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QTL mapping, validation and candidate genes analysis for plant height in maize

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Abstract

Plant height is one of the most important agronomic traits closely associated with biomass, lodging resistance and grain yield in maize. The aim of this study to map QTLs, validate QTL results and analyze candidate genes for plant height based on high density linkage map from a cross between two inbred lines of maize. A set of 199 $F_{2:3}$ progeny derived from the cross between SG5 x SG7 was analysed. Thirty two QTLs associated with plant height (PH) using the least absolute shrinkage selection operator (LASSO) method were identified. QTLs detected in this study were validated to find the stability and consistency in different environments. A total of 12 QTLs detected in this study were similar to earlier reported QTLs, which may be the most reliable and stable. Genes underlying these QTLs (+50 kb from the significant markers) were analyzed and 10 genes were predicted as candidate genes for PH. The work will not only help in underlying the mechanisms that control plant height trait of maize, but also provide a basis for map-based cloning of PH genes and dwarf breeding application in further studies.

Keywords: QTL mapping, maize, quantitative trait loci, plant height, candidate gene

Introduction

Maize is one of the most important crops serves as food, animal feed and raw materials of bioenergy worldwide (Li et al. 2017). Plant height (PH) is one of the most important selection factors in maize architecture because optimal PH is critical for improving plant density to maximize the utilization of fertilizer, moisture and incident photosynthetically active radiation (Li et al. 2017; Mock and Pearce 1975; Rao et al. 2014). Reducing plant height is one of the main strategies to avoid lodging in maize breeding (Ji-hua et al. 2007). Results of studies on classical quantitative genetics show that plant height, the most important factor of plant architecture is a complicated quantitative trait controlled by a large number of genes (Zhou et al. 2016). Recently, studies on genetics of various aspects of maize plant architecture i.e. ear height (EH), and internode number (IN), have been extensively investigated (Ku et al. 2015; Li et al. 2014; Zheng and Liu 2013).

QTL mapping is an efficient strategy to detect underlying genes and elements (Bommert et al. 2013). However, the high complexity of crop genomes and the low-coverage of genetic markers across chromosomes have posed great challenges for dissection of quantitative genetic variation by QTL analysis, especially for detecting small effect QTLs (Wenzl et al. 2006; Yu et al. 2011). Along with the appearance of the first maize genetic linkage map in 1986 based on restriction fragment length polymorphisms (RFLP) technology (Helentjaris et al. 1986), molecular markers based on PCR technology were further developed and applied in constructing maize genetic linkage maps (Davis et al. 1999; Senior et al. 1996; Vuylsteke et al. 1999). Based on the linkage map, QTL mapping have been successfully used for the genetic analysis of PH and obtained great importance (Austin and Lee 1996; Berke and Rocheford 1995; Ji-hua et al. 2007; Khairallah et al. 1998; Salvi et al. 2011; Zhang et al. 2011). To date, using different populations, more than 219 QTLs for maize PH have been identified which are distributed on all 10 maize chromosomes and most of these QTLs are located on chromosomes 1 and 3 (Gramene QTL database). Most identified QTLs on latest linkage map

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always located in large width of the confidence intervals because of low-density linkage map. Ten years ago next-generation sequencing (NGS) technologies appeared on the market and tremendous progress has been made in terms of speed, read length, and throughput, along with a sharp reduction in per-base cost (Van Dijk et al. 2014). Genotyping-by-sequencing (GBS) technology (Elshire et al. 2011) paved the way for the constructing genetic linkage maps with high density SNPs and has achieved great significance for genetic studies in different species (Byrne et al. 2013; Poland et al. 2012; Sonah et al. 2013; Spindel et al. 2013), including maize (Chen et al. 2014; Zhou et al. 2016).

In earlier crop breeding studies, QTLs associated with markers were commonly directly used for marker assisted selection (MAS) but with low efficiency. In fact, QTL mapping results usually different in different studies because of the different environments, genetic backgrounds and different experimental materials (Holland 2007). A QTL detected in multiple environments is a relatively stable QTL and is important for plant breeding (Collins et al. 2008). It has become widely accepted that QTL confirmation/validation and/or fine (high resolution) mapping may be required (Langridge et al. 2001) in different environments and/or genetic backgrounds before being used for marker assisted breeding. One approach for validating QTLs is to map QTLs in different generations (Guan et al. 2011; Su et al. 2010; Wang et al. 2016; Wickneswari et al. 2012). Another approach for validating QTLs is to analyze the similarity of QTLs which closely linked with genetic markers in different genetic populations by developing multiple mapping populations (Gelli et al. 2017, Haussmann et al. 2002, Reddy et al. 2014). With the development of rapid sequencing technologies, the strategy of genome-wide association study (GWAS) is widely used in many species. On the other hand, linkage mapping analysis generates low false positive rates of QTLs (Ding et al. 2015a; Jiang and Zeng 1995). Combining GWAS and linkage mapping could exploit the complementary strengths of both approaches to identify casual loci (Fulker et al. 1999; Motte et al. 2014; Pedergnana et al. 2014).

Great developments have obtained from studies on QTL mapping of PH and genetic identification of dwarf mutants in maize. More than 54 maize dwarf genes for PH have been identified according to MaizeGDB database (http://www.maizegdb.org) and more than 40 maize dwarf genes have been cloned so far. Many of the cloned dwarf genes were reported to be involved in different biosynthesis pathways i.e. BR (brassinosteroid) biosynthetic pathways, gibberellins (GA) biosynthesis pathways and auxin biosynthesis pathways (Fujioka et al. 1988; Hartwig et al. 2011; Lawit et al. 2010; Multani et al. 2003; Peiffer et al. 2014; Spray et al. 1996; Winkler and Freeling 1994; Winkler and Helentjaris 1995). However, most of maize dwarf mutants have less potential applications in maize breeding because of their harmful impacts on grain yield (East 1908). An alternative strategy is to identify moderate alleles (QTLs) reducing plant height, which may be feasible and effective (Li et al. 2017). Xing et al. cloned a major plant height QTL-*qph1*, which contains a naturally occurring rare SNP in *br2. qph1* reduced plant height and ear height with no or very little negative impact on yield when heterozygous (Xing et al. 2015). *ZmGA3ox2* was reported to be a candidate gene for a major QTL-*qPH3.1*, which modify total maize plant height without influence on grain yield and yield related traits (Teng et al. 2013). The purposes of this study were to map QTLs for PH in the $F_{2:3}$ population; validation of the identified PH QTLs with QTLs reported in recent studies and to predict candidate genes for the detected QTLs with comparative genomics strategy by using maize gene annotations.

Materials and methods

Plant materials and phenotyping of plant height

F_1 hybrid seeds were obtained from an intraspecific cross between maize inbred lines SG5 and SG7 in 2013 summer in Liupanshui, Guizhou. A total 199 F_2 plants grew up from F_1 seeds in November 2014 at the Panxian Maize Breeding Station in Sanya, Hainan Island of China. The segregating population of 199 $F_{2:3}$ lines along with P_1, P_2 and F_1 were tested in a randomized block field experiment with three replications, single-row plot with 15 plants, row spacing was 50cm, and plant spacing was 35cm in November 2016 at the Panxian Maize Breeding Station in Sanya, Hainan Island of China. PH of 10 single plants in the middle of each plot were investigated at the period of maturity in the field.

High density linkage map and QTL analysis

Methods of genomic DNA extraction, genotyping by sequencing, sequence data grouping and SNP identification and high density bin map construction were exhibited in our previous study (Su et al. 2017). QTL analysis was performed using least absolute

shrinkage and selection operator (LASSO) method implemented with the GLMNET/R software package (Friedman et al. 2016). For the LASSO method, the p=0.05 was used as the threshold of the p-value, which translates into –log10(p) = 1.3 in this scale. The reason for not using permutation test for the LASSO method is that it is a multiple regression model with severe shrinkage on each marker effect. The nominal level of 0.05 applies to multiple regression analysis (Hu et al. 2012). Significant markers closely linked with the QTLs detected in this study were compared with physical positions or intervals of the QTLs detected for PH in earlier study. QTLs with overlapped intervals, same or similar physical positions will be considered as validated QTLs.

Candidate gene analysis

The +50 kb physical intervals from the significant markers for PH were considered for the search of candidate genes. The 100kb physical intervals of each significant marker were determined on the Zea mays genome (AGPv3.29; ftp://ftp.ensemblgenomes.org/pub/plants/release-29/fasta/zea_mays/dna/Zea_mays.AGPv3.29.dna.toplevel.fa.gz). Genomic positions of the identified QTLs were according to the maize gene annotation database accessible at MaizeGDB (http://www.maizegdb.org)

Results

Evaluation of phenotypic data

Phenotyping data were collected for plant height (PH) in 2016 for the $F_{2:3}$ mapping population (SG5 ×SG7). Significant difference for PH trait was observed within the $F_{2:3}$ population and between the parental genotypes (Supplementary Table 2). The statistical analysis indicated that the frequency distribution of PH among $F_{2:3}$ lines performed as a mixed distribution with transgressive segregations (range, 170.3-280.2cm; mean, 234.0 cm; P1, 197cm; P2, 213cm, standard deviation: 18.08cm). PH trait displayed as bell shaped normal distribution according to observed data (Fig. 1). Significant phenotypic and genetic variations among the lines were found. Therefore, the data is suitable for QTL mapping.

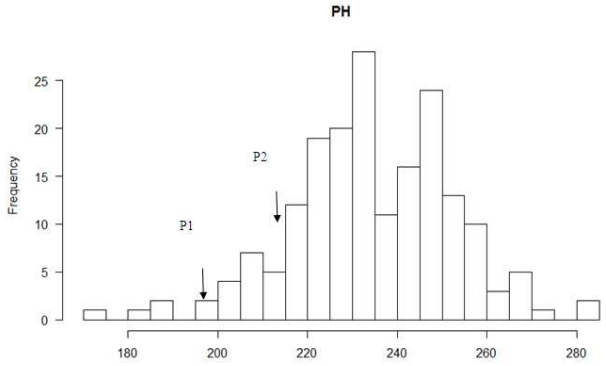

Fig. 1. Distribution of the PH in the $F_{2:3}$ population derived from the cross SG5 X SG7. PH represents plant height. P_1 and P_2 indicate SG5 and SG7, respectively

Genotyping, linkage map construction and QTL analyses

GBS technology was used for maize genome-wide detection of SNPs, the restriction enzyme Mse I and Hae III were used to digest genomic DNA and construct GBS libraries of the F_2 lines and the parents of the intra-specific mapping population (SG5 and SG7). The 144-mer short reads of parents and F_2 individuals were aligned with the Zea_mays. AGPv3.29 sequence to retrieve the physical position of each SNP. The SNPs were found to be distributed across all 10 maize chromosomes. A total of 133,936 polymorphic SNPs between the two parental lines were identified by low-coverage sequencing. The maize genome annotation project database (ftp://ftp. ensembl-genomes.org/pub/plants/release-29/fasta/zea_mays/dna/Zea_mays.AGPv3.29.dna.toplevel. fa.gz) was used to delineate the location of the GBS derived 68,882 SNPs in the genomic regions. After several filter steps, subsequently, a total of 29,927 SNPs were used to infer bins. The recombination maps were divided into skeleton bins (Huang et al. 2009) for further genetic analysis. A total of 3,305 bins were formed as described in our earlier study (S. A high density genetic map was constructed by mapping these 3,305 bin markers onto the 10 maize chromosomes. The total length of the linkage map is 2236.66 cM with LG2 (382.80 cM) being the largest and LG10 (139.51 cM) being the smallest. The average distance between two adjacent markers is 0.68 cM. The number of markers per linkage group varies from 120 (LG10) to 623 (LG1), with an average of 330.5 markers per linkage group. The average marker density with LG1 having the highest marker density (0.497 cM per interval) and LG2 having the lowest density (1.190 cM per interval) (Su et al. 2017).

Using the 3,305 bin-markers mapped on the intra-specific linkage map (Fig. 2), we performed QTL mapping for the PH using the LASSO method. Manhattan plot of the result is shown in Figs. 3a,b for

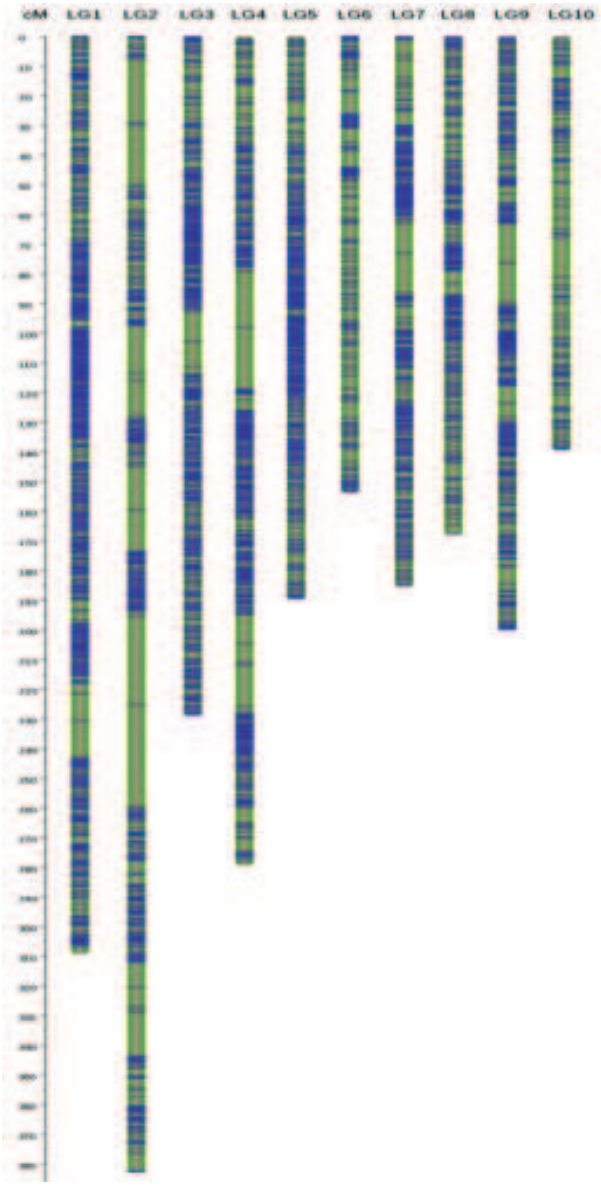

Fig. 2. Intra-species genetic linkage map of maize constructed using the F_2 population derived from the cross of SG7 and SG5

the additive effect test and dominance effect test, respectively. A total of 32 QTLs for the PH trait which distributed over all 10 maize chromosomes were identified: fifteen of them for additive and seventeen for dominance. The –Log10(p)-value ranges from 1.45 (*qPH*-30) to 10.06 (*qPH*-16). Information of the identified QTLs is summarized in Table 1. The additive effect of *qPH-4* is 11.06, which is the highest additive effect among all 15 additive QTLs while *qPH-6* is the smallest with additive effect 1.19. The dominance effect of *qPH-20* is 6.91, which is the highest dominance effect among all 17 dominance QTLs while *qPH-28* is the smallest with dominance effect 0.97.

Validation of QTLs across mapping populations

Based on physical positions of markers linked closely with the target PH trait, QTLs identified in this study were compared with those of earlier reported QTLs on GWAS or linkage mapping studies. Among the 32 QTLs identified in this study, twelve major QTLs were overlapped with the chromosomal regions carrying the QTLs detected in other studies, including Yang et al. (2014), Zhou et al. (2016) and Berke & Rocheford, (1995) (Table 1). These validated QTLs were located on chromosomes 1, 2, 3, 4, 5, 7 and 9. The QTLs that were identified in different genetic background or mapping populations are likely most reliable and stable QTLs across germplasm. These validated QTLs include: QTLs *qPH*-11 and *qPH*-27 on chromosome 1, *qPH-1, qPH-15, qPH*-20 and *qPH*-22 on chromosome 2, *qPH*-19 on chromosome 3, *qPH*-21 on chromosome 4, *qPH-2 on* chromosome 5, *q*PH-13 on chromosome 7, *qPH*-23 on chromosome 9 and *qPH*-24 on chromosome 10 (Fig. 4). The detailed information of overlapped QTLs with earlier study were listed in Table 1. Of these twelve validated QTLs, additive effect phenotypic variation ranged from 4.34cm to 8.48cm while dominance effect phenotypic variation ranged from 3.25cm to 6.91cm.

Candidate genes analysis for PH

In order to identify candidate genes for PH, we considered the +50 kb from the significant markers intervals for the search of candidate genes. The full list of differentially expressed genes (DEGs) included in the 100kb limited physical intervals to PH are reported in Additional file 1: Supplemental Table 1. Genes underlying these 32 mapped QTLs (+50 kb from the significant markers) were analyzed.100kb physical intervals of ten QTLs (*qPH*-11, qPH-20, *qPH*-3, *qPH*-19, *qPH*-12, *qPH*-30, *qPH*-18, *qPH*-13, *qPH*-25, *qPH*-8) contain less protein coding genes, of which four QTLs *qPH*-11, qPH-20, *qPH*-13 and *qPH*-19 were stable QTLs based on above analysis, according to the maize gene annotation database accessible at MaizeGDB (http://www.maizegdb.org). Recent work have shown that the bZIP transcription factor associated strongly with PH trait was found most promising in chickpea (Kujur et al. 2016). GRMZM2G002075 and GRMZM2G027976 genes in *qPH*-20 and *qPH*-3 respectively, both encode the BZIP transcription factor protein. Wang et al. (2017) reported a cytochrome P450 gene is a putative candidate for plant architecture in cucumber, which was the first

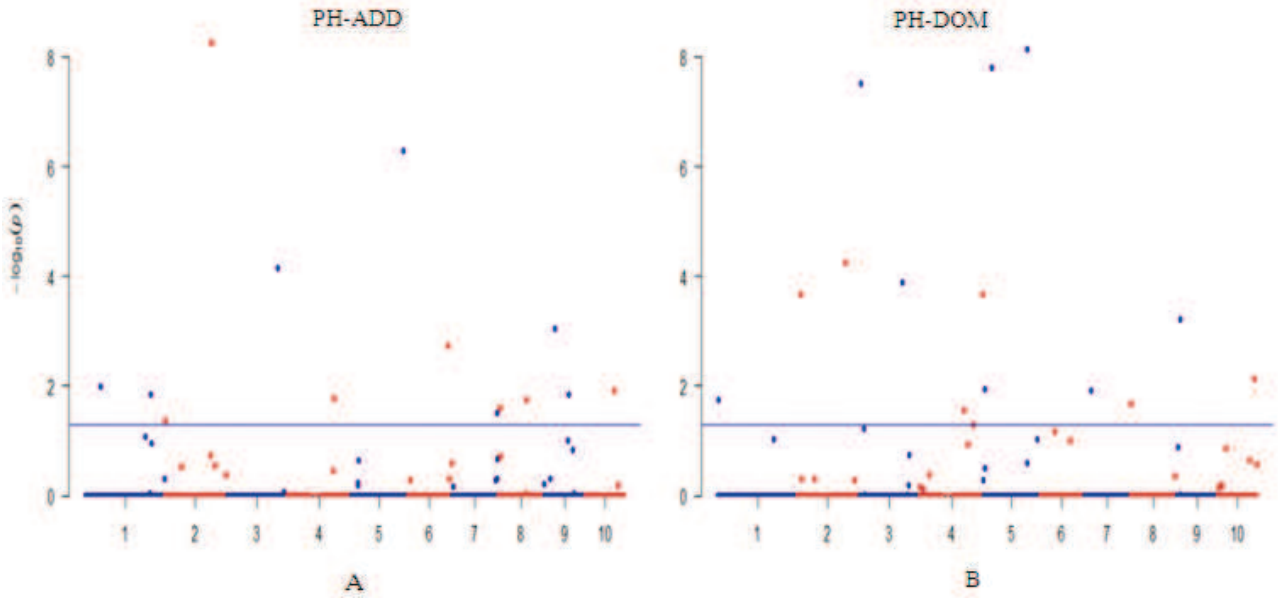

Fig. 3. Plots of the additive effect (a) and dominance effect (b) test statistic –Log10(p) against genome location for PH of maize using the LASSO method. The horizontal blue line is the critical value of the test statistic at the nominal level of –Log10(0.05) = 1.3. Note that LASSO is a multiple marker model and no adjustment for the critical value of test statistic is required

map-based cloning of a plant height gene in cucurbit crops (Wang et al. 2017). GRMZM2G171118 in *qPH*-11 encodes the Cytochrome P450 CYP71W7 gene and GRMZM2G106468 in *qPH*-8 encodes the Putative cytochrome P450 superfamily protein. Concurrent with the bioinformatic identification of DUF266 as putative Glycosyltransferases (GTs), the rice *brittle culm 10* mutant (*Osbc10*) was characterized. The *Osbc10* mutant displayed significant decrease in plant height and tiller number (Zhou et al. 2009). GRMZM2G176576 in *qPH*-19 encodes the Glycosyltransferase gene. Atwood et al. 2014 reported that silencing of a replication protein A (RPA) gene will decreases internode length and plant height in soybean (Atwood et al. 2014). GRMZM2G115013 in *qPH*-12 encoding the replication protein A 1A. Sosso et al. 2012 presented evidence Pentatricopeptide repeat (PPR-like) mutants ppr2263 grow slower than wild-type siblings, exhibit reduced plant height in maize(Sosso et al. 2012). AC205735.3_FG006 in *qPH*-30 encoding the Pentatricopeptide repeat (PPR-like) superfamily protein. Banerjee et al. 2010 reported Germin-like protein could explain its functional role in regulation of plant height and disease resistance in rice plant (Banerjee and Maiti 2010). GRMZM2G115491 in *qPH*-18 encodes the Germin-like protein. Chen et al. 2015 proposed OsNAC2 encoding a NAC transcription factor that affects plant height through mediating the gibberellic acid pathway in rice (Chen et al. 2015). GRMZM2G181605 in *qPH*-13 encodes the NAC transcription factor. The results of Ding et al. 2015 showed that DNL1, encodes cellulose synthase-like D4, is a major QTL for plant height and leaf width in rice (Ding et al. 2015b). GRMZM2G177631 in *qPH*-25 encodes the Cellulose synthase-8.

Discussion

Stable or reliable QTLs are useful for marker assisted selection (MAS) while false positive QTLs are no use (Su et al. 2010). QTLs could be validated by analyzing reproducibility of the target QTL in different generations of same population or in different populations. The detected QTLs in one mapping population may not be the same with those detected in other populations. Validation of QTLs and associated candidate genes across mapping populations is critical for finding stable QTLs and common genes to target for improved PH of crop plants through molecular breeding approaches or gene editing approaches by using CRISPR/Cas9 genome editing tool. The accuracy of QTL mapping relies to some extent on the density of the genetic maps and then requires high numbers of genetic markers. Single-nucleotide polymorphisms (SNPs), which are suitable for high-throughput genotyping

Table 1. QTL identified for PH trait of maize using high-density SNP bin-map from the LASSO method

QTL	Chr[a]	Significant markers[b]	Positions(bp)		Add/ Dom[c]	Effect (cm)	–Log 10(p)	QTL-MI[d]	References
			Start	End					
qPH-1	2	mk818	179707083	179761787	A	–8.48	8.75	177729785	Yang et al (2014)
qPH-2	5	mk2129	199655167	199788188	A	4.34	7.37	175.20–207.50	Zhou et al (2016)
qPH-3	3	mk1297	196261948	196378288	A	4.78	5.00		
qPH-4	9	mk2973	40797767	41052145	A	11.06	3.31		
qPH-5	6	mk2301	152009546	154444909	A	7.39	2.98		
qPH-6	1	mk125	58055198	58578972	A	1.19	2.33		
qPH-7	10	mk3238	109854244	110734370	A	–5.88	2.19		
qPH-8	8	mk2655	7485275	7742393	A	5.37	2.12		
qPH-9	8	mk2761	108981257	109227283	A	3.84	2.05		
qPH-10	9	mk3047	93102400	93881124	A	10.73	1.98		
qPH-11	1	mk473	247148375	248469066	A	–7.16	1.72	250093228	Yang et al (2014)
qPH-12	4	mk1621	177089569	177284993	A	5.70	1.64		
qPH-13	7	mk2616	173650074	173825465	A	–6.23	1.64	168684039	Yang et al (2014)
qPH-14	8	mk2658	8212170	8411837	A	5.57	1.55		
qPH-15	2	mk630	2344967	2496410	A	–5.64	1.54	2629759	Yang et al (2014)
qPH-16	5	mk1911	34609122	34770001	D	3.43	10.06		
qPH-17	3	mk988	7025155	7061793	D	–5.36	9.19		
qPH-18	5	mk2070	168741773	169867694	D	–1.98	9.03		
qPH-19	3	mk1221	166431281	166824490	D	–5.10	5.18	165020886	Yang et al (2014)
qPH-20	2	mk828	186815405	187388746	D	6.91	4.79	186893688	Yang et al (2014)
qPH-21	4	mk1803	239271583	239542220	D	–5.18	4.17	239686959	Yang et al (2014)
qPH-22	2	mk669	15019804	15597564	D	–4.32	3.97	15581220	Yang et al (2014)
qPH-23	9	mk2929	13633632	13989100	D	4.30	3.97	11499461	Yang et al (2014)
qPH-24	10	mk3267	139949935	140342000	D	4.22	2.51	137.3-142.8	Berke, & Rocheford, (1995)
qPH-25	7	mk2381	26219166	26760153	D	–4.02	2.34		
qPH-26	5	mk1840	5977671	6113458	D	–6.65	2.14		
qPH-27	1	mk7	2927187	3155254	D	3.25	2.11	2941675	Yang et al (2014)
qPH-28	3	mk1020	20132946	20461320	D	0.97	2.03		
qPH-29	8	mk2637	2659454	2773805	D	3.93	1.85		
qPH-30	4	mk1601	168385687	168497509	D	–4.21	1.72		
qPH-31	6	mk2237	56840585	58444372	D	3.48	1.50		
qPH-32	4	mk1707	205404410	205629373	D	4.55	1.45		

[a]Chr. = chromosome; [b]significant markers indicate bin-markers closely associated with QTLs detedted; [c]ADD = additive effect; DOM = dominance effect; [d]QTL-MI = positions (Mb) or marker intervals (bp) of QTLs identified in previous studies, indicate whether the QTL is overlapped with the QTL identified in previous studies

methods, turn out to be markers of choice to extensively map large sets of individuals (Simon et al. 2008). The usefulness of genetic maps largely depends on their density (Harushima et al. 1998): a high-density linkage map will promote high-resolution genetic mapping and positional cloning of crucial genes and can also benefit physical map assembly. In our previous study, a high density genetic map was

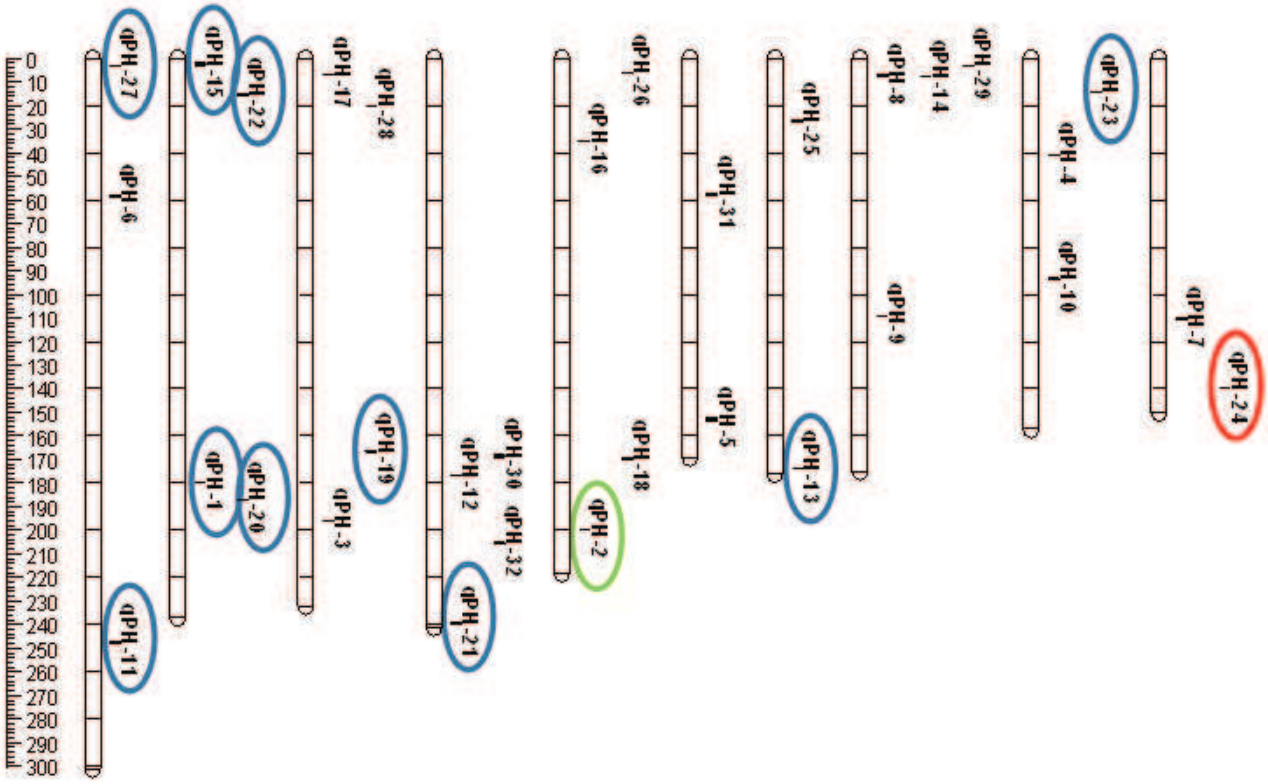

Fig. 4. QTL locations for plant height trait studied in the SG5/SG7 $F_{2:3}$ population. QTLs represented by bars are shown on the right of the linkage groups, close to their corresponding markers. Significant bin-marker intervals for each QTL are indicated by the length of vertical bars. The ten QTLs circled in blue were stably detected at same or similar physical location in this study and study of Yang et al (2014). The one QTL circled each in green and red were detected at same or similar physical location with study of Zhou et al. (2016) and Berke, & Rocheford (1995), respectively

generated by mapping 3,305 bin markers which consist of a total 29,927 filtered SNPs onto the 10 maize chromosomes. The length of bin markers ranged from 50 Kb to 21.65 Mb, 71.5% of the bin markers are less than 0.6 Mb in length. The average distance between two adjacent bin-markers is 0.68 cM, corresponding to a physical distance of about 0.69 Mb. We have shown that the identified QTLs can be narrowed down to relative small physical intervals of the target genome (Su et al. 2017).

Since Ewards et al. (1987) first successfully mapped maize PH QTLs, to date, using different populations, more than 219 QTLs for maize PH have been identified on all 10 maize chromosomes and most of these QTLs are located on chromosome 1 and 3 (Gramene QTL database). Previous QTL mapping results were different from different researchers. Recently, Zhou et al. (2016) identified 14 QTLs distributed on chromosomes 1, 2, 4, 5, and 10 for maize PH trait on the basis of a high density linkage map from a set of 314 RILs derived from inbreds Ye478 and Qi319(Zhou et al. 2016). Another GWAS of maize was carried out by Yang et al. (2014) for 17 agronomic traits with a panel of 513 maize inbred lines. A total of 343 significant loci were detected for the 17 traits included PH (Yang *et al.* 2014). Compared with these results, the majority of QTL identified in this study (*qPH*-1, *qPH*-2, *qPH*-11, *qPH*-13, *qPH*-15, *qPH*-19, *qPH*-20, *qPH*-21, *qPH*-22, *qPH*-23, *qPH*-24 and *qPH*-27) are either overlapping with the QTLs detected by Zhou et al. (2016) and Yang et al. (2014) or in the vicinity of those QTLs (see Tables 1).

Most of cloned dwarf genes for maize PH were reported to be involved in different biosynthesis pathways i.e. BR (brassinosteroid) biosynthetic pathways, Gibberellins (GA) biosynthesis pathways and Auxin biosynthesis pathways (Fujioka et al. 1988, Hartwig et al. 2011, Lawit et al. 2010, Multani et al.

2003, Peiffer et al. 2014, Spray et al. 1996, Winkler and Freeling 1994, Winkler and Helentjaris 1995) on the basis of maize dwarf mutant. These cloned dwarf genes have less benefit for maize breeding because of their harmful impacts on grain yield (East 1908). PH is critical for improving plant density to achieve suitable planting density and reducing plant height is one of the main strategies to avoid lodging in maize breeding (Ji-hua et al. 2007). It is of great significance to identify moderate alleles (QTLs) reducing plant height and with no or very little negative impact on yield related traits.

In this study, comparative genomics strategy (Zhu and Zhao 2007) was conducted to identify and characterize the effect of putative candidates. Candidate genes may be functionally conserved or structurally homologous genes identified from other related species. Comparative genomics strategy can rapidly work if functionally conserved or structurally homologous genes affecting phenotypic variation of interest have already been confirmed in other species. 10 candidate genes underlying ten QTLs (*qPH*-11, *qPH*-20, *qPH*-3, *qPH*-19, *qPH*-12, *qPH*-30, *qPH*-18, *qPH*-13, *qPH*-25, and *qPH*-8) were obtained from comparing genetic studies on other species. In particular, *qPH*-11, *qPH*-13, *qPH*-20 and *qPH*-19 among ten QTLs were overlapped with QTLs detected in previous GWAS study for PH in maize (Yang et al. 2014). The results obtained in this study not only provide basis for further clonging and functional verification these genes, but also paved the way for identifying moderate alleles (QTLs) reducing plant height and without negative impact on yield related traits to achieve suitable planting density in maize.

Acknowledgments

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