J. Amer. Soc. Hort. Sci. 134(5):543–552. 2009.

Modeling the Effects of Drought Stress on Leaf Development in a *Brassica oleracea* Doubled Haploid Population Using Two-phase Linear Functions

Ralf Uptmoor[2], Mildred Osei-Kwarteng, Susanne Gürtler[1], and Hartmut Stützel
Institute of Biological Production Systems, Leibniz Universität Hannover, Herrenhäuser Strasse 2, D-30419 Hannover, Germany

Additional index words. crop growth simulation, quantitative trait loci analysis, in silico breeding

Abstract. **The combination of quantitative trait loci (QTL) analysis and ecophysiological modeling has been suggested as an approach to reveal the genetic basis of complex traits since phenotypes change with time and environmental conditions and the variation within populations can be described by genotype-specific parameterization of response curves on time and influential environmental factors. The objectives of the present study are a genotype-specific parameterization of a model describing leaf area development under well-watered and drought stress conditions, the use of QTL for estimating model input parameters, an evaluation of the model, and a comparison of the genotype-specific and QTL-based model parameterization. We used a two-phase linear function to describe preflowering leaf area development in a *Brassica oleracea* L. doubled haploid population. To illustrate effects of drought on leaf growth, the function was combined with a plateau function, which estimates the soil water status at which stress effects begin to reduce leaf expansion, a genotype-specific slope of the response to soil water status, and the soil water status at which leaf expansion becomes zero. A total number of 14 QTL were detected on the parameters of the two-phase linear function describing preflowering leaf area development and the plateau function describing the effects of drought on leaf area development. Nine of these QTL colocalized to QTL detected on data of static leaf area measurements and osmotic adjustment. The entire model was able to distinguish between genotypes during later growth stages under well-watered and drought stress conditions. However, the predictability was largely reduced when drought stress became more severe at the final measurement dates. Independent evaluation trials showed that the accuracy of the model was on the same level or even higher when genotype specific input parameters were replaced by allele-specific QTL effects.**

The combined application of molecular markers and ecophysiological models can help to speed up the development of improved cultivars and enable the dissection of complex agronomic traits into the underlying physiological factors (e.g., for an efficient selection of candidate genes) (Boote et al., 2001; Hammer et al., 2006; Slafer, 2003). Connecting different levels of biological organization could provide significant impacts on crop improvement beyond single gene traits (Hammer et al., 2004). Dissecting complex traits into the parameters of ecophysiological models could be used for detecting quantitative trait loci (QTL) responsible for the phenotypic variability, while functional genome analysis provides information on the underlying genes and assists in the establishment of stable markers for marker-assisted selection. Theoretically, combining ecophysiological models with QTL models allows the prediction of the behavior of virtual plants of a breeding population under different environmental conditions, including conditions that do not exist or appear at present (Reymond et al., 2004). This could facilitate the in silico selection of favorable allele combinations (e.g., for climate change scenarios) by using computer simulations predicting the development of virtual genotypes under different environmental situations. QTL-based model approaches allow the prediction of any possible genotype of a segregating population in a wide range of environmental conditions (Tardieu, 2003). Because quantitative traits with genotype by environment interactions can be linked to stable underlying genotype characteristics, Yin et al. (2000) and Reymond et al. (2003) proposed that a genetic analysis could be carried out on parameters that describe the responses of genotypes to environmental conditions. In contrast to classical approaches that distinguish between constitutive QTL detected under any environmental condition, adaptive QTL describe adaptation processes and are detected only in specific (e.g., stress) environments. As a consequence adaptive QTL describe, in the present study, the adaptation process itself or the plasticity of a trait and are therefore directly detected on responses to environmental conditions. Genotypes independent of those used for QTL detection and for model parameterization have been used for testing the predictive quality of a combined QTL and ecophysiological model by Reymond et al. (2003).

Quantitative traits are inherently too complex to be described by a single value because their phenotypes change with age, metabolic rate, or environmental conditions (Ma et al., 2002). Due to their simplicity, linear models are widely used (e.g., to describe changes with age). However, even if the data

---

Received for publication 5 Mar. 2009. Accepted for publication 17 Sept. 2009.
We gratefully acknowledge Graham J. King (Rothamsted Research, Harpenden, UK) and Michael J. Kearsey (Birmingham, UK) for providing plant material and marker data. We thank Katharina Meyer and Ilona Napp for excellent technical assistance, and Frauke Urban and Daniel Gerhard for phenotyping.
We gratefully acknowledge the German Academic Exchange Service (DAAD) for providing a scholarship for Mildred Osei-Kwarteng.

[1] Current address: Federal Research Centre for Cultivated Plants, Institute of Horticultural Crops and Fruit Breeding, Erwin-Baur-Strasse 27, D-06484 Quedlinburg, Germany.

[2] Corresponding author. E-mail: uptmoor@gem.uni-hannover.de.

show good linearity throughout large time intervals, they cannot be applied for data showing rate change points (Buchwald, 2007). Exponential, saturating, and sigmoidal functions have been established to describe the growth trajectories that include rate changes. Exponential and saturating functions describe extreme situations, which are generally not realistic during extended time periods. Sigmoidal functions combine both the situations and are well suited to describe most plant developmental processes. QTL analysis was successfully carried out on sigmoidal growth curves (Ma et al., 2002; Malosetti et al., 2006). However, the use of sigmoidal growth curves has several disadvantages: 1) large datasets representing a wide range of plant development stages are needed; 2) many of the widely used functions are somewhat inflexible due to fixed inflexion points, which is the case for the logistic function; and 3) more flexible ones like the Richards function (Richards, 1959) show high covariances between parameters, which leads to convergence problems and instable model parameters.

In the present study, we propose a two-phase linear model to describe preflowering leaf area (LA) development in *B. oleracea*. The advantages are two-fold. First, *n*-linear extensions can easily be generated. Parameterizable transitions between the linear segments by adding additional terms with successive rate-change points result in completely general models with *n* successive slopes, which are separated by *n*-1 rate-change points (Buchwald, 2007). Second, particular growth rates can be estimated for discrete durations, which may give information about the altered activity of QTL during different growth stages. To account for changing environmental conditions, the two-phase linear function describing LA development under optimum growth conditions was combined with a plateau function, which estimates the effects of drought on leaf expansion rates (LER). The plateau function is a special form of an *n*-phase linear function, including periods where the slope equals to zero. Two rate change points reflect the point when the stress effect starts to significantly change LER and the soil water status at which LER becomes zero.

Model parameters are given for each genotype by individual parameter estimates and based on particular QTL allele combinations in the present study. Many combined QTL and ecophysiological modeling approaches show pooled data from different levels of environmental stress (Reymond et al., 2003), sowing dates (Yin et al., 2005), or temperature regimes (Uptmoor et al., 2008). Earlier studies have shown that differences between genotypes in yield and biomass accumulation are only moderately predictive if all plants experienced the same environmental conditions. Yin et al. (2000) found in barley (*Hordeum vulgare* L.) RILs that correlations between predicted and measured values were mainly due to the segregation on one particular locus and that correlations were largely reduced when yield and biomass were predicted on independent datasets. One major reason for the low accuracy of crop models in predicting genotype effects may be that experimental errors blur trait differences between genotypes and that there is no substantial variability for all model input parameters in the segregating population. Therefore, we used a wide cross between chinese kale [*B. oleracea* var. *alboglabra* (L.H. Bailey) Musil] and broccoli (*B. oleracea* var. *italica* Plenck) segregating for both LER under well-watered conditions and adaptation to drought (Sebastian et al., 2000). Experiments for model parameterization and evaluation were carried out under controlled conditions to minimize random experimental errors. Prediction accuracies are shown separately for stress and nonstress conditions at different development stages. The main objectives of the present study were the parameterization of a model illustrating leaf area development of a *B. oleracea* doubled haploid (DH) population under well-watered and drought stress conditions individually for each DH line of the population, the detection of QTL on the parameters of the model followed by a model parameterization based on QTL effects, the evaluation of the model using independent experiments, and a comparison of the two methods used for model parameterization.

Materials and Methods

Plant material

The study was carried out on 46 DH lines developed from F_1 plants of a cross between the rapid cycling chinese kale and broccoli [A12 and GD33 (Bohuon et al., 1998)]. The population was mapped with 432 restricted fragment length polymorphism (RFLP) markers covering 875 cM (Sebastian et al., 2000), of which 92 uniformly distributed markers were selected for QTL analyses.

Experiments

Expt. 1. Plants were sown in trays and transplanted 3 weeks after sowing. Single plants were cultivated in pots (50 × 12.5 cm) with four replications, including well-watered plants and plants under continuously drying soil-water conditions in a randomized complete block design (RCBD). Pots were filled with 9.4 kg of sandy soil (< 10% silt, < 2% clay) and 1.1 kg of a nutrient solution containing 1.5% 16N–2.6P–21.6K (Flory 2 Mega 16–6–26; Euflor, Munich, Germany). The well-watered plants were irrigated every second day to 11.7% soil water content (SWC), which corresponds to 85% water holding capacity. Average daily air temperature was 20.0 °C and ranged from 17.9 to 22.7 °C during growth in the pots. Average daily maximum air temperature was 24.0 °C, and average daily minimum was 16.8 °C. Relative humidity (RH) was, on average, 60.2% and ranged from 46.5% to 77.6%. For the stress treatment, watering was stopped 9 d after transplanting. Leaf length (LL) was measured 32, 38, 45, 52, and 59 d after sowing (DAS). Plants were harvested 64 DAS (Table 1), and leaf area (LA) was measured with a leaf area meter (LI-COR 3100; LI-COR, Lincoln, NE).

Expt. 2. Plants were cultivated in an α-lattice design with two replications. For data analysis, the experiment was treated as RCBD because no significant differences were observed between subplots. Each column (50 × 10 cm) was filled with sandy loam (6.3 kg) and 1.2 kg of the nutrient solution. The control treatment was watered every second day to 19.0% SWC, which, for this soil type, corresponded to 85% water holding capacity. Mean daily air temperature was 20.5 °C and ranged from 18.4 to 27.2 °C. Average daily maximum air temperature was 26.8 °C, and average daily minimum was 17.7 °C. RH was, on average, 49.1% and ranged from 28.0% to 66.0%. Watering was stopped 13 d after transplanting in the stress treatment. LL was measured 36, 41, 45, and 52 DAS. Plants were harvested 52 DAS and LA was measured (Table 1).

Expt. 3. Plants were cultivated with two replications and four plants per replication in a RCBD. The experiment did not include a well-watered control treatment. Mean air temperature was 18.1 °C and ranged from 17.6 to 22.3 °C. Average daily

Table 1. Soil type, average air temperatures (T), and relative humidity (RH) at the beginning of the stress cycle and during leaf area measurements in *Brassica oleracea*.

Soil type	T (°C)	RH (%)		Start stress cycle	Leaf area measurements (no.) 1	2	3	4	5	6
				Expt. 1						
Sand	20.0	60.2	DAS (d)[z]	30	32	38	45	52	59	64
			TS (DD)[y]	703	744	868	1011	1148	1287	1384
			WHC (%)[x]	85.0	81.8	68.3	55.9	43.5	33.4	30.5
				Expt. 2						
Sandy loam	20.5	49.1	DAS (d)	34	36	41	45	52		
			TS (DD)	790	831	921	1005	1159		
			WHC (%)	85.0	81.5	64.7	50.2	35.6		
				Expt. 3						
Sand	18.1	46.8	DAS (d)	32	40	48	55	62		
			TS (DD)	614	757	891	1013	1155		
			WHC (%)	85	62.1	50.6	44.0	23.9		

[z] Days from sowing.
[y] Temperature sum in degree-days.
[x] Percentage of water holding capacity.

maximum air temperature was 19.7 °C and average daily minimum air temperature was 16.1 °C. RH was, on average, 46.8% and ranged from 18.3% to 77.2%. Watering was stopped 11 d after transplanting. Instead of using nondestructive LL measurements, plants were harvested and LA was measured 40, 48, 55, and 62 DAS (Table 1) using a leaf area meter.

Model description and data analysis

Measured LL of each DH line from the final harvest of Expt. 1 was taken for estimating LA, which was ascertained by fitting the following function to single leaves:

$$LA = aLL^{b}, \tag{1}$$

where a and b are parameters of a potential function. The parameters were estimated individually for each DH line because certain variability in length/width ratio exists within the population. On average, 37 leaves per genotype were used for estimating the parameters a and b. R^2 for predicting LA by LL applying the above function on each DH line ranged from 0.75 to 0.99 and averaged 0.94. The need for using a potential function showed that the LL/LA ratio changes during leaf growth. Because plants were harvested before flowering, all leaf growth stages were included in the analysis and there is no hint that the plant age has an effect on the LL/LA ratio.

A two-phase linear regression was fitted to the LA data to obtain LER using the iterative method of proc NLIN of the software package SAS (version 9.1; SAS Institute, Cary, NC). LER per unit thermal time was assumed to be independent from temperature, and 0 °C was used as the base temperature for leaf growth (Olesen and Grevsen, 1997). LER was not corrected for variations in RH. The regression includes an initial LER (LER_{ini}) and a maximum LER (LER_{max}):

$$\begin{array}{ll} LA = LER_{ini} \times TS & if\ TS < TS_{LER} \\ LA = LER_{\max}(TS - TS_{LER}) + LER_{ini} \times TS_{LER} & if\ TS > TS_{LER} \end{array}, \tag{2}$$

where TS is the actual temperature sum, and TS_{LER} is the threshold TS, for switching from LER_{ini} to LER_{max}. Relative LER (LER_{rel}) was calculated for the estimation of drought

Table 2. Parental line (PL) means and doubled haploid (DH) population means and ranges from Expt. 1 of initial leaf expansion rate (LER_{ini}), maximum leaf expansion rate (LER_{max}), temperature sum when leaf expansion switches from LER_{ini} to LER_{max} (TS_{LER}), percentage of water holding capacity when LER starts to decline (WHC_{dr}), rate to which LER declines per unit WHC (S_R), final leaf area averaged overall experiments under well-watered (LA_{ww}) and drought stress conditions (LA_{cd}), and osmotic adjustment (oA) in *Brassica oleracea*. Pearson's correlation coefficients between the traits are shown. An asterisk indicates a significant correlation at $P \leq 0.05$.

Trait	PLs A12[z]	GD33[y]	DH-population Mean	Max	Min	Pearson's correlation coefficients LER_{ini}	TS_{LER}	WHC_{dr}	S_R	LA_{WW}	LA_{CD}	oA
LER_{max} (cm^2/DD)[x]	2.53	2.23	2.28	3.54	1.46	0.55*	0.20	–0.17	–0.13	0.86*	0.49*	0.26
LER_{ini} (cm^2/DD)	0.26	0.18	0.176	0.243	0.107		–0.20	–0.04	0.12	0.61*	0.25	0.12
TS_{LER} (DD)	908	919	899	989	820			–0.22	0.16	–0.04	0.10	–0.14
WHC_{dr} (%)	88.2	89.2	84.2	94.7	55.6				–0.79*	–0.10	0.18	0.02
S_R	0.0134	0.0161	0.0159	0.0280	0.0116					0.11	–0.28*	–0.08
LA_{ww} (cm^2)	1214.4	1176.7	1125.7	1587.2	707.6						0.47*	0.31*
LA_{cd} (cm^2)	462.5	401.1	420.0	525.5	341.0							0.17
oA (MPa)	–0.158	–0.518	–0.311	–0.671	–0.063							

[z] Chinese kale.
[y] Broccoli.
[x] DD = degree-days.

stress effects on LER due to soil water deficits using a plateau function. The parameters of the function were estimated by an iterative method with the following equation:

$$\begin{array}{ll} LER_{rel} = 1 & \text{if } WHC > WHC_{dr} \\ LER_{rel} = 1 + S_R(WHC - WHC_{dr}) & \text{if } WHC < WHC_{dr} \end{array}, \quad (3)$$

where WHC is the actual percentage of the water holding capacity of the soil and WHC_{dr} is the percentage of water holding capacity above which no effects on LER were expected. S_R is the slope of the linear response curve of LER_{rel} to WHC from WHC_{dr} to WHC_0. WHC_0 is the percentage of water holding capacity at which LER_{rel} becomes zero. WHC_0 was estimated as follows:

$$WHC_0 = \frac{-1}{S_R} + WHC_{dr}. \quad (4)$$

The model parameters LER_{ini}, LER_{max}, TS_{LER}, WHC_{dr}, and S_R were computed individually for each DH line based on the results of Expt. 1. The model was evaluated using the results of Expts. 2 and 3. The soil type had a significant effect on WHC_{dr}. In Expt. 2, WHC_{dr} was significantly smaller than in the other experiments. However, S_R was not significantly affected by the soil type (data not shown). WHC_{dr} was adjusted in Expt. 2 by multiplying the estimated values with 0.95 because, on average, a 5% reduction was observed. To replace the original model input parameters by QTL-based parameter estimates, QTL analyses were carried out on the five parameters with a LOD threshold of 3.0 using PlabQTL 1.2 (Utz and Melchinger 1996) and the composite interval mapping method (Jansen and Stam 1994) by multiple regression with cofactors (Haley and Knott 1992). Regressions were carried out on the QTL output of each trait and predicted trait values were produced for each DH line as follows:

$$y_j = m + \sum a_i g_{ij}, \quad (5)$$

where y_j is the predicted value of the j-th DH line, m is the estimated population mean, a_i is the additive effect of the i-th QTL, and g_{ij} is the indicator for the allelic condition of the i-th QTL in the j-th DH line, defined as 1 for the broccoli allele and –1 for the chinese kale allele. For comparison of results, QTL analyses were performed on the averaged final LA data of Expts. 1, 2, and 3.

Because osmotic adjustment (oA) was assumed to be an important adaptation mechanism influencing LER under drought stress conditions, osmotic potential (Ψ_s) of leaves from the well-watered and the stress treatment was measured in Expt. 2 using the psychometric method and Wescor C-52 sample chambers (Wescor, Logan, UT). Ψ_s was corrected for relative leaf water content (RWC) as follows:

$$RWC = \frac{FW - DW}{TW - DW}, \quad (6)$$

where FW and DW are fresh weight and dry weight of the leaves and TW is the turgor weight of the leaf after submergence of leaf samples in distilled water for 24 h. oA was estimated as follows:

$$oA = \Psi_{s,cd} \times RWC_{cd} - \Psi_{s,ww} \times RWC_{ww}, \quad (7)$$

where $\Psi_{s,cd}$ and RWC_{cd} are Ψ_s and RWC under drought stress conditions, and $\Psi_{s,ww}$ and RWC_{ww} are Ψ_s and RWC under well-watered conditions. QTL analysis was carried out as described above.

Results

The parameter a (Eq. 1) for estimating LA from LL averaged 0.30 and ranged from 0.03 to 0.61. The parameter b averaged to 2.3 and ranged from 2.0 to 3.0. Means and ranges for LA_{ww}, LA_{cd}, oA, and the model parameters are shown in Table 2. LER_{max} was significantly correlated to LA_{ww} ($P \leq 0.05$) and S_R was negatively correlated with LA_{cd} and WHC_{dr} (Table 2). LA of the population mean was accurately estimated by the model. R^2 for predicting LA_{ww} was 0.95 and R^2 for LA_{cd} was 0.97 or 0.92, respectively, in the two evaluation trials Expts. 2 and 3 (Fig. 1).

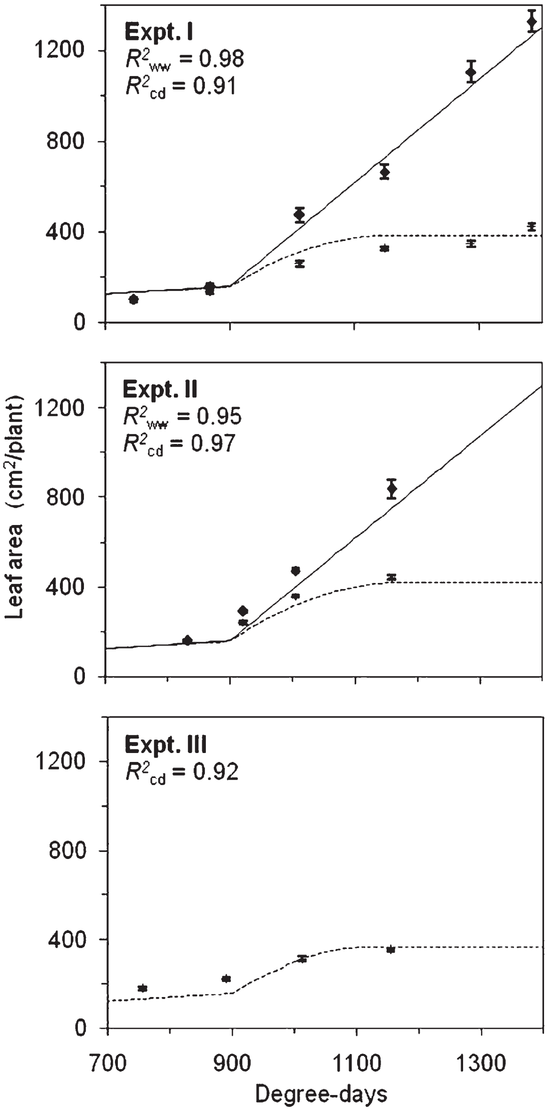

Fig. 1. Population mean of the measured leaf area under well-watered conditions (filled rhombus) and in a continuously drying soil (cross) in *Brassica oleracea*. The solid and dotted lines represent the simulated leaf area under well-watered conditions and in a continuously drying soil. The model was parameterized using the data of Expt. 2, and evaluated using the data of Expts. 2 and 3.

QTL analyses. A total of 21 QTL were detected. Of them, 14 QTL were detected for the five model parameters (Table 3 and Fig. 2), evidence for six QTL was found for LA$_{ww}$ and LA$_{cd}$, and one QTL was detected for oA. Between one (TS$_{LER}$, LER$_{ini}$, and oA) and five (LER$_{max}$) QTL were detected for the other parameters. One additional QTL (LOD = 2.9) for TS$_{LER}$ was used in the model, regardless of the fact that the LOD score was <3. Four QTL each were found for LA$_{cd}$ and S$_R$. With exception of O6, at least one QTL was detected on each chromosome. QTL for LER$_{ini}$, LER$_{max}$, S$_R$, LA$_{ww}$, and LA$_{cd}$ colocalized on O1. From the six QTL with LOD scores >6, three were on O1. Five QTL were detected on two distinct regions of O3 and evidence for four QTL was found in two regions of O2.

Both QTL for LA$_{ww}$ on O1 and O3 colocalized to a QTL for LER$_{max}$ (Fig. 2). Three of the four QTL for S$_R$ were found to be in the same chromosomal regions as QTL for LA$_{cd}$. Two QTL each were detected for the parameters TS$_{LER}$ and WHC$_{dr}$. One QTL of both model parameters colocalized to QTL for S$_R$. The QTL for oA coincided with QTL for LA$_{ww}$ and LER$_{max}$, but not to QTL for stress-related traits. QTL for LA$_{cd}$ and the drought-responsive model parameter S$_R$ colocalized to QTL for LA$_{ww}$ and LER on O1. Another QTL for LA$_{cd}$ was found in the same region as a QTL for LER$_{max}$ on O9. The effect of the broccoli allele of four of the five QTL for LER$_{max}$ reduced the trait, while the effect of the broccoli allele on O3 increased LER$_{max}$. The broccoli allele effect of the LA$_{ww}$ QTL on O3 was positive as well. The broccoli allele effects for all detected QTL for S$_R$ and LA$_{cd}$ were negative.

Modeling leaf area development. Pooling the data from all harvesting dates showed that the model accurately predicts LA$_{ww}$ (Fig. 3). R^2 of the evaluation experiment was only slightly reduced when compared with Expt. 1. Using original and QTL-based model input parameters in Expt. 2 resulted in $R^2 = 0.89$ and $R^2 = 0.90$, respectively. However, the slopes of both regressions (0.84 and 0.82) were significantly different from 1, while both intercepts did not significantly differ from 0. Predicting LA$_{cd}$ resulted in a reduced accuracy (Fig. 4). This held especially true when LA$_{cd}$ of the two evaluation trials was predicted using the original model parameters (Expt. 2: $R^2 = 0.60$, Expt. 3: $R^2 = 0.58$). QTL-based parameters lead to increased prediction accuracies ($R^2 = 0.77$ and $R^2 = 0.72$).

The development stage-specific R^2, using the original model parameters, calculated from genotype-specific measurements, ranged from 0.39 to 0.67 in Expt. 1 and between 0.10 and 0.45 in Expt. 2 (Table 4). Based on QTL parameters, R^2 ranged between 0.26 and 0.57 in Expt. 1 and between 0.10 and 0.38 in Expt. 2. R^2 was observed to be generally higher during later development stages in both experiments. Nearly all estimated intercepts and slopes were statistically different from 0 or 1, respectively. While intercepts were overestimated, slopes were

Table 3. List of all detected quantitative trait loci (QTL) based on the data of Expt. 1 for initial leaf expansion rate (LER$_{ini}$), maximum leaf expansion rate (LER$_{max}$), temperature sum when leaf expansion switches from LER$_{ini}$ to LER$_{max}$ (TS$_{LER}$), percentage of water holding capacity when LER starts to decline (WHC$_{dr}$), rate to which LER declines per unit WHC (S$_R$), final leaf area averaged overall experiments under well-watered (LA$_{ww}$) and drought stress conditions (LA$_{cd}$), and osmotic adjustment (oA) in *Brassica oleracea*.

Trait	LG[z]	Nearest marker	POS (cM)[y]	Range[x]	Additive effect[w]	LOD	R^2i[v]	R^2p[u]
LER$_{max}$ (cm^2/DD)[t]	1	pW239E2	30	22–34	–0.31	8.2	0.20	0.64
	3	pW102J1	92	86–100	0.17	5.3	0.22	
	5	ACCACE02	88	62–88	–0.19	9.24	0.10	
	8	pW138	24	14–32	–0.18	6.6	0.10	
	9	pN52E2	0	0–12	–0.28	3.8	0.16	
TS$_{LER}$ (DD)	1	pN186E1	22	4–26	26.4	2.9	0.24	0.48
	7	pW194E1	16	6–30	–21.5	3.1	0.18	
LER$_{ini}$ (cm^2/DD)	1	pW239E2	30	22–34	–0.023	6.5	0.39	0.39
WHC$_{dr}$ (%)	2	pO119E2	100	96–100	4.74	3.5	0.23	0.44
	4	pN59E1N	54	40–64	–5.01	4.0	0.12	
S$_R$	1	pW239E2	30	26–34	–0.0022	7.6	0.18	0.62
	2	pW116E1	0	0–12	–0.0015	4.6	0.10	
	2	pO119E2	100	96–100	–0.0020	6.9	0.25	
	3	pW102E1	20	16–22	–0.0014	5.9	0.06	
oA (MPa)	3	pW102J1	90	84–98	–0.07	3.14	0.27	0.27
LA$_{ww}$ (cm^2)	1	pW239E2	32	26–42	–92.6	3.19	0.17	0.39
	3	pW102J1	92	86–100	100.9	3.83	0.23	
LA$_{cd}$ (cm^2)	1	pW239E2	28	18–34	–13.5	3.42	0.22	0.62
	2	pW141E1	92	84–100	–18.1	4.96	0.34	
	3	pW153J1	10	0–16	–15.4	3.60	0.37	
	9	pO125E1N	10	4–22	–20.2	4.90	0.29	

[z] Linkage group.

[y] Distance from the first marker of the chromosome.

[x] Supporting log of the odds (LOD) > 2 interval (cM).

[w] Effect of the broccoli allele.

[v] R^2 of the individual QTL.

[u] R^2 of all detected QTL.

[t] DD = degree-days.

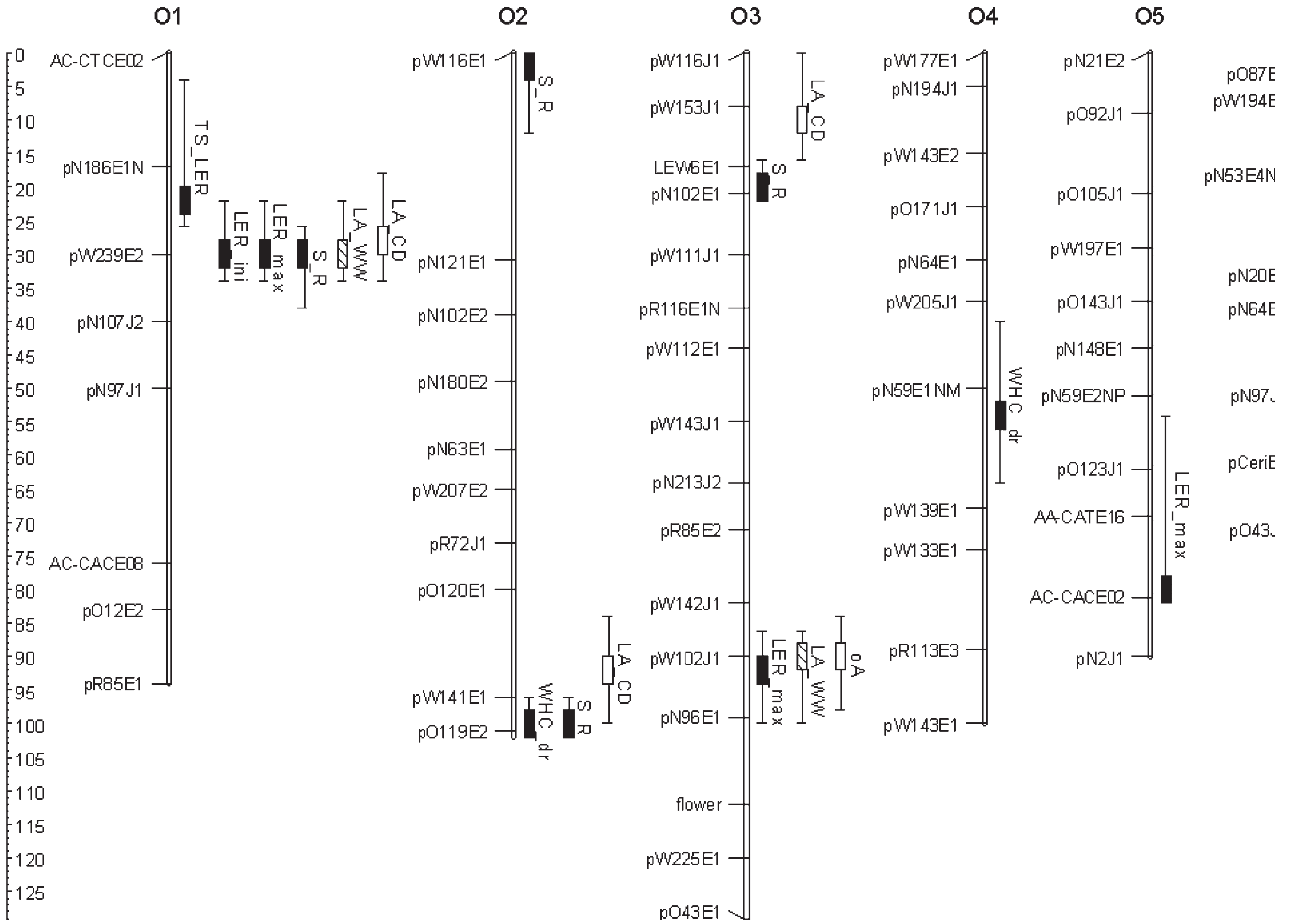

Fig. 2. Linkage map of a *Brassica oleracea* doubled haploid (DH) population. Marker positions are given according to Sebastian et al., (2000). Quantitative trait loci (QTL) positions of initial leaf expansion rate (LER_{ini}), maximum leaf expansion rate (LER_{max}), temperature sum when leaf expansion switches from LER_{ini} to LER_{max} (TS_{LER}), percentage of water holding capacity when LER starts to decline (WHC_{dr}), rate to which LER declines per unit WHC (S_R), final leaf area averaged overall experiments under well-watered (LA_{ww}) and drought stress conditions (LA_{cd}), and osmotic adjustment (oA). Boxes show the position of the maximum logarithm of the odds (LOD) score ± 2 cM, whiskers the LOD > 2 support intervals.

largely underestimated by using both parameterization methods.

The accuracy of the model was considerably reduced under drought stress. By using original model parameters, R^2 varied between 0.20 and 0.47 in Expt. 1, between 0.10 and 0.21 in Expt. 2, and ranged from 0.03 to 0.19 in Expt. 3 (Table 4). In comparison with the original model parameters, R^2 for the QTL parameters were estimated to be notably smaller in Expt. 1 (0.12–0.40) but on a similar level or even higher in Expt. 2 (0.05–0.31) and Expt. 3 (0.01–0.28). Similar to the observations under well-watered conditions, the intercepts of the regressions were regularly overestimated while the slopes were underestimated. Especially in the two evaluation experiments, R^2 continuously increased from the first to the third measurement and decreased remarkably at the fourth measurement.

Discussion

Between one and five QTL were detected for the five model parameters, LA_{ww}, LA_{cd}, and oA. Despite the low number of DH lines phenotyped in the present study, the number of QTL detected for LER_{max}, S_R, and LA_{cd} were comparatively high. Interestingly, four QTL were detected for LA_{cd}, while only two QTL were detected for LA_{ww}. Differences in flowering time within the population at relatively high temperatures (Uptmoor et al., 2008) probably led to an increased ability of the organs involved in flower development to attract assimilates in a way that variability in LA at the end of the experiment was reduced under well-watered conditions. In some plants, initial curd development was visible at that time. However, for both traits, LA_{ww} and LA_{cd}, two QTL colocalized to the parameters of the influencing model parameters, LER_{max} and S_R, which represent LER in a nonstress environment, and the response of LER to changes in soil water status.

QTL analyses on response curves facilitates the detection of adaptive QTL. QTL analyses produced clusters on chromosomes O1, O2, O3, and O9. QTL on O1, O3, and O9 were detected for traits under well-watered and drought stress conditions. Particularly, the clusters on O1 and O3 included QTL for constitutive as well as for adaptive traits. For example, while oA can be considered as an adaptive trait per se (Kamoshita et al., 2002), one approach to distinguish between adaptive and constitutive QTL is to define QTL detected under stress and nonstress conditions as constitutive, while QTL

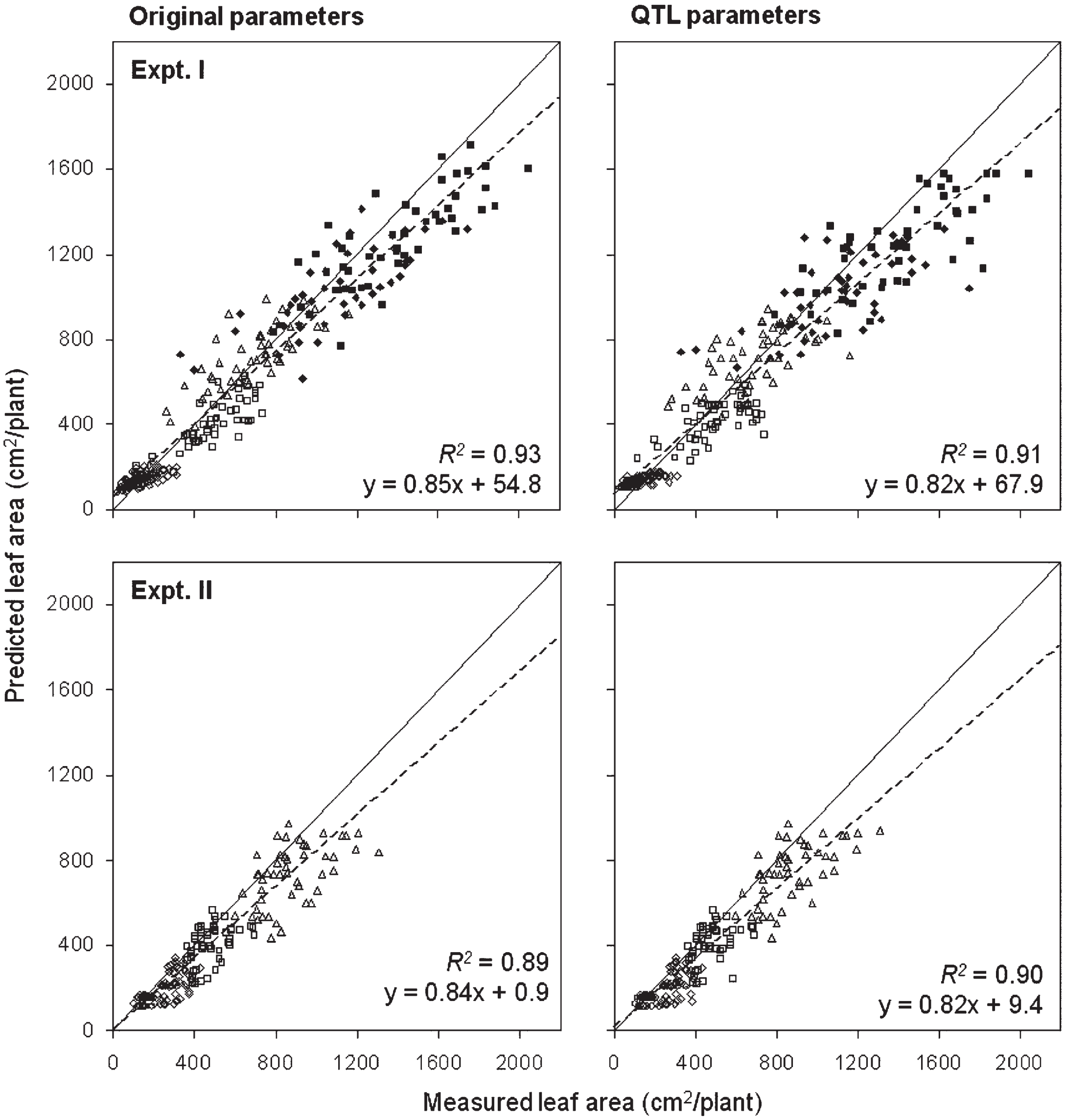

Fig. 3. Measured versus simulated leaf area under well-watered conditions. Model parameters were estimated individually for each genotype using the data of Expt. 1 (original parameters) and based on quantitative trait loci effects (QTL parameters). Expt. 2 was used for model evaluation. The symbols open circle, open rhombus, open square, open triangle, filled rhombus, and filled square represent the measurement dates shown in Table 1 in the ascending order. The 1:1 line (solid), the regression line (dotted), and regression statistics are shown.

detected only under environmental stress are considered to be adaptive (Sadok et al., 2006). Under this hypothesis, QTL analyses carried out on LA under well-watered and drought stress conditions would in the present example lead to the conclusion that the QTL for LA_{cd} on O1 is of constitutive nature due to the colocalization of a QTL for LA_{ww}. In this regard, QTL analyses carried out on response curves of ecophysiological models enables the direct identification of adaptive QTL. The model parameter S_R for instance is a measure of the responsiveness to changes in the soil water status and hence adaptive by definition. The method of carrying out QTL analyses on response curves has been suggested to be more reliable than others for the identification of QTL for adaptive traits (Reymond et al., 2004).

oA was suggested to be an important adaptive drought response mechanism [e.g., in cereals (Teulat et al., 1998)]. Issarakraisila et al. (2007) found that chinese kale does not adjust osmotically while other brassicas do. The variability in oA was high within the population as well as between parental lines. However, only one QTL was detected and there was no coincidence to QTL for other adaptation mechanisms. Different explanations for the missing colocalization of QTL for oA and other adaptive traits are possible: We failed to detect all major QTL for oA, or the constitutive nature of Ψ_s may play an important role. In the present study, Ψ_s was higher in the chinese kale parental line under well-watered and drought stress conditions. Hence, response curves of Ψ_s to environmental conditions may be more meaningful than oA itself.

ARE COMBINED MODELING APPROACHES SUITABLE FOR PREDICTING TRAIT DIFFERENCES BETWEEN GENOTYPES IN DEFINED ENVIRONMENTS? Knowledge about the predictability of genotypic differences in defined environments is important because

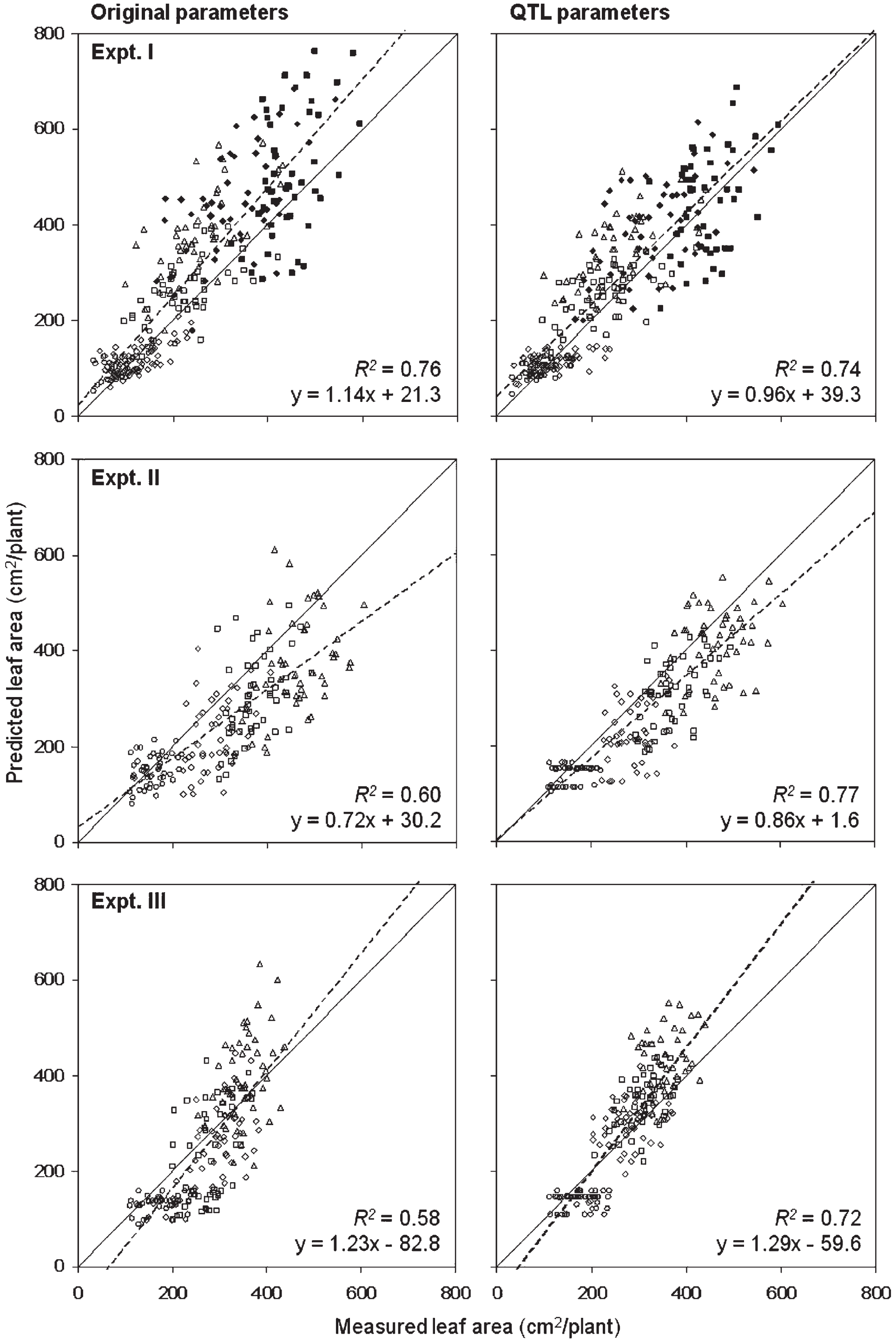

Fig. 4. Measured versus simulated *Brassica oleracea* leaf area in a continuously drying soil. Model parameters were estimated individually for each genotype on the data of Expt. 1 (original parameters) and based on quantitative trait loci effects (QTL parameters), Expts. 2 and 3 were used for model evaluation. The symbols open circle, open rhombus, open square, open triangle, filled rhombus, and filled square represent the measurement dates given in Table 1 in an ascending order. The 1:1 line (solid), the regression line (dotted), and regression statistics are shown.

this is a major prerequisite for using combined QTL and ecophysiological models (e.g., in plant breeding programs). Reymond et al. (2003) assumed that the wide range of environmental conditions under which LER of maize (*Zea mays* L.) RILs was tested partially caused high accuracies of a combined modeling and QTL approach. The present study indeed revealed an only moderate predictability of LA at specific developmental stages under controlled environmental conditions. However, the substantial discrepancies between R^2 values for using original model input traits and QTL-based model parameterization in Expt. 1 disappeared when the model was applied on independent evaluation trials. The decrease in R^2 values in Expt. 1 can be attributed to the additional sources of errors that are resulting from QTL analyses (i.e., to errors due to undetected minor QTL), errors in estimating QTL effects, and epistatic QTL effects, which were not taken into account in the present model. On the contrary, the QTL-based estimation of model parameters may reduce the effects of random experimental errors occurring in trials carried out for model parameterization and resulting in incorrect genotype-specific parameter estimates (cf. Yin et al., 2000). These suggestions are supported by the results obtained from carrying out regression analysis on pooled LA data from different development stages, where a notable increase in R^2 was observed for the QTL-based model parameterization in both evaluation experiments including drought stress conditions. However, similar effects were not observed under well-watered conditions, where experimental errors are assumed to have less influence on plant development in comparison with environmental stress conditions.

The development stage had a major influence on prediction accuracies. For the stress treatments, a decrease in prediction accuracies was observed at the late growth stages, when drought stress was most severe. Under well-watered and drought stress conditions, predictability was lowest at early developmental stages. Although high correlations between the traits LER_{max} and LER_{ini} were observed, only one QTL was detected for LER_{ini}. This may reflect the low power of QTL detection using small sample sizes (Vales et al., 2005), possibly induced by higher experimental errors because plant development may have suffered from transplanting. However, two-phase linear

Table 4. Accuracy statistics for estimating leaf area under well-watered and continuous soil drying conditions in *Brassica oleracea* using the original model input parameters and quantitative trait loci (QTL) parameters. R^2, slope, and intercept of linear regressions are given for each measurement date.

DAS	Well-watered			Continuous drying		
(d)[z]	R^2	Intercept	Slope	R^2	Intercept	Slope
Original parameters Expt. I						
32	0.43	84.6**[y]	0.48**	0.43	57.3**	0.49**
38	0.39	99.2**	0.32**	0.40	66.1**	0.41**
45	0.64	99.4**	0.62**	0.37	131.1**	0.57**
52	0.59	330.6**	0.57**	0.47	179.6**	0.78
59	0.60	476.1**	0.50**	0.36	188.8**	0.78
64	0.67	415.8**	0.61**	0.20	47.1	1.03
QTL parameters Expt. I						
32	0.36	101.7**	0.28**	0.24	76.2**	0.25**
38	0.26	125.5**	0.16**	0.26	82.9**	0.26**
45	0.41	220.4**	0.39**	0.38	142.8**	0.48**
52	0.40	424.2**	0.43**	0.40	203.1**	0.56**
59	0.49	541.9**	0.43**	0.33	177.3**	0.66
64	0.57	494.4**	0.54**	0.12	162.9	0.64
Original parameters Expt. II						
36	0.10	84.8**	0.39**	0.10	96.3**	0.34**
41	0.10	69.2	0.51*	0.15	37.3	0.64
45	0.23	123.6	0.59*	0.21	–14.2	0.84
52	0.45	261.9**	0.57*	0.12	100.8	0.58
QTL parameters Expt. II						
36	0.10	107.4**	0.23**	0.05	121.4**	0.14**
41	0.15	86.2	0.46**	0.16	67.2	0.55*
45	0.25	177.6**	0.49**	0.31	23.7	0.77
52	0.38	291.6**	0.52**	0.16	203.5*	0.48**
Original parameters Expt. III						
40				0.03	116.3**	0.08**
48				0.17	–23.0	0.92
55				0.19	45.4	0.76
62				0.15	90.22	0.87
QTL parameters Expt. III						
40				0.01	131.0**	0.05**
48				0.16	171.5**	0.46**
55				0.28	110.9	0.75
62				0.14	79.6	0.62

[z] Days after sowing.

[y] Statistically different at $P \leq 0.05$ (*) or $P \leq 0.01$ (**), respectively.

regressions produce inaccuracies, especially at the break point when LER switches from the first to the second development stage. More complex sigmoidal functions are generally more appropriate and biologically more meaningful (Ma et al., 2002; Malosetti et al., 2006) especially because they do not show abrupt transitions between development stages. However, an *n*-phase linear function was used because of its several advantages: 1) it can be applied on reduced datasets including, for example, the exponential growth phase only; 2) parameters are more flexible compared with exponential growth functions; 3) linear models turned out to be more robust, especially when minimal experimental data are used (Buchanan et al., 1997); and 4) the model is simple to interpret and can easily be extended or combined with stress effects as described in the present study.

Toward in silico breeding: genotype selection from simulation studies. Yin et al. (2003) highlighted the potential of combined approaches in predicting the performance of a mapping population, but concluded that the weakness of current crop-growth models in predicting differences in complex traits within segregating populations is a major drawback for application of combined QTL and crop models. The present model was able to predict LA at different development stages and under various environmental conditions. However, genotype effects were not fully rebuilt by the model. Slopes were largely underestimated, while intercepts of environment and development stage-specific regression analysis were overestimated at any condition and during most growth stages. Because prediction accuracies using both original input parameters and QTL-based model parameters were at least on the same level, we assume that rather the ecophysiological model itself than the QTL-based model parameterization was the major drawback for genotype-specific LA estimations. Although the experiments were conducted in controlled environments, specific conditions are seldom under full experimental control, which may be one major reason for the lack in accurate discriminations between genotypes. This is reflected by the strong differences observed between the well-watered and drought stress conditions under which plants were grown. As described above, in the latter case, random effects are assumed to have more influence on the variability within DH lines than under optimal growing conditions.

One major question arises from the present study because selection of superior genotypes for specific groups of environmental conditions is one of the most interesting future applications of combined modeling approaches: how accurate have simulation models to be for the use in plant breeding programs? We selected the top 20% of the DH lines, which had the highest LAs averaged overall development stages under well-watered conditions in Expt. 2, based on predicted and measured LAs. The coincidence between genotypes selected phenotypically and based on the combined QTL and ecophysiological model was 40%. The coincidence increased to 70%, considering 50% of the genotypes with the highest LAs. Under drought stress, the coincidence was 55% when selecting the top 20%, or 58% when selecting the 50% best-performing genotypes. Thirty-five percent of the DH lines with the highest LAs under well-watered conditions were also among the best-performing genotypes under drought stress.

Results imply that further advancements in this research area may have a substantial impact on progress in crop improvement, especially in breeding for drought-prone environments. However, breeding for drought-related traits remains difficult because strong adaptation processes (e.g., by excessively reducing leaf growth) are important traits when severe stress continues for long periods, but the same behavior may be contraproductive under conditions of erratic rainfall with short drought periods (Ben Haj Salah and Tardieu, 1997; Sambatti and Caylor, 2007). In this regard, simulation studies may help in finding the best compromise between maximizing growth or yield potentials and maximizing yield stability under varying environmental conditions. However, the most important step for the application of combined QTL and ecophysiological models is the identification of stable genotype-dependent parameters that account for the growth potential and for its responses to environmental conditions (Sadok et al., 2007). Because the genotype-specific evaluation of response curves on environmental stress requires large datasets to be analyzed, a refinement of current crop growth models by including parameters that are easy to measure and give stable characteristics is needed.

Literature Cited

Ben Haj Salah, H. and F. Tardieu. 1997. Control of leaf expansion rate of droughted maize plants under fluctuating evaporative demand. Plant Physiol. 114:893–900.

Bohuon, E.J.R., L.D. Ramsay, J.A. Craft, A.E. Arthur, D.F. Marshall, D.J. Lydiate, and M.J. Kearsey. 1998. The association of flowering time quantitative trait loci with duplicated regions and candidate loci in *Brassica oleracea*. Genetics 150:393–401.

Boote, K.J., M.J. Kropff, and B.S. Bindraban. 2001. Physiology and modeling of traits in crop plants: Implications for genetic improvement. Agr. Syst. 70:395–420.

Buchanan, R.L., R.C. Whiting, and W.C. Damert. 1997. When is simple good enough: A comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves. Food Microbiol. 14:313–326.

Buchwald, P. 2007. A general bilinear model to describe growth or decline time profiles. Math. Biosci. 205:108–136.

Haley, C.S. and S.A. Knott. 1992. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69:315–324.

Hammer, G., M. Cooper, F. Tardieu, S. Welch, B. Walsh, F. van Eeuwijk, S. Chapman, and D. Podlich. 2006. Models for navigating biological complexity in breeding improved crop plants. Trends Plant Sci. 11:587–593.

Hammer, G.L., T.R. Sinclair, S. Chapman, and E. van Oosterom. 2004. On systems thinking, systems biology and the in silico plant. Plant Physiol. 134:909–911.

Issarakraisila, M., Q. Ma, and D.W. Turner. 2007. Photosynthetic and growth responses of juvenile chinese kale (*Brassica oleracea* var. *alboglabra*) and caisin (*Brassica rapa* subsp. *parachinensis*) to waterlogging and water deficit. Scientia Hort. 111:107–113.

Jansen, R.C. and P. Stam. 1994. High resolution of quantitative traits into multiple loci via interval mapping. Genetics 136:1447–1455.

Kamoshita, A., Jingxian Zhang, J. Siopongco, S. Sarkarung, H.T. Nguyen, and L.J. Wade. 2002. Effects of phenotyping environment on identification of quantitative trait loci for rice root morphology under anaerobic conditions. Crop Sci. 42:255–265.

Ma, C.X., G. Casella, and R. Wu. 2002. Functional mapping of quantitative trait loci underlying the character process: A theoretical framework. Genetics 161:1751–1762.

Malosetti, M., R.G.F. Visser, C. Celis-Gamboa, and F.A. van Eeuwijk. 2006. QTL methodology for response curves on the basis of non-linear mixed models, with an illustration to senescence in potato. Theor. Appl. Genet. 113:288–300.

Olesen, J.E. and K. Grevsen. 1997. Effects of temperature and irradiance on vegetative growth of cauliflower (*Brassica oleracea* L. *botrytis*) and broccoli (*Brassica oleracea* L. *italica*). J. Expt. Bot. 48:1591–1598.

Reymond, M., B. Muller, A. Leonardi, A. Charcosset, and F. Tardieu. 2003. Combining QTL analysis and an ecophysiological model to analyze the genetic variability of the responses of leaf growth to temperature and water deficit. Plant Physiol. 131:664–675.

Reymond, M., B. Muller, and F. Tardieu. 2004. Dealing with the genotype × environment interaction via a modeling approach: A comparison of QTLs of maize leaf length or width with QTLs of model parameters. J. Expt. Bot. 55:2461–2472.

Richards, F.J. 1959. A flexible growth curve for empirical use. J. Expt. Bot. 10:290–300.

Sadok, W., B. Boussuge, C. Welcker, and F. Tardieu. 2006. A modeling approach to genotype × environment interaction, p. 77–91. In: J.H.J Spiertz, P.C. Struik, and H.H. van Laar (eds.). Scale and complexity in plant systems research. Springer, Dordrecht, The Netherlands.

Sadok, W., P. Naudin, B. Boussuge, B. Muller, C. Welcker, and F. Tardieu. 2007. Leaf growth rate per unit thermal time follows QTL-dependent daily patterns in hundreds of maize lines under naturally fluctuating conditions. Plant Cell Environ. 30:135–146.

Sambatti, J.B.M. and K.K. Caylor. 2007. When is breeding for drought tolerance optimal if drought is random? New Phytol. 175:70–80.

Sebastian, R.L., E.C. Howell, G.J. King, D.F. Marshall, and M.J. Kearsey. 2000. An integrated AFLP and RFLP *Brassica oleracea* linkage map from two morphologically distinct doubled haploid mapping populations. Theor. Appl. Genet. 100:75–81.

Slafer, G.A. 2003. Genetic basis of yield as viewed from a crop physiologist's perspective. Ann. Appl. Biol. 142:117–128.

Tardieu, F. 2003. Virtual plants: Modeling as a tool for the genomics of tolerance to water deficit. Trends Plant Sci. 8:9–14.

Teulat, B., D. This, M. Khairallah, C. Borries, C. Ragot, P. Sourdille, P. Leroy, P. Monneveux, and A. Charrier. 1998. Several QTLs involved in osmotic-adjustment trait variation in barley (*Hordeum vulgare* L.). Theor. Appl. Genet. 96:688–698.

Uptmoor, R., S. Schrag, E. Esch, and H. Stützel. 2008. Crop based QTL analysis across environments and QTL-based modeling of time to flowering in *Brassica oleracea*. Mol. Breed. 21:205–216.

Utz, H.F. and A.E. Melchinger. 1996. PLABQTL: A program for composite interval mapping of QTL. J. Quantitative Trait Loci 2:1–5.

Vales, M.I., C.C. Schön, F. Capettini, X.M. Chen, A.E. Corey, D.E. Mather, C.C. Mundt, K.L. Richardson, J.S. Sandoval-Islas, H.F. Utz, and P.M. Hayes. 2005. Effect of population size on the estimation of QTL: A test using resistance to barley stripe rust. Theor. Appl. Genet. 111:1260–1270.

Yin, X., P. Stam, M.J. Kropff, and A.H.C.M. Schapendonk. 2003. Crop modeling, QTL mapping, and their complementary role in plant breeding. Agron. J. 95:90–98.

Yin, X., P.C. Struik, F.A. van Eeuwijk, P. Stam, and J. Tang. 2005. QTL analysis and QTL-based prediction of flowering phenology in recombinant inbred lines of barley. J. Expt. Bot. 56:967–976.

Yin, X., S. Chasalow, C.J. Dourleijn, P. Stam, and M.J. Kropff. 2000. Coupling estimated effects of QTLs for physiological traits to a crop growth model: Predicting yield variation among recombinant inbred lines in barley. Heredity 85:539–549.