Identification of significant loci for drought resistance and root traits at seedling stage with a set of maize introgression lines

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Abstract

Genetic dissection of the genetic basis of drought resistance is important for crop improvement. In this study, significant loci for drought resistance related traits at seedling stage were identified with a set of maize introgression lines under two environments. Two drought resistance indicators, leaf-drying degree under medium drought stress (LDM) and severe drought stress (LDS), and three root traits, root dry weight under drought stress (RWS), root length under drought stress (RLS), and root number under drought stress (RNS) were investigated. A total of 31 and 25 significant loci for LDM and LDS were identified, respectively, and 20 of them were commonly detected under both environments. For the three root traits, 41 significant loci were detected with 20 loci identified under both environments. Of the 97 significant loci, only 19 loci related to both leaf-drying degree and root traits under stress simultaneously, suggesting that these root traits had limited contribution to drought resistance at seedling stage. The significant loci detected in this study would be useful in molecular breeding for drought resistance in maize.

Keywords: introgression lines, drought resistance loci, root traits, seedling stage, maize

Abbreviations: ABA - Abscisic acid, ASI - Anthesis-silking interval, DA - Drought avoidance, DE - Drought escape, DSI - Yield performance under stress/yield under control, DT - Drought tolerance, ILs - Introgression lines, LDM - Leaf-drying degree under medium drought stress condition, LDS - Leaf-drying degree under severe drought stress condition, QTL - Quantitative trait loci, RLS - Root length under drought stress, RNS - Root number under drought stress, RWS - Root dry weight under drought stress

Introduction

Drought is one of the major abiotic stresses affecting crop production. As an upland crop, maize is more frequently attacked by drought stress especially in the area without irrigation device. On the other hand, domestication, extensive selection and breeding for high yield has narrowed the genetic basis of maize, and this decreased the drought resistance of modern varieties in turn (Ali et al, 2006). Understanding the genetic and molecular basis of drought resistance would aid to improve their resistance to drought.

It is critical to select suitable indicators in the genetic study and breeding for drought resistance. Drought resistance index (DSI, yield performance under stress/yield under control) is the direct indicator for drought resistance at reproductive stage, and leaf-drying area or degree under stress is a suitable one at seedling stage (Mitchell et al, 1998). In addition, scientists have put great effort on selecting morph-physiological traits related to drought resistance in the past few decades, they found canopy temperature, stay-green, anthesis-silking interval (ASI), leaf area, abscisic acid (ABA) content, osmotic adjustment and root traits can be used as secondary indices in varied crops (Lebreton et al, 1995; Walter and Shurr, 2005; Turner et al, 2007; Liu et al, 2011; Jiang et al, 2012). Among these traits, ASI and stay-green have been used in the breeding practice to select drought resistant lines in maize (Ribaut and Ragot, 2007; Campos et al, 2004). Leaf-drying can be caused by drought stress (Masclaux et al, 2001; Borrás et al, 2003), and leaf-drying was found to be negatively correlated with yield under water-stressed environments (Lafitte et al, 2004). Thus leaf-drying degree can be used as a good indicator for drought resistance at seedling stage.

With the development of molecular markers, QTL mapping for drought resistance has been conducted in different crops (Ribaut et al, 1996; Zhang et al, 2006), and further used in molecular-assisted selection (MAS) (Serraj et al, 2005; Courtois et al, 2003; Harris et al, 2007). In maize, favorable alleles at five QTL for ASI (Ribaut et al, 1996) were introgressed into a maize hybrid with MAS, and the yield increased more than 50% under drought stress condition (Ribaut and Ragot, 2007).

To date, most genetic study on drought resistance was conducted at reproductive stage in field conditions (Lafitte et al, 2004). However, it is hard to control the degree of drought stress and apply the same

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stress level on each line at certain growing stage in the field condition especially for crops with big plants. On the contrary, precise drought stress could be applied uniformly on each line when growing in pots. In addition, seedling stage is one of the critical phases affecting yield. Thus in this study, leaf-drying degree and root traits at seedling stage in a set of introgression lines were investigated under the same level of drought stress, the aims are to identify significant loci for drought resistance and root traits, and to compare the genetic basis of drought resistance and the root traits.

Materials and Methods

Plant materials and stress treatment

An IL population including 124 lines was used in this study. The ILs were developed from a cross between Huangzao 4 (HZ4) and CML343. HZ4, an elite inbred extensively used in China, was used as recurrent parent. CML343 introduced from International Maize and Wheat Improvement Center with the character of drought resistance was used as donor. Pollens from 20 BC_1F_1 plants were mixed and pollinated to HZ4 and a BC_2F_1 population containing 170 lines was generated. Then the lines were selfed for four generations, and a total of 124 BC_2F_5 lines were obtained and subsequently used to conduct experiments for two times at an experimental station with a glass rain-off shelter on the campus of Huazhong Agricultural University, China in 2012.

On April 18 (drought stress was treated in the late spring), the 124 ILs and HZ4 were planted in plastic pots that laid out following a design of randomized complete blocks with two replicates, and each replicate contained two pots. The volume of the pots is about 3.8 dm^3 with a hole on the bottom, and 3 kilograms of dried river sand was loaded in each pot. At 2-leaf stage, 10 uniform seedlings were kept in each pot, and started watering with ½ Hoagland cultural solution. At about 3-leaf stage, stress treatment was then applied by stop watering. To apply the same stress degree in each pot, the pots were weighted at 6:00 PM everyday, and the water content in the pots was controlled at the same level (at a deduce rate of 10% field moisture capacity per day) by adding the cultural solution. Drought stress was stopped till the water content at the pots reached to 20% of field moisture capacity.

For the second experiment, seeds were sowed on May 17 (drought stress was treated in the early summer), and the experimental design and stress treatment were the same as the first experiment.

Traits and measurements

When the field moisture capacity reached at 80%, 5 plants in each pot were sampled to measure upground dry mass. The rest 5 plants were kept to score the degree of leaf-drying under medium drought stress condition (LDM, 50% field moisture capacity) and severe drought stress condition (LDS, 20% field moisture capacity), respectively. Leaf-drying degree was recorded as 0 (no leaf-drying) to 9 (>90% of the leaf area was drying). After scoring LDS, sand in the pots was poured out and the roots of each plant were washed carefully. Then three roots traits, root number under stress (RNS), root length under stress (RLS, cm) and root dry weight under stress (RWS, g) were measured.

Genotyping and marker-trait association analysis

A total of 201 polymorphic SSRs evenly distributed on the map of IBM2 2008 Neighbors (http://maizegdb.org) were employed to genotype the population.

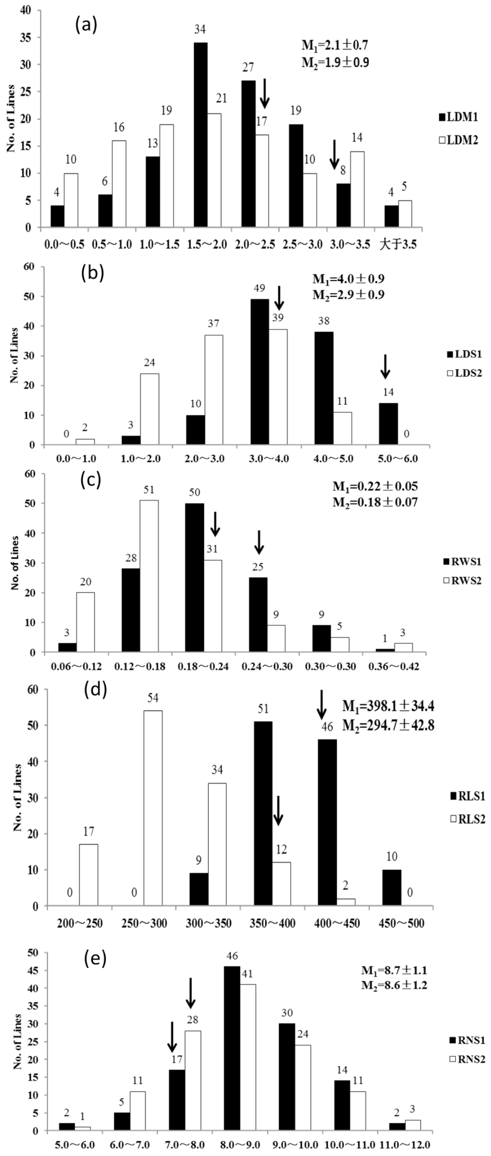

Figure 1 - Distribution of LDM (a), LDS (b), RWS (c), RLS (d), RNS (e) in the ILs. The black and white histograms show the phenotypic distributions of the trait in the late spring and early summer experiments, respectively. Means for HZ4 are labeled with arrows.

Marker-trait associations were calculated with software GGT 2.0 (http://www.dpw.wau.nl/pv/pub/ggt/). Since a number of fragments remains in the ILs, other programs are not suitable to detect QTL with this population. The squared correlation coefficients between marker data and trait values (R^2 values) and the associated probabilities of the correlation values were calculated. As these probabilities were very small, the $^{-10}$LOG (-lg) values were reported, such as a value of $^{-10}$LOG =3 indicated a correlation probability value of 0.001. A false discovery rate (FDR) threshold for the p-values of individual association tests was also calculated by GGT (Storey, 2002), and FDR-controlling was very effective in QTL analysis (Benjamini and Yekutieli, 2005). For the significant loci clustered in a chromosomal region, only the locus with the largest value (-lgp) was selected. Then single factor ANOVA for the data from different genotypes at each significant locus was conducted to verify the associations and calculate means of the traits in each group of genotypes.

Results and Discussion

Performance of drought resistance and root traits in the ILs under stress condition

Phenotyping is the most important work in study on understanding the genetic basis and breeding for drought resistance because it is hard to apply uniform stress on each line. In this study, precise and consistent water stress was applied on each line by growing the plants in pots and weighting the pots on each day.

Performance of leaf-drying degree under medium and severe stress and the three root traits in the ILs and HZ4 is given in Figure 1. LDM and LDS in HZ4 were higher than the means of the ILs in both experiments, suggesting that introgression fragments from CML343 had positive effects on improving the drought resistance in general. For the root traits, RNS in HZ4 showed consistently lower than the means of the ILs in both experiments. The values of drought resistance indices from the spring experiment were higher than that in the summer experiment, this could be explained by the fact that a shading net was covered on the shelter roof during the drought stress in the summer experiment, and this caused the stress developed slower in the summer experiment (Figure 1). In addition, all the traits investigated varied largely in the ILs especially for the traits of LDM, LDS and RWS, and they all fit in a normal distribution, indicating the polygenic characteristic of these traits (Figure 1).

Table 1 - Paired correlation coefficients among the five traits investigated.

	LDM	LDS	RWS	RLS
LDS	$0.55^{**}/0.80^{**}$			
RWS	0.00/-0.14	$-0.03/-0.21^{*}$		
RLS	$-0.18^{*}/-0.16$	$-0.13/-0.18^{*}$	$0.06/0.27^{**}$	
RNS	$-0.10/-0.29^{**}$	$-0.09/-0.23^{**}$	$0.29^{**}/0.22^{*}$	0.16/0.10

The values on the left side of the slash sign were from the spring experiment and that on the right were from the summer experiment. *, ** significant at p = 0.05 and p = 0.01, respectively.

Two-factor ANOVA for the traits was conducted. Significant variations of all the traits were observed among the ILs (p<0.01), however, the variations between the environments (experiments) were not significant for all the traits (data not shown).

Correlation analysis among the traits

Coefficients of pairwise correlations of the traits are given in Table 1. LDM was positively and strongly correlated to LDS in both experiments (r = 0.55 and 0.80, respectively). RWS was positively and significantly correlated to RLS and RNS in general. However, correlations between leaf-drying degree and the root traits were not significant or not strong (-0.29 < r < 0.00) (Table 1), similar results were also reported in rice (Yue et al, 2006), suggesting that the root traits under stress were not related to the drought resistance indices at the seedling stage.

Genotyping and identification of significant loci for leaf-drying degree and root traits

Genotyping with 201 SSRs revealed that the number of introgressed segments in each IL varied from 14 to 38 with an average of 22.5. On the average 6.04% of the markers were heterozygous. The percentage of recipient genome ranged from 61.1% to 86.1% with an average of 75.5%. The total length of introgressed segments in each IL varied from 846.03 cM to 2,635.19 cM with an average of 1,626.97 cM. On the average, length of the introgressed segments was 72.34 cM.

A total of 97 significant loci including 56 loci for leaf-drying degree (Table 2) and 41 loci for root traits (Table 3) were identified.

Totally 31 significant loci for LDM were detected, and they were distributed on all the chromosomes of maize. Of them, 9 were identified in the both experiments with -lgp values ranging from 2.1 to 15.0. Alleles from CML343 at 15 loci decreased leaf-drying degree under medium stress condition (Table 2).

A total of 25 significant loci for LDS were identified with 8 of them were detected in the both experiments. Alleles from CML343 at 8 loci decreased leaf-drying degree under severe stress condition (Table 2).

Only two significant loci for RWS were detected in the early summer experiment with -lgp values ranging from 2.4 to 2.5. Alleles from CML343 at one of the loci increased root weight under drought stress condition (Table 3). Twenty significant loci for RLS were detected with -lgp values ranging from 1.9 to 4.5, and 8 of them were identified in the both experiments. Alleles from CML343 at three of the loci increased root length under sever stress condition (Table 3). A total of 19 significant loci for RNS were detected, and 12 were identified in the both experiments with -lgp val-

Table 2 - Significant loci for leaf-drying degree detected in the two experiments.

Traits	Chr	Loci	Position (cM)		Late spring			Early summer	
				-lgp	Effect‡	p	-lgp	Effect‡	p
LDM	1	umc1177	10.50				1.5	-0.59	0.026
		bnlg1083	198.00	2.2	0.37	0.007	6.8	0.93	0.000
		bnlg1016	328.48				3.5	1.03	0.009
		bnlg2086	401.20	2.4	-0.45	0.004			
		umc1035	587.00				1.6	-0.61	0.036
		umc1774	936.00	4.2	0.64	0.000	15.0	1.49	0.000
		umc1797	1141.00				2.3	-0.57	0.020
	2	bnlg1017	65.70				8.4	1.49	0.000
		umc1516	584.30				2.3	0.56	0.005
	3	umc2257	30.50				7.4	1.44	0.000
		umc1012	159.00	2.1	0.43	0.013	3.8	0.82	0.000
		umc1300	299.16	3.0	0.47	0.001			
		umc1489	567.60	3.6	0.62	0.000	6.1	1.08	0.000
	4	umc1821	211.00				2.0	-0.60	0.006
		bnlg1917	724.00				6.1	1.02	0.000
	5	bnlg1382	148.18				5.4	1.09	0.000
		umc2161	295.00				4.5	-0.99	0.000
		umc1687	411.09	2.5	-0.45	0.007			
	6	umc1083	127.80				5.1	0.87	0.000
		umc1063	452.70	5.1	0.95	0.000	7.2	1.42	0.000
	7	umc1301	405.50	2.8	-0.56	0.001	5.6	-1.04	0.000
		umc1407	600.20	4.4	0.57	0.000	13.7	1.44	0.000
	8	umc1075	49.40				5.3	-0.90	0.000
		bnlg162	367.00				2.7	-0.66	0.003
		umc2218	547.20				4.1	-0.91	0.000
	9	bnlg1272	12.00				6.0	0.91	0.000
		umc1893	162.93				2.1	-0.54	0.009
		nc134	247.60	2.6	-0.43	0.004	7.6	-1.02	0.000
		umc2343	385.30				6.1	-0.96	0.000
	10	phi052	100.56	4.2	0.61	0.000	12.9	1.37	0.000
		umc1061	386.65				2.0	-0.54	0.009
LDS	1	bnlg1083	198.00				4.6	0.70	0.000
		umc1297	435.50				1.6	-0.61	0.019
		umc1774	936.00	4.4	0.74	0.000	11.4	1.23	0.000
		umc1797	1141.00				2.4	-0.56	0.013
	2	bnlg1017	65.70	3.1	0.83	0.001	5.7	1.14	0.000
	3	umc2257	30.50	2.5	0.70	0.009	4.7	1.13	0.000
		umc1012	159.00				2.7	0.65	0.001
		umc1489	567.60	2.7	0.59	0.003	4.2	0.83	0.000
	4	bnlg1917	724.00				3.7	0.74	0.000
	5	bnlg1382	148.18				3.4	0.82	0.000
		umc2588	268.76				6.2	0.87	0.000
		umc1687	411.09				2.5	-0.52	0.011
		bnlg386	661.94	2.7	0.51	0.003			
	6	umc1083	127.80				6.3	0.94	0.000
		umc1063	452.70	3.1	0.89	0.001	5.6	1.23	0.000
	7	umc1301	405.50				3.1	-0.70	0.001
		umc1407	600.20	3.6	0.64	0.000	10.6	1.18	0.000
	8	umc1075	49.40				3.1	-0.63	0.001
		umc1069	571.50	2.5	0.53	0.003			
	9	bnlg1272	12.00				5.7	0.86	0.000
		bnlg1583	62.30	3.0	0.57	0.001			
		nc134	247.60				4.8	-0.75	0.000
		umc2343	385.30	2.8	-0.54	0.002	3.2	-0.66	0.001
	10	phi052	100.56	3.1	0.59	0.001	9.9	1.14	0.000
		umc1061	386.65				1.8	-0.47	0.013

‡Effects are the means of genotypes with the introgressed allele minus the means of genotypes with the HZ4 allele at the significant locus.

ues ranging from 2.1 to 5.1. Alleles from CML343 at 8 loci increased root number under sever stress condition (Table 3).

Most favorable alleles at the loci for LDM, LDS and RNS were from HZ4, however, performance of drought resistance and RNS in HZ4 was below the average of the ILs. This could be explained by the fact that there are multiple segments in each IL, so epistasis may also play an important role in the genetic basis of these traits, especially for the trait of LDS. For instance, a large number of epistatic QTLs for drought tolerance related traits were identified in groundnut (Ravi et al, 2011).

Introgression lines have been employed to map agronomically important traits (Eshed and Zamir, 1995; Hao et al, 2009; Salvi et al, 2011). In comparison to F_2, DH or RIL populations, mapping with ILs should increase the detection power due to its less complex background (Eshed and Zamir, 1995). In addition, NILs and ILs can be used as mapping populations for QTL analysis and breeding materials for inbreds development simultaneously. The marker locus bnlg1083 we found to be associated with LDM, LDS and RNS in this study was also related to a maize

Table 3 - Significant loci for the three root traits detected in the two experiments.

Traits	Chr	Loci	Position	Late spring			Early summer		
			(cM)	-lgp	Effect‡	p	-lgp	Effect‡	p
RWS	6	umc1083	127.80				2.5	-0.04	0.009
	8	umc2218	547.20				2.4	0.05	0.004
RLS	1	bnlg1016	328.48	2.2	-30.57	0.036	2.0	-39.71	0.029
		umc1774	936.00	2.7	-21.68	0.003	2.8	-28.40	0.002
	2	bnlg1017	65.70	2.2	-28.87	0.003			
		umc1516	584.30				2.2	-23.35	0.006
	3	umc1012	159.00				3.0	-31.30	0.001
		nc030	189.00	2.3	-19.58	0.007			
		umc1489	567.60	2.2	-20.35	0.011	2.6	-31.89	0.001
	4	umc1821	211.00	3.6	27.52	0.000	2.2	24.28	0.011
		bnlg1917	724.00				2.3	-27.05	0.006
	5	umc1687	411.09	2.9	24.01	0.002	2.9	30.61	0.001
	6	umc1083	127.80				2.3	-24.44	0.014
		umc1520	410.00				1.9	-32.80	0.017
		umc1063	452.70	2.6	-29.23	0.003			
	7	umc1426	47.80	3.3	24.69	0.000	4.5	35.89	0.000
		umc1407	600.20	3.7	-29.06	0.000	2.8	-28.33	0.002
	8	umc1075	49.40				2.2	23.03	0.006
		bnlg162	367.00	3.2	23.56	0.001			
		umc1663	608.10				3.1	31.67	0.001
	9	umc1519	322.59	3.1	-21.59	0.000	3.6	-28.69	0.000
	10	phi052	100.56				2.9	-28.36	0.001
RNS	1	bnlg1083	198.00	3.4	-0.74	0.000	3.4	-0.81	0.000
		umc1035	587.00	2.9	0.84	0.013	4.3	1.34	0.000
		phi265454	973.00				3.8	-1.09	0.001
	2	bnlg1909	306.30	2.1	0.68	0.012	4.4	1.13	0.000
	3	umc1012	159.00	2.6	-0.74	0.003	3.0	-0.90	0.001
		umc1489	567.60	2.4	-0.79	0.002			
	4	umc1821	211.00	3.7	0.93	0.000	2.6	0.81	0.003
	5	bnlg1382	148.18	2.5	-0.65	0.020	3.3	-0.91	0.002
		umc1687	411.09				2.5	0.79	0.005
	6	umc1595	153.70				4.3	1.61	0.000
		umc2165	502.90				4.5	1.39	0.000
	7	umc2368	564.77	3.0	-1.16	0.002	2.4	-1.14	0.007
	8	umc1075	49.40				2.6	0.73	0.003
		bnlg162	367.00	3.4	0.83	0.002	5.1	1.10	0.000
		umc1663	608.10	3.4	0.80	0.001	3.5	0.91	0.001
	9	umc1170	101.10	2.2	0.60	0.007	2.4	0.73	0.005
		umc2343	385.30	2.1	0.55	0.014	3.9	0.92	0.000
	10	umc1507	308.98	3.1	1.17	0.001	3.8	1.41	0.000
		umc1569	505.50				3.7	1.29	0.001

‡Effects are the means of genotypes with the introgressed allele minus the means of genotypes with the HZ4 allele at the significant locus.

QTL for plant height under stress condition (Guo et al, 2008). In the region bnlg1904-umc1772 of 3.04 bin where Hao et al (2009) identified some QTLs for plant survival rate under drought condition at seedling stage, significant locus (umc1012) for LDM, LDS, RLS and RNS was also detected in this study. In addition, in the region bnlg1451-umc2067 of 10.02bin, a significant locus phi052 for LDM, LDS and RLS detected, Cai et al (2012) also identified a QTL for axial root length. These results demonstrate the usefulness of ILs on the study of genetic basis of drought resistance in maize. The 25 significant loci commonly identified in the both experiments (Tables 2 and 3), especially that also detected in other reported should be useful in molecular breeding.

Pleiotropism of the traits

Of the 97 significant loci detected, 20 loci were associated with LDS and LDM simultaneously, 8 loci were found controlling at least two root related traits simultaneously. However, only 19 loci were detected to be associated with the traits related to both leaf-drying degree and root traits (Tables 2 and 3). Among these loci, phi052 and umc1063 associated with both LDM and LDS, umc1407, umc1489 and umc1774 related to LDM, LDS and RLS, and umc1821 related to both RLS and RNS, were especially useful in maize genetic improvement for drought resistance.

Drought resistance is a complex trait that includes drought escape (DE) via a short life cycle or developmental plasticity, drought avoidance (DA) via enhanced water uptake and reduced water loss and drought tolerance (DT) mainly via osmotic adjustment and antioxidant capacity. Thus, under field condition it is impossible to apply the same drought stress level on all experimental units due to inconsistent flowering time and plant size besides the complex status of genetic components (Yue et al, 2006). In this study, the correlations between leaf-drying degree (LDM, LDS) and upground dry mass were not significant (r ranged from -0.04 to 0.13), suggesting that DA was eliminated. In addition, LDM and LDS investigated in this study should be mainly related to drought tolerance considering the fact that the water content in each pot was adjusted to the same level. Root plays an important role under drought stress by uptaking sufficient water and preventing plants from suffering water stress (Lebreton et al, 1995), and root traits measured under stress in this study were mainly related to drought avoidance. In this study, only 19 loci were associated with leaf-drying degree and root traits simultaneously, suggesting that DT and DA at seedling stage in maize had different genetic basis in general. This also explained the lack of strong correlation between leaf-drying degree and root traits in this study (Table 1). Similar result was also reported in rice (Yue et al, 2006). Thus genetic improvement of drought resistance can be achieved by pyramiding the favorable alleles at these DT and DA loci.

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References

Ali AJ, Xu J, Ismail AM, Fu B, Vijaykumar CHM, Gao Y, Domingo J, Maghirang R, Yu S, Gregorio G, Yanaghihara S, Cohen M, Carmen B, Mackill D, and Li Z, 2006. Hidden diversity for abiotic and biotic stress tolerances in the primary gene pool of rice revealed by a large backcross breeding program. Field Crops Res 97: 66-76

Benjamini Y, and Yekutieli D, 2005. Quantitative trait loci analysis using the false discovery rate. Genetics 171: 783-790

Borrás L, Maddonni GA, and Otegui ME, 2003. Leaf senescence in maize hybrids: plant population, row spacing and kernel effects. Field Crops Res 82: 13-26

Cai H, Chen F, Mi G, Zhang F, Maurer HP, Liu W, Reif J, and Yuan L, 2012. Mapping QTLs for root system architecture of maize (*Zea mays* L) in the field at different developmental stages. Theor Appl Gen 125: 1313-1324

Campos H, Cooper M, Habben JE, Edmeades GO, Schussler JR, 2004. Improving drought tolerance in maize: a view from industry. Field Crop Res 90: 19-34

Courtois B, Shen L, Petalcorin W, Carandang S, Mauleon R, Li Z, 2003. Locating QTLs controlling constitutive root traits in the rice population IAC165 ×Co39. Euphytica 134: 335-345

Eshed Y, and Zamir D, 1995. An introgression line population of *Lycopersicon pernellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics 141: 1147-1162

Guo J, Su G, Zhang J, and Wang G, 2008. Genetic analysis and QTL mapping of maize yield and associate agronomic traits under semi-arid land condition. Afri J Biotec 7: 1829-1838

Hao Z, Liu X, Li X, Xie C, Li M, Zhang D, Zhang S, and Xu Y, 2009. Identification of quantitative trait loci for drought tolerance at seedling stage by screening a large number of introgression lines in maize. Plant Breed 128: 337-341

Harris K, Subudhi PK, Borrel A, Jordan D, Rosenow D, Nguyen H, Klein P, Klein R, and Mullet J, 2007. Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence. J Exp Bot 58: 327-338

Jiang T, Fountain J, Davis G, Kemerait R, Scully B, Lee RD, and Guo B, 2012. Root morphology and gene expression analysis in response to drought stress in maize (*Zea mays*). Plant Mol Biol Rep 30: 360-369

Lafitte HR, Price AH, and Courtois B, 2004. Yield response to water deficit in an upland rice mapping population: associations among traits and genetic markers. Theor Appl Genet 109: 1237-1246

Lebreton C, Lazic-Jancic V, Steed A, Pekic S, and Quarrie SA, 1995. Identification of QTL for drought responses in maize and their use in testing causal relationships between traits. J Exp Bot 46: 853-865

Liu Y, Subhash C, Yan J, Song C, Zhao J, and Li J, 2011. Maize leaf temperature responses to drought: Thermal imaging and quantitative trait loci (QTL) mapping. Environ Exp Bot 71: 158-165

Masclaux C, Quillere I, Gallais A, and Hirel B, 2001. The challenge of remobilisation in plant nitrogen economy. A survey of physio-agronomic and molecular approaches. Ann Appl Biol 138: 69-81

Mitchell JH, Siamhan D, Wamala MH, Risimeri JB, Chinyamakobvu E, Henderson SA, and Fukai S, 1998. The use of seedling leaf death score for evaluation of drought resistance of rice. Field Crop Res 55: 129-139

Ravi K, Vadez V, Isobe S, Mir RR, Guo Y, Nigam SN, Gowda MVC, Radhakrishnan T, Bertioli DJ, Knapp SJ, Varshney RK, 2011. Identification of several small main-effect QTLs and a large number of epistatic QTLs for drought tolerance related traits in groundnut (*Arachis hypogaea* L). Theor Appl Genet 122(6): 1119-1132

Ribaut JM, and Ragot M, 2007. Marker-assisted selection to improve drought adaptation in maize: the backcross approach, perspectives, limitations, and alternatives. J Exp Bot 58: 351-360

Ribaut JM, Hoisington DA, Deutsch JA, Jiang C, and Gonzalez-de-Leon D, 1996. Identification of quantitative trait loci under drought conditions in tropical maize. I. Flowering parameters and the anthesis-silking interval. Theor Appl Genet 92: 905-914

Salvi S, Corneti S, Bellotti M, Carraro N, Sanguineti MC, Castelletti S, Tuberosa R, 2011. Genetic dissection of maize phenology using an intraspecific introgression library. BMC Plant Biol 11: 1471-

2229

Serraj R, Hash CT, Rizvi SM, Sharma A, Yadav RS, Bidinger FR, 2005. Recent advances in marker-assisted selection for drought tolerance in pearl millet. Plant Prod Sci 8: 334-337

Storey JD, 2002. A direct approach to false discovery rates. J Roy Sta B 64: 479-498

Turner NC, Abbo S, Berger JD, Chaturvedi SK, French RJ, Ludwig C, Mannur DM, Singh SJ, and Yadava HS, 2007. Osmotic adjustment in chickpea (*Cicer arietinum* L) results in no yield benefit under terminal drought. J Exp Bot 58:187-194

Walter A, Shurr U, 2005. Dynamics of leaf and root growth: endogenous control versus environmental impact. Ann Bot 95: 891-900

Yue B, Xue W, Xiong L, Yu X, Luo L, Cui K, Jin D, Xing Y, and Zhang Q, 2006. Genetic basis of drought resistance at reproductive stage in rice: separation of drought tolerance from drought avoidance. Genetics 172: 1213-1228

Zhang X, Zhou S, Fu Y, Su Z, Wang X, and Sun C, 2006. Identification of a drought tolerant introgression line derived from Dongxiang common wild rice (*O rufipogon* Griff). Plant Mol Biol 62: 247-259