World Journal of Agricultural Research, 2017, Vol. 5, No. 3, 126-134
Available online at http://pubs.sciepub.com/wjar/5/3/2

DOI:10.12691/wjar-5-3-2

Identification of Major QTLs in an Advanced Backcross Lines Associated with Waterlogging Tolerance at Maize Seedling Stage

Khalid A. Osman[1,2], Bin Tang[1], Fazhan Qiu[1], Ahmed M. El Naim[3,*]

[1]National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, China
[2]Agricultural Research Corporation, P.O Box 126 Wed Medani, Sudan
[3]Department of Crop Sciences, Faculty of Natural Resources and Environmental Studies, University of Kordofan, Elobied, Sudan
*Corresponding author: naim17amn@yahoo.com

Abstract Waterlogging strongly affects agronomic performance and yield of maize. In order to investigate the genetic basis of maize seedling response, remapping of the majors quantitative trait loci (QTL) associated with waterlogging tolerance (WT) related traits were subjected, including plant height, root length, shoot fresh weight, root fresh weight, root dry weight, shoot dry weight, total dry weight, during maize seedling stage by using advanced backcross QTL (AB-QTL) analysis approach in a mixed linear model and inclusive composite interval mapping method under waterlogging and control conditions. A 266 BC_2F_2 population derived from a cross between a waterlogging-tolerant line 'HZ32' and a susceptible line 'K12' was used. A new linkage map constructed, consisting of 167 polymorphic SSR markers, spanned 1797.6 cM in length across a maize genome, with an average distance of 10.8 cM between adjacent markers. A total of 44 and 25 putative QTLs were detected under waterlogging treatment and control conditions, respectively. These QTLs were distributed over all 10 chromosomes, and had LOD scores ranging from 2.58 to 14.74, explaining 3.46 to 24.37% phenotypic variation in the individual traits. Out of which, thirty one major QTLs individually accounted for more than 10% of the phenotypic variation; they were governed traits associated with RL, PH, SDW, RDW, TDW and RFW were mapped in the different genomic region on chromosomes 1, 2, 3, 4, 6, 7 and 9. The results reveal that the former major QTL mapped by AB-QTL, could be selected in backcross population for fine mapping of waterlogging tolerance. The results also may provide new insight into the molecular basis of the waterlogging response of seedlings stage and useful markers for MAS and further genetic studies on maize waterlogging tolerance.

Keywords: *AB-QTL mapping, Maize (Zea Mays), SSR marker, MAS, waterlogging stress*

Cite This Article: Khalid A. Osman, Bin Tang, Fazhan Qiu, and Ahmed M. El Naim, "Identification of Major QTLs in an Advanced Backcross Lines Associated with Waterlogging Tolerance at Maize Seedling Stage." *World Journal of Agricultural Research*, vol. 5, no. 3 (2017): 126-134. doi: 10.12691/wjar-5-3-2.

1. Introduction

Waterlogging is one of the most important constraint factors for maize production and productivity in tropical and subtropical regions around the world [1]. As the global climate is continuously changing, waterlogging is becoming a matter of prime importance for agricultural productivity and global food security [2]. The main cause of damage under waterlogging is oxygen deprivation, which affect nutrient and water uptake, so the plants show wilting even when surrounded by excess of water. Lack of oxygen shift the energy metabolism from aerobic mode to anaerobic mode[3]. In some part of Africa South-East Asia, China, Australia and united states, about 30% of all maize growing areas have been affected via flooding problems during the seedling stage and causing 20-30% yield losses [4]. Hence, new maize varieties with greater adaptation to waterlogging are essential to increase maize productivity in waterlogged soil. The development of waterlogging-tolerant varieties with high yield potential should be one of the main aims of many maize breeders [5].

In recent years, more knowledge has been accumulated on the molecular, biochemical, physiological, morphological, anatomical and metabolic responses to waterlogging and oxygen deficiency in plants [6]. Waterlogging tolerance was found to be a quantitative trait and mainly governed by additive genetic variation [7]. Different traits have been used as indirect selection indices for waterlogging tolerance, used by scientists in maize [8]. In addition, significant genetic variability has been observed in the tolerance of maize to waterlogging stress [5]. This variability could be exploited to develop maize varieties tolerant for waterlogging stress during the rainy season in the tropics region. With the development of DNA markers and QTL mapping methodologies, QTL analyses of waterlogging tolerance have been studied in several crops [9]. Recently, several QTL mapping studies of waterlogging tolerance have been reported in maize and its wild relative, Zea luxurians and Zea nicaraguensis.

Previous studies showed that the early stage of maize development is the most sensitive stage to waterlogging, especially from the second leaf stage (V2) to the seventh leaf stage (V7) [10]. A larger number of QTLs for waterlogging tolerance related traits have been identified during the maize seedling stage, such as root and shoot development-associated traits [4], capacity for root aerenchyma formation [11], adventitious root formation [12], tolerance to toxins under reducing soil conditions and leaf injury [13].

To date, only two major QTLs in rice, Sub1A and Snorkel have been map-based cloned, which were found to encode ethylene-responsive factor-type transcription factors involved in gibberellin biosynthesis or signal transduction. Waterlogging tolerance of maize seedlings is a polygenic trait and is highly influenced by environment. Significant genotype by environment interaction could be detected by comparing QTLs identified in multiple environments and QTL with consistent expression across environments is required for MAS breeding [14]. In order to achieve this goal, they conducted the markers and phenotype analysis in advanced backcross generations. It is expected that through the introgression of new exotic QTL alleles, the AB strategy will contribute to an increased level of genetic diversity in their modern crop varieties. To date, several reports on the application of the AB-QTL strategy are available for tomato, rice and many other crops [15]. A BC_2F_2 population developed from an interspecific cross between waterlogging tolerant (HZ32) and sensitive (K12) was used in an advanced backcross QTL analysis to identify and introduce waterlogging tolerance useful genes from tolerant line into the sensitive one. The information generated in the present study will be useful to developing waterlogging-tolerant elite maize lines through molecular marker assisted selection and fine mapping.

2. Materials and Methods

2.1. Plant materials and Genotyping

A BC_2F_2 mapping population was developed from a cross between two maize inbred lines, 'HZ32' (waterlogging tolerant) and 'K12' (highly waterlogging-sensitive) as the donor and recurrent parent respectively. The two parents were selected based on their morphological and physiological criteria, [34]. F_1 plants were grown in the experiment field, and the several most vigorous F_1 plants were backcrossed to ' K12 ' (as the male). BC_1F_1 plants were obtained, which were grown in the field. The best individuals based on phenotypic selection were backcrossed a second time to K12 to produce BC_2F_1 seeds. They were grown in the field to produce BC_2F_2 and subsequently families. The seeds of the 266 $BC_2F_{2:3}$ families derived from the corresponding BC_2F_2 selfed-plants were utilized to conduct the waterlogging pot experiments. Total genomic DNA from BC_2F_2 plants and two parental lines were isolated from young leaf tissue following a standard CTAB extraction method [35] with minor modifications according to Qiu *et al.* [4]. The modifications in the procedure were addition of boiled CTAB extraction buffer to the 50 mL polypropylene centrifuge tube, and a reduction of the incubation time to 30 minutes.

2.2. Pot Experiments and Phenotypic Measurements

The pot experiments were carried out under glasshouse conditions at Huazhong Agricultural University, Wuhan, China (114°36′E and 30°47′N) in 2012. The day/night temperatures were 33/17°C, with a photoperiod of 13/11 h. Two experiments, EXP.1 and EXP.2, were arranged in a randomized complete-block design, in the spring and autumn at maize-seedling growing seasons. Two pots were included for each replication per genotype with one the control and the other of waterlogged treatment. Twelve seedlings per pot were included in each replication. The average values of 12 seedlings of the $BC_2F_{2:3}$ lines were considered to represent the phenotypes of the BC_2F_2 plants. The seeds of the $BC_2F_{2:3}$ lines were planted in each pot of 20 cm in diameter and 30 cm in depth filled with 3.5 kg of sieved, sterilized dry field soil. The waterlogging treatments were conducted at the second leaf stage after 7 d of normal growth, and each pot was filled with 2-3 cm water above the soil surface and this water level was maintained until harvest. The controls were irrigated as needed to avoid drought stress or waterlogging stress. Twelve seedlings of each replication per genotype were harvested for trait scoring under waterlogged and control conditions. Phenotypic data of both pots experiments were evaluated at 6 days after the initiation of waterlogging treatment. The sampling method, drying, treatment and traits measured were performed. Seven waterlogging-related traits, including plant height (PH), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW) root dry weight (RDW), and total dry weight (TDW = SDW + RDW) were measured on a family basis under the waterlogging and control conditions. PH was measured in a centimeter (cm) from the base of the culm to the tip of the longest leaf. RL was also measured in a centimeter (cm) from the base of the culm to the tip of the longest root. Roots fresh and shoots fresh of each pot were measured at electronic balance (MP500B), then separately bulked together and put them 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 at electronic balance.

2.3. Markers Development and (SSR) GENOTYPING

Primer sequences of 1052 SSR markers were obtained from the Maize Genetics and Genomics Database (www.maizegdb.org). Polymerase chain reaction (PCR) amplifications of DNA markers were performed in a T1 Thermocycler Module 96 (Biometro, Goettingen, Germany). Each amplification reaction contained a volume of 20 µl, consisting of 6 µl of genomic DNA (10 ng/µl), 1.5 µl of $MgCl_2$ (25 mM), 0.5 µl of dNTP mixtures (10 mM), 2 µl of 10×PCR buffer, 1.2 µl each primer pair (5 µM), 0.12 µl of Taq polymerase (5 units/µl) and 7.48 µl of double-distilled water. PCR parameters were as follows: 94 °C for 5 min, and 31 cycles of 40 s at 94 °C, 45 s at

58°C, 50 s at 72°C, then 5 min at 72°C. PCR products of the amplified DNA fragments were separated on 6% denatured polyacrylamide gel electrophoresis (PAGE) in 0.5 × TBE buffer, followed by silver staining.

2.4. Statistical analysis

Analyses of variance (ANOVAs) were performed using the general linear model (GLM) procedure of the SAS program 'PROC MIXED' ver. 8.02. To calculate the adjusted means and the broad-sense heritability (h^2) of the families, the heritability was computed as:

$$h^2 = \delta_g^2 / \left(\delta_g^2 + \delta_e^2 / n\right)$$

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 $BC_2F_{2:3}$ families for all traits were performed using the univariate procedure of SAS and normal distributions were checked using for skewness and kurtosis. Simple Pearson correlation coefficient (r) was calculated using the 'PROC CORR' option of the SAS program among seven different seedling traits.

2.5. Linkage Map Construction

Genotypic data of the BC_2F_2 population were collected with 167 SSR markers possessed clear and stable polymorphism in both parents, molecular linkage maps were constructed using Mapmakers 3.0 [18] at a cutoff recombination fraction of 0.375, threshold logarithm of odds (LOD) score of 3.0 and the Kosambi function for estimation of map distances (cM). QTL mapping was using a method of ICIM-ADD for additive mapping described by Li *et al. [16]*. The LOD score for declaring a QTL was 2.5 for each trait through a permutation of 1000 times and the walking speed for all QTLs was 1 cM. For each trait, some marker intervals were revised if the position of multiple QTL peaks was < 10 cM apart and regarded as a single QTL. Graphical representation of linkage maps was drawn using MapDraw Version 2-2 [17].

3. Results

3.1. Phenotypic Variation of $BC_2F_{2:3}$ Families

The values of PH, RL, SFW, RFW, SDW, RDW and TDW under waterlogging stress were significantly lower than those for controls. Genetic variation was significant ($P < 0.05$) for all traits investigated under different moisture regimes, with the exception of the PH, SFW, RFW, RDW and TDW. ANOVAs showed significant difference in environmental variation for all traits under waterlogging stress; a variation was due to waterlogging treatment, that indicating strong genetic effects (Table 1).

The broad sense heritability's (h^2) of the various phenotypic traits were relatively high across the two pot experiments (Spring and Autumn seasons), ranging from 0.65 to 0.83 for the control of SDW and RFW respectively and for .66 to .77 under waterlogging stress (Table 1). The high h^2 implied that most of the phenotypic variance for each trait was genetic and could be effectively improved by selective breeding programs. Comparing h^2 of the seven investigated traits showed that most of the measured traits under control conditions were always very similar or high to the waterlogging treatments, In other words, usually less than those calculated for phenotypic traits of SFW, SDW and TDW under waterlogging treatments.

Correlation coefficients between pairs of all seven seedling traits were calculated under waterlogged, normal conditions in the two seasons separately (Table 2). Significant positive associations ($P < 0.01$) occurred among seven seedling traits measured for waterlogging and control conditions. In addition, highly significant positive correlations were observed in both Experiments, for RDW, RFW, and SDW (Table 2). However, a weak relationship was observed between RL and RDW and no relationship was found between RL and RFW, SDW, RDW and TDW in Experiment 2 under control and waterlogging treatment. Suggested that an expression of RL is sensitively to environmental factor.

Table 1. Broad sense heritability (h^2) of seedling traits observed under waterlogging, normal condition and their WT condition in the two experiments

Treatments	Control			WT[a)]		
Traits	VG[c)]	VE[d)]	$h^{2e)}$	VG	VE	h^2
Plant height(cm)	8.57^{***}	5.081^{ns}	0.77	4.632^{*}	3.685^{***}	0.72
Root length(cm)	15.947^{***}	9.206^{***}	0.78	7.286^{**}	5.238^{***}	0.74
Shoot fresh weight(g)	0.109^{***}	0.081^{ns}	0.73	0.041^{***}	0.029^{***}	0.74
Root fresh weight(g)	0.072^{*}	0.030^{**}	0.83	0.032^{**}	0.019^{***}	0.77
Shoot dry weight(g)	0.001^{***}	0.001^{***}	0.65	0.001^{***}	0.001^{***}	0.66
Root dry weight(g)	0.001^{*}	0.001^{***}	0.80	0.001^{**}	0.001^{***}	0.76

WT = waterlogging tolerance; [d-e)] ANOVA results for the effect of $Bc_2F_{2:3}$ families(Genotypes variance (VG), seasonal variance (VE)). Differences between the mean values were significant at $P<0.05$ (*), 0.01 (**), 0.001 (***), or not significant. [f)] The heritability was computed as $h^2=\delta^2_g/(\delta^2_g+\delta^2_e/n)$, where δ^2_g andδ^2_e were the estimates of genetic and residual variances and n was the number of replications.

Table 2. Simple correlation coefficients among traits measured in the $BC_2F_{2:3}$ families, the above diagonal referred to Exp.1 and the below diagonal noted to Exp.2 for control, WT and WTC

conditions	traits	PH	RL	SFW	RFW	SDW	RDW	TD
Control	PH		0.414**	0.391**	0.745**	0.173**	0.702**	0.463**
	RL	-0.077^{ns}		0.299**	0.240**	0.159*	0.292**	0.271**
	SFW	0.525**	0.149*		0.563**	0.746**	0.565**	0.801**
	RFW	0.744**	-0.167^{ns}	0.689**		0.285**	0.859**	0.628**
	SDW	0.427**	0.106^{ns}	0.741**	0.602**		0.305**	0.843**
	RDW	0.728**	-0.122^{ns}	0.743**	0.836**	0.626**		0.720**
	TDW	0.606**	0.023^{ns}	0.808**	0.777**	0.918**	0.851**	
WT	PH		0.417**	0.429**	0.550**	0.193**	0.554**	0.393**
	RL	0.030^{ns}		0.219**	0.086^{ns}	0.015^{ns}	0.136*	0.049^{ns}
	SFW	0.554**	0.149*		0.568**	0.737**	0.582**	0.784**
	RFW	0.771**	-0.14^{ns}	0.629**		0.352**	0.769**	0.644**
	SDW	0.494**	0.068^{ns}	0.639**	0.535**		0.432**	0.847**
	RDW	0.717**	-0.164^{ns}	0.601**	0.862**	0.513**		0.826**
	TDW	0.669**	-0.011^{ns}	0.733**	0.750**	0.915**	0.798**	

The significance of correlation coefficient at P＜0.05 (*), 0.01 (**), 0.001 (***), and non-significant (ns). PH = plant height; RL = root length; SFW = shoot fresh weight; RFW = root fresh weight; SDW = shoot dry weight; RDW = root dry weight; TDW =total dry weight. WT = waterlogging treatment.

3.2. Construction of Genetic Maps

The linkage map constructed for the BC_2F_2 population of HZ32 × K12, consisting of one hundred and sixty-seven polymorphic markers between the parental lines, spanned 1797.6 cM with an average distance of 10.8cM among markers (Figure 1). Comparing them with the physical positions of the maize chromosome bin map, polymorphic markers resulted in coverage of 90 bins, indicating the map had good coverage of maize's 10 chromosomes. The linear order of SSR markers on the linkage map was in good agreement with previously published maize IBM 2008 Neighbors maps, and no inversion in marker order was observed. The parental genotypes in the 266 BC_2F_2 mapping population showed a normal distribution, indicating the suitability of this population for genetic map construction and QTL mapping.

3.3. QTLs Detection

Considering QTL mapping for the seven waterlogging-response traits by ICIM-ADD mapping, in both experiments (Figure 1). Forty-four and 25 putative QTLs were detected under waterlogging treatment and control conditions, respectively. These QTLs were distributed over all 10 chromosomes, and had LOD scores ranging from 2.58 to 14.74, explaining 3.46 to 24.37% of the phenotypic variation in the individual traits. The effect of individual QTL was generally medium to major and 20 QTLs individual accounted for more than 10% of phenotypic variance, Out of these 11 QTL individually accounted for more than 15 % of the phenotypic variation.

Plant height (PH) in BC_2F_2 population was influenced by 10 genomic regions under waterlogging treatment and control conditions, on six chromosomes; 3(*qph3-1* and *qph3-2*), 4(*qph4*), 5(*qph5*), 7(*qph7*), 9(*qph9-1, qph9-2, qph9-3* and *qph9-4)* and *10(qph10*). Out of these 10 QTLs identified, four and six were detected in EXP.1 and EXP.2, separately. Individual QTL accounted for 5.48–24.37% of the phenotypic variation. For three of the QTL (*qph4, qph5* and *ph7*), alleles from 'K12' contributed towards an increase of the trait values. For the other seven QTL alleles from 'HZ32' tended to increase the trait value. Two major QTLs i.e., *qph9-3* and *qph9-2* were identified at LOD value >11.0 explaining 16.16 and 24.37% of phenotypic variation respectively, in both experiments, 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.

Root length (RL) was governed by 14 QTLs were identified under waterlogging treatment and control conditions on all 10 chromosomes, except for chromosomes 3 and 10. Among them, four QTLs expressed over two experiments and 10 QTLs were located specifically. These QTLs were accounting for 3.50-9.54% of the phenotypic variation. HZ32 contributed trait-enhancing alleles for three QTLs and four QTLs for K12 under waterlogging treatment. Of 14 QTL associated with RL only two QTLs (*qrl8-2* and *qrl8-3*) were mapped in the same genomic region for both waterlogging and control conditions, at marker interval umc1777-umc1149, their positive alleles were from HZ32; indicating that the same genetic elements may control gene expression.

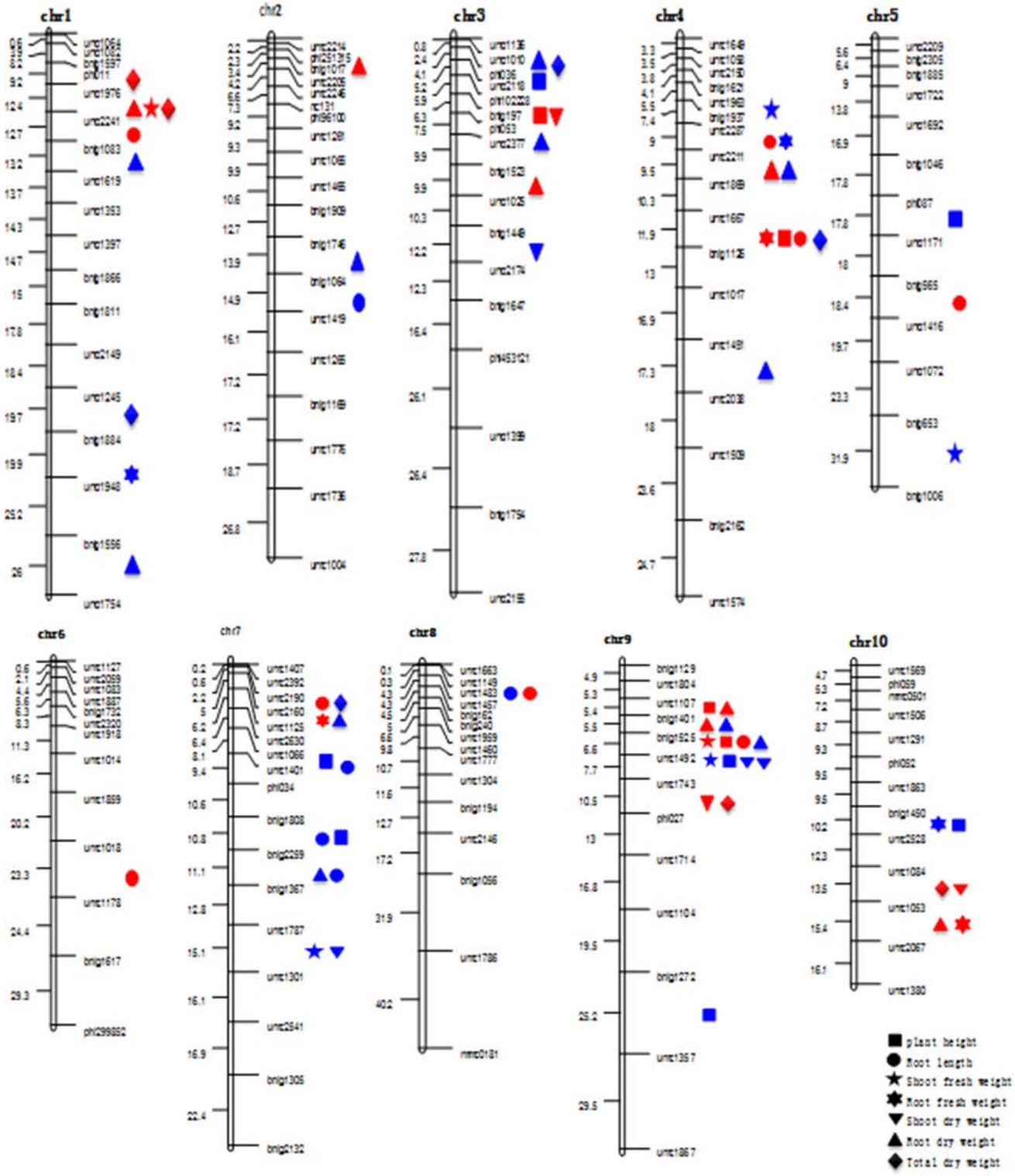

Figure 1. Molecular linkage map of the BC_2F_2 population derived from a cross between 'HZ32' and 'K12', and summary of QTL for all traits responsive to waterlogging in the mapping population. Which are shown at the right side of each chromosome in different shape of a traits and different colors for different experiments (red: Exp.1; blue: Exp.2 of each trait). The distances between markers (cM) are listed to the left of each figure part

Seven putative QTLs for Shoot fresh weight (SFW) were detected, five under waterlogging treatment and two under normal conditions. These QTLs were distributed over five of the 10 chromosomes. Of the seven QTL associated with SFW, two and five were found in EXP.1, EXP.2, separately. Individually accounting for 4.48 to 9.15% of the phenotypic variation. The parent K12 contributed enhanced effect at four QTLs whereas HZ32 made positive contribution at three QTLs positions that tended to increase the SFW. Five QTLs affecting Root fresh weight (RFW) were located on three different chromosomes, under waterlogging and control conditions. Explaining 4.00 - 14.72% of phenotypic variation. Out of these QTLs identified, two loci (*qrfw4-1*, and *qrfw10-1*) with negative-additive effects were contributed by the parent K12 whereas, HZ32 contributed three QTLs (*qrfw1*, *qrfw4-2* and *qrfw10-2*), tended to increase the trait values. Shoot dry weight (SDW) were influenced by six QTLs under waterlogging treatment and one under normal condition; on four chromosomes 3(*qsdw3-1* and *qsdw3-2*),

7(*qsdw7*), 9(*qsdw9-1*, *qsdw9-2* and *qsdw9-3*) and 10 (*qsdw10*); were explained variances in the range of 7.35–14.57%. Out of seven QTLs influencing the trait, 'HZ32' alleles contributed to increase the SDW at five loci (*qsdw3-1, qsdw3-2, qsdw9-1, qsdw9-2,* and *qsdw9-3*), 'K12' alleles contributed to increases at the other loci. A total of eighteen QTLs influencing root dry weight (RDW) were mapped under waterlogging treatment and control conditions on seven chromosomes (1, 2, 3, 4, 7, 9 and 10) in the present study. Out of which, 11 QTLs expressed in experiment two, indicating their sensitivity to environmental changes. Out of the remaining six were detected in experiment 1 (Figure 1). The HZ32 allele contributed increased effect at five QTLs under waterlogging condition and the K12 allele at six QTL positions. The QTL affecting RDW, *qrdw1-3, qrdw3-3, qrdw4-3, qrdw7, qrdw2* and *qrdw9-3*, explained 17.61%, 18.02%, 20.05%, 20.33%, 21.87% and 23.81% 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 a good agreement with the high heritability estimates of this trait in Table 1. The HZ32 allele contributed to trait increase at nine loci. Single QTL explained 4.42-23.81% of phenotypic variation and the K12 allele at nine loci explaining 8.75%-21.87% of phenotypic variation.

Total dry weight (TDW) was influenced by three QTLs under normal condition, distributed on chromosomes 1, 4, and 7, were detected in both experiments. Individual QTL had values of R^2 ranging from 4.29–8.20%, and the primary effect was positive additive, meaning that alleles from 'HZ32' at all three QTLs operate in the direction of increasing the trait values. There were five QTLs controlling TDW under waterlogging treatment, and the additive effects were positive at two loci, mapped on chromosomes 1(*qtdw1-2*) and *9*(*qtdw9*). Out of which, three loci were involved in TDW with negative-additive effects located on chromosomes 1(*qtdw1-2*), 3(*qtdw3*) and 10(*qtdw10*), meaning that parental contribution of allele tended to increase the trait values. The contributions to phenotypic variations for a single QTL varied between 4.49 and 10.22%, with *qtdw10* recording the highest contribution. Out of the eight, two QTLs were detected in experiment 2, the other six QTLs were found in experiment 1, no common QTL was found between the two seasons, indicating their low sensitivity to environmental changes.

3.4. Co-localization of QTLs for Different Traits

Taken together, about 85 % of these QTL were co-located with at least one other QTL, forming eight QTL hotspots that controlled part of the variation for at least two different traits (Figure 1). The highest concentration of QTL was in the marker interval bnlg1126–umc117 on chromosome 4. Other impressive clusters of QTL were found on chromosomes 3, 7 and 9. For example, one genomic region with QTL co-localized for more than three traits were detected on chromosomes 3 under control conditions. Three QTL hotspots were found on chromosomes 4 and 7 under waterlogging treatment, where QTL for more than three traits each were detected. The results indicated that these regions were under related genetic control. These traits all had highly significant positive associations with each other. In general, the favorable alleles from most QTLs clusters had the same direction of effect in all traits, but had opposite genetic effects at the other loci on several chromosomes. Although common genetic mechanisms could exist for these traits, selection for beneficial alleles at all loci might be intricate due to the variability of various QTLs' effects.

4. Discussion

4.1. Phenotypic Variation

The present study reports on the genetic dissection of seven waterlogging related traits. An advanced backcross population was utilized for a straight-forward detection and introgression of favorable exotic alleles in the 'K12' background according to [15]. Our data showed a significant variation of traits among the BC_2F_2 lines in both experiments. An attempt was made to investigate the correlations coefficient (*r*) among traits responsive to waterlogging stress that were observed in the $BC_2F_{2:3}$ families. The strongest relationship among the trait indicated common mechanisms for waterlogging tolerance. 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 work, PH, RL, SFW, SDW, RDW and TDW, possess common genomic regions in chromosomes 1, 3, 4, 6, 7 and 9. These morphological characters were significantly positive correlated, which is in agreement with the observations of the QTLs of these characters were mapped in genomically similar regions. This finding is on line with the opinions expressed by Ali *et al.*, [19].

4.2. Main-effect QTL Detected for Waterlogging Tolerance

For declaring the significant association of the chromosomal region with trait, thresholds levels for each trait at each experiment were separately computed by conducting permutation test with 1000 a permutations and used for intensive composite interval mapping (ICIM). A total of 69 QTLs were detected including several QTLs expressing across two experiments, the phenotypic variance explained by these QTLs ranged from 3.46% for root dry weight to 24.37% for plant height. The extent of complex nature of the traits was evident from the observation on number of significant QTLs per trait [20]. The percent contribution of individual QTLs to total phenotypic variation for respective traits suggested a complex inheritance pattern of the traits under study.

According to a major QTL explaining > 15% of the phenotypic variance in primary mapping [21], a major QTLs controlling traits associated with PH and RDW were mapped in a different genomic region on chromosomes 1, 2, 3, 4, 7 and 9. Which consistently identified in the both experiments the expression of waterlogging tolerance is known to be environmentally

dependent and genetically complex [22]. 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 [23]. However, the consistently detected major QTL indicated that these regions on 9 chromosomes were important in the waterlogging response in BC_2F_2 maize population; indeed, the most important waterlogging-tolerance QTL in this study.

Nine out of 11 major QTLs were only for dry matter accumulation (RDW), most of them were detected under waterlogging-treatment conditions in both experiments. This result was online with Qiu et al [4], so we presume that there is a specific waterlogging-tolerance responsive gene. It is sufficiently 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. Most major QTLs affected RDW under waterlogging tolerance condition were detected on chromosomes 2,4 and 9 explained highly of the phenotypic variance and two on chromosomes 9 bin (9.02), account in total 48.18% of phenotype variation, all with positive-additive effect allele from tolerance parent 'HZ32', this confirm the result was found by Zhang *et al.,[24]*, reported that according to the IBM 2008 Neighbour's consensus genetic map, the major QTL on chromosome 9 is located near *sucrose synthase 1*, a known anaerobic response gene [25]. The gene product sucrose synthase 1 was up-regulated as a result of the anaerobic treatment in maize seedlings. Subbaiah and Sachs [25] 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.

Two major QTLs (*qph9-2* and *qph9-3*) governed plant height trait, located on chromosome 9, explaining 24.37% and 16.16 of phenotypic variation respectively, with HZ32 allele was detected under normal and waterlogging treatment conditions. This result suggested that plant height is an important stable selection criterion for maize waterlogging tolerance, which can be used practically in breeding programs.

Many earlier studies have also found that QTL alleles enhancing a trait value originated from a phenotypically inferior parent, in maize under various abiotic stresses, such as waterlogging [4], drought [26], low nitrogen [9], low phosphorus [27], cold [28]. Advanced backcross-QTL analysis in Tomato [15], [15]; [29] and QTL mapping in rice [19]. These loci would provide the needed diversity for the trait in maize breeding programs.

4.3. QTL Associated with Multiple Traits for Waterlogging Tolerance

Previous maize studies showed that correlated traits shared regions associated with QTL [16]. In this study, a total of 69 QTLs were detected for the seven waterlogging related traits in both experiments (Figure 1). Of these loci, nine almost 85% were associated with QTL for two to eight traits, and localized on chromosome region bins 1.06, 3.05, 4.03, 7.02, 7.03and 9.2-3, 9.04, 9.05 (Figure 1). It is very interesting to examine co-locating QTLs in biological and breeding perspective while considering phenotypic and genetic correlations. One of the central concepts in genetical genomics is the existence of QTL hotspots, where a single polymorphism leads to widespread downstream changes in the expression of distant genes, which are all mapping to the same genomic locus [30]. The incidence of QTL clusters in similar genomic regions reflected trait associations. Cai and Morishima [31] suggesting that possibility of the pleiotropic effects a single or closely linked genes might control plant development under waterlogging conditions and make an important contribution to enhancing tolerance to waterlogging. The QTL clusters could be deployed for improving waterlogging tolerance in maize through MAS. By comparing locations within chromosome bins of these QTLs clusters, several major QTLs for waterlogging tolerance-related traits were identified in previous studies, they located near or the same chromosome regions in the present study.

The co-localized QTLs for PH and RFW, with positive additive effect mapped to the umc1754- bnlg1556 interval on chromosome 1 bin (1.06) were located on the same region of a major QTL (*Qaer1.06*) for aerenchyma formation under non-flooded conditions [14] Using an F_2 population, most of the QTLs identified by the waterlogging-response traits were also clustered in the chromosome region bins 4.03-4.05, 7.02 and 9.04 [4]. Also Mano *et al.,* [13] detected three QTLs for aerenchyma formation under flooding stress on two regions of chromosome 1 (*Qaer1.06*, bin 1.06 and *Qaer1.11*, bin 1.11) and one on chromosome 5 (*Qaer5.09*, bin 5.09) in BC2 population, in our study, five major QTLs were detected, related to root trait in the region (bin 1.11), umc2149-umc2241 interval markers, out of these, *qrl1-1* and *qrdw1-2* with alleles from 'HZ32'. The results indicated that these regions were under related genetic control.

Four QTL On the chromosome 3, region (bin 3.6) clustered to the phi102228-bnlg197 interval markers and other three QTLs were co-located in the umc2155-bnlg1449 interval markers, which related to five traits RL, RDW, SDW, PH and SFW were found to share the same map location with a major locus for adventitious root formation under flooding conditions (Figure 1). In the other words, several important root-QTL clusters were localized on chromosome region bins 4.08 7.02, and 9.04 (*qrl9-1* and *qrl9-2*) under waterlogging treatment conditions in both experiments (Figure 1), this finding on line with previous reported of Salvi *et al.* [21], Mano *et al.* [12] and Qiu *et al.* [4]. Indicated that, specific stress-responsive gene to increase root tolerance to waterlogging in these regions. Recently, Zhang *et al.,* [24] identified a major QTL in bin 5.04 that controls waterlogging response in PH, using a genome-wide association study of 144 maize inbred lines. In our study, there three QTLs co-localized for PH, RL and SFW were also detected in the marker interval bnlg653-umc1171 on chromosome 5 (bin 5.04). That indicated specific stress-responsive gene to increase plant height and root tolerance to waterlogging in this region. In this study, multiple QTL clusters affecting

many traits were identified of which; some are either genetically correlated or all ometrically related. It is very difficult to speculate the causative mechanism between all these traits in a hotspot as correlations do not suggest link between them. It is possible that these clusters represent more than one gene but the present mapping population resolution is not sufficient to differentiate whether it is due to either linkage or pleiotropy. It is observed that some hotspots contain QTLs that are not all ometrically linked. It may possible that these loci represent *trans* acting QTL (most likely transcription factors) where the effect of alterations in regulation or structural characteristics would be expected to have smaller effects on many traits [32]. It can be concluded that each QTL within a QTL hotspot might only contribute a small positive effect, however co-location of multiple traits indicate that selection for beneficial allele at these loci will result in a cumulative increase in waterlogging tolerance due to the integrative positive effect of various QTLs. The result demonstrated that AB-QTL analysis of waterlogging responses traits in maize not only confirmed known waterlogging-tolerance loci, although highlighted the utility of this approach in mapping novel tolerance loci. With the increase of QTL numbers identified for waterlogging-response traits in different experiments, the genetic basis of maize waterlogging tolerance will be become much clearer with confirmed the previous works.

The expression of waterlogging tolerance in maize is genetically complex and influenced by environmental factors and it is difficult to accurately estimate epistatic QTLs and QTL × environment interaction effects in the present study, owing to lack of repeated waterlogging stress. Because analysis of BC_2F_2 populations does not provide much information about the real nature of epistatic interactions because of the confounding effect of background loci or other QTLs with larger effect interfere with detection of interactions [33]. The present work reveals that favorable alleles for waterlogging tolerance contributing traits were distributed among BC_2F_2 population and major QTLs were co-localized in different genomic regions. QTL hotspots will be useful for understanding the common genetic control mechanism of the co-localized traits and selection for beneficial allele at these loci will result in a cumulative increase in tolerant due to the integrative positive effect of various QTLs, however the information generated in the present study will be useful for fine mapping and to identify the genes underlying major robust QTLs and transfer all favourable QTLs into one genetic background to break genetic barriers of waterlogging tolerance.

5. Conclusion

Beyond the AB-QTL and an inclusive composite interval mapping method, we identified multiple QTL clusters affecting many traits on across of the ten chromosomes, suggesting that our approach was useful in elucidating the genetic mechanisms underlying maize waterlogging tolerance. Furthermore, the study confirmed and emphasis the previous finding of novel favorable alleles for waterlogging-response traits among F2 population. The major robust QTLs are useful for transfer to different genetic backgrounds through marker assisted backcross breeding to break genetic barriers to waterlogging tolerance. Considering the effect and distribution of novel waterlogging tolerance influencing QTLs among two cultivated species, further research is needed to unearth and use novel genomic regions influencing waterlogging tolerance contributing traits to attain food security.

References

[1] Rathore TR, Warsi MZ. *Production of maize under excess soil moisture (waterlogging) conditions*. In: Proceedings of the 2nd Asian Regional Maize Workshop PACARD. Laos Banos, Phillipines, Feb 23-27, 1998. p.23.

[2] Ghassemi F, Jakeman AJ, Nix HA. *Salinisation of Land and Water Resources, Human Causes, Extent, Management and Case Studies*. University of New South Wales Press, Sydney, 1995. pp. 1-526.

[3] Ahmed F, Rafii MY, Ismail MR, Juraimi AS, Rahim HA, Asfaliza R, Latif MA. Waterlogging Tolerance of Crops: Breeding, Mechanism of Tolerance, Molecular Approaches, and Future Prospects. *BioMed Research International*, 2013.

[4] Qiu FZ, Zheng YL, Zhang ZL, Xu SZ. "Mapping of QTL associated with waterlogging tolerance during the seedling stage in maize". *Ann Bot-London*, 2007, 6:1067-1081.

[5] Zaidi PH, Rafique S, Rai P, Singh N, Srinivasan G. "Tolerance to excess moisture in maize (Zea mays L.): susceptible crop stages and identification of tolerant genotypes", *Field Crop Res*, 90:189-202. 2004.

[6] Mustroph A, Boamfa EI, Laarhoven LJJ, Harren FJM, Pors Y, Grimm B. "Organ specific analysis of the anaerobic primary metabolism in rice and wheat seedlings II: Light exposure reduces needs for fermentation and extends survival during anaerobiosis". *Planta* 225:139-152. 2006.

[7] Zhou L, Wang J, Yi Q, Wang Y, Zhu Y, Zhang Z. "Quantitative trait loci for seedling vigor in rice under field conditions". *Field Crops Res*., 100:294-301. 2007.

[8] Li HM, Liang H, Tang ZX, Zhang HQ, Yan BJ, Ren ZL. "QTL Analysis for grain pentosans and hardness index in a Chinese 1RS.1BL × non-1RS.1BL wheat cross". *Plant Mol Biol Report*, 2012.

[9] Liu J, Li J, Chen F, Zhang F, Ren T, Zhuang Z, Mi G. "Mapping QTLs for root traits under different nitrate levels at the seedling stage in maize (*Zea mays* L.) ". *Plant Soil*, 305:253-265. 2008.

[10] Liu YZ, Tang B, Zheng YL, Ma KJ, Xu SZ, Qiu FZ. "Screening methods for waterlogging tolerance at Maize (*Zea mays* L.) seedling stage. *Agric Sci China*, 9: 362-369. 2010.

[11] Mano Y, Omori F, Takeda K. "Construction of intraspecific linkage maps, detection of a chromosome inversion, and mapping of QTL for constitutive root aerenchyma formation in the teosinte "Zea nicaraguensis". *Mol Breeding*, 29: 137-146. 2012.

[12] Mano Y, Muraki M, Fujimori M, Takamizo T, Kindiger B. "AFLP–SSR maps of maize × teosinte and maize × maize: comparison of map length and segregation distortion". *Plant Breeding*, 124:432-439. 2005.

[13] Mano Y, Muraki M, Fujimori M, Takamizo T. "Identification of QTL controlling flooding tolerance in reducing soil conditions in maize (*Zea mays* L.) Seedlings". *Plant Production Science*, 9: 176-181. 2006.

[14] Fukao T, Harris T, Bailey-Serres J. "Evolutionary analysis of the sub1 gene cluster that confers submergence tolerance to domesticated rice". *Ann Bot*, 2009, 103:143-150.

[15] Tanksley SD, Nelson JC. "Advanced backcross QTL analysis: a method for simultaneous discovery and transfer of valuable QTL from unadapted germplasm into elite breeding lines". *Theor Appl Genet*, 92: 191-203. 1996.

[16] Li H, Ye G, Wang J. "A modified algorithm for the improvement of composite interval mapping". *Genetics*,2007, 175, 361-374.

[17] Liu RH, Meng JL. "Map Draw: a microsoft excel macro for drawing genetic linkage maps based on given genetic linkage data". *Yi Chuan*, 25, 317-321. 2003.

[18] Lander ES, Green P, Abrahamson J, Barlow A, "Daly MJ, Lincoln SE, Newberg LA. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations". *Genomics*, 1:174-181. 1987.

[19] Ali ML, Pathan MS, Zhang J, Bai G, Sarkarung S, Nguyen HT. "Mapping QTLs for root traits in a recombinant inbred population from two indicia ecotypes in rice". *Theoretical and Applied Genetics*, 101: 756-766. 2000.

[20] Marathi B, Guleria S, Mohapatra T, Parsad R, Mariappan N, Kurungara VK, Atwal SS, Prabhu KV, Singh NK, Singh AK. "QTL analysis of novel genomic regions associated with yield and yield related traits in new plant type based recombinant inbred lines of rice (Oryza sativa L.)". *BMC Plant Biol*, 2012, 12:137.

[21] Salvi S, Tuberosa R, Chiapparino E, Maccaferri M, Veillet S, van Beuningen L, Issac P, Edwards K, Phillips RL. "Towards positional cloning of Vgt1, a QTL controlling the transition from the vegetative to the reproductive phase in maize". *Plant Mol Biol.*, 48: 601-613. 2002.

[22] Liao CT, Lin CH. "Physiological adaptation of crop plants to flooding stress". *Proceedings of the National Science Council ROC (B)*, 25:148-157. 2001.

[23] Toojinda T, Siangliw M, Tragoonrung S, Vanavichit A. "Molecular genetics of submergence tolerance in rice: QTL analysis of key traits". *Ann Bot*., 91:243-253. 2003.

[24] Zhang X, Tang B, Yu F, Liu L, Wang M, Xue Y, Zhang Z, Yan J, Bing Y, zheng Y, Qiu F. *Identification of Major QTL for Waterlogging Tolerance Using Genome-Wide Association and Linkage Mapping of Maize Seedlings*. Plant Molecular Biology *Report*, 2012.

[25] Subbaiah CC, Sachs MM. "Molecular and cellular adaptations of maize to flooding stress". *Annals of Botany*, 91: 119-127. 2003.

[26] Rahman H, Pekic S, Lazic-Jancic V, Quarrie SA, Shah SM, Pervez A, Shah MM. "Molecular mapping of quantitative trait loci for drought tolerance in maize plants". *Genetics and Molecular Research*, 10: 889-901. 2011.

[27] Zhu J, Mickelson SM, Kaeppler SM, Lynch JP. "Detection of quantitative trait loci for seminal root traits in maize (Zea mays L.) seedlings grown under differential phosphorus levels". Theor Appl Genet., 113: 1-10. 2006.

[28] Hund A, Fracheboud Y, Soldati A, Frascaroli E, Salvi S, Stamp P. "QTL controlling root and shoot traits of maize seedlings under cold stress". *Theoretical and Applied Genetics*, 109, 618-629. 2004.

[29] Bernacchi D, Beck Bunn T, Emmatty D, Eshed Y, Inai S, Lopez J, Petiard V, Sayama H, Uhlig J, Zamir D, Tanksley S. "Advanced backcross QTL analysis of tomato. II. Evaluation of near-isogenic lines carrying single-donor introgressions for desirable wild QTL-alleles derived from *Lycopersicon hirsutum* and *L. pimpinellifolium*". *Theor Appl Genet.*, 97: 170-180. 1998.

[30] Schadt EE, Monks SA, Drake TA, Lusis AJ, Che N, Collnayo V, Ruff TG, Milligan SB, Lamb JR, Cavet G, Linsley PA, Mao M, Stoughton RB, Friend SH. "Genetics of gene expression surveyed in maize, mouse and man". *Nature*, 422:297-302. 2003.

[31] Cai W, Morishima H. "QTL clusters reflect character associations in wild and cultivated rice". *Theoretical and Applied Genetics*, 104:1217-1228. 2002.

[32] Rae AM, Street NR, Robinson KM, Harris N, Taylor G: "Five QTL hotspots for yield in short rotation coppice bioenergy poplar: The poplar biomass loci". *BMC Pl Bio.* 9:23. 2009.

[33] Yano M, Sasaki T: "Genetic and molecular dissection of quantitative traits in rice". *Pl Mol Bio*, 35:145-153. 1997.

[34] Tang B, Xu SZ, Zou XL, Zheng YL, Qiu FZ. "Changes of Antioxidative Enzymes and Lipid Peroxidation in Leaves and Roots of Waterlogging-Tolerant and Waterlogging-Sensitive Maize Genotypes at Seedling Stage". *Agricultural Sciences in China*, 9:651-661. 2010.

[35] Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW. "Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics". Proceeding of the National Academy of Sciences United States of America, 81: 8014-8018. 1984.