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Mapping of QTL Associated with Waterlogging Tolerance during the Seedling Stage in Maize

FAZHAN QIU, YONGLIAN ZHENG, ZILI ZHANG and SHANGZHONG XU*

College of Plant Science and Technology, National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China

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• *Background and Aims* Soil waterlogging is a major environmental stress that suppresses maize (*Zea mays*) growth and yield. To identify quantitative trait loci (QTL) associated with waterlogging tolerance at the maize seedling stage, a F_2 population consisting of 288 $F_{2:3}$ lines was created from a cross between two maize genotypes, 'HZ32' (waterlogging-tolerant) and 'K12' (waterlogging-sensitive).
• *Methods* The F_2 population was genotyped and a base-map of 1710·5 cM length was constructed with an average marker space of 11·5 cM based on 177 SSR (simple sequence repeat) markers. QTL associated with root length, root dry weight, plant height, shoot dry weight, total dry weight and waterlogging tolerance coefficient were identified via composite interval mapping (CIM) under waterlogging and control conditions in 2004 (EXP.1) and 2005 (EXP.2), respectively.
• *Key Results and Conclusions* Twenty-five and thirty-four QTL were detected in EXP.1 and EXP.2, respectively. The effects of each QTL were moderate, ranging from 3·9 to 37·3 %. Several major QTL determining shoot dry weight, root dry weight, total dry weight, plant height and their waterlogging tolerance coefficient each mapped on chromosomes 4 and 9. These QTL were detected consistently in both experiments. Secondary QTL influencing tolerance were also identified and located on chromosomes 1, 2, 3, 6, 7 and 10. These QTL were specific to particular traits or environments. Although the detected regions need to be mapped more precisely, the findings and QTL found in this study may provide useful information for marker-assisted selection (MAS) and further genetic studies on maize waterlogging tolerance.

Key words: Maize (*Zea mays*), waterlogging tolerance, genome mapping, SSR marker, QTL, epistasis effect.

INTRODUCTION

Waterlogging is one of the most important constraint factors for maize production and productivity in tropical and subtropical regions. In South-East Asia, 15 % of all maize-growing areas are affected by waterlogging problems, causing losses in maize production of 25–30 % almost every year (Rathore and Warsi, 1998). In China, the most common situation is that maize yield is limited by lack of available water, but there are large areas that are subject to waterlogging at the maize seedling stage, which is one of the most serious constraints for maize productivity, especially in south-eastern China. In these areas, high spring rainfall over a short period can lead to waterlogging of the soil for periods extending to weeks. This often causes severe damage to maize seedlings due to their poor adaptation to waterlogging. Waterlogging is becoming a matter of worldwide concern in many agricultural areas, where similar conditions have led to the spread of this environmental threat (Ghassemi *et al.*, 1995). Predictions are that global warming will result in more erratic weather patterns, which could further exaggerate the problem. In order to increase maize productivity in waterlogged soils, new maize varieties with greater adaptation to waterlogging are essential. Hence, the development of waterlogging-tolerant varieties with a high yield potential is one of the main objectives of many maize breeding programs in the region (Anon., 2003; Zaidi *et al.*, 2004).

The degree of stress in waterlogged soils is associated with growth stage, duration of flooding, soil type, soil acidity/alkalinity, climatic factors, growth conditions and genotypes (Rathore and Warsi, 1998; Mano *et al.*, 2002). In a previous study (Zaidi *et al.*, 2004), the early stages of maize development were shown to be the most sensitive to waterlogging, especially from the second leaf stage (V2) to the seventh leaf stage (V7), and roots are the first to be affected under waterlogged conditions. When the waterlogging treatment was continued for 6 d, most roots except for some adventitious ones were found to be decomposing, and plants were unable to take up the required atmospheric and edaphic nutrients, resulting in leaching and denitrification as a result of nitrogen deficiency. The latter is observed as a yellowing of the older leaves. Nitrogen deficiency itself then further increases plant stress. During waterlogging, gas exchange between soil and air decreases as gas diffusion in water is decreased 10^4-fold (Armstrong, 1979; Armstrong and Drew, 2002), O_2 in the soil is rapidly depleted, and the soil may become hypoxic or anoxic within a few hours (Gambrell and Patrick, 1978; Malik *et al.*, 2002). The anaerobic response of maize has been extensively reviewed previously (Sachs, 1993, 1994; Sachs *et al.*, 1996; Mustroph and Albrecht, 2003). The

* For correspondence. E-mail shangzhongxu@gmail.com

inability of maize to withstand low oxygen conditions in the root zone results in substantial yield losses (Dennis *et al.*, 2000).

In recent years, more and more information has been accumulated on the molecular, biochemical, physiological, morphological, anatomical and metabolic responses to waterlogging and oxygen deficiency in plants (Kennedy *et al.*, 1992; Vartapetian and Jackson, 1997; Baxter-Burrell *et al.*, 2003; Greenway *et al.*, 2006; Mustroph *et al.*, 2006). Tolerance to waterlogging has been studied in other crops such as wheat, soybean, *Arabidopsis* and rice, where it appears to be oligogenically inherited (Setter *et al.*, 1997; Boru *et al.*, 2001; VanToai *et al.*, 2001; Kolk *et al.*, 2002; Xu *et al.*, 2006). It is interesting to note that ethylene has been reported as being involved in gene regulation after prolonged oxygen deprivation in maize (He *et al.*, 1996), rice (Fukao *et al.*, 2006; Xu *et al.*, 2006) and *Arabidopsis thaliana* (Peng *et al.*, 2001; Baxter-Burrell *et al.*, 2003; McGrath *et al.*, 2005). Saab and Sachs (1996) reported that an ethylene-signalling pathway is required for the induction of the xyloglucan endo-transglycosylase gene and that an ethylene-independent pathway is mainly responsible for the induction of *ADH*1 in flooded maize roots. Programmed cell death and aerenchyma formation in maize roots is regulated by ethylene, which is one important adaptation mechanism to tolerate a low-oxygen soil environment (Drew *et al.*, 1979; Justin and Armstrong, 1991; Drew *et al.*, 2000). In addition, Xu *et al.* (2006) reported that the submergence tolerance gene, *Sub1A*, is an ethylene-response-factor-like gene that confers submergence tolerance to rice. In *Arabidopsis thaliana*, ethylene was also involved in the hypoxic induction of the *ADH* gene (Peng *et al.*, 2001).

Some recent studies have documented variation in the anaerobic response of maize to flooding (Sachs *et al.*, 1996) and several morphological responses during waterlogging have been also reported (Subbaiah and Sachs, 2003). According to previous studies, the inheritance and expression of traits associated with waterlogging tolerance in maize seedlings are physiologically and genetically complex (Sachs, 1993, 1994; Liao and Lin, 2001; Subbaiah and Sachs, 2003). Complicated responses to waterlogging, such as anaerobic proteins synthesis, alterations of gene expression, metabolic (switch to a fermentative pathway) and structural changes (e.g. aerenchyma formation) have been observed. There appears to be inherent genetic variability in maize with regard to waterlogging tolerance (Sachs *et al.*, 1996). However, manipulating waterlogging tolerance in maize is still hampered by inadequate knowledge of the molecular and physiological basis of the process.

Progress in developing improved waterlogging-tolerant cultivars would be accelerated if the genes controlling the various underlying processes could be identified and tagged with molecular markers. The goals of the current study were to create a genetic map of the traits associated with waterlogging tolerance, and to identify the QTL controlling tolerance to waterlogging at the seedling stage under controlled conditions by using segregating F_2 populations derived from a cross between a waterlogging-tolerant accession ('HZ32') and a susceptible accession ('K12'). The QTL analysis of physiological traits and the identification of potential candidate genes may help in understanding the genetic mechanisms of waterlogging tolerance, as well as in developing waterlogging-tolerant elite maize lines through molecular marker-assisted selection.

MATERIALS AND METHODS

Plant material and population development

An F_2 mapping population was developed from a cross between two maize inbred lines, 'HZ32' (highly waterlogging-tolerant) and 'K12' (highly waterlogging-sensitive) (Zhang *et al.*, 2003; Tang *et al.*, 2005). These lines had previously been identified (unpubl. res.) using the waterlogging tolerance coefficient (WTC = waterlogging treatment of each trait/control of each trait; described in detail below). Three hundred and forty-one F_2 seeds derived from a single F_1 parent were planted and 288 of the subsequent F_2 plants were successfully self-pollinated at the experimental farm of Huazhong Agricultural University. The seeds of the 288 $F_{2:3}$ ears (families) were harvested from the F_2 selfed-plants in the 2003 maize-growing season. The F_2 plants were used for genotyping SSR (simple sequence repeat) loci, and the $F_{2:3}$ seeds (families) harvested from each F_2 plants were utilized to conduct the waterlogging experiments.

Plant growth conditions

To avoid the influence of rainfall, the experiment was carried out under glasshouse conditions. The day/night temperatures were 30/22 °C, relative humidity was 55–75 % and the photoperiod was 13/11 h (day/night).

Two experiments, EXP.1 and EXP.2, were conducted in the 2004 and 2005 maize-seedling growing seasons, respectively. The same $F_{2:3}$ families together with the two parents and the F_1 hybrid were exposed to control (no flooding) and waterlogging treatments. The experiments were laid out in a randomized complete-block design with three replications. Two plastic pots were included for each replication per genotype: one pot for the control and the other for the waterlogging treatment. Fifteen seedlings were grown in each pot of 32 cm diameter and 32 cm depth filled with 10 kg of sieved, sterilized dry field soil (the basic physical–chemical properties of the selected soil were the same as those of the cultivated fields of the Huazhong Agricultural University) amended with 1·0 g $(NH_4)_2SO_4$, 0·8 g P_2O_5, and 0·6 g K_2O per kilogram of soil until the second leaf had fully expanded (V2 stage). The pots intended for the waterlogging treatment were then filled with water to 2–3 cm above the soil surface for 6 d. The controls were irrigated as needed to avoid drought stress or waterlogging stress.

Sampling, drying and weighing methods

After undergoing the waterlogging treatment for 6 d in both experiments, plant height (PH), root length (RL), root dry weight (RDW) and shoot dry weight (SDW) of each replicate per genotype under control and waterlogging

treatment were measured. The measurements were considered to represent the phenotypes of the F_2 plants.

Fifteen plants of each genotype per replicate were used for trait scoring under control and waterlogged conditions. Plants were carefully taken out of the pot and immersed in water. Roots were gently washed under running water; root loss during cleaning was kept to a minimum. Root length was measured from the coleoptilar node to the tip of the longest root and plant height was measured from the coleoptilar node to the tip of the longest leaf. Roots were then seperated from the plant. Roots and shoots of each replicate per genotype for control and waterlogging stress were put into separate paper bags, which were then rapidly transferred into ovens and dried at 65 °C until a constant weight was achieved. Root dry weight and shoot dry weight were measured using an electronic balance (MP500B, Ashiba). The waterlogging tolerance coefficient (WTC) was calculated for each pair of pots grown at the same time, with the averaged values for the fifteen plants of each pot being used. The WTC of RL, RDW, PH, SDW and total dry weight (TDW; TDW = SDW + RDW) of each replicate per genotype was calculated using the following formula:

$$\text{WTC} = \text{waterlogging treatment of each trait/control of each trait}$$

Because the control and waterlogging treatment plants were paired for each genotype, we obtained 288 WTC values for each trait.

DNA isolation and SSR analysis

Genomic DNA from each of the F_2 plants and the parental lines was isolated from fresh leaf tissue following a procedure similar to that used by Saghai-Maroof *et al.* (1984). The modifications in the procedure were (1) addition of boiled CTAB extraction buffer to the 50 mL polypropylene centrifuge tube, and (2) a reduction of the incubation time to 30 min.

Genotyping of the F_2 individuals was performed with 177 SSR markers. Sequences of all SSR markers were obtained from the MaizeGDB database (http://www.maizegdb.org/ssr.php). Each amplification reaction contained 20 μL, consisting of 1× reaction buffer, 10 % Glycerol, 2 mmol of $MgCl_2$, 150 μmol of each dNTP mix, 0·3 μmol of each SSR primer, 0·75 U of Taq DNA polymerase, and 50 ng of genomic DNA. The reaction mixture was overlaid with one drop of mineral oil. Amplifications were performed in a PTC-100 Programmable Thermal Controller (MJ Research, Inc., Watertown, MA, USA) and T1 Thermocycler Module 96 (Biometro, Goettingen, Germany) programmed for the first denaturation step to last 2 min at 94 °C, followed by 30 cycles of 1 min at 94 °C, 40 s at 58 °C, and 50 s at 72 °C, with a final extension for 5 min at 72 °C. The amplified fragments were separated on 6 % polyacrylamide sequence gel containing 7 mol urea and visualized using the following silver-staining procedure. The polyacrylamide gel was fixed twice in 10 % ethanol + 0·5 % glacial acetic acid for 6 min, or fixed once for 12 min, then rinsed with ddH_2O for 6 min, dipped in 0·2 % $AgNO_3$ for 12 min of staining, rinsed with ddH_2O for about 12 s, and placed in 1·5 % NaOH + 0·4 % formaldehyde (37 %) until DNA bands were displayed clearly. Later the gel was placed in 0·75 % Na_2CO_3 for 3 min to end the staining, and finally rinsed with tap water for about 3 min and then air-dried.

Linkage analysis and map construction

A molecular linkage map was constructed using Mapmaker Version 3·0 (Lander *et al.*, 1987; Lincoln *et al.*, 1993). All the markers were assigned at LOD ≥ 2·5 to ten linkage groups. By means of the Kosambi mapping function (Kosambi, 1944), the values of recombination fractions were converted into genetic map distances (cM). Linkage groups were determined using the 'group' command; the order of the markers for each linkage group was determined using the command 'first order'. Ungrouped/unlinked markers were assigned to the respective linkage groups using the 'try' command. The map was drawn according to Liu and Meng (2003).

Statistical analysis

All the QTL analyses for the individual environments were performed using Windows QTL Cartographer Version 2·0 (North Carolina State University, Raleigh, NC) programmed by Wang *et al.* (2002). Composite interval mapping (CIM) was used to map the QTLs. The parameters were set as follows. Map function: Kosambi; distance units: centimorgan (cM); distance type: position; cross-information: SF3 (self-cross F3); walk speed: 2 cM; LR (likelihood ratio) threshold: 11·50 under H0:H3 (H0: $a = 0$, $d = 0$; H3: $a <> 0$, $d <> 0$), i.e., LOD = 2·5; CIM mode selection: model 6, i.e., standard model; background controls: 5 of control marker numbers and 10·0 cM of window size. Significance thresholds were determined by permutation tests (n =1000 permutations; Churchill and Doerge, 1994); considering a significant and a suggestive locus when the LOD statistic exceeded the 95th ($P < 0·05$) and the 63rd ($P < 0·37$) percentile of the permutation distribution, respectively (Wittenburg *et al.*, 2002; Rodrigo *et al.*, 2006). QTL × Environment (Q × E) interaction and digenic epistatic QTLs analysis were conducted by using QTLMapper V2·0 based on a mixed model approach (Wang *et al.*, 1999). $P \leq 0·005$ for Type-I errors and a $\log_{10}$ likelihood ratio (LOD) value of 2·5 were used as criteria to declare the putative main effect QTL position, digenic epistatic QTLs and QTL × Environment (Q × E) interaction. Epistasis effect was estimated according to the definition of Mather and Jinks (1982). The R^2 value (coefficient of determination) from this analysis indicated the percentage of phenotypic variance explained by the marker genotypes at the locus.

The PROC MIXED procedure of SAS ver. 8·02 (SAS Institute Inc., Cary, North Carolina, 1991–2001) was used to calculated the adjusted means and the broad-sense heritability (h^2) of the families. The heritability was

computed as $h^2 = \delta_g^2/(\delta_g^2 + \delta_e^2/n)$, where δ_g^2 and δ_e^2 were the estimates of genetic and residual variances, respectively, derived from the expected mean-squares of the analysis of variance, and n was the number of replications. Analysis of variance was done using the general linear model (GLM) procedure of the SAS program. The frequency distribution of the $F_{2:3}$ famililies for all traits was performed using the univariate procedure of SAS and normal distributions were checked using the Shapiro–Wilk Test. Simple Pearson correlation coefficients (r) were calculated between the traits using the adjusted means of the $F_{2:3}$ families. The significance of the correlation coefficient at $P \leq 0{\cdot}05$, 0·01, and 0·001 are indicated as *, ** and ***, respectively.

RESULTS

Phenotypic variation and phenotypic data

The frequency distribution of the $F_{2:3}$ families for five waterlogging responsive traits and their WTC were normal, as determined by the Shapiro–Wilk test (data not shown). Values of the mean and range for the five traits are shown in Table 1. The parents showed statistically significant differences for the traits RL, RDW, PH, SDW and TDW under waterlogging conditions, but there were no differences under normal conditions. As shown in Table 1, the five responsive traits of all the genotypes were significantly reduced (5–58 %) in the waterlogged conditions compared with the controls in both experiments. In other words, the WTC for the five waterlogging response traits were greater for 'HZ32' than for 'K12', which indicated that the traits were more greatly affected by waterlogging in 'K12' than in 'HZ32'. The results suggest that 'HZ32' exhibited consistently and significantly superior waterlogging tolerance for the waterlogging response traits. Moreover, the effects of waterlogging were much more severe on RL and RDW than on PH and SDW (Table 1).

The $F_{2:3}$ families exhibited a wide range of variation for the traits studied (Table 1). Transgressive segregation in both directions was observed for most traits under

TABLE 1. *Trait mean values for 288* $F_{2:3}$ *families and two parents along with broad-sense heritability* (h^2) *in the two experiments*

Traits	Root length (cm)	Root dry weight (g)	Plant height (cm)	Shoot dry weight (g)	Total dry weight (g)
EXP.1 (2004)					
Control					
'HZ32' (P_1)	33·66 ± 1·21	0·16 ± 0·02	28·43 ± 1·62	0·41 ± 0·01	0·57 ± 0·02
'K12' (P_2)	32·56 ± 1·21	0·12 ± 0·02	24·23 ± 1·62	0·38 ± 0·01	0·50 ± 0·02
P_1 vs $P_2^{\dagger}$	ns	ns	ns	ns	ns
$F_{2:3}$ families (mean)	33·95 ± 2·62	0·17 ± 0·02	24·52 ± 1·31	0·24 ± 0·01	0·41 ± 0·03
$F_{2:3}$ families (range)	11·7–47·2	0·04–0·48	13·5–35·5	0·08–0·57	0·14–1·05
h^2	0·89	0·86	0·82	0·78	0·84
Waterlogging stress					
'HZ32' (P_1) stress	19·40 ± 1·40	0·11 ± 0·01	27·19 ± 3·21	0·35 ± 0·02	0·46 ± 0·02
'K12' (P_2) stress	14·95 ± 1·40	0·05 ± 0·01	20·5 ± 3·21	0·19 ± 0·02	0·24 ± 0·02
P_1 vs P_2	*	**	**	**	**
$F_{2:3}$ families (mean)	16·7 ± 1·34	0·06 ± 0·01	21·10 ± 1·96	0·20 ± 0·03	0·28 ± 0·01
$F_{2:3}$ families (range)	3·9–34·25	0·01–0·24	8·96–31·06	0·04–0·39	0·06–0·96
h^2	0·28	0·43	0·74	0·71	0·82
WTC of P_1	0·58	0·68	0·95	0·85	0·81
WTC of P_2	0·46	0·42	0·85	0·50	0·48
EXP.2 (2005)					
Control					
'HZ32' (P_1)	26·32 ± 1·41	0·23 ± 0·01	36·81 ± 1·13	0·77 ± 0·02	1·00 ± 0·01
'K12' (P_2)	29·76 ± 1·41	0·17 ± 0·01	33·72 ± 1·13	0·69 ± 0·02	0·86 ± 0·01
P_1 vs P_2	ns	ns	ns	ns	ns
$F_{2:3}$ families (mean)	39·1 ± 1·52	0·42 ± 0·01	35·39 ± 1·92	0·59 ± 0·02	1·03 ± 0·01
$F_{2:3}$ families (range)	26–55·2	0·15–0·83	24·1–44·94	0·14–1·2	0·42–2·03
h^2	0·72	0·83	0·51	0·62	0·67
Waterlogging stress					
'HZ32' (P_1)	19·12 ± 1·23	0·16 ± 0·01	26·84 ± 1·62	0·62 ± 0·02	0·78 ± 0·03
'K12' (P_2)	14·85 ± 1·23	0·09 ± 0·01	18·30 ± 1·62	0·51 ± 0·02	0·60 ± 0·02
P_1 vs P_2	*	**	**	**	**
$F_{2:3}$ families (mean)	14·69 ± 1·30	0·14 ± 0·02	25·96 ± 1·54	0·39 ± 0·01	0·54 ± 0·01
$F_{2:3}$ families (range)	5·8–22·5	0·03–0·53	15·3–40·1	0·14–0·8	0·1–1·08
h^2	0·32	0·57	0·69	0·58	0·61
WTC of P_1	0·73	0·70	0·73	0·80	0·78
WTC of P_2	0·50	0·52	0·54	0·73	0·70

The heritability was computed as $h^2 = \delta_g^2/(\delta_g^2 + \delta_e^2/n)$, where δ_g^2 and δ_e^2 were the estimates of genetic and residual variances, respectively, derived from the expected mean-squares of the analysis of variance, and n was the number of replications. WTC (waterlogging tolerance coefficient) was computed as WTC = waterlogging treatment of each trait/control of each trait. Data are mean ± s.e.m.

† Statistical test for difference between two parents at 0·05 (*) and 0·01 (**) levels of probability; ns, not significant.

TABLE 2. *Simple correlation coefficients between waterlogging-response traits in maize obtained in a F_2 mapping population derived from a cross between waterlogging-tolerant 'HZ32' and waterlogging-sensitive 'K12' in EXP.1 (2004 season) and EXP.2 (2005 season)*

	EXP.2 (2005)				
	Total dry weight	Plant height	Root length	Shoot dry weight	Root dry weight
EXP.1 (2004)					
Total dry weight	*0·8456****	0·7163***	0·3430**	0·8768***	0·8925***
Plant height	0·74826***	*0·6785****	0·3854*	0·6360***	0·5609***
Root length	0·31448**	0·3401*	*0·2345*^{ns}	0·2782*	0·6788***
Shoot dry weight	0·8631***	0·7327**	0·3723**	*0·7843****	0·2863*
Root dry weight	0·8358***	0·27291**	0·7843***	0·6538**	*0·4786****

Values in italics denote a correlation between the two experiments.
*, significant at $P < 0·05$; **, $P < 0·01$; ***, $P < 0·001$; ^{ns}, not significant.

waterlogging conditions, indicating that both parents transmitted favourable alleles for each trait. Broad-sense heritabilities (h^2) computed across the five traits mentioned were relatively moderate (Table 1). PH and TDW had the highest h^2 at 0·74 and 0·82, while RL had the lowest at 0·28 and 0·32 in EXP.1 and EXP.2, respectively, under waterlogging-stress conditions. Comparing with h^2 under control conditions, the results indicated that RL was more prone to be affected by waterlogging stress than other traits (Table 1).

Correlations between measured traits and WTC were evaluated for statistical significance in both experiments as shown in Tables 2 and 3. Highly significant positive correlations between TDW and PH, TDW and SDW, TDW and RDW, and RL and RDW were found in both experiments. Positive correlations between PH and SDW, and PH and RDW were also highly significant in EXP.2 (Table 2). In addition, highly significant positive correlations among WTC of the five response traits assessed were found in both two experiments (Table 3), indicating that these traits were not expressed independently of one another. Highly significant correlations were observed between EXP.1 and EXP.2 for TDW, PH, SDW and their WTC (Tables 2 and 3). However, a weak relationship was observed for RDW ($r = 0·4786$) and no relationship was found for RL between the two experiments (Table 2). Similar results were found for WTC of RDW and RL (Table 3), suggesting that the expression of RDW and RL is sensitive to the growing environment.

Construction of molecular marker linkage map

One hundred and seventy-seven SSR markers showing co-dominant segregation were employed to construct a linkage map (Fig. 1), of which 148 informative markers were assigned to ten chromosomes based on LOD values exceeding 11·5. The linkage map had a total length of 1710·5 cM with an average interval of 11·5 cM between adjacent markers.

QTL detection for PH, SDW, RL, RDW and TDW

QTL for traits responsive to waterlogging in both experiments mapped to maize chromosomes 1, 2, 3, 4, 6, 7, 9 and 10 (Tables 4 and 5, Fig. 1). A total of 59 putative QTL were found to be associated with the five waterlogging-response

TABLE 3. *Simple correlation coefficients between the waterlogging tolerance coefficient (WTC = waterlogging treatment of each trait/control of each trait) for five waterlogging-response traits in maize obtained in a F_2 mapping population derived from a cross between waterlogging-tolerant 'HZ32' and waterlogging-sensitive 'K12' in EXP.1 (2004 season) and EXP.2 (2005 season)*

	EXP.2 (2005)				
	Total dry weight	Plant height	Root length	Shoot dry weight	Root dry weight
EXP.1 (2004)					
Total dry weight	*0·836****	0·72073***	0·34648***	0·89889***	0·72193***
Plant height	0·64826***	*0·683****	0·3783***	0·70882***	0·45982***
Root length	0·51448***	0·64001***	*0·345*^{ns}	0·30955***	0·27884***
Shoot dry weight	0·66317***	0·52703***	0·35719***	*0·820****	0·3863***
Root dry weight	0·72258***	0·47291***	0·4111***	0·69853***	*0·453****

Values in italics denote a correlation between the two experiments.
***, significant at $P < 0·001$; ^{ns}, not significant.

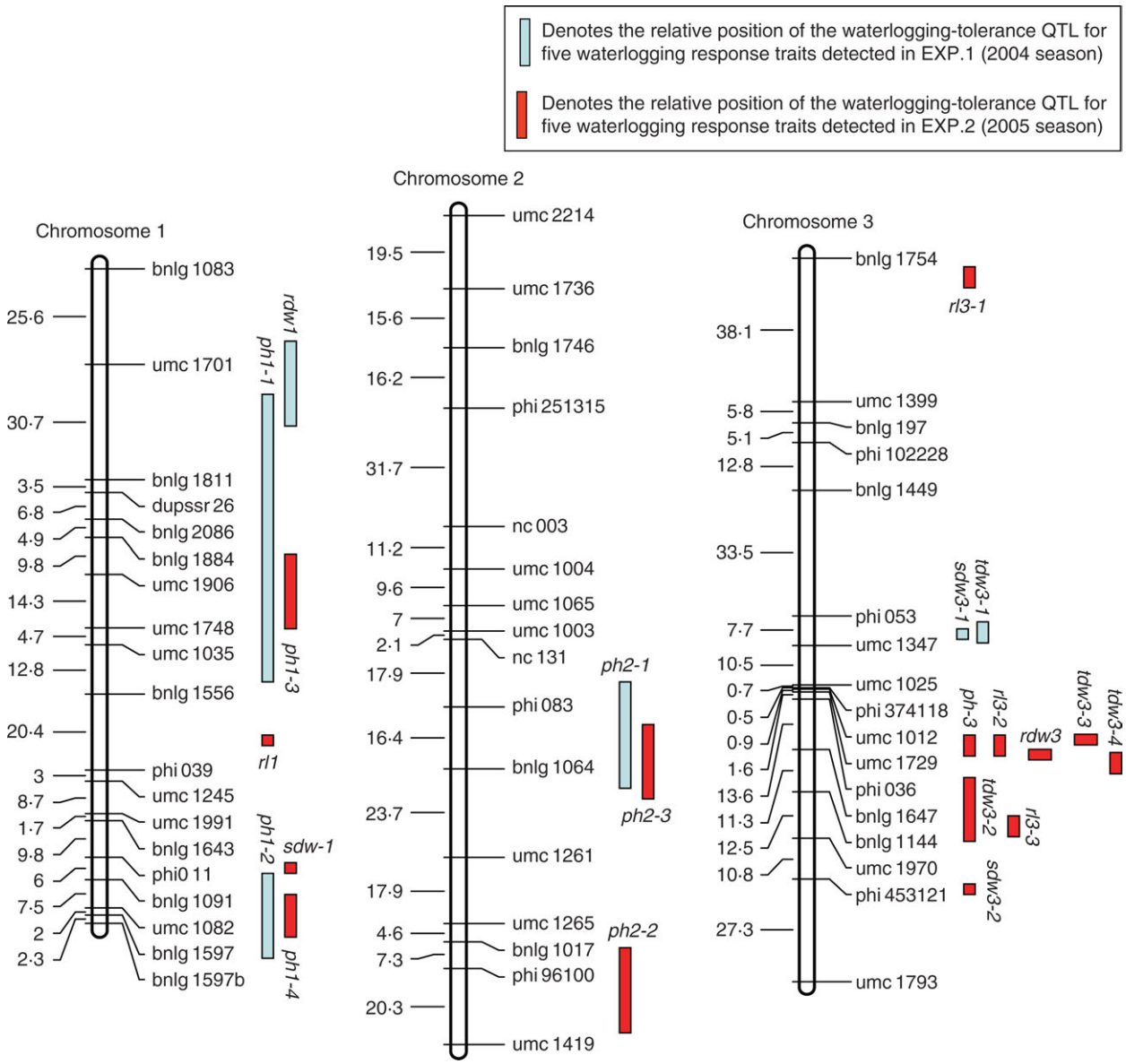

FIG. 1. Molecular linkage map of the F_2 population derived from a cross between 'HZ32' and 'K12', and summary of QTL for all traits responsive to waterlogging in the mapping population of maize in EXP.1 and EXP.2. *sdw* = shoot dry weight; *ph* = plant height; *rl* = root length; *rdw* = root dry weight; *tdw* = total dry weight. For all the QTL names, the first number following the letters represents the chromosome locations of the QTL and the second number represents the orders of the QTL located on the same chromosome by the same trait. The distances between markers (cM) are listed to the left of each figure part.

traits and their WTC when the results of the two experiments were considered together. Fourteen and 25 QTL were detected under control and waterlogging treatment conditions, respectively. Twenty QTL were identified for WTC (Tables 4 and 5). The detected QTL individually accounted for 3·9–37·3 % of the phenotypic variation. Out of the total of 59, 13 QTL individually accounted for more than 10 % of the phenotypic variation. A list of the putative QTL flanked by SSR markers along with their phenotypic variance, additive effects and peak LOD scores, are presented in Tables 4 and 5. A graphical presentation of QTL locations on the linkage map is shown in Fig. 1.

For PH, 15 QTL were detected under control and waterlogging treatment conditions on chromosomes 1 (*ph1-1*, *ph1-2*, *ph1-3* and *ph1-4*), 2 (*ph2-1*, *ph2-2* and *ph2-3*), 4 (*ph4-1*, *ph4-2*, *ph4-3* and *ph4-4*), 6 (*ph6*), 7 (*ph7*) and 10 (*ph10-3* and *ph10-4*) (Tables 4 and 5). Out of these 15, six and nine QTL were detected in EXP.1 and EXP.2, respectively. Individual QTL accounted for 4·2–14·3 % of the phenotypic variation. For six of the QTL (*ph1-2*, *ph2-1*, *ph2-2*, *ph4-2*, *ph1-4* and *ph2-3*), alleles from 'K12' contributed towards an increase of the trait values. For the other nine QTL, alleles from 'HZ32' tended to increase the trait value.

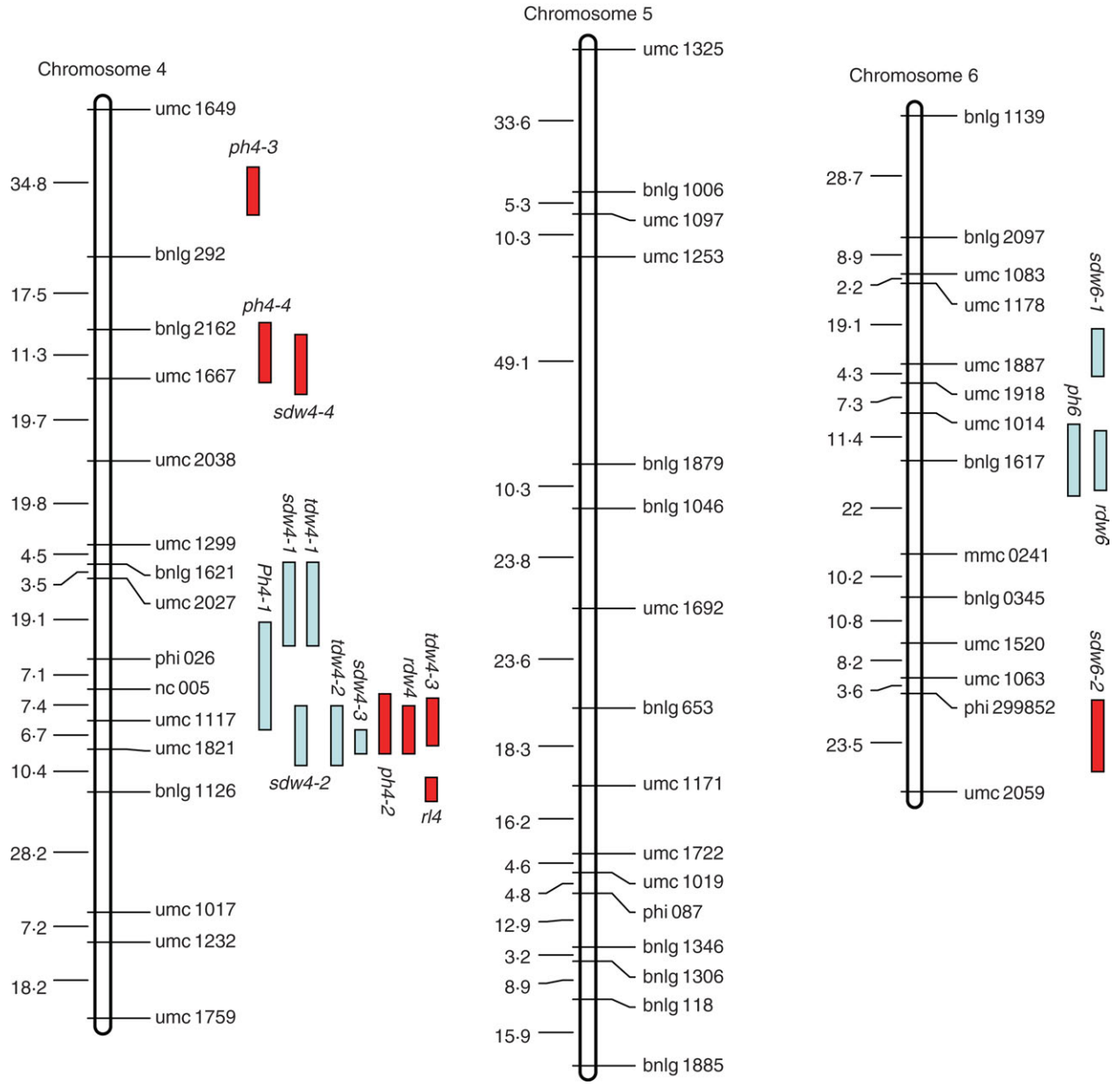

FIG. 1. Continued

Nine QTL were mapped for SDW on chromosomes 1 (*sdw1*), 3 (*sdw3*), 4 (*sdw4-1*, *sdw4-2*, *sdw4-3* and *sdw4-4*), 6 (*sdw6-2*) and 9 (*sdw9-1*, *sdw9-2* and *sdw9-3*) (Tables 4 and 5). Individual QTL accounted for 3·9–37·2 % of the phenotypic variation. For six of these QTL (*sdw4-1*, *sdw4-4*, *sdw6-2*, *sdw9-1*, *sdw9-2* and *sdw9-3*), alleles from 'HZ32' tended to increase the trait values, whereas for the other three QTL the allele from 'K12' contributed to the increase in the trait score (Tables 4 and 5).

Only one QTL for RL, *rl7-1*, was detected under control conditions. Three QTL (*rl4*, *rl7-2* and *rl7-4*) were detected under waterlogged conditions (Tables 4 and 5). Individual QTL had values of R^2 ranging from 5·2–5·5 % and 6·3–7·4 % for EXP.1 and EXP.2, respectively. Except for QTL *rl7-2*, all the alleles were from 'HZ32'.

Of the four QTL associated with RDW, two (*rdw9-1* and *rdw9-2*) were found in both experiments. Out of the remaining two QTL, one (*rdw4*) on chromosome 4 was detected only in EXP.2 whereas the other (*rdw6*) on chromosome 6 was found only in EXP.1 (Tables 4 and 5). The QTL affecting RDW, *rdw9-1* and *rdw9-2*, explained 26·3 % and 36·3 % of the phenotypic variation in both experiments, respectively, suggesting that most of the major QTL for this trait have been identified. This finding is in good agreement with the high heritability estimates of this trait in both experiments (Table 1).

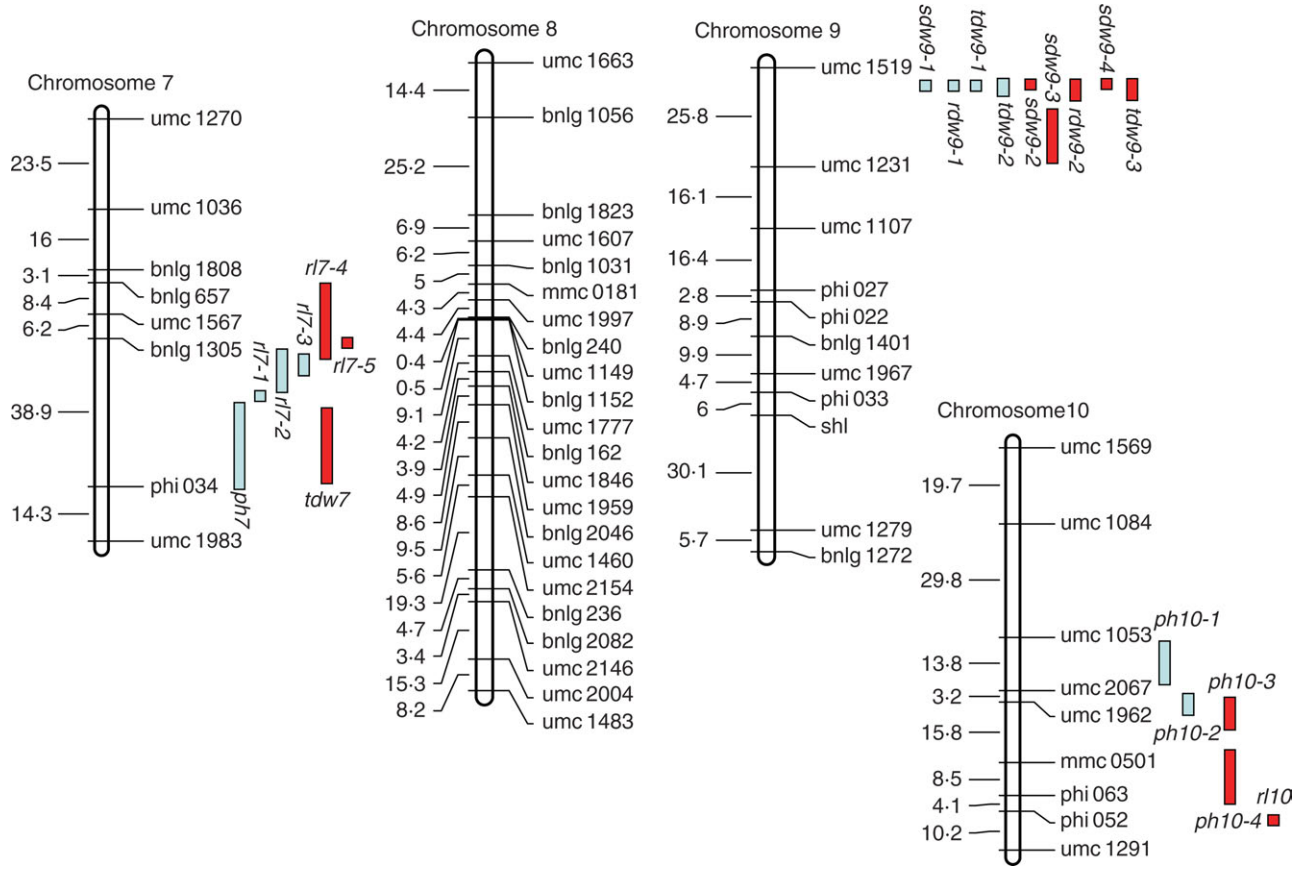

FIG. 1. Continued

Seven genomic regions were detected to be associated with TDW. Out of the seven, two QTL, *tdw4-2* and *tdw4-3* on chromosome 4, were common in both experiments, indicating their low sensitivity to environmental changes, which is in good agreement with the observed phenotypic correlation between the two experiments for this trait ($r = 0{\cdot}8456$). Out of the remaining five QTL, three (*tdw3-1*, *tdw4-1* and *tdw9-1*) on chromosomes 3, 4 and 9 were detected only in EXP.1, whereas the other two (*tdw3-2* and *tdw7*) were found only in EXP.2. Individual QTL had values of R^2 ranging from 4·1–33·3 % and 5·4–9·5 % for EXP.1 and EXP.2, respectively. Four QTL had alleles from 'K12', the exceptions being *tdw4-1*, *tdw7* and *tdw9-1* (Tables 4 and 5).

QTL detection for WTC

A total of 20 putative QTL were found to be associated with the WTC of the five waterlogging-response traits when the results of the two experiments were considered together. The detected QTL individually accounted for 4·0–31·7 % of the phenotypic variation. Out of the 20, five QTL individually accounted for more than 10 % of the phenotypic variation (Tables 4 and 5).

Two QTL (*ph10-1* and *ph10-2*) for the WTC of PH were detected on chromosome 10 in EXP.1. They could explain 3·06 % and 3·23 % of the total phenotypic variation and the primary effect was negative-additive, meaning that alleles from 'K12' at *ph10-1* and *ph10-2* operate in the direction of increasing the WTC of PH (Tables 4 and 5). In addition, only one QTL (*ph3*) for the WTC of PH was detected on chromosome 3, which could explain 5·4 % of the phenotypic variation. The 'K12' alleles at *ph3* increased the WTC of PH.

Out of four QTL associated with the WTC of SDW, two (*sdw4-3* and *sdw6-1*) were detected in EXP.1 and the other two (*sdw3-2* and *sdw9-4*) were detected in EXP.2. No common QTL were found between the two seasons (Tables 4 and 5).

Seven QTL were detected for the WTC of RL on chromosomes 1, 3, 7 and 10 in the two experiments (Tables 4 and 5). The QTL *rl7-3* was detected on chromosome 7 and explained 4·0 % of the phenotypic variation in EXP.1. In EXP.2, six QTL (*rl1*, *rl3-1*, *rl3-2*, *rl3-3*, *rl7-5* and *rl10*) individually had R^2 values ranging from 5·1 to 6·7 %. No common QTL were found between the two seasons. Except for *rl7-5* and *rl10*, all alleles were from 'K12'.

Only two QTL (*rdw1* and *rdw3*) were detected for the WTC of RDW in the two experiments. One (*rdw1*) was detected in EXP.1 while the other (*rdw3*) was detected in EXP.2, They only explained 4·4 % and 5·2 % of the total phenotypic variation and the primary effect was negative-additive, meaning that alleles from 'K12' at

TABLE 4. *Map position and main characteristics of QTL with a LOD score ≥2·5 for plant height (*ph*), shoot dry weight (*sdw*), root length (*rl*), root dry weight (*rdw*), total dry weight (*tdw = sdw + rdw*) and waterlogging tolerance coefficient (WTC = waterlogging treatment of each trait/control of each trait) detected in a F_2 population of maize derived from a cross between waterlogging-tolerant 'HZ32' and waterlogging-sensitive 'K12' in EXP.1 (2004 season)*

Traits	QTL[a]	Chromosome number	cM[b]	Range[c]	Nearest marker	LOD[d]	R^2 (%)[e]	Additivity[f]
Control								
Plant height	*ph1-1*	1	75	27–109	bnlg1556	9·04	13·5	1·374
	ph1-2	1	166	160–174	bnlg1643	3·83	6·0	−1·459
	ph4-1	4	141	136–149	umc2027	3·03	4·7	0·743
	ph7	7	98	85–105	umc1567	3·06	4·9	1·552
Shoot dry weight	*sdw4-1*	4	111	109–118	umc1299	2·73	4·2	2·196
Root length	*rl7-1*	7	79	77–82	umc1567	2·53	7·4	1·892
Root dry weight	–	–	–	–	–	–	–	–
Total dry weight	*tdw4-1*	4	111	109–118	umc1299	3·02	4·6	0·319
Waterlogging treatment								
Plant height	*ph2-1*	2	147	140–154	nc131	2·86	4·2	−0·054
	ph6	6	81	76–90	umc1918	3·85	5·8	1·831
Shoot dry weight	*sdw3-1*	3	95	95	umc1347	2·50	3·9	−0·647
	sdw4-2	4	148	143–158	phi026	3·34	5·6	−1·747
	sdw9-1	9	2·0	2·0	umc1519	15·19	37·3	0·385
Root length	*rl7-2*	7	61	53–76	bnlg657	3·33	6·3	−2·243
Root dry weight	*rdw6*	6	80	78–82	umc1918	2·56	4·2	0·978
	rdw9-1	9	2·0	2·0	umc1519	3·29	26·3	0·366
Total dry weight	*tdw3-1*	3	95	93–96	umc1347	2·63	4·1	-0·073
	tdw4-2	4	148	143–158	phi026	3·21	5·5	-0·223
	tdw9-1	9	2·0	2·0	umc1519	10·17	33·3	0·072
WTC								
Plant height	*ph10-1*	10	57	53–62	umc2067	3·06	7·1	−0·061
	ph10-2	10	66	65–71	umc1053	3·23	5·2	−0·062
Shoot dry weight	*sdw4-3*	4	150	150–152	nc005	2·60	5·1	−0·104
	sdw6-1	6	58	49–59	umc1918	2·96	5·4	0·024
Root length	*rl7-3*	7	57	55–62	bnlg657	2·68	4·0	−0·060
Root dry weight	*rdw1*	1	25	18–34	bnlg1811	2·89	4·4	−0·627
Total dry weight	*tdw9-2*	9	2·0	2·0–4·0	umc1519	5·92	31·7	0·045

[a]The first number following the letters represents the chromosome locations of the QTL and the second number represents the orders of the QTL located on the same chromosome by the same trait.
[b]Position of the peak of the QTL in centimorgans.
[c]Range of the QTL above the threshold LOD score.
[d]LOD score calculated by WinQTLCart 2·0.
[e]Percentage of the phenotypic variance explained by genotype class at QTL peak.
[f]Additivity: positive additivity indicates that the high values of the trait were inherited from the tolerant parent ('HZ32'); negative additivity indicatess that the high values of the trait were inherited from the sensitive parent ('K12').

rdw1 and *rdw3* operate in the direction of increasing the WTC of RDW (Tables 4 and 5).

Four QTL were detected to be associated with TDW. Out of the four, two (*tdw9-2* and *tdw9-3*) were common in both experiments. They could explain 31·7 % and 30·7 % of the total phenotypic variation and the primary effect was additive, meaning that alleles from 'HZ32' at *tdw9-2* and *tdw9-3* operate in the direction of increasing the WTC of TDW (Tables 4 and 5). The other two QTL (*tdw3-3* and *tdw3-4*) located on chromosome 3 were detected in EXP.2. They could explain 12·2 % and 12·8 % of the phenotypic variation. The 'K12' alleles at *tdw3-3* and *tdw3-4* increased the WTC of TDW.

QTL groups

Taken together, only six QTL (*rl1*, *ph2-2*, *rl3-1*, *ph4-3*, *sdw6-1* and *sdw6-2*) were not mapped close to other QTL (Fig. 1). The remaining 53 QTL were detected in the same chromosome regions, forming 11 groups. The highest concentration of QTL was found in the *umc1519–umc1231* marker interval on chromosome 9. Other important groups that were found were on chromosomes 3, 4 and 7, where QTL for more than two traits each were detected (Fig. 1). The results indicate that these regions are under related genetic control.

Epistatic effect

Significant epistatic loci ($P \leq 0{\cdot}005$) for WTC and all target traits under control and waterlogging-stress conditions were detected by the QTLmapper (Wang *et al.*, 1999; Table 6). Means of the results from 2004 and 2005 were used as input data for the analysis. In control conditions, a total of eight epistatic interactions were detected for all traits. One significant QTL pair for each trait was detected in *sdw*, *rl* and *tdw*. The epistatic effect (*AAij*) explained 6·78 % to 7·46 % of total variance. Five QTL

TABLE 5. *Map position and main characteristics of QTLs with a LOD score ≥2·5 for plant height (*ph*), shoot dry weight (*sdw*), root length (*rl*), root dry weight (*rdw*), total dry weight (*tdw = sdw + rdw*) and waterlogging tolerance coefficient (WTC = waterlogging treatment of each trait/control of each trait) detected in a F_2 population of maize derived from a cross between waterlogging-tolerant 'HZ32' and waterlogging-sensitive 'K12' in EXP.2 (2005 season)*

Traits	QTL[a]	Chromosome number	cM[b]	Range[c]	Nearest marker	LOD[d]	R^2 (%)[e]	Additivity[f]
Control								
Plant height	*ph1-3*	1	89	68–95	umc1748	4·81	11	1·977
	ph2-2	2	212	199–221	umc1265	3·26	9·4	−1·738
	ph4-2	4	141	136–151	umc1821	3·08	6·5	−0·105
Shoot dry weight	*sdw1*	1	157	157	bnlg1643	2·76	5·7	−0·020
	sdw6-2	6	158	146–159	umc1063	3·82	8·5	0·092
Root length	–	–	–	–	–	–	–	–
Root dry weight	*rdw4*	4	143	141–147	phi026	2·91	6·5	−0·089
Total dry weight	*tdw4-3*	4	143	139–149	phi026	3·24	7·5	−0·320
Waterlogging treatment								
Plant height	*ph1-4*	1	174	164–175	phi011	3·74	7·0	−1·140
	ph2-3	2	161	147–179	phi083	5·10	14·3	−1·661
	ph4-3	4	18	16–20	bnlg292	2·58	10·3	0·130
	ph4-4	4	60	56–64	bnlg292	2·76	6·1	0·239
	ph10-3	10	72	66–79	umc1053	2·67	5·5	0·496
	ph10-4	10	88	84–91	phi063	2·65	5·3	0·347
Shoot dry weight	*sdw4-4*	4	63	60–68	bnlg292	3·06	6·3	0·006
	sdw9-2	9	2·0	2·0	umc1519	2·68	7·2	0·029
	sdw9-3	9	24	12–24	umc1231	3·13	6·7	0·047
Root length	*rl4*	4	161	161–162	umc1117	2·58	5·2	0·004
	rl7-4	7	55	50–62	bnlg1808	2·64	5·5	1·737
Root dry weight	*rdw9-2*	9	2·0	2·0–4·0	umc1519	6·7	36·3	2·609
Total dry weight	*tdw3-2*	3	125	119–133	umc1729	3·61	9·5	−0·754
	tdw7	7	96	87–100	umc1983	2·73	5·4	0·704
WTC								
Plant height	*ph3*	3	114	114–115	umc1729	2·66	5·4	−0·439
Shoot dry weight	*sdw3-2*	3	154	154–155	phi453121	2·68	5·6	−0·087
	sdw9-4	9	0	0–2	umc1519	7·03	20·8	−0·034
Root length	*rl1*	1	136	135–137	bnlg1556	2·74	5·1	−0·037
	rl3-1	3	0	0–6	umc1399	2·80	5·3	−0·702
	rl3-2	3	114	114–116	umc1025	3·49	6·7	−0·035
	rl3-3	3	125	125–128	bnlg1647	2·5	6·1	−0·034
	rl7-5	7	51	50–51	bnlg1305	2·79	5·5	0·031
	rl10	10	102	102–105	mmc0501	2·77	5·6	0·027
Root dry weight	*rdw3*	3	117	117	umc1729	2·67	5·2	−1·955
Total dry weight	*tdw3-3*	3	114	114	phi374118	2·64	12·2	−0·652
	tdw3-4	3	119	117–124	umc1729	2·77	12·8	−0·749
	tdw9-3	9	2·0	2·0–4·0	umc1519	5·91	30·7	0·065

[a]The first number following the letters represents the chromosome locations of the QTL and the second number represents the orders of the QTLs located on the same chromosome by the same trait.
[b]Position of the peak of the QTL in centimorgans.
[c]Range of the QTL above the threshold LOD score.
[d]LOD score calculated by WinQTLCart 2·0.
[e]Percentage of the phenotypic variance explained by genotype class at QTL peak.
[f]Additivity: positive additivity indicates that the high values of the trait were inherited from the tolerance parent ('HZ32'); negative additivity means that the high values of the trait were inherited from the sensitive parent ('K12').

pairs were detected for *ph*, contributing from 4·62 % to 11·81 % of the variance. Three pairs of epistatic loci involved two intervals, both having a significant putative QT locus while the other five QTL pairs involved one putative QT locus. No espistatic QT locus was detected for *rdw*. In waterlogging-stress conditions, ten epistatic QTL pairs were detected. The contribution rates of a single QTL pair varied from 7·6 to 33·47 %. Two pairs of epistatic loci in *sdw* and *tdw* involved two intervals both having a significant putative QT locus, and eight epistatic QTL involved one interval having a significant putative QT locus (Table 6). For the WTC of the used traits, ten epistatic QTL pairs were detected. The contribution rates of a single QTL pair varied from 5·98 to 25·49 %. Four pairs of epistatic loci (one for *ph*, one for *sdw* and two for *rl*) involved two intervals both having a significant putative QT locus, while the other six QTL pairs involved one putative QT locus.

DISCUSSION

Phenotypic variation

Traits of the two parental lines responsive to waterlogging differed considerably when the plants were exposed to waterlogging-stress conditions. These differences disappeared when the plants developed under control conditions.

TABLE 6. *Epistatic loci for plant height (PH), shoot dry weight (SDW), root length (RL), root dry weight (RDW), total dry weight (TDW = SDW + RDW) and the waterlogging tolerance coefficient (WTC = waterlogging treatment of each trait/control of each trait) under control and waterlogging-stress conditions in maize*

Trait	Chromosome interval *i*	Interval *i*	Chromosome interval *j*	Interval *j*	LOD[a]	A^b *i*[c]	R^2 (*Ai*)[d]	*Aj*[e]	R^2 (*Aj*)	AA^f *ij*	R^2 (*AAij*)[g]
Epistasis loci detected under control											
ph	1–6	bnlg1884-umc1906	4–8	umc2027-phi026	9·3	1·97	4·5	0·4	5·4	2·09	8·92
	1–6	bnlg1884-umc1906	4–14	umc1017-umc1232	8·57	1·96	13·0			1·43	11·81
	1–7	umc1906-umc1748	4–8	umc2027-phi026	4·09	2·07	3·45	0·54	4·1	2·43	4·62
	2–14	bnlg1017-phi96100	5–3	umc1097-umc1253	4·28	−1·43	6·35			−1·44	7·18
	2–1	umc2214-umc1736	4–11	umc1117-umc1821	6·04			−0·89	10·2	−2·22	9·42
sdw	1–14	bnlg1643-phi011	4–7	bnlg1621-umc2027	3·59	−0·01	6·2	1·34	5·8	−1·21	6·78
rl	7–6	bnlg1305-phi034	8–3	bnlg1823-umc1607	3·42	1·23	8·4			2·17	7·46
tdw	1–18	bnlg1597-bnlg1597b	4–8	umc2027-phi026	4·95			0·23	6·52	0·28	7·12
Epistasis loci detected under waterlogging stress											
ph	2–10	phi083-bnlg1064	5–9	umc1171-umc1722	7·98	−2·1	8·3			−3·44	11·9
	5–15	bnlg118-bnlg1885	4–3	bnlg2162-umc1667	7·59			0·49	10·56	0·34	11·9
sdw	9–1	umc1519-umc1231	7–5	umc1567-bnlg1305	8·12	0·03	18·9	−0·54	8·9	−0·81	16·8
	5–4	umc1253-bnlg1879	4–11	umc1117-umc1821	5·32			−1·02	9·47	1·26	8·69
	9–1	umc1519-umc1231	10–1	umc1569-umc1084	13·29	0·1	21·89			0·15	19·87
rl	7–6	bnlg1305-phi034	3–2	umc1399-bnlg197	3·56	1·56	8·23			1·87	7·63
rdw	6–7	umc1014-bnlg1617	9–2	umc1231-umc1107	8·66	0·01	7·78			0·02	8·16
	9–1	umc1519-umc1231	10–1	umc1569-umc1084	9·69	0·03	29·6			0·13	30·3
tdw	4–8	umc2027-phi026	4–11	umc1117-umc1821	11·7	0·77	10·81	−0·76	9·45	−0·78	11·46
	9–1	umc1519-umc1231	10–4	umc2067-umc1962	11·05	0·16	29·46			0·21	33·47
Epistasis loci detected by WTC											
ph	4–11	umc1117-umc1821	8–15	bnlg2046-umc1460	7·91	−0·04	7·64			−0·09	6·98
	4–3	bnlg2162-umc1667	8–2	bnlg1056-bnlg1823	5·74	0·35	7·21			0·37	7·86
	3–10	umc1012-umc1729	1–7	umc1906-umc1748	4·82	0·02	9·86	−0·07	5·78	−0·12	8·79
	10–4	umc2067-umc1962	9–4	phi027-phi022	3·56	0·41	5·68			0·45	6·78
sdw	4–3	bnlg2162-umc1667	9–1	umc1519-umc1231	9·58	0·05	24·2	0·03	17·37	0·22	25·49
rl	7–5	umc1567-bnlg1305	1–2	umc1701-bnlg1811	4·23	0·32	6·34	−0·61	5·67	−0·82	7·23
	1–10	bnlg1556-phi039	3–10	umc1012-umc1729	3·89	−0·06	5·96	−0·09	6·89	−0·14	8·37
rdw	3–10	umc1012-umc1729	5–8	bnlg653-umc1171	2·81	−0·09	6·46			−0·19	5·98
tdw	9–1	umc1519-umc1231	10–8	phi052-umc1291	8·7	0·01	19·8			0·02	17·8
	2–4	phi251315-nc003	9–1	umc1519-umc1231	11·7			0·38	19·8	0·97	20·6

The means of 2004 and 2005 were used as input data.

[a]LOD score calculated by QTLmapper 2·0 at $P \leq 0{\cdot}005$ level of probability.

[b]Additive effect.

[c]The estimates of additive effect for testing point *i*.

[d]Percentage of the phenotypic variance explained by the marker genotypes at the locus.

[e]Additive effect for testing point *j*.

[f]Additive × additive epistasis.

[g]Additive by additive effects between the two testing points *i* and *j*.

The data in Table 1 suggest that 'K12' was more severely affected by waterlogging whilst 'HZ32' was more tolerant to waterlogging. In other words, higher values for the traits investigated indicated higher waterlogging tolerance. Since the parental lines were selected on the basis of high or low WTC under artificial control conditions during the maize seedling growing season in our previous study (unpubl. data), this observation indicates that the selection method was efficient in this respect. Moreover, the fact that the favourable alleles for most of the identified QTL were inherited from the waterlogging-tolerant parent also shows the efficacy of the selection method.

The strong phenotypic correlations among traits responsive to waterlogging stress that were observed in the two parental lines and the $F_{2:3}$ families indicated common mechanisms for waterlogging tolerance (Tables 2 and 3). Classical quantitative genetics assumes that trait correlation is a causal effect of pleiotropy or an effect of closely linked genes. Therefore, it would be expected that the QTL for the correlated traits would be mapped in similar genomic regions. In the present study, PH and SDW possess three common genomic regions in chromosomes 1 and 4 (Fig. 1). These morphological characters were also mapped in regions very close to the QTL for TDW in chromosomes 3, 4, 7 and 9 (Fig. 1). PH, SDW and TDW were significantly positive correlated (Tables 2 and 3), which is in agreement with the observations as the QTLs of these characters were mapped in genomically similar regions (Fig. 1). The positive relationships between the other responsive traits also support this hypothesis (Tables 2 and 3). This finding is in line with the opinions expressed by Materechera *et al.* (1992) and Ali *et al.* (2000).

Significant variation and a normal distribution for the traits studied made this population suitable for QTL analysis. Except for RL in EXP.2, the mean values of the population were close to the mid-parental values for all traits in both experiments (Table 1). Although the phenotypic data for $F_{2:3}$ were distributed normally, transgressive segregation was observed in both directions for all traits, indicating that neither of the parents carried all the positive or all negative alleles.

A major QTL for waterlogging tolerance is located on chromosome 9

Major QTL controlling traits associated with SDW, RDW, TDW, the WTC of TDW and the WTC of SDW all mapped to the same region of chromosome 9 and were consistently identified in the both experiments. The expression of waterlogging tolerance is known to be environmentally dependent and genetically complex (Sachs, 1993, 1994; Liao and Lin, 2001; Subbaiah and Sachs, 2003). For other crops, such as rice, in different years and seasons and with different mapping populations, the QTL controlling traits related to waterlogging tolerance have been mapped on many genomic regions (Xu and Mackill, 1996; Toojinda *et al.*, 2003). However, the consistently detected major QTL indicated that this region on chromosome 9 is important in the waterlogging response in this maize population; indeed, the most important waterlogging-tolerance QTL in this study. Moreover, these QTL were only for dry matter accumulation and were not associated with root length and plant height. The QTL were only detected under waterlogging-stress conditions in both experiments, so we presume that there is a specific waterlogging-tolerance responsive gene. It is worthwhile considering the association between the identified QTL controlling waterlogging tolerance and genes known to be regulated by anoxia, which provides us with some genetic evidence that some genes responsive to anoxia may be involved in minor pathways of waterlogging tolerance. According to the IBM2 Neighbour's consensus genetic map, the major QTL on chromosome 9 is located near *sucrose synthase 1*, a known anaerobic response gene (McCarty *et al.*, 1986; Springer *et al.*, 1986; Gupta *et al.*, 1988; Huang *et al.*, 1994; Subbaiah and Sachs, 2003). The gene product sucrose synthase 1 was upregulated as a result of the anaerobic treatment in maize seedlings. Subbaiah and Sachs (2003) demonstrated how a simple post-translational modification of sucrose synthase by the addition/removal of phosphate can lead to potent changes in the tolerance of maize seedlings to anoxia. However, much finer mapping and a gene-specific marker are needed to prove that if this QTL actually is sucrose synthase.

Secondary QTL for waterlogging tolerance

The contributions to waterlogging tolerance of secondary QTL on chromosomes 1, 2, 3, 4, 6, 7 and 10 were all moderate with R^2 values ranging from 3·9 to 14·3 % (Tables 4 and 5). Although the effects of these QTL were small, they were often detected by the CIM procedure for several traits in both environments (Fig. 1).

Eleven QTL for RL were detected in this study. Ten of the 11 QTL were detected under waterlogging-stress conditions, the exception being *rl7-1* in both experiments (Fig. 1). Because RL was more sensitive to waterlogging than other traits (see Results) and its heritability was low, only one common QTL region was consistently detected under waterlogging-stress condition on chromosome 7. The results indicated that the root length QTL did not appear to contribute to root length under control conditions, and it is thus a specifically stress-responsive gene to increase root tolerance to waterlogging.

Eleven common genomic regions were associated with more than one trait in the two experimental seasons (Fig. 1, Tables 1 and 2). Seven of the eleven QTL were detected under waterlogging-stress conditions. The results indicated that the QTL region for numerous coincident traits increase plant growth under waterlogging conditions, whereas loci for only one trait may indicated a more specialized response to waterlogging.

Three of the four QTL for the WTC of SDW received positive alleles from 'K12', which generally has poorer phenotypic values than 'HZ32' (Table 1). This fact indicates that although 'K12' is phenotypically poor, it possesses some QTL alleles capable of increasing the trait value. Similarly, Tanksley and Nelson (1996), Bernacchi *et al.* (1998) and Ali *et al.* (2000) detected QTL alleles

enhancing the trait value from a phenotypically inferior parent in tomato and rice.

Ten QTL mapped to the *umc1299–umc1017* interval on chromosome 4 were detected by PH, SDW, TDW, RDW, RL and the WTC of SDW in EXP.1 and EXP.2 (Fig. 1). Out of the ten, six QTL (*sdw4-2*, *tdw4-2*, *sdw4-3*, *ph4-2*, *rdw4* and *tdw4-3*) were found located in the same regions in both experiments. Although statistical analysis indicated that they appear to be slightly different loci, it may be possible that these QTL in fact represent the same locus because their mode of gene action was similar (Tables 4 and 5), and the peak position of a LOD score can be altered in QTL with moderate (minor) effects due to environmental interactions or statistical error. Tuberosa *et al.* (2002*a*) reported QTL for root traits in a hydroponic system using the maize cross 'Lo964' × 'Lo1016'. They identified three QTL for primary root length and one for adventitious root weight located on chromosome 4. In addition, one QTL for root pulling force located on chromosome 4 was identified by Lebreton *et al.* (1995). The QTL on chromosome 4 for primary root length, root weight and root pulling force are adjacent to the QTL *rdw4* and *rl4*. Mano *et al.* (2005) identified that the QTL controlling adventitious root formation under waterlogged conditions was also located on chromosome 4. This position overlaps with the proposed QTL for maize seedling tolerance to waterlogging in our research (Fig. 1). It appears possible that the QTL identified in our study on chromosome 4 are similar or the same as those in the region controlling these root traits. Wei and Li (2000) reported that waterlogging strongly reduced the growth of adventitious roots and dry matter accumulation of the whole root system. These results are in agreement with the root traits studied in our research (Table 1). The *rl4* locus was only detected under waterlogging conditions, which may indicate that it is a specialized waterlogging-stress-responsive gene controlling root traits. However, *rdw4* was detected under normal conditions, which indicated that it may be unrelated to waterlogging tolerance in this population. Moreover, Tuberosa *et al.* (2002*b*) reported that *bin 1·03* harboured the major QTL for root biomass and leaf growth rate in a drain-pipe experiment carried out on the maize cross 'DTP79' × 'B73'. Mano *et al.* (2006) also reported QTL controlling flooding tolerance in reducing soil conditions in maize seedlings using a cross of the maize inbred lines 'F1649' (tolerant) and 'H84' (sensitive). They identified a single QTL for degree of leaf injury and dry matter production located on chromosome 1 (*bin 1·03–1·04*). One QTL (*rdw1*) associated with the WTC of RDW and two QTL associated with PH were also detected on chromosome 1 (*bin 1·03–1·05*) in the present study (Fig. 1).The identification of similar genetic regions suggests that similarity exists among these crosses in the genetic control of root dry matter accumulation. Further research is needed to test this possibility.

Seven QTL associated with PH, RL and TDW were found within the *bnlg1808* and *phi034* interval on chromosome 7. Out of the seven, four QTL only associated with RL were identified under waterlogging-stress conditions. Although statistical analysis suggested that these QTL appear to be slightly separated at different loci, it may be possible that they actually represent the same locus, as also their mode of gene action was very similar. A similar result was also found on chromosome 10 (Fig. 1, Tables 4 and 5). Several genomic regions were found where the different traits measured were under related genetic control. Five QTL (*ph-3*, *rl3-2*, *rdw3*, *tdw3-3* and *tdw3-4* on chromosome 3) for PH, RL, RDW and TDW were identified under waterlogging-stress condition in EXP.2, and all appeared to be located on the same region on chromosome 3. Most of these expressed considerable negative-additivity, indicating that favourable alleles originated from 'K12'. Although no co-location of QTL was detected between EXP.1 and EXP.2, the results indicated that QTL controlling some of the traits were on the same linkage group and sometimes close together, and genetic correlations between the traits also confirm these relations (Tables 2 and 3).

Epistasis, or interlocus interaction, is a kind of gene interaction whereby one gene interferes with the phenotypic expression of another non-allelic gene. A considerable body of classical work has strongly suggested the prevalence of an epistatic effect on quantitative traits in genetic populations (Spickett and Thoday, 1966; Allard, 1988). In the present study, most of QTL were detected in both of the two experimental years and no epistasis-by-environment interaction was detected. This indicates that a highly coherent detection of QTL was achieved between the experiments conducted in the two years. In addition, no identical epistatic QTL pairs were found to be responsible for the target traits. These results suggest that different epistatic systems control the target traits under different water-supply conditions, and that epistasis explained a considerable portion of the total genotypic variances of the measured traits and their WTC (Table 6).

In this study, most of the QTL identified by the waterlogging-response traits were located in clusters on chromosomes 3, 4, 7 and 9. Fine-mapping and trait dissection using near-isogenic lines should make it possible to determine a detailed mapping position for waterlogging tolerance QTL on chromosome 9. Once confirmed, such markers – tightly linked to waterlogging tolerance, especially to the major QTL on chromosome 9 – would facilitate the development of waterlogging-tolerant elite maize varieties and positional cloning. A concern is that the overall percentage of phenotypic variation explained by these markers remains relatively low and that other genomic regions may well also play a major role. However, this research indicates that the first steps have been set.

CONCLUSIONS

To the best of our knowledge, there has been no other report on QTL analysis of maize seedling waterlogging tolerance except for Mano *et al.* (2006). We have identified several QTL with genome-wide significance, suggesting that our approach is useful to elucidate the genetic mechanisms underlying maize waterlogging tolerance. The use of different populations and/or evaluation methods should allow us

to detect novel QTL in further studies. Although the detected regions need to be mapped more precisely, the findings and QTL found in this study may provide useful information for marker-assisted selection (MAS) and for further genetic studies on maize waterlogging tolerance.

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