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ORIGINAL PAPER

Mapping QTL controlling maize deep-seeding tolerance-related traits and confirmation of a major QTL for mesocotyl length

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Abstract Deep-seeding tolerant seeds can emerge from deep soil where the moisture is suitable for seed germination. Breeding deep-seeding tolerant cultivars is becoming increasingly important in arid and semi-arid regions. To dissect the quantitative trait loci (QTL) controlling deep-seeding tolerance traits, we selected a tolerant maize inbred line 3681-4 and crossed it with the elite inbred line-X178 to generate an F_2 population and the derivative $F_{2:3}$ families. A molecular linkage map composed of 179 molecular markers was constructed, and 25 QTL were detected including 10 QTL for sowing at 10 cm depth and 15 QTL for sowing at 20 cm depth. The QTL analysis results confirmed that deep-seeding tolerance was mainly caused by mesocotyl elongation and also revealed considerable overlap among QTL for different traits. To confirm a major QTL on chromosome 10 for mesocotyl length measured at 20 cm depth, we selected and self-pollinated a BC_3F_2 plant that was heterozygous at the markers around the target QTL and homozygous at other QTL to generate a BC_3F_3 population. We found that this QTL explained more phenotypic variance in the BC_3F_3 population than that in the F_2 population, which laid the foundation for fine mapping and NIL (near-isogenic line) construction.

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Introduction

In low precipitation areas, it is necessary to sow seeds deep to reach the moist soil required for better seedling emergence. However, when sown deep, the juvenile plant needs to elongate its organs to push the plumule to the soil surface. The elongating organs differ among various crops, rice and oat elongate both the mesocotyl and the first internode (Hoshikaw 1969; Turner et al. 1982), barley and wheat elongate the coleoptile and the first internode (Schillinger et al. 1998; Takahashi et al. 2001; Takeda and Takahashi 1999), maize and sorghum elongate their

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mesocotyl to push the shoots to the surface (Dungan 1950; Hoshikaw 1969).

In low precipitation areas of the inland Pacific Northwest, the outdated winter wheat cultivar Moro, which showed poor resistance to disease and modest grain yield potential, was still widely sown due to its excellent emergence from deep sowing conditions in dry soil (Schillinger et al. 1998). In the Loess Plateau of China, local wheat variety Hong Mang Mai was usually sown 10 cm below the surface to achieve normal emergence (Takeda et al. 1995). In the arid southwestern regions of the US and western Mexico, local Native Americans planted corn under approximately 30 cm depth to reach adequate soil moisture for germination (Troyer 1997). Polthanee (2001) demonstrated that although deep sowing of peanuts resulted in poor root development and lower leaf area index at the early growth stage, deep sowing not only increased root density, leaf area index and total dry weight at 60 and 90 days after seeding, but also raised seed yield and 100-seed weight. Rebetzke et al. (2005) found that deep-seeding tolerant wheat varieties Halberd and HM14bS were less affected by increasing stubble mass and produced higher seedling biomass and more tillers per plant in a non-tillage field. Brown et al. (2003) suggested that wheat and field peas should be sown deeper to decrease the damage done by mice in southern Australia. Deeper sowing could also diminish the negative effects of parasitic weed (Van Delft et al. 2000).

Deep-seeding tolerant crops need to be bred specifically for arid and semi-arid regions, but the genetic analysis of deep-seeding tolerance traits is still very limited in most crops. Suge et al. (1998) found that the deep-seeding tolerance of wheat was closely related to coleoptile length. Rebetzke et al. (2007b) reported that coleoptile length was linearly correlated with the presence of the Rht-B1b and Rht-D1b alleles. Spielmeyer et al. (2007) found two QTL on wheat chromosomes 6AS and 3AS controlled coleoptile length. In barley, Takeda and Takahashi (1999) found that deep-seeding tolerance was closely related to coleoptile length and the first internode length. Takahashi et al. (2001) mapped the QTL for deep seedling emergence, coleoptile length and the first internode length on chromosomes 1H, 5H and 7H. In maize, Troyer (1997) and Zhao et al. (2009) found that deep-seeding tolerance was associated with mesocotyl elongation. Troyer (1997) found three regions on chromosomes 3, 6 and 9 that controlled the mesocotyl length using translocation tester stocks. However, QTL analysis has not been performed for deep-seeding tolerance traits in maize.

Seedling vigor refers to the ability and potential of seeds to develop rapid and uniform emergence (AOSA 1983; Perry 1987). Plants with greater seedling vigor grow to the autotrophic growth stage earlier, cover the ground rapidly and minimize water loss. Maize cultivars that showed early seedling vigor could not only enhance crop establishment in central Europe and northern Mediterranean areas, where low temperature retarded seedling growth, but they could also ensure uniform seedling establishment (Hund et al. 2004). Several traits were used to measure seedling vigor in rice and maize, including seed germination rate, seedling shoot length, seedling fresh weight, seedling dry weight, root length, root dry weight, dry matter weight, etc. (Asghar and Khan, 2005; Cervantes-Ortiz et al. 2007; Cui et al. 2002; Han et al. 2007; Molatudi and Mariga 2009; Redonna and Mackill 1996; Zhang et al. 2005a, b; Zhou et al. 2007). Moreover, many of these traits were related to each other (Zhang et al. 2005a, b; Mehdi and Ahsan 2000). There were many genetic researches on rice seedling vigor, but only a few related articles relating to the genetics of maize seedling vigor (Hund et al. 2004; Presterl et al. 2007; Qiu et al. 2007). Generally, the seedling vigor related traits were evaluated under low temperature and were seldom analyzed under deep-seeding conditions, although the need to breed cultivars that emerged fast under deep-seeding conditions was becoming imperative (Schillinger et al. 1998).

The development of molecular markers provided convenient tools for genetic analysis of maize deep-seeding tolerance. Many QTL analyses have been previously reported in maize, which facilitated the construction of substitution lines by continual backcrossing and marker-assisted selection (MAS). The selected lines provide invaluable materials for further dissecting QTL and isolating the candidate genes. Several QTL have been isolated by map-based cloning in maize including Tga1 (Wang et al. 2005), DGAT1 (Zheng et al. 2008) and Vgt1 (Salvi et al. 2007). The Maize Genome Sequencing project (http://www.maizegdb.org) has generated a large amount of data that are convenient for the mapping process, thus paving the way for QTL fine mapping and map-based cloning.

To determine the genetic basis of deep-seeding tolerance in maize, we conducted QTL analysis based on an F_2 population and the $F_{2:3}$ families. Through continual backcrossing and MAS, we produced a BC_3F_3 population segregating at the genomic region around a major QTL on chromosome 10, and used this population to confirm the existence of the major QTL. The detected loci for deep-seeding traits in our research might be useful for breeding cultivars tolerant to deep sowing.

Materials and methods

Population construction

The parents of the mapping population were selected based on a previous report that, among 12 maize inbred lines,

inbred line 3681-4 had the largest emergence rate and the longest mesocotyl, and X178 had the shortest meosocotyl at 25 cm depth (Zhao et al. 2009). The deep-seeding tolerant parent, 3681-4, was developed from Hopi Indian corn germplasm (Zhao and Wang 2008) and had the highest emergence rate and longest mesocotyl. The intolerance parent, X178, had the shortest mesocotyl and was a parent of an elite hybrid Nongda 108, which was a major cultivar from 2001 to 2004 in China. The F_1 plant derived by crossing maize inbred lines 3681-4 with X178 was self-pollinated at the Sanya experimental station of China Agricultural University in Hainan province, China. The 221 unselected F_2 plants and the 2 parents were sown by hand in the Dongbeiwang experimental station of China Agricultural University. The rows were 5 m long with 0.67 m space between rows. Standard cultivation management practices were used. All F_2 plants were self-pollinated to produce 221 $F_{2:3}$ families. The $F_{2:3}$ seeds harvested from each F_2 plant were dried and used for measuring deep-seeding related traits in the culture room, where the temperature was 25°C.

We obtained BC_1F_1, BC_2F_1 and BC_3F_1 seeds by continual backcrossing with X178. These backcrossed progenies were sown at depths of 20–30 cm to ensure that a higher proportion of the seeds retained the positive QTL. We performed MAS for 170 BC_3F_2 plants and selected a BC_3F_2 plant (Suppl. Fig. 2), which was then selfed to get one BC_3F_3 population. The $BC_3F_{3:4}$ seeds were harvested from individual BC_3F_3 plants and were used to measure mesocotyl length at 20 cm depth. The phenotypic and genotypic data for the BC_3F_3 population were used to confirm the existence of the QTL on chromosome 10 (qMES20-10).

Measurement of deep-seeding traits

We first studied the response of the two parents (X178 and 3681-4) to different sowing depths ranging from 2 to 20 cm. For every 1 kg dry sand screened with a sieve (1.2-mm sieve pore), 44.8 ml tap water was added and manually mixed with the sand. PVC tubes (height: 50 cm, diameter: 20 cm) were loaded with the evenly mixed sand to a depth of 15 cm, and 30 seeds were then sown evenly onto the surface. After this, mixed sand was added to the tubes to reach 2, 4, 8, 10, 12, 16 and 20 cm depth, respectively. The PVC tubes were masked with one layer of black plastic and one layer of black cloth to keep dark environment for seed germination and they were kept in a culture room at 25°C (Flint 1944). The seedling traits including mesocotyl length, coleoptile length and seedling length were measured after 14 days.

To measure the phenotypes of the $F_{2:3}$ families sown at 20 cm depth, the PVC tubes were first loaded with 15 cm mixed sand (as stated above), and 30 seeds for each $F_{2:3}$ family were sown evenly onto the surface, afterward, another 20 cm of mixed sand was used to cover the seeds. Two families were sown in each PVC tube, which was divided into two parts by a wooden sheet and masked with one layer of black plastic and one layer of black cloth. After keeping at 25°C for 14 days, we first counted the number of seedlings above the surface, and then took the seedlings out to measure the length of the mesocotyl, coleoptile and seedling length. The emergence rate on the 14th day was calculated as (the number of emerged seedlings on the 14th day/the number of seedlings that had visible root or shoot on the 14th day) × 100. The mean values of the three seedling traits for each of the 221 $F_{2:3}$ families were calculated to represent the phenotypes of the F_2 plant. The measurements of these four traits were replicated twice for the 221 $F_{2:3}$ families. The $BC_3F_{3:4}$ families used to confirm qMES20-10 were sown in the same way as the $F_{2:3}$ families, and only the mesocotyl length was measured.

For the phenotype evaluation at 10 cm depth, 5 cm of mixed sand was added to the bottom of a box (width: 40 cm, length: 60 cm, height: 16 cm), and after sowing 30 seeds of each $F_{2:3}$ family, another 10 cm of mixed sand was added to cover the seeds, 15 families were sown in each box, which was then masked with one layer of black plastic and one layer of black cloth and kept in a culture room at 25°C. The deep-seeding tolerant parent 3681-4 had 100% seedling emergence rate on the 5th day if sown at 10 cm depth and X178 did not show any seeding emergence until the 7th day, so we measured the emergence rate on the 5th day for the $F_{2:3}$ families. On the 12th day, the seedlings of the 221 $F_{2:3}$ families were taken out to measure the length of mesocotyl, coleoptile and seedling length. The emergence rate was calculated as (the number of emerged seedlings on the 5th day/the number of seedlings that had visible root or shoot on the 12th day) × 100.

We referred to the four traits sown at 20 cm depth as MES20, COL20, SDL20 and RAT20 for mesocotyl length, coleoptile length, seedling length and emergence rate on the 14th day. The four traits for seedlings at 10 cm depth were referred to as MES10, COL10, SDL10 and RAT10 for mesocotyl length, coleoptile length, seedling length on the 12th day, and emergence rate on the 5th day.

The mean, standard error, skewness, kurtosis, correlation coefficient and heritability were computed using SAS (SAS 2000). The broad sense heritability was calculated according to Holland et al. (2003).

DNA extraction and SSR analysis

Leaf samples were collected from each F2 and BC3F3 plant and the two parents at the seedling stage and stored at

−80°C. DNA was extracted using a CTAB method. SSR analysis was conducted as described by Senior and Heun (1993).

Construction of a molecular linkage map and QTL analysis

A total of 580 SSR primer pairs downloaded from MaizeGDB (http://www.maizegdb.org) and 66 IDP primer pairs (Fu et al. 2006) were initially used to screen for polymorphisms between the X178 and 3681-4. 179 co-dominant markers including 158 SSR and 21 IDP amplified polymorphic bands between the parents, and were used to survey the 221 F_2 plants. The crude marker order was determined by the QTL IciMapping version 3.0 software (Li et al. 2008) with the distance threshold set at 40 cM. The crude marker order was adjusted using MAPMAKER/EXP3.0 (Lincoln et al. 1992) and the ultimate genetic map was constructed with GGT 2.0 software (VanBerloo, 2008). A chi-squared test was applied to identify any distorted segregation of markers from the expected 1:2:1 ratio.

QTL analysis was carried out using Windows QTL Cartographer 2.5 (Wang et al. 2010). CIM was run using CIM model 6 of the program and the window size was set at 10 cM when analyzing the eight traits. Five markers were used for background control and these markers were detected through backward regression method. For each trait, the empirical threshold level for detecting QTL was determined by 1,000 permutations. The QTL denotation followed the rules suggested by McCouch et al. (1997), the QTL name was started with a lowercase "q", then the trait name in capital letters, followed by a dash and the chromosome number where the QTL was detected. If there were more than one QTL for the same trait on the same chromosome, another dash and number were added after the chromosome number to distinguish them. For the BC_3F_3 population, five SSR markers (umc1280, umc1506, bnlg1028, bnlg153 and bnlg2190) around the QTL on chromosome 10 were used to survey the population composed of 158 individuals. The genetic distance between each marker was calculated using MAPMAKER/EXP3.0 and this QTL was confirmed using CIM in Windows QTL Cartographer 2.5 (Wang et al. 2010).

Results

Variation and correlation of deep-seeding tolerance-related traits

Four traits were respectively measured at 10 and 20 cm depths. A summary of the phenotypic data was listed in Table 1, from which we can see three aspects. First, all eight traits followed normal distribution, as the absolute value of skewness and kurtosis of each of the eight traits were less than 1. This might indicate the traits were controlled by several QTL, each with a small effect. Second, the range of mesocotyl length and emergence rates at 10 and 20 cm depth showed minor transgressive segregation, whereas coleoptile and seedling length showed evident transgressive segregation. This might suggest that most QTL from the 3681-4 had positive effects on the MES10, RAT10, MES20, and RAT20 phenotypes. Third, the heritability of most of the traits seemed slightly high. The trait with the highest heritability was MES20 and the lowest was SDL10 (Table 1), which meant that MES20 was least affected by the environment compared to other traits.

RAT10 could be seen as an index of the speed of seedling emergence or seedling vigor under deep-seeding conditions. SDL10 and MES10 were significantly correlated with RAT10. The correlation coefficient was larger for SDL10 compared with MES10 (Table 2), which

Table 1 Deep-seeding tolerance-related traits of X178, 3681-4 and $F_{2:3}$ families and their broad-sense heritability

	Parents		$F_{2:3}$ families				
Traits	X178	3681-4	Range	Mean ± SE	Skewness	Kurtosis	Heritability
MES10 (cm)	5.90	16.61	10.38–16.89	13.80 ± 1.16	−0.08	−0.26	0.75
COL10 (cm)	4.92	5.54	3.79–5.75	4.71 ± 0.42	0.37	−0.49	0.77
SDL10 (cm)	25.37	29.42	16.43–31.91	23.36 ± 2.67	0.36	−0.20	0.56
RAT10	0	100	0–89.66	40.92 ± 22.42	0.08	−0.96	0.79
MES20 (cm)	6.28	22.27	10.39–21.50	15.54 ± 2.23	0.10	−0.57	0.90
COL20 (cm)	5.11	5.56	3.76–6.71	5.10 ± 0.52	0.04	−0.10	0.81
SDL20 (cm)	23.20	30.02	16.14–36.77	26.30 ± 3.10	0.42	0.64	0.79
RAT20	0	100	19.76–98.33	70.71 ± 16.63	−0.79	−0.01	0.61

MES10, COL10, SDL10 and RAT10 represent mesocotyl length, coleoptile length, seedling length on the 12th day and emergence rate on the 5th day at 10 cm depth. MES20, COL20, SDL20 and RAT20 represent mesocotyl length, coleoptile length, seedling length and emergence rate on the 14th day at 20 cm depth

Table 2 Phenotypic correlation coefficients of deep-seeding tolerance-related traits

	MES10	COL10	SDL10	RAT10	MES20	COL20	SDL20
MES10	1						
COL10	0.115	1					
SDL10	−0.022	0.107	1				
RAT10	0.281**	−0.024	0.451**	1			
MES20	0.791**				1		
COL20		0.608**			−0.206*	1	
SDL20			0.503**		0.073	0.361**	1
RAT20				0.487**	0.560**	0.079	0.401**

* Significant at the 0.01 level, ** Significant at the 0.001 level

MES10, COL10, SDL10 and RAT10 represent mesocotyl length, coleoptile length, seedling length on the 12th day and emergence rate on the 5th day at 10 cm depth. MES20, COL20, SDL20 and RAT20 represent mesocotyl length, coleoptile length, seedling length and emergence rate on the 14th day at 20 cm depth

corresponded to the view that seedling length was a trait related to seedling vigor (Asghar and Khan 2005; Cervantes-Ortiz et al. 2007; Cui et al. 2002; Han et al. 2007; Molatudi and Mariga 2009; Redonna and Mackill 1996; Zhang et al. 2005a, b; Zhou et al. 2007). RAT20, which directly measured deep-seeding tolerance, had the largest correlation coefficient with MES20 and the second largest with SDL20. This suggested that mesocotyl length was the main contributor to deep-seeding tolerance (Table 2). In addition, we found that the correlation between the corresponding traits under the two depth conditions was significant, the correlation coefficients between MES10 and MES20, COL10 and COL20, SDL10 and SDL20 were 0.791, 0.608, 0.503 respectively, indicating that the corresponding traits at different depths might be controlled by the same genetic factors.

Construction of a molecular linkage map

The chi-squared test showed that 28 markers on chromosomes 1, 2, 3, 4, 5, 6, 7 and 9 deviated from the expected ratio of 1:2:1 when the significance level was set at 0.05, and when the P-value was set at 0.01, only 11 markers on chromosomes 2, 3, 4, 5, 7 and 9 were significant. We included all of the markers in our linkage and QTL analysis. The 179 co-dominant SSR and IDP markers were used to construct 10 linkage groups when the distance threshold was set at 40 cM. The total length of the linkage map was 1,865.54 cM with an average interval of 10.42 cM (Fig. 1). All markers except umc1968 were located in the similar region of the MaizeGDB maps (http://www.maizegdb.org).

QTL analysis for deep-seeding tolerance-related traits

QTL were detected based on LOD thresholds after permutation tests. The LOD thresholds for MES10, COL10, SDL10, RAT10, MES20, COL20, SDL20 and RAT20 were 3.73, 3.91, 4.34, 3.82, 3.73, 3.87, 3.78 and 4.04, respectively. A total of 25 QTL, including ten QTL for traits at 10 cm depth and 15 QTL for traits at 20 cm depth, were detected on all chromosomes except for chromosome 5 and 9. These QTL explained 3.81–17.95% of the phenotypic variance observed (Fig. 1; Table 3). In total, six QTL clusters containing more than one QTL were identified based on whether the 1 LOD supporting interval overlapped or not, including two on chromosomes 1, and one on chromosomes 3, 4, 6 and 10.

10 cm depth

Ten QTL were detected for traits at 10 cm depth, including five for MES10, two for COL10, one for SDL10 and two for RAT10. Two QTL on chromosome 6 and 10 for MES10 and one QTL on chromosome 3 for RAT10 explained more than 10% of the phenotypic variance. Six of the ten QTL were found to be located in the QTL clusters mentioned above. It was interesting to note that all the QTL regions from the 3681-4 background had positive effects on the traits at 10 cm depth (Table 3).

Five QTL were detected for MES10 on chromosomes 1, 3, 6, 6 and 10, three of them lay in the QTL clusters among them. These QTL explained 5.27, 7.10, 8.48, 11.02 and 17.95% of the phenotypic variance, respectively, qMES10-10 had the largest LOD value, additive effect and phenotypic variance explained (PVE) (Table 3).

Two QTL were detected for COL10 and one for SDL10 on chromosomes 1, 2 and 4, respectively, and they explained 6.33, 9.01 and 9.35% of the phenotypic variance, qSDL10-4 for SDL10 was in the QTL cluster on chromosome 4 (Table 3).

The two QTL for RAT10 were in the QTL clusters on chromosomes 1 and 3, explaining 7.19 and 15.31% of the phenotypic variance, qRAT10-3 had the largest LOD value,

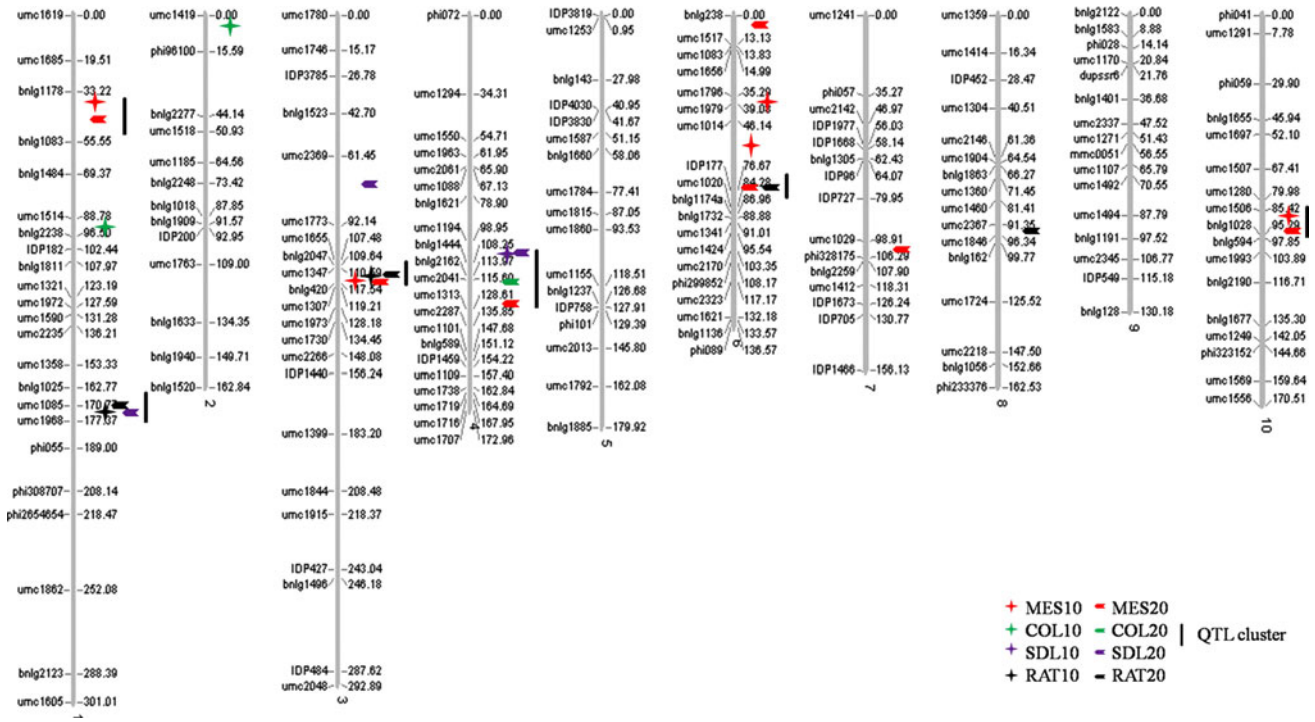


Fig. 1 Molecular linkage map based on the F_2 population and QTL detected for the eight traits at 10 cm and 20 cm depths. The number at the bottom of each linkage map correspond to the chromosome name of the maizegdb maps. MES10, COL10, SDL10 and RAT10 represent mesocotyl length, coleoptile length, seedling length on the 12th day and emergence rate on the 5th day at 10 cm depth. MES20, COL20, SDL20 and RAT20 represent mesocotyl length, coleoptile length, seedling length and emergence rate on the 14th day at 20 cm depth

additive effect and PVE. The collocation of qMES10-3 and qRAT10-3 indicated that mesocotyl length might be associated with seedling emergence at 10 cm depth. Unexpectedly, the only QTL for SDL10 was not in the same region with any QTL for RAT10, although the correlation between the two traits was significant (Tables 2, 3).

20 cm depth

Fifteen QTL were detected for the four traits at 20 cm sowing depth. These included seven QTL for MES20, one for COL20, three for SDL20 and four for RAT20. Among them, six QTL explained more than 10% of the phenotypic variance, including three for MES20, one for COL20, and two for RAT20 (Table 3).

Among the seven QTL for MES20, five lay in the QTL clusters on chromosomes 1, 3, 4, 6 and 10, and six QTL from the 3681-4 background increased mesocotyl length except for qMES20-4. Three QTL on chromosomes 3, 6 and 10, which had the largest LOD value and additive effect, explained more than 10% of the phenotypic variance and could be considered the major QTL according to the standard mentioned by Collard et al. (2005) (Table 3).

The QTL for COL20 was situated in the QTL cluster one chromosome 4 and explained16.01% of the phenotypic variance. The allele from 3681-4 background for qCOL20-4 increased coleoptile length (Table 3). Two of the three QTL for SDL20 were in the QTL clusters on chromosomes 1 and 4. The alleles from 3681-4 for all three QTL increased seedling length, qSDL20-4 had the largest LOD value, additive effect and PVE (Table 3).

Three of the four QTL for RAT20 were in the common QTL clusters, and these three QTL from the 3681-4 background were positive contributors for seedling emergence, qRAT20-3 had the largest LOD, additive effect and PVE. We found that, among the four QTL for RAT20, two QTL on chromosomes 3 and 6 overlapped with two QTL for MES20, and one QTL on chromosomes 1 overlapped with one QTL for SDL20. This comparison might indicate that MES20 and SDL20 were related to deep-seeding tolerance.

Confirmation of qMES20-10

After backcross and MAS using the 179 markers on the linkage map, one BC_3F_2 plant heterozygous at qMES20-10 and homozygous at all the other QTL controlling MES20 (Suppl. Fig. 2) was selected and selfed to form a BC_3F_3 population. This BC_3F_3 population was scanned using five markers surrounding the qMES20-10 locus and the mesocotyl length of the $BC_3F_{3:4}$ families were measured at 20 cm depth (Fig. 2). The distribution of mesocotyl length

Table 3 QTL detected based on deep-seeding tolerance-related traits in the $F_{2:3}$ families

Trait/threshold[a]	QTL[b]	Flanking markers	LOD	A[c]	PVE[d] (%)
MES10/3.73	qMES10-1	bnlg1178-bnlg1083	3.92	−0.41	5.27
	qMES10-3	umc1347-bnlg420	4.93	−0.53	7.10
	qMES10-6-1	umc1796-umc1979	5.18	−0.52	8.48
	qMES10-6-2	umc1014-IDP177	5.28	−0.62	11.02
	qMES10-10	umc1506-bnlg1028	11.57	−0.76	17.95
COL10/3.91	qCOL10-1	umc1514-bnlg2238	3.97	−0.20	6.33
	qCOL10-2	umc1419-phi96100	4.34	−0.11	9.01
SDL10/4.34	qSDL10-4	bnlg1444-bnlg2162	5.54	−1.35	9.35
RAT10/3.82	qRAT10-1	umc1085-umc1968	4.52	−9.73	7.19
	qRAT10-3	umc1347-bnlg420	9.32	−14.67	15.31
MES20/3.73	qMES20-1	bnlg1178-bnlg1083	4.75	−0.63	5.25
	qMES20-3	umc1347-bnlg420	14.21	−1.68	17.56
	qMES20-4	umc1313-umc2287	4.06	0.70	4.19
	qMES20-6-1	umc238-umc1517	7.92	−0.81	10.98
	qMES20-6-2	umc1020-bnlg1174a	6.62	−0.78	7.43
	qMES20-7	umc1029-phi328175	3.77	−0.78	3.81
	qMES20-10	bnlg1028-bnlg594	9.41	−1.11	11.41
COL20/3.87	qCOL20-4	umc2041-umc1313	10.05	−0.32	16.01
SDL20/3.78	qSDL20-1	umc1085-umc1968	4.75	−0.56	7.27
	qSDL20-3	umc2369-umc1773	4.33	−1.51	7.71
	qSDL20-4	bnlg1444-bnlg2162	5.74	−1.36	9.08
RAT20/4.04	qRAT20-1	umc1968-phi055	7.83	−10.81	13.16
	qRAT20-3	umc1347-bnlg420	6.75	−9.91	12.22
	qRAT20-6	umc1020-bnlg1174a	4.42	−6.77	6.40
	qRAT20-8	umc2367-umc1846	4.63	4.80	6.64

[a] MES10, COL10, SDL10 and RAT10 represent mesocotyl length, coleoptile length, seedling length on the 12th day and emergence rate on the 5th day at 10 cm depth. MES20, COL20, SDL20 and RAT20 represent mesocotyl length, coleoptile length, seedling length and emergence rate on the 14th day at 20 cm depth. The numbers to the right of the trait name abbreviations mean the LOD threshold level after permutation tests

[b] The QTL names are denoted following the rules suggested by McCouch et al. (1997), where the number after the first dash means the chromosome number and the number after the second dash is used to distinguish the two QTL detected on the same chromosome for the same trait

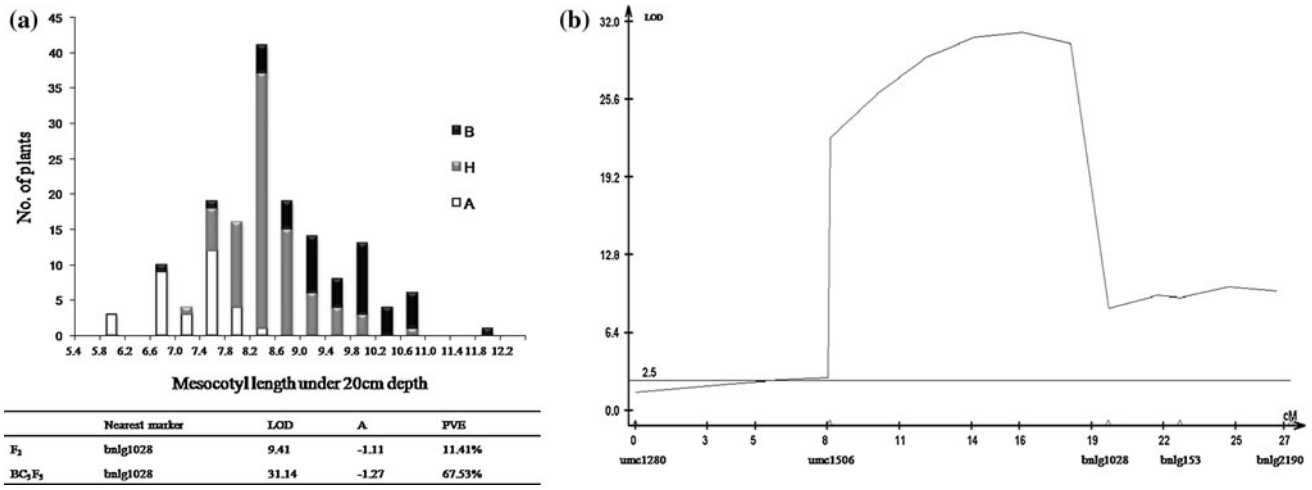
[c] A indicates additive effects estimated with Windows QTL Cartographer 2.5, negative value means that the allele from 3681-4 is positive contributor

[d] PVE represents phenotypic variance explained

was divided into three groups based on the genotypic differences at the bnlg1028 SSR marker, where B means homozygous 3681-4 background, H means heterozygous genotype, and A means homozygous X178 background (Fig. 2a). This marker was the nearest marker to the LOD peak in both the primary mapping (based on the F_2 population) and the advanced mapping (based on the BC_3F_3 population). The results confirmed that the qMES20-10 locus was between SSR markers umc1506 and bnlg1028 and that the genetic distance of this interval was 9.87 cM in the F_2 population and 11.6 cM in the BC_3F_3 population (Fig. 2b). The physical distance between the two markers was 5.24 Mb according to the B73 sequence (http://www.maizegdb.org). This qMES20-10 explained 67.53% of the phenotypic variance in the BC_3F_3 population (Fig. 2). The larger phenotypic effect and LOD value compared with those in F2 population confirmed that this QTL did exist. Although we could not get a 3:1 or 1:2:1 ratio from this BC_3F_3 population, the result still gave us confidence for further dissecting this locus.

Discussion

Researches on deep-seeding tolerance have been previously performed in several crops, including wheat, barley, oat and maize (Hoshikaw 1969; Suge et al. 1998; Takahashi et al. 2001; Troyer 1997), but the reports on maize were quite limited. Troyer (1997) demonstrated that mesocotyl elongation was the cause of deep-seeding tolerance. Zhao et al.



	Nearest marker	LOD	A	PVE
F_2	bnlg1028	9.41	-1.11	11.41%
BC_3F_3	bnlg1028	31.14	-1.27	67.53%

Fig. 2 Confirmation of qMES20-10. Confirmation of qMES20-10 using a BC_3F_3 population: **a** Distribution of mesocotyl length at 20 cm depth based on the genotype of SSR marker bnlg1028 and the effect of qMES20-10 in this population. In the upper part of **a**: *B* means homozygous 3681-4, *H* means heterozygous, *A* means homozygous X178. In the lower part of **a**: *A* means additive effect, *PVE* means phenotypic variance explained; **b** Confirmation of qMES20-10 based on QTL Cartographer Version 2.5, the genetic distance and physical distance between umc1506 and bnlg1028 was 11.6 cM and 5.24 Mb, respectively

(2009) also gave similar conclusion. Consistent with these results, we found that mesocotyl length seemed to be the only organ that responded to sowing depth (Suppl. Fig. 1). However, the correlation analysis based on our 221 $F_{2:3}$ families showed that RAT20 correlated with both MES20 and SDL20, and the correlation coefficients were 0.560 and 0.401, respectively, which might indicate that mesocotyl was the major contributors to deep-seeding tolerance and seedling length could also improve deep-seeding tolerance. The overlap of the QTL for MES20 and SDL20 with the QTL for RAT20 might also validate this prediction. Our analysis not only reconfirmed the conclusion made by Troyer (1997) and Zhao et al. (2009), but also revealed that seedling length might also be related to deep-seeding tolerance, although the contribution might be smaller than that of mesocotyl length.

We measured three seedling traits under two depth conditions to compare the QTL for the corresponding traits (e.g. MES10 and MES20) because the correlation coefficients were significant between the corresponding traits at different sowing depths (Table 2). The comparison would tell us whether the genetic basis of the corresponding trait at different depth was similar. Three QTL for MES10 were in the same regions with three QTL for MES20 on chromosomes 1, 3 and 10, which confirmed the existence of these QTL for mesocotyl length under the two depth conditions. The increased additive effects of the three QTL at 20 cm depth might reflect the result that mesocotyl length became longer under deeper conditions (Suppl. Fig. 1). This might be caused by endogenous IAA and GA increase, as deep-seeding treatment increased the content of endogenous IAA and GA and treatment with TIBA and UCZ (IAA: Indole-3-acetic acid; GA: gibberellic acid; TIBA: IAA transport inhibitor; UCZ: GA biosynthesis inhibitor) decreased mesocotyl length (Zhao and Wang 2008, 2010). The sole QTL for SDL10 on chromosome 4 was mapped to the same region of qSDL20-4 for SDL20, suggesting that this locus had consistent effect on seedling length at different sowing depths. Unexpectedly, we failed to detect common QTL for coleoptile length under the two depth conditions. But the LOD value for COL10 in the genomic region of qCOL20-4 (umc2041-umc1313) was 3.67, slight lower than the LOD threshold for COL10 (3.91), indicating that a QTL for COL10 might exist between umc2041 and umc1313.

RAT10 was considered more of an indicator for early seedling vigor than an indicator for deep-seeding tolerance because it reflected the speed of seedling emergence. The higher correlation coefficient of SDL10 with RAT10 compared to that of MES10 might validate our view because seedling length was used as a seedling vigor related trait (Redonna and Mackill 1996; Zhou et al. 2007). However, we failed to detect common QTL for SDL10 and RAT10, this discrepancy might be caused by the low heritability of SDL10 which led to less QTL detection, or because the genetic loci for the two traits were not detected due to the probability of Type-II error, or because epistasis played a significant role for both traits. Meanwhile, qSDL20-1 for SDL20, which was considered to be a seedling vigor related trait under deeper condition, was in the same QTL cluster with qRAT10-1 for RAT10. In addition, qSDL20-4 for SDL20 was in the same region with the only QTL for SDL10, suggesting that the two loci on chromosome 1 and 4 had consistent effect on seedling

vigor. Seedling vigor-related traits and deep-seedling tolerance-related traits were considered to be correlated with each other (Rebetzke et al. 2007a; Spielmeyer et al. 2007), Rebetzke et al. (2007a) found that coleoptile length of wheat was genetically correlated with seedling vigor at different depths, they also found (2001) that the QTL on chromosome 4B for early vigor overlapped with one QTL for coleoptile length. Spielmeyer et al. (2007) detected a common QTL on chromosome 6A that was responsible for increased leaf width and coleoptile length. The phenotypic clues for relationships between them in our experiment were the significant correlations between RAT10 and RAT20, and between SDL20 and RAT20 (Table 2). Through QTL analysis, we found that two QTL for RAT10 on chromosomes 1 and 3 were in the same QTL cluster with two QTL for RAT20, and one QTL for SDL20 on chromosomes 1 overlapped with one QTL for RAT20. Therefore, we considered that deep-seedling tolerance might be genetically correlated with early seedling vigor.

The overlap of QTL loci in our research reflected the significant correlations between traits, similar results on related traits were also obtained in barley and wheat. For example, Takahashi et al. (2001) reported a QTL that controlled coleoptile length, first internode length and deep-seeding tolerance on chromosome 5H, and another QTL that controlled deep-seeding tolerance and first internode length on chromosome 7H in barley. Spielmeyer et al. (2007) found a QTL controlling leaf width, coleoptile length and final height on chromosome 6A in wheat. These coinciding loci might justify their value for breeding deep-seeding tolerant varieties.

Using translocation tester stocks, Troyer (1997) found three loci on chromosomes 3, 6 and 9 that controlled the mesocotyl length under deep-seeding conditions, the two loci on the short arm of chromosome 3 and the centromeric region of chromosome 6 detected by Troyer (1997) might correspond to the qMES20-3, qMES20-6-2 found in our analysis. Troyer (1997) did not find any loci on the long arm of chromosome 10, although translocation tester stocks breaking at the short arm of chromosome 10 were used. We used the BC_3F_3 population developed in our experiment to investigate the existence of qMES20-10. The relatively larger LOD value and the greater phenotypic variance (Fig. 2) explained by this QTL in our BC_3F_3 population helped us to conclude that there was one QTL on chromosome10 that controlled MES20. We also used another BC_3F_3 population segregating at qMES20-10 locus to confirm that qMES20-10 was in the interval between umc1506 and bnlg1028 (Suppl. Fig. 3). High-resolution mapping of this QTL would not only contribute to the understanding of the molecular basis of deep-seeding tolerance but also help breed deep-seeding tolerant cultivars through MAS.

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