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GENETIC EVALUATION OF ROOT COMPLEXITY IN MAIZE

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Root architecture is strongly linked to plant survival under abiotic and biotic stress conditions. The objective of this study was to investigate the inheritance of the primary root system complexity in maize (*Zea mays* L.). For a total of 231 recombinant inbred lines (RIL) derived from the IBM (B73×Mo17) population multiple primary root systems were produced by applying a replicated alpha lattice experimental design. Digital images of each root system were taken at days four and eight after germination. For each root system image, the fractal dimension (FD) was computed. Significant differences between RILs were found in the FD calculated after four (FD1) and eight (FD2) days. For FD1 22 QTLs, for FD2 13, and for FD change over time (ΔFD) 12 QTLs were found on all ten maize chromosomes explaining between 24.6 and 46.8% of the phenotypic variation. Both parental inbreds contributed FD-increasing QTL alleles. FD1 and FD2 had five chromosomal BIN locations in common. Four unique QTLs were identified for the dynamics of root growth between days four and eight. Maize root mutants involved in root morphology were located in chromosomal BINs carrying QTLs for FD1 or FD2. This study demonstrated the usefulness of the IBM population as a maize community resource to investigate the genetic basis of root complexity in maize.

Key words: maize, QTL, primary root system, complexity, fractal dimension

Introduction

The development of a healthy root system is an important part of the overall plant development programme. Root branching and architecture are strongly linked to plant survival under abiotic (e.g. drought, flooding, nutrient deficiencies) and biotic (e.g. competition among plants, diseases, pests) stress conditions. A limited number of genetic studies is available relating maize root architecture and development with yield, root lodging, and tolerance to stresses under field conditions. This lack of in-depth knowledge is mainly due to the labour-intensive digging required to obtain root samples, the destructive nature of this procedure, and the highly heterogeneous root systems within and among

different maize cultivars as a response to a complex soil matrix. Additionally, traditional measures such as root length and biomass do not provide an accurate quantification of root branching or complexity. In addition, root complexity and root development depend on genetic and environmental factors and their interactions (O'Toole and Bland, 1987)

The ability of plants to grow and produce seeds is governed by a functional and efficient root system. Complex root systems are characterized by a high number of branching points, having a higher probability of finding adequate resources by exploring a large portion of the soil face than root systems with less complex root systems. The complexity of root systems can be determined by applying the mathematical fractal dimensions (FD). A key feature of fractals is self-similarity at varying scales, i.e. a small part of the structure resembles the whole structure. Fractals are dimensionless and fractal geometry allows for the description, study and analysis of complex shapes found in nature. In general, FD is more suitable to describe complex natural objects than standard Euclidian geometry (Mandelbrot, 1983).

Multiple studies demonstrated that various objects in nature are self-similar and can, therefore, be analysed employing fractal dimensions; examples include root systems (Tatsumi et al., 1989; Eghball et al., 1993; Lynch et al., 1993; Nielsen et al., 1997; Masi and Maranville, 1998; Oppelt et al., 2000; Walk et al., 2004), shoot systems and canopies of young trees (Morse et al., 1985; Foroutan-pour et al., 1999), seaweeds (Kubler and Dugeon, 1996), sponges (Abraham, 2001), neurons (Fernandez et al., 1994) and fungal mycelia (Mihail et al., 1995). The availability of adequate computer power and image analysis software packages allows for the application of FD to study root characteristics in more detail (Costa et al., 2001). Images for the study of FD have been previously acquired by video camera (Ottman and Timm, 1984; Cunningham et al., 1989) and optical scanner (Arsenault et al., 1995; Box, 1996; Kaspar and Ewing, 1997) as well as by photographic images (Tatsumi et al., 1989; Eghball et al., 1993; Masi and Maranville, 1998; Abraham, 2001), image transparencies (Nielsen et al., 1999) and SimRoot (Lynch et al., 1997). Despite this previous work on FDs there was to our knowledge no software package available that allows for an efficient large-scale screening of segregating QTL mapping populations.

The overall goal was to contribute to the scarce information about root morphology, complexity and development using images of primary root systems of maize. To achieve this goal a novel software package was developed for root image processing and complexity calculations. The specific objectives of this study were to (1) evaluate a large set of maize recombinant inbred lines (RIL) derived from the four times random-mated IBM (B73×Mo17) population for their fractal dimensions from root images and their variation over time, (2) determine quantitative genetic parameters for this characteristic, (3) map and characterize quantitative trait loci (QTL) affecting the complexity of primary root systems in maize, and (4) compare the root complexity QTLs with QTLs identified for other root morphology traits.

Materials and methods

Plant materials

A set of 240 recombinant inbred lines (RILs) selected from the IBM population were used. These RILs were developed by continuous selfing of randomly selected individuals from a four times random mated F_2 population developed from cross B73×Mo17 (Lee et al., 2002). Inbred B73 belongs to the Stiff Stalk Synthetic heterotic pool, whereas Mo17 is a non-Stiff Stalk inbred derived from the Lancaster pool.

Experimental design

The 240 RILs were subdivided into five sets. Each set comprised parental inbreds B73 and Mo17 as single entries and 48 RILs. The sets were evaluated in separate experiments. The experimental design for all experiments was a 10×5 alpha design with two replications.

Germination procedure

All seeds were surface sterilized with a commercial 6% Clorox® solution for 10 minutes. After this treatment, the seeds were washed three times with distilled and sterilized water. For each genotype, five seeds of the same genotype were placed in the upper third of a non-toxic germination paper. The embryo faced the bottom of the germination paper. The space between the seeds was maximized to prevent contact between different root systems. Each germination paper was moisturized with a Captan® (BAYER) 2.5 g l^{-1} solution, and afterwards rolled up vertically. Five rolled germination papers were placed vertically in one 2.5 l plastic bucket with 750 ml of distilled water plus 20 ml of Captan solution. All experiments were conducted in a germination chamber without illumination at 28ºC and 100% relative humidity. According to the field experiment terminology, each germination paper was regarded as a single plot and each bucket as an incomplete block.

Image processing

Images were taken four (Time 1) and eight days (Time 2) after germination with a Sony Cybershot 5.0 megapixels digital camera. The camera was mounted on a stand to standardize the image process. The background surface and the light in the room were taken into consideration to optimize the image quality. The images were acquired and saved in the JPEG format. A software package was developed to process the digital images of the maize primary root systems. The software package consisted of Matlab® (MATHWORKS) subroutines with the following six-step procedure.

Step 1: The RGB image was converted into a grey scale image.

Step 2: A square region of interest of the image containing the root was singled out to accommodate the requirements of the program used to calculate the FD.

Step 3: Thresholding was used to convert the grey-scale images into binary images.

Step 4: Histogram equalization was applied to further improve the image.

Step 5: The image was smoothed to remove noise by applying a median filter. Among all filters of equal size the median filter has an excellent noise reduction capability (Gonzalez and Woods, 2002).

Step 6: A Matlab subroutine was developed to measure FDs with a calculation program based on the box counting method as described by Mandelbrot (1983). According to this method the images were sequentially divided into grids of descending size and for each grid two values were recorded: N_s, the number of squares intersected by the image, and s, the side length of grid squares. FD is the regression coefficient describing the association between log (N_s) and log (1/s), which ranges from 1 to 2. It was expected that more complex embryonic root systems would have a larger FD. The variation of FD values between Time 1 (FD1) and Time 2 (FD2) was calculated as $\Delta FD=(FD1-FD2)$.

Statistical analysis

All data sets of the plant material evaluated were combined and an analysis of variance was performed applying the following model:

$$y = \mu + \alpha_i + \beta_{j(i)} + \delta_{k(ij)} + \gamma_l + \varepsilon_{(ijkl)m}$$

where y represents the phenotypic mean of a genotype, α_i is the effect of the i^{th} set, $\beta_{j(i)}$ is the effect of the j^{th} replication in the i^{th} set, $\delta_{k(ij}$ is the k^{th} block effect in the j^{th} replication of the i^{th} set, γ_l is the effect of the l^{th} genotype, and ε represents the residual error. All effects in the model were considered random. Estimates of the genotypic variance (σ_g^2), error variance (σ^2), and phenotypic variance (σ_p^2) and their standard errors were calculated as described by Searle (1971). Heritability estimates (h^2) for the RILs were calculated on an entry-mean basis as described by Hallauer and Miranda (1988). Phenotypic (r_p) correlation coefficients were calculated between traits by applying standard methods (Mode and Robinson, 1959). PLABSTAT (Utz, 1998) and SAS 9.1 (SAS Institute, 1996) software packages were used for all calculations.

QTL analysis

Linear unbiased predictors for 231 of 240 RILs were used in a single marker QTL analysis. Both molecular and phenotypic data were available only for this subset of RILs. The map for the random mated B73×Mo17 (IBM) population (Davis et al., 2001) is populated with > 1,000 RFLP and > 850 simple sequence repeat (SSR) markers. In this study, the genotypic data consisted of 1,167 markers that were evenly distributed across the maize genome. Composite interval mapping (CIM) was employed for QTL detection and the estimation of QTL effects. A LOD threshold of 3.6 was chosen for declaring a presumed QTL significant, ensuring a comparison-wise error rate of $P < 0.0003$ and an experiment-wise error rate of $P < 0.30$. Estimates of QTL positions were obtained where the LOD score reached its maximum in the region under consideration. All putative QTLs were examined for the presence of digenic epistatic effects. The portion of the phenotypic variance explained by all QTLs was determined by the adjusted coefficient of determination of regression (R_{adj}^2) fitting a model including all detected QTLs. All necessary calculations were performed with the PLABQTL (Utz and Melchinger, 1996) and SAS 9.1 (SAS Institute, 1996) software packages.

Results

Phenotypic evaluation

Histograms of the progeny means of 231 RILs are shown in Figure 1. Fractal dimensions determined four days after germination (FD1) varied between 1.084 and 1.157. Progeny means for FD determined eight days after germination (FD2) ranged from 1.080 to 1.202. Means of parental inbreds B73 and Mo17 were significantly ($P < 0.05$) different for FD2 but not for FD1. Heritability estimates were low for FD1 (0.32) and intermediate for FD2 (0.51) (Fig. 1). The phenotypic correlation of FD1 with FD2 was highly significant ($P < 0.01$) and of moderate size ($r_p = 0.70$).

QTL analysis for root complexity

A total of 21 QTLs were found for FD1 on all chromosomes; these QTLs explained between 2.0 and 17.8% of $\hat{\sigma}_P^2$ (Table 1). Significant QTL interactions were identified between QTLs on chromosomes 1 (BIN 1.04) and 10 (BIN 10.07) as well as between QTLs on chromosomes 2 (BIN 2.04) and 7 (BIN 7.01/2) (see also Fig. 4). In a model fitting simultaneously all significant additive

QTL effects and additive×additive epistatic interactions, the two digenic interactions accounted for 3.5 and 3.7% of the phenotypic variation, respectively. In total, the simultaneous fit explained 46.8% of the phenotypic variation for FD1. Both parental inbreds contributed alleles that increased FD1.

A total of 13 QTLs were found for FD2. These QTLs were located on all chromosomes, except for chromosomes 6 and 9, explaining between 2.5 and 7.6% of $\hat{\sigma}_P^2$ (Table 3). A significant additive×additive epistatic interaction was found between BINs 5.03 and 7.00 (see also Fig. 4). In a model fitting all significant additive QTL effects and additive×additive epistatic interactions simultaneously, this digenic interaction accounted for 5.0% of the phenotypic variation. All QTLs explained 37.5% of the phenotypic variation for FD2. Both parental inbreds contributed alleles that increased FD2 values. Although the QTL in BIN 7.00 was detected to be significant (LOD < 3.6), the simultaneous fit showed a non-significant main effect.

As to ΔFD, 12 significant QTLs explaining between 1.8 and 10.2% of $\hat{\sigma}_P^2$ were found on chromosomes 1 (2 QTLs), 2, 4 (2 QTLs), 5, 7 (2QTLs), 8 (2QTLs) and 9 (2QTLs) (Table 3). No significant digenic interactions were identified among these QTLs (Fig. 4). Nine of 12 marker intervals showed a negative genetic effect. In a model fitting all significant additive QTL effects simultaneously in one model, 24.6% of the phenotypic variation for ΔFD was accounted for. Parental inbred B73 contributed the alleles increasing ΔFD for nine out of 12 QTLs.

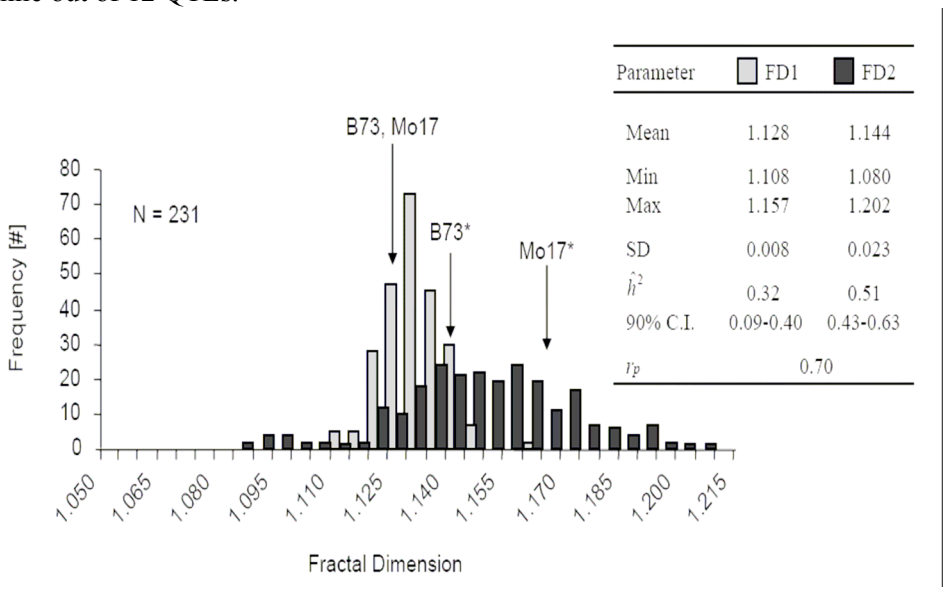

Fig. 1. Histogram for fractal dimensions of primary root systems of 231 RILs derived from the cross B73×Mo17 after four (FD1) and eight (FD2) days of germination. Arrows indicate the means of parental lines B73 and Mo17. Stars indicate parental means for FD2.

Table 1
Parameters associated with QTL for FD of primary root systems measured four days after germination (FD1). Parameters were estimated from phenotypic data of 231 RILs derived from the cross B73×Mo17

BIN[†]	Pos. cM	Marker interval		*LOD*	Genetic effect[‡]	$R^2_{partial}$[§]	$R^2_{(total)adj}$
		Left	Right				
1.01	2.8	MO005	MO006	3.78	0.001*	2.0	
1.04[#]	*40.0*	*MO059*	*MO060*	*7.44*	*Ns*		
1.07	68.2	MO103	MO104	4.04	0.001*	3.2	
1.09	82.0	MO127	MO128	8.79	–0.002**	5.5	
1.10	90.4	MO136	MO137	8.65	0.001**	4.5	
1.11	100.8	MO150	MO151	11.92	0.002**	13.3	
2.04	28.6	MO209	MO210	6.41	–0.001**	3.8	
3.06	45.4	MO372	MO373	7.62	0.002**	8.3	
4.09	63.2	MO527	MO528	5.95	0.002**	10.0	
5.03	24.8	MO583	MO584	7.93	–0.002**	8.6	
5.05	38.6	MO617	MO618	14.84	–0.003**	17.8	
6.04	20.8	MO697	MO698	5.75	0.001*	2.2	
6.05	26.8	MO707	MO708	5.01	–0.001**	4.0	
7.01/2	12.8	MO779	MO780	7.16	0.001**	3.6	
7.02	22.8	MO797	MO798	4.27	–0.001*	3.2	
8.01	4.6	MO874	MO875	4.67	–0.001*	2.8	
8.03	23.2	MO901	MO902	7.73	0.002**	11.5	
8.07	46.6	MO935	MO936	13.49	0.001**	5.1	
9.01	1.0	MO962	MO963	5.08	0.002**	10.1	
10.00	2.2	MO1075	MO1076	5.61	0.001**	5.4	
10.07	45.0	MO1157	MO1158	4.35	0.001*	3.2	
10.07	48.2	MO1160	MO1161	5.77	–0.001**	5.2	
		1/400	10/482		0.001**	3.5	
		2/286	7/128		–0.001**	3.7	46.8

[†]BIN locations are designated by an X.Y code, where X is the linkage group containing the BIN and Y is the location of the BIN within the linkage group (Gardiner et al., 1993). [‡]Additive genetic effects were estimated in a simultaneous fit using multiple regression.[§] R^2 = Proportion of phenotypic variance explained by the respective QTL. Total adjusted R^2 was calculated in a simultaneous fit using multiple regression.[#]QTL with LOD value larger than 3.5 but with non-significant genetic effect, as determined by a simultaneous fit using multiple regression

Common QTLs across traits

For FD1 and FD2, four common QTL regions were found (1.11, 5.03, 8.07 and 10.00). BINs 7.02 and 9.01 harboured QTLs for FD1 and ΔFD. Traits FD2 and ΔFD shared no common QTLs (Fig. 2).

Table 2
Parameters associated with QTLs for FD of primary root systems measured eight days after germination (FD2). Parameters were estimated from phenotypic data of 231 RILs derived from the cross B73×Mo17

BIN[†]	Pos. cM	Marker interval		*LOD*	Genetic effect[‡]	$R^2_{partial}$[§]	$R^2_{(total)adj}$
		Left	Right				
1.02	12.2	MO019	MO020	7.28	0.005**	7.6	
1.11	100.8	MO150	MO151	6.60	0.004**	4.6	
2.01	4.6	MO169	MO170	8.21	0.004**	5.8	
2.07	42.8	MO240	MO241	8.34	–0.004**	6.1	
3.04	21.2	MO319	MO320	8.3	–0.005**	6.2	
3.05	32.4	MO348	MO349	4.21	0.005**	6.8	
4.06	39.2	MO484	MO485	11.01	–0.003**	3.2	
4.11	74.4	MO542	MO543	4.77	–0.004**	4.7	
5.01	12.8	MO560	MO561	8.77	0.004**	3.8	
5.03	22.6	MO577	MO578	6.22	–0.004**	4.5	
7.00[#]	*4.8*	*MO766*	*MO767*	*5.09*	*ns*		
7.03	34.0	MO816	MO817	6.24	–0.003*	2.8	
8.07	46.4	MO935	MO936	4.64	0.003*	2.5	
10.00	2.2	MO1075	MO1076	7.34	0.004**	4.0	
		5/226	7/48		0.004**	5.0	37.5

[†]BIN locations are designated by an X.Y code, where X is the linkage group containing the BIN and Y is the location of the BIN within the linkage group (Gardiner et al., 1993).[‡]Additive genetic effects were estimated in a simultaneous fit using multiple regression.[§]R^2 = Proportion of phenotypic variance explained by the respective QTL.Total adjusted R^2 was calculated in a simultaneous fit using multiple regression.[#]QTL with LOD value larger than 3.5 but non-significant genetic effect, as determined by a simultaneous fit using multiple regression.

Table 3
Parameters associated with QTLs for the change of FDs over time (ΔFD). Parameters were estimated from phenotypic data of 231 RILs derived from the cross B73×Mo17

BIN[†]	Pos. cM	Marker interval		*LOD*	Genetic effect[‡]	$R^2_{partial}$[§]	$R^2_{(total)adj}$
		Left	Right				
1.03	30.2	MO045	MO045	3.73	–0.007**	3.1	
1.06	60.6	MO096	MO097	3.78	–0.005*	1.8	
2.09	69.0	MO276	MO277	12.24	–0.009**	5.5	
4.01	0.8	MO423	MO424	3.97	0.007**	3.6	
4.05	29.6	MO470	MO471	3.62	–0.005*	2.5	
5.08	62.2	MO645	MO646	6.47	–0.013**	10.1	
7.02	16.8	MO786	MO787	8.81	0.014**	8.0	
7.02	18.0	MO790	MO791	6.40	–0.011**	5.1	
8.04	32.4	MO916	MO917	5.04	–0.013**	10.2	
8.05	36.6	MO924	MO925	6.72	0.010**	5.8	
9.01	1.8	MO964	MO965	8.81	–0.008**	4.6	
9.02	10.0	MO977	MO978	3.61	–0.006**	3.1	24.6

[†]BIN locations are designated by an X.Y code, where X is the linkage group containing the BIN and Y is the location of the BIN within the linkage group (Gardiner et al., 1993).[‡]Additive genetic effects were estimated in a simultaneous fit using multiple regression.[§]R^2 = Proportion of phenotypic variance explained by the respective QTL.Total adjusted R^2 was calculated in a simultaneous fit using multiple regression.[#]QTL with LOD value larger than 3.5 but non-significant genetic effect, as determined by a simultaneous fit using multiple regression.

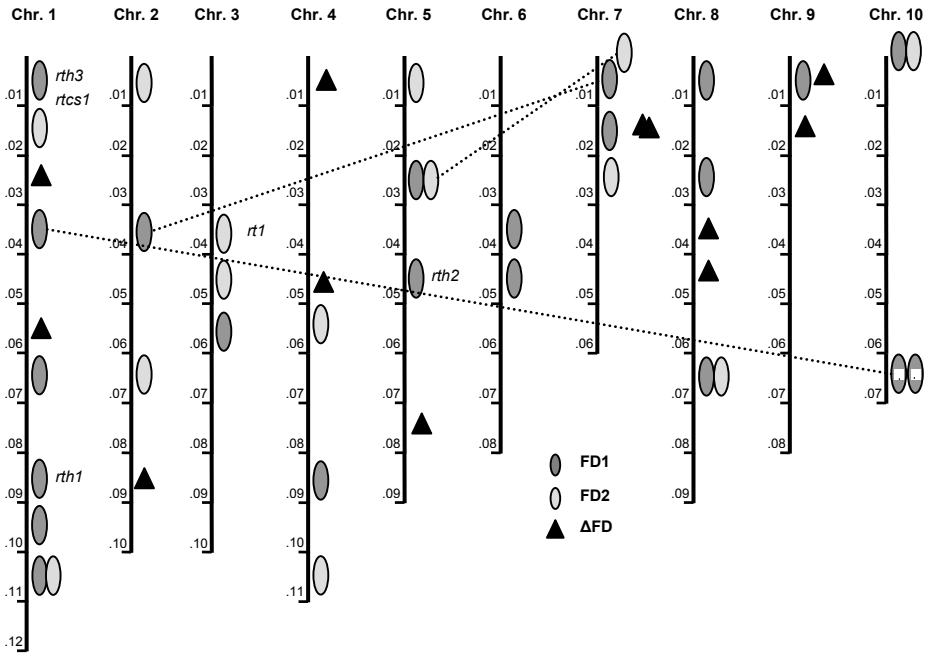

Fig. 2. Schematic linkage map reporting QTLs for FD of primary roots determined 4 and 8 days after germination from the cross B73×Mo17. Numbers to the left of the chromosome indicate the respective BIN. Chromosomal regions carrying QTLs for FD of primary roots determined 4 and 8 days after germination are represented by ovals and QTL locations for the change of FD over time are indicated by triangles; the dashed line indicates significant digenic additive×additive epistatic interactions; plus and minus signs in ovals describe signs of QTL effect

Discussion

The overall mean of FD for the primary root system of the parental inbreds B73 and Mo17 and the RILs increased over time. This observation is in agreement with the increased number and length of lateral and seminal roots (data not presented) found eight days after germination, which resulted in an increased root complexity. However, the difference between the overall population means for FD1 and FD2 were small. This finding could be explained by the short time interval between image acquisitions. However, based on a previous study (Fonseca, 2005) evaluating a diverse set of maize inbreds for primary root complexity, the four-day interval between image acquisition was sufficient for observing a significant increase in root length, width and branching. According to fractal geometry, a line has a dimension of one independently of its length. Therefore, if the system expands just in one direction without further branching the FD of a root system will not change. The analysis of the dynamic change of root complexity over time, as determined by ΔFD, in the IBM population revealed significant differences among RILs for

their root development. RILs with negative ΔFD values were also observed**,** a finding that might indicate that after an initial complex development only the primary root continued to grow.

The significant genotypic variation for FD1 and FD2 observed among the IBM RILs is in agreement with findings in other species. Tatsumi et al. (1989) determined FD values for root systems of different plant species varying between 1.48 and 1.58. FD values of maize root systems ranged from 1.40 to 1.73 if the plants were subjected to nitrogen stress (Eghball et al., 1993). Estimates of FD for different sorghum (*Sorghum bicolor* (L.) Moench) genotypes varied between 1.44 and 1.89 and FD values for soybean roots (*Glycine max* (L.) Merr.) changed with increasing planting densities (low density: $1.30 < FD < 1.67$; high density: $1.15 < FD < 1.36$) (Foroutan-pour et al., 1999). Oppelt et al. (2000) reported FD values ranging between 1.17 and 1.66 for the fruit tree species *Strychnos spinosa*, *Vangueria infausta* and *Strychnos cocculaides* and for the shrub *Grewia flava*.

In this study, the complexity of the primary root system of maize in the two-dimensional space was determined by restricting the growth of the root system in germination paper rolls. In order to capture the complexity of adult maize root systems this approach must be extended into the three-dimensional space. One possible solution to this complex problem might be to count the number of root tips. Abraham (2001) studied the fractal branching of the sponge species *Raspailia inaequalis* and found a high correlation between the number of tips/end points and the FD values of the three-dimensional structure. For plant species no studies are yet available to confirm this relationship.

Applying composite interval mapping, a large number of QTLs ($N \geq 12$ QTL) were found for FD and its change over time. Even though these FD1, FD2 and ΔFD were significantly correlated with each other and the number of detected QTLs was large, only 7 out of 40 chromosomal BINs carrying QTLs were found to contain QTLs for at least two traits. If QTLs in adjacent BINs are also regarded as common, 12 chromosomal regions with multiple QTLs were identified. This moderate level of congruency could be accounted for by the small proportion of the phenotypic variation explained by the identified QTL**,** indicating that several additional QTLs with small effects remain to be detected. Possible ways to increase the power of QTL detection are to consider larger population sizes ($N > 500$) and to improve the accuracy of the phenotyping. Another possible explanation for the limited QTL overlap across traits is that FD values measured at different primary root development stages capture different sets of uncorrelated root complexity-defining characteristics. In agreement with this hypothesis, Fonseca (2005) reported only moderate to low associations between root characteristics determined 6 and 12 days after germination for a set of 45 diverse maize inbreds. The presence of different root growth patterns as described by Gao and Bohn (2004) might also explain the observed lack of QTL congruency.

Mutants affecting root morphology have been studied in *Arabidopsis*, soybeans (Kosslak et al., 1997), and tomato (Zobel, 1991). Also for maize, several mutants affecting the formation of root hairs (*rth1*, *rth2*, *rth3*), lateral roots (*lrt1*, *slr1*, *slr2*) and shoot-borne roots (*rtcs*, *rt1*) were found (Jenkins, 1930; Hetz et al., 1996; Hochholdinger et al., 2004). Evaluation of these mutants demonstrated that primary, lateral and adventitious root formation was partly controlled by different sets of genes. For six maize root mutants their chromosomal BIN location is known. It is worth noting that root mutants have been mapped to all the BINs that have been shown to carry QTL for FD1 or FD2 (Fig. 1) in this study. Following the hypothesis of Robertson (1985) it can be speculated that such mutants represent extreme alleles for the genes underlying the QTL identified in this study.

Transgressive segregation was observed for FD1, FD2 and ΔFD. In agreement with this observation, both parental inbreds contributed QTL alleles that increased FD. It is interesting to note that for ΔFD, 9 out of 12 QTLs with increasing effects were contributed by parent Mo17. This is in accordance with the significantly higher FD2 and ΔFD means found for parent Mo17 than for parent B73. Previous observations confirm significant growth rate differences for the primary root between parents Mo17 and B73 (Fonseca, 2005).

Tuberosa et al. (2002) summarized QTL results found in the literature for the primary root characteristics, including length and diameter, of maize plants grown in hydroponics. About half of the QTL locations identified in this study aligned with QTL locations reported for primary root length and diameter. However, new putative QTL locations were also identified due to the use of different parental germplasm and the application of new traits that take into account root complexity and its dynamics.

Conclusions

This study demonstrated that FD differences between RILs of the IBM population have a genetic basis. Applying a QTL mapping approach, it was possible to identify chromosomal regions in the maize genome carrying putative genes for root complexity. Some of these QTL regions were associated with root mutants providing logical candidate genes for root complexity. However, only limited information is available about the genes underlying these mutants. In addition, most QTLs were located in regions with no further information about possible candidate genes explaining the observed genotypic variation for root complexity.

This study also provides the technical basis for a systematic investigation of maize root complexity. All images used to determine fractal dimensions are available to investigate the usefulness of other complexity measures, such as entropy. Root complexity needs to be correlated with conventional root characteristics, such as root length, number of seminal roots, root angle, root hair

density or root biomass. Within this project first ideas were developed to determine the complexity of adult root systems in all three dimensions. Consequently, the complexity of primary and secondary root systems needs to be associated with important agronomic traits, such as root lodging, drought tolerance, N-uptake efficiency, tolerance/resistance to insects and/or diseases. The root evaluation system deployed herein may help maize breeders to more efficiently screen maize germplasm for important root characteristics. In addition, knowledge about the genetic relationship between root complexity and agronomic traits could accelerate the development of improved maize cultivars.

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