plant height) in relation to ET also was reported lately [29]. However, these results do not provide enough data to clarify maize genomic regions of PH-related traits response to ET.

In the present study, to explore the genetic architecture for PH-related (PH, ear height, and internode length above the uppermost ear) traits response to ET in maize plant, QTL mapping of these traits (with ET treatment or without) was conducted using two recombinant inbred line (RIL) populations derived from the cross K22 × BY815 and KUI3 × B77. The objectives of this study were: (1) to determine QTLs of additional genome regions of traits relating to PH, ear height (EH), and internode length above the uppermost ear (ILAU) under ET treatment conditions; (2) to estimate their differences in QTLs detected under both ET treatment and untreated control; and (3) to characterize and analyze the QTLs and candidate gene and compare the differences between the two RILs population associated with ET response.

Materials and methods

Plant materials and field experiments

The two sets of RIL populations for this research, Pop. 1 (N = 197 RILs) and Pop. 2 (N = 177 RILs), were derived from the cross K22 × BY815 and KUI3 × B77, and were developed by China Agricultural University in a single-seed descent method. Parental inbred lines BY815, K22, and B77 were derived from a Chinese Non-Stiff Stalk germplasm, whereas the inbred line KUI3 was derived from CIMMYT and tropical germplasm. By815 and B77 is sensitive to ET treatment, while not K22 and KU13.

A field experiment was conducted during the growing seasons in 2015, the two populations and four parents were evaluated under two treatments, with and without ethylene administration, and in two locations, the Wuqiao test station (Hebei Province, WQ) and the Lishu test station (Jilin Province, LS). No specific permissions were required in the two experimental site. The field studies did not involve wildlife or any endangered or protected species. All the maize inbred lines of flat planting were hand-sown on April 26 in 2015 at Wuqiao (WQ), and

ridge planting on May 6 in 2015 at Lishu (LS), respectively. A split–split–plot design was used in the field experiments with two replications. The main plot was ET treatment or control (CK) (with two levels), the sub-plot factor was populations (with two levels), and the sub-sub-plot factor was genotype [24, 30]. Each plot consisted of a row of 3m × 0.67m with a density of 67,500 plants/ha. For ET treatment, ethephon (270 g/ha at a 600 mg/L concentration, as used by Wei [31]) was applied by foliar-spraying with an agricultural manual sprayer at the 8-leaf stage (V8, according to Abendroth et al.) [32, 33]. In the control group, an equal volume of water was applied by foliar-spraying at the same stage of growth. Treatment was performed quarantine by a membrane or baffle and applied on June 16 (2015) at Wuqiao (WQ) and on June 22 (2015) at Lishu (LS), respectively.

Twenty days after pollen shedding, eight consecutive plants from the plot center were selected to evaluate PH, EH, and ILAU. PH was measured from ground to tassel top, EH from ground to ear node, and ILAU from the node above the uppermost ear to tassel top. The eight-plant average in each replication is reported as the trait values per family, while the average under the two experimental environments in the treatment conditions is described as the overall performance. Broad-sense heritability (h^2) was calculated as follows: $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_{ge}^2/n + \sigma_e^2/nr)$, where σ_g^2 is the genetic variance, σ_{ge}^2 is interaction variance of genotype and environment, σ_e^2 is error variance, n represents the number of environments, r is replication number. Estimation of σ_g^2, σ_{ge}^2, and σ_e^2, and descriptive statistics and simple Pearson correlation coefficients (r), and analysis of variance (ANOVA) were performed using SPSS 21.0 [34–36].

Molecular linkage map construction and QTL analysis

Genomic DNA was obtained from leaves of seedling stage plants from the two RIL populations using the cetyltrimethylammonium bromide method (CTAB) [37]. Genotype analysis of each SNP marker was conducted with the Illumina MaizeSNP50 BeadChip and analyzed using Genome Studio Data analysis software (Illumina Inc., San Diego, CA, USA), generating clusters of homozygous and heterozygous genotypes. In total, 3072 SNP markers were selected to analyze polymorphisms between KUI3×B77 and K22×BY815. A total of 2126 and 2263 SNP markers had polymorphisms between parent pairs [38, 39]. After excluding SNP markers with major segregation distortion, 2126 and 2263 SNP markers were used to generate two genetic linkage maps by Joinmap 4.0 software [34]. The maps were 1744 cM in length (average mapping interval of 0.82 cM) for Pop. 1 and 1640.4 cM (average mapping interval of 0.74 cM) for Pop. 2. A total of 4136 SNP loci were consistent with maize database chromosome bin locations.

QTL mapping for each location was conducted by composite-interval mapping (CIM) in Windows QTL cartographer version 2.5 [40, 41]. For CIM, Model 6 of Zmaoqtl module was applied for detecting QTL and their effects. The genome was scanned every 1 cM between markers and putative QTLs with a window size of 10 cM. Maximum cofactors were utilized to manipulate trait genetic backgrounds. Five control markers were determined by forward and backward regression. Empirical threshold levels for declaring QTL significance at the 5% genome-wide type I error level were achieved via 1000 random permutations [34, 42]. Estimates of phenotypic variance and effect were based on expressed values of QTL peak.

Results

Phenotypic variance in plant height, ear height, and internode length above the uppermost ear under ethylene treatment

According to the combined ANOVA analysis across the two locations, all components were highly significant variance in all measured traits of RIL populations, except for replication

Table 1. Combined analysis of variance for plant height-related traits in the ethylene treated and control RIL populations grown in two different locations (*F*-values).

Variation sources	KUI3×B77		
	PH[a]	EH[b]	ILAU[c]
Family	9.87**	8.33**	7.99**
Location	3.96**	4.12**	3.22**
Replication	1.43	1.33	0.95
Treatment	556.77**	229.81**	29.39**
Family×location	1.30	1.27	0.77
Family×treatment	2.67**	2.73**	3.58**

[a] *PH* plant height.

[b] *EH* ear height.

[c] *ILAU* the internode length above the uppermost ear.

** Significant at $P < 0.01$.

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variance (Table 1). PH-related trait values for the parents and RIL population in the two treatment groups in Table 2. PH, EH, and ILAU for parent KUI3 were less than for B77. The trait values of the population demonstrated high variance, showing continuous distribution around average and transgressive segregations that exceeded high or low parent values (Table 2). ET treatment was effective ($P < 0.01$) and led to a decrease in PH, EH, and ILAU (except ILAU of KUI3). Using the population KUI3×B77 and parent B77 as examples, the average effect of ET treatment on the population was a decrease in PH from 170.16 to 150.52 cm, EH from 71.53 to 54.95 cm, and ILAU from 98.59 to 95.71 cm ($P < 0.01$). The average effect of ET treatment on B77 was a decrease in PH from 191.33 to 160.29 cm, EH from 73.18 to 53.23 cm, and ILAU from 116.60 to 107.06 cm ($P < 0.01$). The broad-sense heritability for all traits in the ET treated and control groups was from 0.45 to 0.84 (Table 2).

Table 2. Phenotypic performance of plant height-related traits for two RIL populations and their parents under two ethylene treatments.

Population		PH[a]		EH[b]		ILAU[c]	
		CK[d]	ET[e]	CK[d]	ET[e]	CK[d]	ET[e]
B77	Mean±SD	191.33±4.70	160.29±6.26	73.18±2.22	53.23±2.95	116.60±3.83	107.06±4.78
KUI3	Mean±SD	157.50±4.67	145.50±5.72	68.00±3.12	56.91±2.76	89.50±2.54	90.11±3.87
KUI3×B77	Mean±SD	170.16±1.03	150.52±0.94	71.53±0.66	54.95±0.47	98.59±0.65	95.71±0.58
	Range	135.24–212.87	116.49–182.4	51.8–90.76	40.57–71.81	77.68–124.79	76.88–120.89
	Kurtosis	-0.07	-0.11	-0.53	-0.12	0.04	0.20
	Skewness	-0.21	-0.30	-0.01	0.08	0.06	0.02
	H_B^2	0.75	0.71	0.80	0.73	0.65	0.57
	CI	0.67–0.80	0.62–0.77	0.74–0.84	0.65–0.79	0.55–0.73	0.45–0.67

[a] *PH* plant height.

[b] *EH* ear height.

[c] *ILAU* the internode length above the uppermost ear.

[d] *CK* without ethylene (CK) treatments.

[e] *ET* with ethylene (ET) treatments.

h^2 the broad-sense heritability.

CI Confidence interval, the confidence intervals of broad-sense heritability between 5 and 95% significance levels.

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The traits showed phenotypic correlations under the treatment conditions (Table 3). In all treatment conditions, pairwise correlations were significant with the exception of CK_ILAU, CK_EH, and ET_EH.

QTL identification for the objective agronomic traits

Sixty QTLs related to the three traits were identified in both populations (Table 4) by CIM, locating 30 QTLs in Pop. 1 (Fig 1), and 30 QTLs in Pop. 2 (Fig 2). Twenty-two QTLs were simultaneously detected in ET-treated and control groups, while five QTLs were detected at two locations under ET treatment only. Individual QTL explained 3.87 to 17.71% of phenotypic variance.

QTL analysis for plant height

Twenty-five QTLs were identified for PH under ET-treated and control groups in the two populations (Table 4), with 11 QTLs in Pop. 1 (Fig 1), and 14 QTLs in Pop. 2 (Fig 2) respectively. These QTLs were mapped onto all chromosomes except for chromosomes 5 and 10, and an individual QTL explained 4.45 to 14.91% of the phenotypic variance. Six of the 25 QTLs from KUI3 (*q2PH2-3*, *q2PH3*, *q2PH5*, *q2PH7*, *q2PH9-1*, *q2PH9-2*) caused PH values to rise. Four QTLs (*q2PH2-1*, *q2PH2-3*, *q2PH2-5*, *q2PH9-1*) were identified in both ET-treated and control conditions, while one QTL (*q2PH8-1*) were observed under ET treatment, and three (*q2PH2-2*, *q2PH5*, *q2PH8-3*) under control conditions. Indeed, QTL *q2PH9-1* was detected under both ET treatment and untreated control, contributing 10% to phenotypic variance in PH. QTL *q2PH8-1* was identified only under ET treatment, and it explained over 10% of PH phenotypic variance.

QTL analysis for ear height

For EH, 17 QTLs in Pop. 1, and 8 in Pop. 2 were identified on all chromosomes except for chromosome 10. An individual QTL explained 4.46 to17.71% of phenotypic variance. Four of the 17 QTLs from KUI3 (*q2EH2-3*, *q2EH5*, *q2EH7*, *q2EH9*) resulted in an increase in trait values. Four QTLs (*q2EH2-3*, *q2EH6*, *q2EH7*, *q2EH9*) were detected under ET-treated and control

Table 3. Correlation coefficients between plant height-related traits in the two RIL populations.

Trait	CK_PH [a]	ET_PH [b]	CK_EH [c]	ET_EH [d]	CK_ILAU [e]	ET_ILAU [f]
CK_PH [a]						
ET_PH [b]	0.85**					
CK_EH [c]	0.67**	0.62**				
ET_EH [d]	0.53**	0.68**	0.83**			
CK_ILAU [e]	0.80**	0.65**	0.10	0.04		
ET_ILAU [f]	0.78**	0.87**	0.27**	0.23**	0.83**	

Correlation coefficients below the diagonal line in each quadrant of the table are for the KUI3×B77 population.

[a] *CK_PH* plant height without ethylene (CK) treatments.

[b] *ET_PH* plant height with ethylene (ET) treatments.

[c] *CK_EH* ear height without ethylene (CK) treatments.

[d] *ET_EH* ear height with ethylene (ET) treatments.

[e] *CK_ILAU* the internode length above the uppermost ear without ethylene (CK) treatments.

[f] *ET_ILAU* the internode length above the uppermost ear with ethylene (ET) treatments.

** Significant at $P < 0.01$.

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Table 4. QTLs detected for plant height-related traits in the two RIL populations across two environments and two ethylene treatments.

Traits	QTL	Chr.	Location [a]	Treatment [b]	Position [c] (cM)	Marker Interval [d]	Support interval [e] (cM)	Physical interval [f] (bp)	LOD [g]	R^2 (%) [h]	A [i]	Positive allele [j]
KUI3 × B77												
PH	*q2PH2-1*	2	LS	CK	49.2	PZE-102038161-SYN29038	47.1–51.7	18,316,951–20,724,795	3.47	6.45	-3.48	B77
			LS	ET					8.23	14.91	-5.06	
			WQ	CK					4.26	7.96	-4.23	
			WQ	ET					4.01	7.38	-3.99	
	q2PH2-2	2	WQ	CK	68.7	PZE-102056514-ZM012191-1494	66.9–69.1	34,539,814–37,198,403	5.92	11.31	-6.43	B77
			LS	CK					3.68	6.97	-3.69	
	q2PH2-3	2	WQ	CK	118.9	PZE-102132131- PZE-102134800	119.2–126.3	182,620,244–184,968,231	4.23	8.12	5.41	KUI3
			WQ	ET					3.62	6.97	3.85	
			LS	CK					5.24	9.25	5.63	
			LS	ET					3.26	5.84	2.97	
	q2PH2-4	2	WQ	CK	157.2	PZE-102169752- PZE-102170996	156.6–157.8	213,205,091–214,210,573	2.59	4.8	-4.05	B77
	q2PH2-5	2	LS	CK	180.9	PZE-102184387-SYN15855	178.5–180.9	227,531,180–228,895,610	2.63	4.81	-3	B77
			LS	ET					2.89	5.12	-2.68	
			WQ	CK					3.12	5.01	-4.16	
			WQ	ET					2.67	6.23	-3.37	
	q2PH3	3	LS	ET	118.2	SYN37387- PZE-103144159	116.5–119.8	195,935,474–199,484,261	3.9	6.74	3.41	KUI3
	q2PH5	5	WQ	CK	164.4	PZE-105156919- PZE-105158393	162.3–165.8	205,567,299–206,188,028	3.66	6.93	4.77	KUI3
			LS	CK					4.02	7.71	3.95	
	q2PH6	6	WQ	CK	97.2	PZE-106094294- PZE-106098403	95.2–101.2	149,598,681–152,167,281	5.37	10.31	-5.67	B77
	q2PH7	7	LS	ET	78.5	PZE-107058976- PZE-107060141	78.2–78.5	112,980,785–115,691,953	2.95	4.6	2.84	KUI3
	q2PH8-1	8	LS	ET	45.8	PZE-108015015- PZE-108018250	43.9–50	14,753,943–17,383,010	6.67	12.15	-4.52	B77
			WQ	ET					5.36	11.47	-3.37	
	q2PH8-2	8	WQ	ET	63	PZE-108046118- PZE-108054764	59.9–63.4	76,039,534–97,777,183	5	8.98	-4.24	B77
	q2PH8-3	8	LS	CK	71.7	PZE-108064061-SYNGENTA14910	70.8–72	114,297,738–118,401,344	7.22	14.47	-5.2	B77
			WQ	CK					6.35	11.51	-6.32	
	q2PH9-1	9	LS	ET	48.3	PZE-109022525- PZE-109024455	46.3–49.6	23,008,509–24,487,068	6.12	10.77	4.33	KUI3
			WQ	ET					4.21	7.06	3.84	
			LS	CK					6.38	12.14	4.2	
			WQ	CK					5.41	10.12	5.32	
	q2PH9-2	9	LS	CK	62.9	PZB02480.2- PZE-109071675	62.1–64.5	107,416,972–116,353,876	3.97	7.56	3.77	KUI3
EH	*q2EH2-1*	2	LS	ET	47.4	SYN29778- SYN29038	46.5–51.7	17,793,282–20,724,795	4.76	8.69	-2.47	B77
	q2EH2-2	2	WQ	CK	66.9	PZE-102054526-SYN34233	66.1–67.5	32,516,815–36,590,474	5.08	9.12	-3.22	B77
			LS	CK					3.04	5.81	-2.47	

(*Continued*)

Table 4. (Continued)

Traits	QTL	Chr.	Location [a]	Treatment [b]	Position [c] (cM)	Marker Interval [d]	Support interval [e] (cM)	Physical interval [f] (bp)	LOD [g]	R^2 (%) [h]	A[i]	Positive allele [j]
	q2EH2-3	2	WQ	CK	129.2	PZE-102134800- PZE-102139383	126.3–130.1	184,968,231–187,633,354	3.08	5.75	1.51	KUI3
			WQ	ET					2.89	5.07	1.72	
			LS	CK					3.56	6.24	2.97	
			LS	ET					3.25	5.33	2.86	
	q2EH3	3	WQ	ET	1.8	PZE-103000307- PZE-103001968	1.5–3.9	1,233,964–1,978,736	2.52	4.55	-1.35	B77
	q2EH5	5	WQ	CK	164.4	PZE-105156970- PZE-105158756	163.7–166.8	205,776,348–206,557,530	5.45	9.98	2.93	KUI3
			LS	CK					3.69	7.18	2.8	
	q2EH6	6	LS	CK	116.1	SYN23640- PZE-106112056	113.6–117.8	156,879,515–159,464,206	3.95	7.6	-2.8	B77
			WQ	CK					4.81	8.56	-2.77	
			WQ	ET					9.18	17.71	-2.67	
			LS	ET					2.74	4.47	-1.72	
	q2EH7	7	LS	ET	96.1	PZE-107074506- SYN32297	94.6–99.1	130,360,641–133,374,241	5.64	10.16	2.63	KUI3
			WQ	ET					2.64	4.46	1.37	
			LS	CK					3.11	5.93	2.52	
			WQ	CK					5.32	9.54	2.41	
	q2EH9	9	LS	CK	62.5	PZE-109065808- PZE-109066235	62.5–62.9	108,522,923–109,304,376	3.28	6.28	2.6	KUI3
			WQ	ET					3.51	6.41	1.62	
			LS	ET					5.1	9.18	2.55	
			WQ	CK					4.32	7.54	2.01	
ILAU	*q2ILAU1-1*	1	LS	ET	19	SYN13185- SYN13395	19.0–20.3	6,844,116–7,565,560	2.82	5.49	-2.56	B77
	q2ILAU1-2	1	LS	CK	74	PZE-101046080- SYN23289	71.3–75.6	31,850,441–38,871,055	5.06	10.28	-3.48	B77
	q2ILAU2-1	2	WQ	ET	49.8	PZE-102040312- PZE-102043154	48.9–52	20,103,068–21,874,416	4.08	7.54	-2.9	B77
	q2ILAU2-2	2	LS	CK	180.9	SYN28307- PZE-102186367	176.9–181.5	225,537,147–230,291,137	4.09	7.92	-3.01	B77
			LS	ET					4.5	9.06	-3.42	
			WQ	CK					4.09	7.92	-3.01	
			WQ	ET					4.5	9.06	-3.42	
	q2ILAU6	6	LS	ET	141.7	PHM5361.13- PZE-106129549	139.8–141.7	166,592,099–167,325,854	2.86	5.63	2.61	B77
	q2ILAU8-1	8	WQ	ET	63	PZE-108049474- PZE-108055459	61.1–64.2	85,149,829–99,749,632	6.05	11.34	-3.42	B77
	q2ILAU8-2	8	WQ	CK	69.5	SYN39345- PZE-108066080	67.1–71.7	106,271,779–117,871,384	4.65	9.74	-3.5	B77
			LS	CK					6.82	13.8	-3.99	
	q2ILAU9	9	WQ	ET	47.3	PZE-1090212740 SYN11956	45.4–50.7	21,678,811–25,817,148	4.8	10.87	2.9	KUI3
			LS	ET					4.77	8.67	3.22	
			WQ	CK					4.72	9.94	3.98	
			LS	CK					6.94	13.02	4.27	
K22 × BY815												

(*Continued*)

Table 4. (Continued)

Traits	QTL	Chr.	Location [a]	Treatment [b]	Position [c] (cM)	Marker Interval [d]	Support interval [e] (cM)	Physical interval [f] (bp)	LOD [g]	R^2 (%) [h]	A [i]	Positive allele [j]
ILAU	*q1ILAU2-1*	2	WQ	CK	41.5	PZE-102027559- PZE-102028305	41.5–41.8	12,801,930–13,300,441	4.48	7.46	2.7	BY815
			LS	CK					2.98	4.92	2.29	
	q1ILAU2-2	2	WQ	CK	180.5	PZE-102192366- PZE-102193904	179.2–182.7	234,718,008–236,615,130	4.61	10.82	-2.73	K22
			LS	ET					5.37	8.71	-4.05	
			LS	CK					4.63	8.12	-2.95	
			WQ	ET					6.07	11.07	-3.03	
	q1ILAU3	3	WQ	CK	70.5	SYN12568- PZE-103027704	61–71.1	12,189,577–20,378,743	3.13	4.76	-2.1	K22
	q1ILAU6	6	LS	CK	21.9	PZE-106020696- PZE-106013766	18.3–28.8	16,795,395–34,863,238	4.58	7.7	3.75	BY815
			LS	ET					4.86	7.65	3.73	
			WQ	CK					4	6.58	2.48	
			WQ	ET					3.92	6.74	2.35	
	q1ILAU7-1	7	LS	ET	54.3	PZE-107015084- SYN27395	49.7–55.2	11,799,137–17,697,488	3.53	6.19	3.34	BY815
			LS	CK					3.85	6.65	3.73	
			WQ	ET					3.68	5.81	2.48	
			WQ	CK					3.23	5.7	2.35	
	q1ILAU7-2	7	WQ	CK	70.1	PZE-107068961- SYN28758	68–70.8	125,861,581–129,967,283	3.32	5.48	2.29	BY815
			LS	CK					4.06	6.9	2.75	
	q1ILAU8	8	WQ	CK	94.8	PZE-108099425- PZE-108105216	90.3–98.3	155,643,006–159,952,552	3.7	6.12	-2.47	K22
			LS	CK					4.35	7.35	-2.89	
			WQ	ET					2.77	4.73	-2.1	
			LS	ET					5.03	8.43	-3.62	
	q1ILAU9-1	9	LS	CK	50.9	PZE-109027610- PZE-109055561	46.8–52.4	28,005,182–89,325,234	3.6	6.41	2.55	BY815
			WQ	ET					3.59	6.26	2.29	
			LS	ET					5.57	9.04	4.03	
			WQ	CK					3.81	7.22	2.72	
	q1ILAU9-3	9	WQ	CK	66.3	PZE-109077680- SYN12671	66.3–69.4	125,169,744–128,964,637	2.53	3.87	1.91	BY815
	q1ILAU10	10	WQ	CK	113.4	PZE-110103320- PZE-110103696	113.4–113.9	146,173,534–146,292,761	2.67	4.08	-1.96	K22

PH plant height, *EH* ear height, *ILAU* the internode length above the uppermost ear.

[a] *Location* QTL detected in Wuqiao (WQ) and Lishu (LS).

[b] *Treatment CK*, without ethylene (CK) treatments; *ET*, with ethylene (ET) treatments.

[c] *Position* The peak position with the highest LOD of each QTL.

[d] *Marker Interval* Flanking markers, the left and right markers of the QTL.

[e] *Support interval* Genetic position interval of each QTL.

[f] *Physical interval* Physical location of the QTL in the maize genome (MaizeDB; http://www.maizegdb.org/).

[g] *LOD scores* logarithm of the odds (to the base 10).

[h] R^2 *(%)* rate of contribution were calculated for each QTL.

[i] *A* additive effect, positive values indicated that BY815 or KUI3 carries the allele for an increase in the traits, while negative values(-) indicated that K22 or B77 contributed the allele for an increase in the trait value.

[j] *Positive allele* effect carries of parental.

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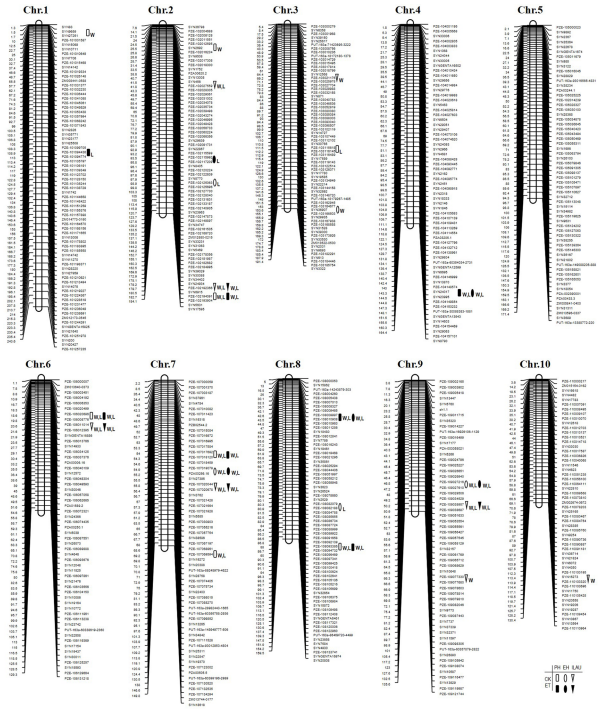

Fig 1. Chromosomal locations of QTLs for plant height-related traits in Pop. 1 (K22 × BY815, N = 197 RILs) population across two environments and two ethylene treatments. Note: Rectangle QTL detected for plant height (PH) with ethylene treatment (Solid) or without (Hollow), Oval QTL detected for ear height (EH) with ethylene treatment (Solid) or without (Hollow), and Inverted triangle QTL detected for internode length above the uppermost ear (ILAU) with ethylene treatment (Solid) or without (Hollow). W indicates a QTL detected in Wuqiao test station, and L indicates a QTL detected in Lishu test station.

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conditions, with two QTLs (*q2EH6*, *q2EH7*) explaining about 10% of phenotypic variance. Two QTLs (*q2EH2-2*, *q2EH5*) were determined only under control conditions.

QTL analysis for internode length above the uppermost ear

Eighteen QTLs were detected for ILAU: 10 in Pop. 1, and 8 in Pop. 2. These QTLs were mapped to every chromosome except for chromosomes 4 and 5. The contributions to phenotypic variance for an individual QTL ranged from 3.87 to 13.80%, and with four QTLs contributing about 10%. Five alleles from 18 QTLs were inherited by K22 (*q1ILAU2-2*, *q1ILAU3*, *q1ILAU8*, *q1ILAU10*) and KUI3 (*q2ILAU9*), and led to a rise in trait values. Seven QTLs (*q1ILAU2-2*, *q1ILAU6*, *q1ILAU7-1*, *q1ILAU8*, *q1ILAU9-1*, *q2ILAU2-2*, *q2ILAU9*) were identified in ET-treated and control conditions, while three QTLs (*q1ILAU2-1*, *q1ILAU7-2*, *q2ILAU8-2*) were identified only under control conditions. QTLs *q1ILAU2-2* and *q2ILAU9*, detected under both ET-treated and control conditions, contributed 10% of phenotypic variance. QTL *q2ILAU8-2*, detected only in control conditions, accounted for more than 10% of the phenotypic variance in ILAU.

Mapping results comparison

By comparing mapping results, one QTL for three measured traits (*q2PH9-1*, *q1PH9*, *q1EH9*/*q1ILAU9-1*, *q2ILAU9* and *q2EH9*) was consistent across two populations, and two QTLs were consistent for one or two traits (*q2PH2-5*, *q2ILAU2-2*, *q1PH2-2* and *q1ILAU2-2*; *q1PH8-1*, *q1EH8-1*, *q2PH8-1*) across two populations at two locations under both ET-treated and control

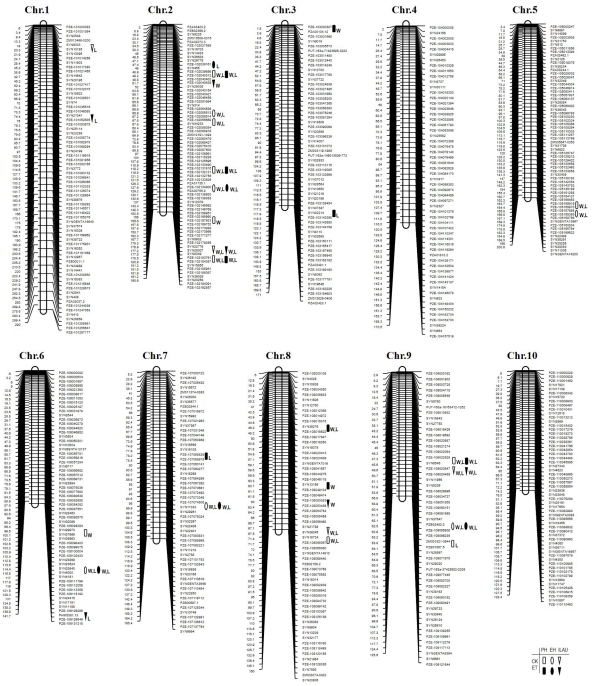

Fig 2. Chromosomal locations of QTLs for plant height-related traits in Pop. 2 (KUI3 × B77, N = 177 RILs) population across two environments and two ethylene treatments. Note: Rectangle QTL detected for plant height (PH) with ethylene treatment (Solid) or without (Hollow), Oval QTL detected for ear height (EH) with ethylene treatment (Solid) or without (Hollow), and Inverted triangle QTL detected for internode length above the uppermost ear (ILAU) with ethylene treatment (Solid) or without (Hollow). W indicates a QTL detected in Wuqiao test station, and L indicates a QTL detected in Lishu test station.

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conditions. Moreover, three QTLs for two or all traits were consistent across one population at both locations under both treatment conditions, four QTLs for two traits across one population at both locations under control conditions, and three QTLs for two traits across one or both populations at both locations under ET treatment (Table 5, Figs 1 and 2).

Discussion

PH is in highly correlation with biomass yield as it has a large impact on grain yield, shorter plants are more lodging-resistant and have an improved per unit yield [18, 33, 43]. However, even though PH is an important agronomic trait the molecular mechanisms that underlie natural variation remains very elusive in experimental population genetics. A number of studies have shown that hormonally mediated pathways and their interactions are major determinants of PH [44], however, further study of ET-treated PH change is required to uncover the molecular genetic basis of this relationship. Although ethephon application is used in maize to prevent lodging by decreasing PH and EH, the genetic basis of treatments that affect PH is yet unknown. In the present study, PH-related traits with lodging resistance were examined with and without ET treatment using two maize RIL populations. Our results indicate that ET treatment has such a great impact on PH-related traits by decreasing phenotypic performance of PH, EH, and ILAU ($P < 0.01$). Sixty trait-related QTLs were identified under ET-treated and control groups in two populations by CIM, locating 30 QTLs in Pop. 1 and 30 in Pop. 2 (Table 4). Twenty-two QTLs were simultaneously detected in both ET-treated and control conditions, and five QTLs were detected at two geographic locations only under ET treatment. An individual QTL explained 3.87 to 17.71% of the phenotypic variance. One QTL for three

Table 5. Comparison of mapping results across the two RIL populations or ethylene treatments in two locations.

QTL	Location [a]	Treatment [b]	Marker Interval [c]	Support interval [d] (cM)	Physical interval [e] (bp)	Rang of R^2 (%)	Candidate Gene/Pevious Studies for PH-related traits
q1ILAU2-1	WQ, LS	CK	PZE-102027559- PZE-102028305	41.5–41.8	12,801,930–13,300,441	4.92–7.46	AT4G00880,
q2PH2-1	WQ, LS	CK, ET	PZE-102038161-SYN29038	47.1–51.7	18,316,951–20,724,795	6.45–14.91	Wang, 2014; AT2G35700
q2EH2-2/q2PH2-2	WQ, LS	CK	PZE-102054526-SYN34233	66.1–67.5	32,516,815–36,590,474	5.81–11.31	
q2PH2-3/q2EH2-3	WQ, LS	ET	PZE-102132131-PZE-102139383	119.2–130.1	182,620,244–187,633,354	5.07–9.25	AT1G72360
q2PH2-5/q2ILAU2-2/q1PH2-2/ q1ILAU2-2	WQ, LS	CK, ET	PZE-102184387- PZE-102193904	178.5–182.7	227,531,180–228,895,610	4.81–11.07	
q1PH4/q1EH4	WQ, LS	ET	PZE-104148574- PZE-104148574	142.4–147.3	235,704,291–236,882,332	5.54–5.99	
q2PH5/q2EH5	WQ, LS	CK	PZE-105156919- PZE-105158393	162.3–165.8	205,567,299–206,188,028	6.93–9.98	Nikolic et al. 2011; Tang et al. 2012; Wang, 2014; Yang et al. 2008; Zhang et al. 2010; Ku et al. 2014; Ajmone-Marsan et al. 1994; Lübberstedt et al. 1997; Tang et al. 2007; Gonzalo et al. 2010
q1PH6-1/q1ILAU6	WQ, LS	CK, ET	PZE-106020696- PZE-106013766	18.3–21.9	16,795,395–34,863,238	5.21–10.99	AT1G74930
q2EH6	WQ, LS	CK, ET	SYN23640- PZE-106112056	113.6–117.8	156,879,515–159,464,206	4.47–8.56	AT1G78440
q1PH7/q1EH7/ q1ILAU7-1	WQ, LS	CK, ET	PZE-107016972- PZE-107021928	52–57.3	14,462,136–21,509,620	4.48–10.99	
q1PH7-2/q1ILAU7-2	WQ, LS	CK	PZE-107069594-SYN28758	68.6–70.8	126,402,635–129,967,283	4.69–6.90	Wang, 2014; Yang et al. 2008; Lima et al. 2006
q2EH7	WQ, LS	CK, ET	PZE-107074506- PZE-107080831	94.6–98.5	130,360,641–133,374,241	4.46–10.16	AT1G73730
q1PH8-1/q1EH8-1/ q2PH8-1	WQ, LS	ET	PZE-108009997-SYN3278	42.1–44.8	10,393,147–13,203,852	5.1–12.15	
q2PH8-3/q2ILAU8-2	WQ, LS	CK	PZE-108064061-SYNGENTA14910	70.8–72	114,297,738–118,401,344	9.74–14.47	
q1PH8-2/q1ILAU8	WQ, LS	CK, ET	PZE-108099425- PZE-108105216	90.3–98.3	155,643,006–159,952,552	4.73–9.27	
q2PH9-1/q1PH9/ q1EH9/q1ILAU9-1/ q2ILAU9/q2EH9	WQ, LS	CK, ET	PZE-109022525- PZE-109027610	46.3–50.6	23,008,509–28,005,182	5.62–13.01	Wang, 2014; Sibov et al. 2003; Yang et al. 2008; Gonzalo et al. 2010; dwarf3

PH plant height, *EH* ear height, *ILAU* the internode length above the uppermost ear.

[a] *Location* QTL detected in Wuqiao (WQ) and Lishu (LS).

[b] *Treatment CK*, without ethylene (CK) treatments; *ET*, with ethylene (ET) treatments.

[c] *Marker Interval* Flanking markers, the left and right markers of the QTL.

[d] *Support interval* Genetic position interval of each QTL.

[e] *Physical interval* Physical location of the QTL in the maize genome (MaizeDB; http://www.maizegdb.org/).

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measured traits (*q2PH9-1*, *q1PH9*, *q1EH9*/*q1ILAU9-1*, *q2ILAU9* and *q2EH9*) was consistent across both populations, and two QTLs for one or two traits (*q2PH2-5*, *q2ILAU2-2*, *q1PH2-2*, and *q1ILAU2-2*, *q1PH8-1*, *q1EH8-1*, *q2PH8-1*) were identified in both RIL populations at both locations under ET-treated and control conditions. These consistent and stable regions are important QTLs indicating potential hot spots for location of genes of PH, EH, and ILAU responses to ET in maize; therefore, fine-mapping of QTLs and of putative candidate gene

validation should enable the cloning of PH, EH, and ILAU related genes to ET response. The data produced in the present study will be of value for further fine-mapping, determination of quantitative trait nucleotides (QTNs), and elucidation of the molecular mechanisms underpinning PH, EH, and ILAU responses to ET.

Mapping results comparison with previous studies

Many of the QTLs exhibited in this study consistency and stability detected across with differently genetic populations of previously studies in the same locus or adjacent bins (Table 5): alleles for PH in the interval between PZE-102038161 and SYN29038 on chromosome 2 for PH identified across Pop. 1 under both treatment conditions examined here and in a previous study [44]; alleles for PH in the interval between PZE-105156919 and PZE-105158393 on chromosome 5 were identified here in Pop. 2 under control conditions and previous studies [24, 30, 45–51]; between PZE-107069594 and SYN28758 on chromosome 7 for PH and ILAU in Pop. 1 under control conditions in this and previous studies [45, 52]; and alleles for the three measured traits in the interval between PZE-109022525 and PZE-109027610 on chromosome 4 were identified in the two population under both ET-treated and control conditions and in previous studies [30, 45, 48, 53]. Taken together, these findings show that many QTLs influence PH, EH and ILAU in maize, suggesting a common origin among some traits. In addition, three clustered QTLs regions (*q2PH2-5*/*q2ILAU2-2*/*q1PH2-2*/*q1ILAU2-2*, *q1PH7*/*q1EH7*/*q1ILAU7-1*, and *q1PH8-2*/*q1ILAU8*) detected under at both locations and both ET-treated and control conditions was not identified in previous studies, and contributed to more than 10% of phenotypic variance. These novel and stable robust QTLs for PH, EH, and ILAU, further indicated that the genetic structures of the PH-related trait in response to ET treatment was affected by many different minor effective QTLs.

Comparison of the mapping results across the measured traits in the two RIL populations

We verified three clustered QTLs (*q2PH2-5*/*q2ILAU2-2*/*q1PH2-2*/q1ILAU2-2, *q1PH8-1*/*q1EH8-1*/*q2PH8-1*, and *q2PH9-1*/*q1PH9*/*q1EH9*/*q1ILAU9-1*/*q2ILAU9*/*q2EH9*) located in the same or similar chromosomes regions of the two populations (Pop. 1, and Pop. 2, Table 5), demonstrate that the traits may also be regulated by one QTL or several of the same QTLs. At the same time, the several other QTLs associated with PH-related traits in response to ET treatment were identified among the different populations. Six QTLs (e.g., *q1ILAU2-1*, *q1PH4*/*q1EH4*) were identified for one or more measured trait in Pop. 1, while other seven QTLs (e.g., *q2PH2-1*, *q2EH2-2*/*q2PH2-2*) only detected in Pop. 2. The results demonstrate that PH-related traits in response to ET treatment in maize can be affected by population-specific QTLs, which attributed to differences in the genetic backgrounds of two populations due to the parental line different.

Genetic architecture of the ethylene response

As a phytohormone, ET has been shown to be involved in stem elongation in deepwater rice [54]. However, some literatures indicated that exogenous ET with moderate to high concentration inhibited stem elongation of maize, as well as other cereal crops such as wheat, oat, and barley etc. [17, 18]. Our results showed that ET treatment applied to maize had similar the physiological responses (i.e. decreasing phenotypic performance of PH, EH, and ILAU) to abiotic stress factors (plant density, waterlogging, etc.). For the three agronomic traits, 19 genetic loci of mapping QTLs were identified as ET-responsive loci in Pop. 1, and 22 genetic loci sensitive to ET in Pop. 2. Further, 7 ET-specific QTLs (11.67%) identified in the two RIL

populations. Thus, the maize ET responses assessed here were consistent and specific, as those described in other studies such stressors such as plant density, nitrogen deficiency and water-logging [34, 55–58]. The results of present study could provide a valuable reference for finding specific genes and elucidating the molecular mechanism involved in ET responses.

Associations among QTLs and candidate genes in maize

To explore further the molecular mechanism of PH-related trait variance, the association from QTLs to genes known to be found in PH-related traits in *Arabidopsis* (a model dicotyledonous plant), rice (a monocotyledonous plant), and maize were studied by the bioinformatics approach using the *Zea mays* genome (Table 5). Sequences for candidate genes of *Arabidopsis*, and crops e.g. maize and rice were downloaded from the National Center for Biotechnology Information (NCBI), and their homologs in maize inbred line B73 were investigated using maizeGDB blast with an *E*-value cutoff of 10^{-10} and coverage longer than 60% [42]. Seven candidate genes controlling PH-related traits were located in 7 consistent QTL intervals (Table 5). *AT2G35700*, *AT1G72360*, and *AT1G74930* were found to be located in the *q2PH2-1*, *q2PH2-3*/*q2EH2-3*, and *q1PH6-1*/*q1ILAU6* intervals, respectively. The two genes encode a member of the subfamily of ERF/AP2 transcription factors. Indeed, the *AP2* genes belong to a large gene family that encode a highly conserved AP2/ERF DNA binding domain and are importance in the regulation of development and in responses to abiotic and biotic stresses [59–61]. Mutations near the exon-intron boundaries in these genes cause misspliced transcript variants, and result in phenotypic changes to the plants, specifically shorter internodes and wrinkled leaves [61]. *AT4G00880* was located in the *q1ILAU2-1* interval. The gene encodes a small size auxin-induced protein initially identified in *Arabidopsis*, soybean, and later in other plants [62–65]. A few SAUR proteins are shown to bind CaM [61], alter apical hook development, and negatively regulate auxin synthesis and transport [64, 66]. Proteins *SAUR76*, *77*, and *78* integrate auxin into ethylene signaling to regulate ET response and plant growth [64]. *AT1G78440* was located in the *q2EH6* interval. The gene encodes a gibberellin 2-oxidase (*GA2ox*) that acts on C19 gibberellins. *GA2ox* hydrolyzes carbon-2 positions of bioactive gibberellic acids (GA1, GA4) and immediate precursors (GA9, GA12, GA20, and GA53) to inactive these proteins [67]. In *Arabidopsis thaliana* and *Nicotiana tabacum*, overexpression of *AtGA2ox7* or *AtGA2ox8* has been shown to result in decreased levels of active gibberellic acids (GAs) and to induce extremely dwarf phenotypes [68]. Moreover, transgenic *Torenia fournieri* plants overexpressing *TfGA2ox* showed dwarf phenotypes as well. However, a mutant of the *SLENDER* gene of *Pisum sativum* encoding *GA2ox*, had higher PH and accumulated GA precursors of high concentration in seeds [69]. Therefore, manipulation of gibberellic acid metabolism by *GA2ox* overexpression might be effective to modify PH. *AT1G73730* was located in the *q1PH7-2*/*q1ILAU7-2* interval. This gene encodes ethylene insensitive 3-like protein (*EIN3*), a transcription factor involving ET signal transduction pathway in *Arabidopsis* [70]. *EIN3* and various *EIN3-like* proteins are not ET-induced but are regulated at post-transcriptional level. Transcription factors can act as activators or repressors of additional downstream ET-responsive genes. Transgenic rice plants overexpressing *OsEIL1* (an *EIN3-like* gene) have been shown to exhibit a short shoot phenotype, coiled primary root, short root, and elevated response to exogenous ET [3]. *Dwarf3* was located in the *q2PH9-1*/*q1PH9*/*q1EH9*/*q1ILAU9-1*/*q2ILAU9*/*q2EH9* interval. This gene encodes a cytochrome P450-mediated early step in gibberellin biosynthesis in maize [61]. Allelic variation at the *Dwarf 3* locus is proposed as basis of a QTL, which was defined for a natural maize height variant [71, 72]. The functional analysis of homologous genes confirm that the maize ET responses QTLs involves ET signal transduction cascade and also interacting with other plant hormones.

The QTLs and candidate genes identified will be of great value for fine-mapping and quantitative nucleotide determination to QTLs cloning of ET-responsive PH-related traits in maize [29, 73–74]. Therefore, further deep understanding for the mapping alleles may contribute to enhancing efficiency for plant height-related traits genetic improvement and elucidating the molecular mechanism involved in ET responses.

Supporting information

S1 Table. Phenotypic data in Pop. 1 for the study.
(XLSX)

S2 Table. Phenotypic data in Pop. 2 for the study.
(XLSX)

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Supervision: Liusheng Duan.

Validation: Weiqiang Zhang.

Writing – original draft: Weiqiang Zhang.

Writing – review & editing: Liusheng Duan.

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