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RESEARCH PAPER

Analysing the genetic control of peach fruit quality through an ecophysiological model combined with a QTL approach

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Abstract

Ecophysiological models are increasingly expected to include genetic information via genotype-dependent parameters. These parameters could be considered as quantitative traits and submitted to analysis. A pre-existing ecophysiological model of fruit quality was used and the distribution of the genotypic parameters in a second backcross population derived from a clone of a wild peach (*Prunus davidiana*) and commercial nectarine varieties (*P. persica* (L.) Batsch) was analysed. The correlations between the two years of experimentation were higher for the genotypic parameters than for the quality traits commonly studied by breeders. The correlations between the genotypic parameters and the quality traits were low. Quantitative trait loci (QTLs) for the genotypic key parameters of the ecophysiological model were detected by linear regression. Co-locations of QTLs for parameters were observed as well as co-locations of QTLs for parameters and quality traits. The ecophysiological model and the results of the QTL analysis were combined by substituting each parameter in the model by the sum of QTL effects. This combined model can simulate the behaviour of genotypes carrying diverse combinations of alleles. The quality of this combined model was moderately suitable, but had some shortcomings. Improvements are suggested and further use of this combined model as a tool for breeders is discussed.

Key words: Ecophysiology, fruit quality, genotypic variation, modelling, peach, QTL.

Introduction

Fruit breeders must satisfy two requests concurrently: the production of high quality fruits and the use of sustainable practices. Wild germplasm is commonly used as a source of resistance to pests and diseases, but its use is limited because it is of low agronomic value. First, it is difficult to achieve the required agronomic improvement because selection is on quantitative traits, such as fruit mass or flesh sugar concentration, which result from several linked processes, such as carbon assimilation or fruit sink strength. Second, it is difficult to select for traits that are sensitive to environmental factors. QTLs controlling these traits often show low stability (Veldboom and Lee, 1996).

To overcome these difficulties, an interdisciplinary approach has been developed by ecophysiological modellers and geneticists (Shorter *et al*., 1991; Boote *et al*., 1996; Hammer *et al*., 1996). Molecular markers make it possible to carry out QTL analyses, which study the genetic variation of a character, locate the genes responsible for this variation, and quantify their effects and interactions. It is then possible to predict the behaviour of genotypes with any given combination of alleles, but only under environmental conditions similar to those where the QTLs were detected. Conversely, an ecophysiological model predicts the behaviour of one genotype in many environments. It decomposes the development of a trait into various processes subjected to environmental factors, with model parameters independent of the environment. An interdisciplinary approach consists of including genetic information in ecophysiological models via genotype-dependent parameters. These parameters could be considered as quantitative traits and characterize a genotype.

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Such an approach was applied to peach (*Prunus persica*) fruit quality because it results from many controlled processes and because it is highly sensitive to environment. Indeed, few QTLs associated with organoleptic fruit quality have been mapped (Abbott *et al*., 1998; Quarta *et al*., 1998) and genes controlling organoleptic fruit quality often remain unknown (Saliba-Colombani *et al*., 2001; Etienne *et al*., 2002). Microclimatic gradients (Corelli-Grappadelli and Coston, 1991; Marini *et al*., 1991), leaf area near the fruit (Kliewer and Weaver, 1971; Génard, 1992) and vegetative vigour of shoots bearing fruit (Génard and Bruchou, 1992) may cause within-plant variation in quality.

The present study was carried out on a population of genotypes derived from a clone of a wild peach (*P. davidiana*) by three generations of crosses with commercial nectarine varieties. The ecophysiological model used was described by Quilot *et al*. (2005) who identified genotypic key parameters of the model. These parameters can be analysed with QTL methods. First, they are estimated for numerous genotypes of the population. Second, they are highly variable from one genotype to another, and mostly independent of the environment. Lastly, they appeared to explain much of the variation in fruit quality in the population.

The distribution of the genotypic key parameters in the population and their stability through two years of experimentation was analysed. The correlations between these parameters and the quality traits commonly studied by breeders was also studied. A QTL analysis of the genotypic key parameters (QTL model) was then performed. An attempt has been made to explain the co-locations of QTLs for parameters and quality traits in order to interpret the functions of the QTLs detected. The QTL model was used to predict, for each genotype of the studied population, the values of each genotypic key parameter of the ecophysiological model. The goodness-of-fit of this combination of models was tested. Finally, the importance of such an approach for selection and for biological understanding was discussed.

Materials and methods

Description of the ecophysiological model

Our ecophysiological model simulates carbon assimilation, its partitioning at the 'shoot-bearing fruit' level, water flux, and sugar accumulation in the flesh during fruit growth, under the influence of environmental factors. Its mathematical formulation and the definition of its parameters have been described previously (Quilot *et al*., 2005). The outputs relevant for this study are dry and fresh fruit masses, stone fresh mass, dry matter content, and total sugar concentration in the flesh.

In addition to this ecophysiological model that is only concerned with fruit growth after the end of the stage of active cell division, the early growth of fruit, during which cells divide, was considered in an empirical way. The fruit size at the end of cell division is an indicator of fruit sink size and, consequently, of its potential expansion (Scorza *et al*., 1991). Cell division was reported to stop around 50–80 d after bloom (DAB), (Ognjanov *et al*., 1995; Yamaguchi *et al*., 2002), depending on the variety. Accordingly, it was assumed that cell division was fully completed at 590 degree-days (dd), which closely corresponds to 80 DAB. Early fruit growth was only considered after 321 dd, as it is not possible to measure diameters without causing fruit damage. Early fruit dry matter growth between 321 and 590 dd was roughly described by a linear function of degree-days after bloom (dd):

$$W_{\text{fruit}}^{\text{early}}(\text{dd}) = W_{\text{fruit}}^{321} + GR_{\text{fruit}}^{\text{early}} \times (\text{dd} - 321)$$

where W_{fruit}^{321} corresponds to fruit dry mass at 321 dd and $GR_{\text{fruit}}^{\text{early}}$ is the fruit early growth rate (g dd^{-1}). The initial fruit dry mass, $W_{\text{fruit}}^{\text{ini}} = W_{\text{fruit}}^{\text{early}}(590)$, input for the ecophysiological model, is computed from the early growth model with dd=590.

The genotypic key parameters of the ecophysiological model

When fruit loads were light, nine of the 40 parameters of the model were identified as genotypic key parameters by Quilot *et al*. (2005). These parameters satisfied three main conditions: the model was sensitive to their variation with respect to potential fruit growth; they varied widely in the population, and their value was accurately estimated. However, the parameter involved in fruit growth limitation close to maturity ($m_{\text{flesh}}^{\max}$) was estimated for only 18 genotypes. Since to analyse genetic variation of a trait is not reliable on so few genotypes, this parameter was not considered in the following study. Consequently, eight genotypic key parameters were studied further. In addition to these eight parameters, the initial fruit dry mass at 590 dd ($W_{\text{fruit}}^{\text{ini}}$, an initial state value of the model) and growth duration from full bloom to maturity ($dd_{\max}$), were important in this study based on model sensitivity and variability in the population. By extension, they were dealt with as parameters. Two parameters of the early growth model, W_{fruit}^{321} and $GR_{\text{fruit}}^{\text{early}}$, were also considered as possible genotypic key parameters. A description of these 12 parameters is given in Table 1.

Plant material

The breeding population is a second backross progeny derived from clone P1908 of *Prunus davidiana* as follows (Pascal *et al*., 1998). Initially, P1908 with small green fruit, was crossed with *P. persica* 'Summergrand' (S) and an F_1 progeny was obtained. One F_1 hybrid resistant to powdery mildew was then back-crossed to S to produce a BC_1 progeny. Finally, BC_1 individuals were used to pollinate *P. persica* 'Zéphir' (Z) to derive the breeding population (BC_2). S and Z are, respectively, yellow and white nectarine cultivars with large tasty fruits.

The study was conducted at the INRA Research Centre of Avignon (France). BC_2 genotypes and the three parents were planted in a completely randomized design with one tree per genotype. Trees were 3 years old in 2001. All genotypes were grafted on GF305 seedling rootstocks and were grown under optimal conditions of irrigation, fertilization, and pest control.

Experiments

Experimental observations were carried out in 2002 on 139 genotypes of BC_2 and on S, Z, and P1908 ($BC_2$02 dataset) and in 2001 on 87 genotypes of the BC_2 population common to both years and S and Z ($BC_2$01 dataset). A very light fruit load was left on each tree (only five fruits per tree) to ensure that all fruits were under non-limiting source conditions (i.e. under maximum growth conditions). However, these non-limiting source conditions appeared to be hardly met for numerous genotypes in 2001.

Diametric fruit growth was monitored from fruitlet thinning to maturity. At maturity, dry and fresh fruit and stone masses were measured. The total amount of sugar (gC) and total flesh sugar

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concentration were also determined. Details on these measurements have been described by Quilot *et al*. (2005).

These data were used by Quilot *et al*. (2004) to detect QTLs for quality traits commonly studied by breeders. These data ($BC_2$02 dataset) were also used to estimate the values of the ecophysiological model parameters (Quilot *et al*., 2005) and the values of the two parameters, W^{321}_{fruit} and GR^{early}_{fruit}, of the early growth model.

QTL analysis

The interspecific map for BC_2 progenies developed by Foulongne *et al*. (2003) and complemented by Quilot *et al*. (2004) was used. QTL detection was performed using a forward multiple linear regression of the phenotypic values of the genotype at each of the molecular markers, with Splus (Splus software, MathSoft Inc., Cambridge, MA). The most likely QTL position corresponded to the locus with the strongest association with the trait. A threshold of significance of 5% was chosen to declare a putative QTL. This method was described by Quilot *et al*. (2004) to detect QTLs for quality traits. QTL detection was carried out for the 12 parameters and for fruit dry mass.

Combination of ecophysiological and QTL models

The approach consists of introducing, in the ecophysiological model, the values estimated from the QTL model instead of the measured values of the parameters. The QTL model takes into account both the origin of the allele at a detected locus and the effect of the alleles at this locus on the parameter value. With a marker from P1908, the effect of the P1908 allele presence (scored 1) is determined by comparison with the presence of an allele coming from S (scored 0). With a marker of S (Z) genome, the effect of a S (Z) allele (scored 1) is determined by comparison with the presence of the other S (Z) allele (scored 0). The effects of the allele scored 0 are set to 0. The parameter value is estimated as the sum of the allele effects, either positive, negative or null, added to an intercept, μ. The intercept corresponds to the parameter value when the genotype only possesses the alleles set to 0. The epistatic effects between two loci were added in the same way to the QTL model.

Accordingly, the value of a parameter X for which N QTLs and M epistatic interactions were detected is estimated for an individual i by:

$$X_i = \mu + \sum_{n=1}^{N} a_n \times G_{i,n} + \sum_{m=1}^{M} e_m \times G_{i,m}$$

where a_n corresponds to the additive effect of the QTL, n and e_m to the effect of the epistatic interaction m. $G_{i,n}$ and $G_{i,m}$ are genetic QTL scores of the individual i that take the value 0 or 1 depending, respectively, on the allele of the corresponding QTL n and on the combination of alleles of the loci involved in the epistatic interaction m.

Statistical analysis

Most of the parameter values have been estimated by Quilot *et al*. (2005) on the $BC_2$02 dataset. However, the hydraulic conductance per unit of fruit surface (aL) was estimated again, setting the value of the permeation coefficient of the fruit surface to water vapour (ρ) constant to the mean observed value for all genotypes, in order to avoid distortions between genotypes. Indeed, ρ was estimated for only 41 genotypes of the population and the estimated aL value may depend on the ρ value. The parameter values from $BC_2$01 dataset were estimated as described by Quilot *et al*. (2005).

Goodness-of-fit of the combined model for each genotype was evaluated using the root mean squared error (RMSE), a common criterion to quantify the mean difference between simulation and measurement in the case of non-linear models (Kobayashi and Us Salam, 2000). The global goodness-of-fit of the model was computed by averaging the relative RMSE (RRMSE) values of all genotypes (see Quilot *et al*., 2004*a*, for details).

All data analyses were performed with the Splus software.

Results

Distribution of the key parameter values estimated on the $BC_2$02 dataset

The distributions of the 12 key parameter values were very similar for the two years. The parameter values of S ('Summergrand') and Z ('Zéphir') were nearly identical for seven parameters and only slightly different for five parameters including the growth duration dd_{max}, the coefficient of the transfer function between sugars and other compounds k_{sugar}, and the hydraulic conductance per unit of fruit surface aL (Fig. 1). By contrast, values of P1908 were clearly different from those of S and Z for five parameters. They were greater for k_{sugar} and s_1, and lower

Table 1. *Symbols, definitions and units of the parameters in the QTL analysis*

The parameter values were estimated either separately in 2001 and 2002 or jointly, depending on the parameters.

Parameter	Definition	Unit	Number of genotypes observed[a]		
			2001	2001/2002	2002
dd_{max}	Growth duration from full bloom to maturity	Degree-days	87		136
W^{321}_{fruit}	Fruit dry mass at 321 dd	g	87		136
GR^{early}_{fruit}	Dry fruit mass growth rate between 321 and 590 dd	g degree-days^{-1}	87		136
W^{ini}_{fruit}	Initial fruit dry mass at 590 dd	g	87		136
RGR^{ini}_{flesh}	Initial relative dry flesh mass growth rate	Degree-days^{-1}	87		136
w^{matu}_{stone}	Potential maximal stone dry mass at maturity	g		149	
df^{1}_{stone}	Concerns the allometric equation relating stone fresh mass to stone dry mass	Dimensionless		155	
k_{sugar}	Coefficient of the transfer function between sugars and compounds other than sugars	Day^{-1}	87		134
r_{su}	Concerns the calculation along growth of the proportion of sucrose in the total amount of sugar in the flesh	Dimensionless		154	
ρ	Permeation coefficient of the fruit surface to water vapour	cm h^{-1}		41	
s_1	Concerns the allometric equation relating fruit area to fruit fresh mass	Dimensionless		149	
aL	Hydraulic conductance per unit of fruit surface	g cm^{-2} bar^{-1} h^{-1}	87		134

[a] Number of BC_2 genotypes for which the parameter values were estimated in 2001 and in 2002 or jointly in both years.

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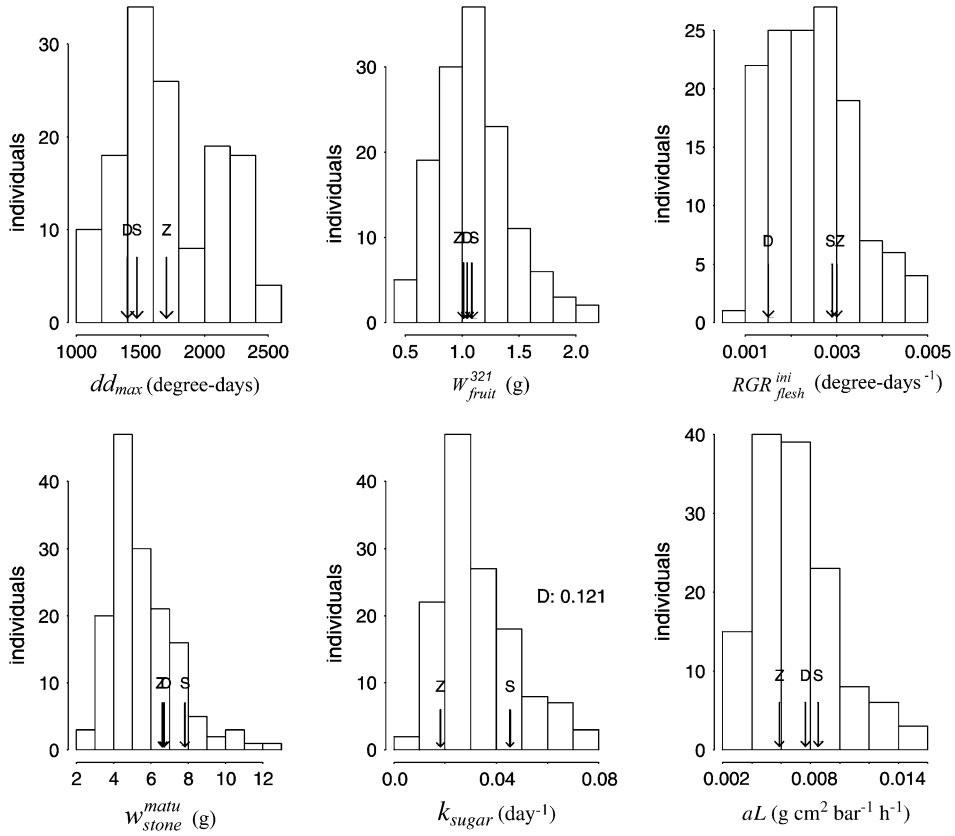

Fig. 1. Distribution of the 12 key parameters of the ecophysiological model estimated on the 2002 dataset. The values of the parents 'Summergrand' (S), 'Zéphir' (Z), and *P. davidiana* (D) are indicated by arrows.

for the three fruit growth parameters $GR^{\text{early}}_{\text{fruit}}$, $W^{\text{ini}}_{\text{fruit}}$, and $RGR^{\text{ini}}_{\text{flesh}}$.

The population exhibited considerable genotypic variation in parameters. Most of the parameters were nearly normally distributed, apart from dd_{max} for which the distribution was bimodal (Fig. 1). Transgressive segregants were observed for high and/or low levels of all parameters. For example, transgressive segregants were very frequent for high levels of growth duration, dd_{max}, since most of the genotypes showed a value higher than the values of the three parents. Trangressions for high levels were also observed for fruit growth parameters (W^{321}_{fruit}, $GR^{\text{early}}_{\text{fruit}}$, $W^{\text{ini}}_{\text{fruit}}$, and $RGR^{\text{ini}}_{\text{flesh}}$), the parameter concerning the calculation of the sucrose to total sugar ratio, r_{su}, and aL and df^{1}_{stone}. For $w^{\text{matu}}_{\text{stone}}$, transgressive segregants towards low values were observed. Conversely, for k_{sugar} and s_1 none of the genotypes in the population showed higher values than the parents.

Stability of the trait and the key parameter values between 2001 and 2002

Seven (W^{321}_{fruit}, $GR^{\text{early}}_{\text{fruit}}$, $W^{\text{ini}}_{\text{fruit}}$, dd_{max}, $RGR^{\text{ini}}_{\text{flesh}}$, k_{sugar}, and aL) of the 12 key parameters were estimated separately from 2001 and 2002 data. The correlations between 2001 and 2002 values were highly significant for all the key parameters (Table 2) and were higher overall than for quality traits. The stone fresh mass was the most stable trait over years. The highest correlations between years for the parameters were observed for growth duration (dd_{max}) and the two parameters of dry matter growth rate, $GR^{\text{early}}_{\text{fruit}}$ and $RGR^{\text{ini}}_{\text{flesh}}$. The sugar concentration in the flesh and the parameter related to sugar metabolism, k_{sugar}, showed least stability.

Table 2. *Correlation coefficients between 2001 and 2002 fruit traits at maturity and parameter values for the 87 genotypes common to the two years*

All correlations appeared highly significant (P <0.001).

Fruit trait		Parameter	
Fruit dry mass	0.52	dd_{max}	0.96
Fruit fresh mass	0.47	W^{321}_{fruit}	0.52
Stone fresh mass	0.60	$GR^{\text{early}}_{\text{fruit}}$	0.81
Flesh dry matter content	0.49	$W^{\text{ini}}_{\text{fruit}}$	0.74
Total flesh sugar concentration	0.35	$RGR^{\text{ini}}_{\text{flesh}}$	0.90
		K_{sugar}	0.37
		aL	0.66

Correlations between traits and key parameters

Among the correlations between the 12 parameters and five traits of interest at maturity (Table 3), the strongest

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Table 3. *Correlation coefficients between fruit traits and parameter values measured for the BC_2 progeny in 2002*

	Fruit dry mass[a]	Fruit fresh mass[a]	Stone fresh mass[a]	Flesh dry matter content[a]	Total flesh sugar concentration[a]
dd_{max}	−0.02	−0.008	−0.01	−0.14	0.12
W^{321}_{fruit}	0.33***	0.30***	0.64***	0.10	−0.04
GR^{early}_{fruit}	0.47***	0.50***	0.58***	0.09	0.02
W^{ini}_{fruit}	0.52***	0.53***	0.70***	0.10	0.01
RGR^{ini}_{flesh}	0.20*	0.14*	0.10	0.11	0.09
w^{matu}_{stone}	0.36***	0.34***	0.81***	0.19*	−0.2
df^{1}_{stone}	0.21*	0.30***	0.47***	−0.22*	−0.12
k_{sugar}	−0.10	−0.08	0.09	−0.06	−0.41***
r_{su}	0.04	0.02	0.09	0.05	0.02
ρ	−0.10	−0.17	0.14	0.09	−0.03
s_1	−0.19*	−0.11	−0.07	−0.22*	−0.08
aL	−0.26**	−0.13	0.04	−0.41***	−0.59***

[a] * P <0.05; ** P <0.01; *** P <0.001; others not significant.

were between stone fresh mass and the three early growth parameters, W^{321}_{fruit}, GR^{early}_{fruit}, and W^{ini}_{fruit} and, as expected, w^{matu}_{stone}, the potential maximal stone dry mass at maturity. Other correlations were significant but not strong: dry and fresh fruit masses appeared correlated to the three early growth parameters and to w^{matu}_{stone}. Fruit fresh mass correlated with the parameter df^{1}_{stone}, which was also correlated with stone mass. Surprisingly, the correlation between fruit dry mass and the initial relative flesh growth rate RGR^{ini}_{flesh} was low and only just significant. The low but significant negative correlation between aL and the fruit dry mass was not expected since the water flux submodel does not influence the carbon submodel. Flesh dry matter content was negatively correlated with the parameter aL, a water uptake parameter, but no correlation was found with the parameter ρ which also interacts in the water fluxes of the fruit. As expected, total sugar concentration was

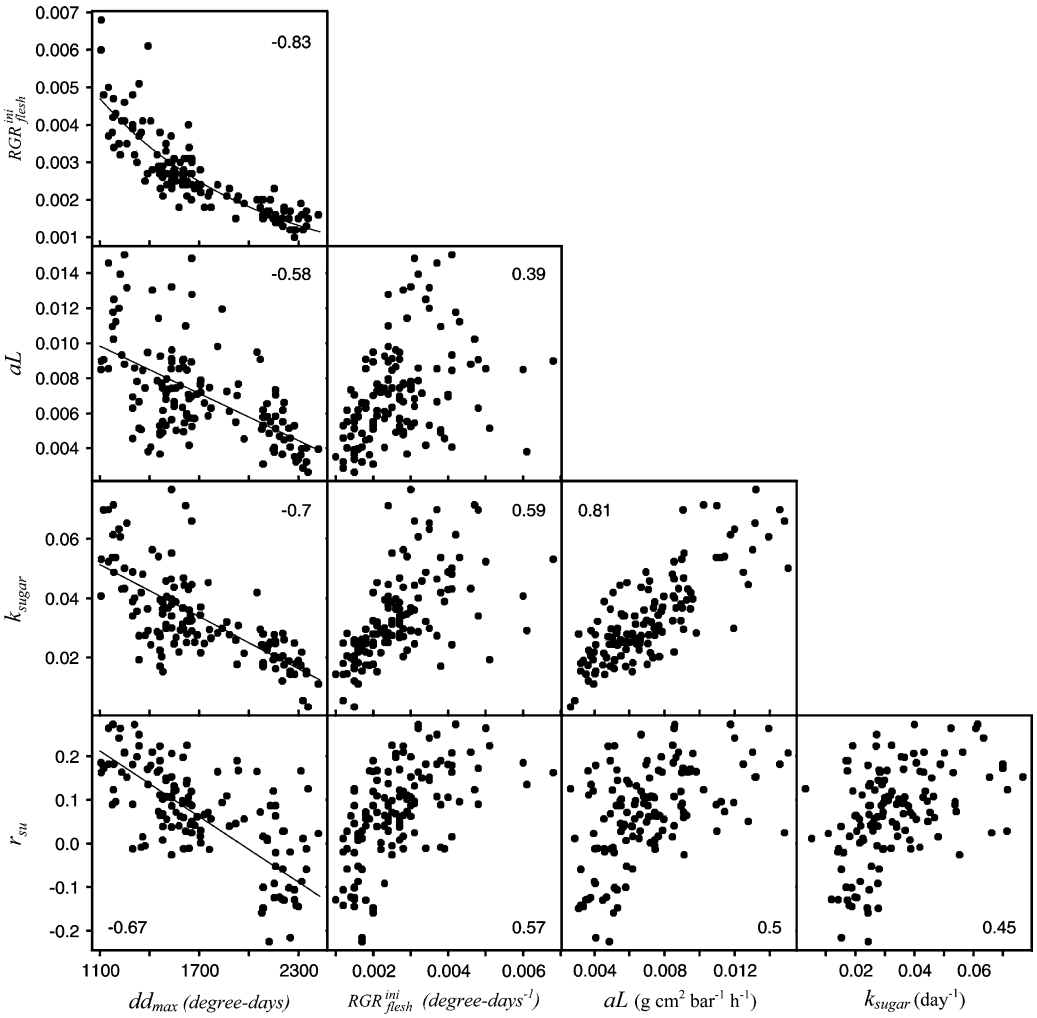

Fig. 2. Relationships between the values of the five parameters RGR^{ini}_{flesh}, aL, k_{sugar}, r_{su}, and dd_{max} for the BC_2 population. The lines represent the global adjustments for the relationships between dd_{max} and the four other parameters. For the relationship between dd_{max} and RGR^{ini}_{flesh} a curve was adjusted, whereas for the others a linear adjustment was done. Correlation is indicated. All correlations appeared highly significant (P <0.001).

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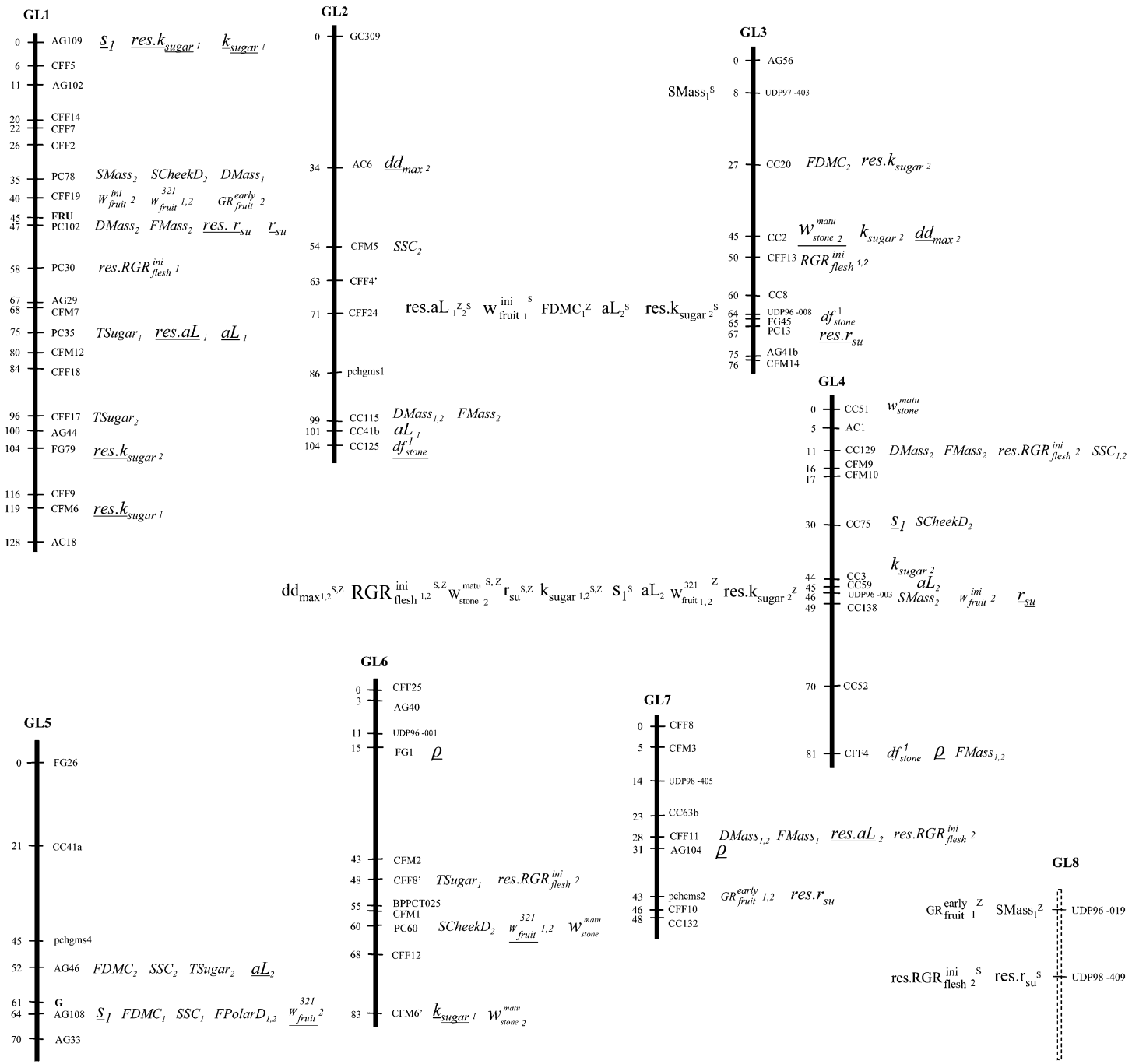

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significantly correlated with the two parameters k_{sugar} and *aL*. Lastly, no correlation was found between the maturity date and any of the traits.

Relationships between the key parameters

Pairwise correlations between RGR^{ini}_{flesh}, *aL*, k_{sugar}, and r_{su}, were strong (Fig. 2). Parameters *aL* and k_{sugar} displayed a particularly tight linear relationship (correlation=0.81). These four parameters were also highly negatively correlated to growth duration dd_{max} (correlation coefficient ranging from –0.58 to –0.83). A non-linear and three linear equations described the relationships between dd_{max} and RGR^{ini}_{flesh}, *aL*, k_{sugar} and r_{su}, respectively (Fig. 2).

Detection of QTLs for traits of interest and key parameters

QTLs were detected for the 12 parameters and for fruit dry mass (see Table SP in the supplementary data available at JXB online). QTLs accounted for between 7% and 67% of the observed variation. Main QTLs were detected for both years, but the fraction of total variation of each trait explained by the QTL was generally lower in 2001 than in 2002. The location of the QTLs on the linkage map is presented in Fig. 3 together with the QTLs detected by Quilot *et al*. (2004) for the traits of interest: fruit fresh mass, stone cheek diameter and fresh mass, total flesh sugar concentration, and flesh soluble solid content.

QTLs with the highest individual contribution were detected for dd_{max} (38% in 2002). For both years, QTLs were detected for dd_{max} and associated with SSR marker UDP96-003 on LG4, with differences between both S alleles and Z alleles. However, the global R^2 only reached 0.39 and 0.54, respectively, in 2001 and 2002.

Most QTLs for the four parameters RGR^{ini}_{flesh}, *aL*, k_{sugar}, and r_{su} were also detected at the same loci as those for dd_{max}. Indeed, for both years, the same three QTLs were detected for the dry flesh growth rate, RGR^{ini}_{flesh}, at the markers UDP96-003 (LG4, S, and Z) and CFF13 (LG3). Three of the four QTLs detected in 2002 for the parameter related to sugar metabolism, k_{sugar}, also co-located with QTLs for dd_{max}. Considering the tight links between dd_{max} and the four parameters RGR^{ini}_{flesh}, *aL*, k_{sugar}, and r_{su}, a QTL analysis was performed on the residuals of the relationships ($res.RGR^{ini}_{flesh}$, *res.aL*, $res.k_{sugar}$, and $res.r_{su}$) linking dd_{max} and the parameters RGR^{ini}_{flesh}, *aL*, $k_{sugar,}$ and r_{su} (Fig. 2). Some QTLs for these residuals were co-located with QTLs for the associated parameters. However, no QTL was detected for the residuals at the same markers as those for dd_{max}, except for a QTL detected by marker UDP96-003 (LG4) for $res.k_{sugar}$ in 2002 (R^2=0.05).

QTLs with high individual contribution were detected for the early growth parameter W^{321}_{fruit} and the potential maximal stone dry mass w^{matu}_{stone} at the PC60 marker, on LG6. QTLs for W^{321}_{fruit} and for W^{ini}_{fruit} and GR^{early}_{fruit} were co-located on LG1. These two regions of LG1 and LG6 and the regions of LG4 and LG8, where QTLs were detected for W^{ini}_{fruit} and GR^{early}_{fruit}, respectively, also harboured QTLs for stone mass (w^{matu}_{stone} and SMass). Alleles coming from P1908 enhanced the values of these parameters at the QTL on LG6 and decreased them at the QTL on LG1 and 4. QTLs for *res.aL*, $res.k_{sugar}$, and $res.r_{su}$ were detected on LG1, each co-located with QTLs for *aL*, k_{sugar}, and r_{su}. Three QTLs (LG4, 6, and 7) for the permeation coefficient of fruit surface to water vapour, ρ, were detected; however, this parameter was observed for 36 genotypes only.

QTLs detected for the parameters and residuals were often co-located with QTLs for quality traits. Most QTLs for fresh and dry fruit mass appeared co-located with QTLs for the fruit dry growth parameters, W^{321}_{fruit}, GR^{early}_{fruit}, W^{ini}_{fruit} (LG1), RGR^{ini}_{flesh} (LG4 and 7). They were also co-located with QTLs for $res.r_{su}$ and r_{su} (LG1), *aL* (LG2), $res.RGR^{ini}_{flesh}$ (LG4), ρ (LG4), *res.aL*, $res.RGR^{ini}_{flesh}$, and ρ (LG7). QTLs for total sugar concentration were detected in the same region of LG1 as QTLs for $res.k_{sugar}$ and *res.aL* and in the same region of LG6 as QTL for $res.RGR^{ini}_{flesh}$. Last, QTLs for flesh dry matter content and $res.k_{sugar}$ were co-located on LG3.

Combination of the ecophysiological and genetic models

Parameters of the ecophysiological model W^{ini}_{fruit}, w^{matu}_{stone}, RGR^{ini}_{flesh}, k_{sugar}, s_1, r_{su}, *aL*, and df^{1}_{stone} were estimated using the QTL results (see Table SP in the supplementary data that can be found at JXB online), concerning 2002 data only. The observed value of dd_{max} for each genotype was used since the model is highly sensitive to this parameter and QTLs detected for dd_{max} only explained a small fraction of the total variation observed, despite a high correlation between the 2001 and 2002 values. For the four parameters for which QTLs were detected on the residuals

Fig. 3. Location of putative QTLs controlling genotypic key parameters of the ecophysiological model and fruit quality traits analysed for two successive years: W^{321}_{fruit}, GR^{early}_{fruit}, W^{ini}_{fruit}, w^{matu}_{stone}, RGR^{ini}_{flesh}, k_{sugar}, ρ, s_1, r_{su}, *aL*, $df^{'}_{stone}$, dd_{max}, fruit dry mass (DMass), fruit fresh mass (FMass), flesh dry matter content (FDMC), fruit polar diameter (FPolarD), stone cheek diameter (SCheekD), stone fresh mass (SMass), soluble solid content (SSC), total sugar (TSugar) concentrations, and of putative QTLs for the residuals ($res.RGR^{ini}_{flesh}$, *res.aL*, $res.k_{sugar}$, and $res.r_{su}$) of the relationships described in Fig. 2, linking dd_{max} and the four parameters RGR^{ini}_{flesh}, k_{sugar}, *aL*, and r_{su}. Markers are listed on the right of each linkage group and genetic distances on the left. QTLs associated with markers of S or Z (superscript) genomes that could be assigned to linkage groups are listed on the left of each linkage group. QTLs associated with markers of the P1908 genome are listed in italics on the right of each linkage group. Underlined QTLs are those for which the P1908 allele confers a positive effect for a horticulture perspective. Year of observation is denoted by 1 and 2 for 2001 and 2002, respectively. When co-located, QTLs are ordered by decreasing individual contribution from left to right on each side of the linkage group.

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of the relationship with dd_{max} (RGR^{ini}_{flesh}, aL, k_{sugar}, and r_{su}), the effects of the QTL were added to the equation of this relationship. For example, the estimated value of aL for an individual i was computed as follows:

$$aL_i = f(dd_{max}) + \mu + a_{UDP96\ -008} \times G_{i,UDP96\ -008} + a_{CFF11} \times G_{i,CFF11} + a_{CFM8} \times G_{i,CFM8} + e_{PPCT025_CFF1} \times G_{i,PPCT025_CFF1}$$

Consequently:

$$aL_i = (0.01478 - 4.498 \times 10^{-06} \times dd_{max}) - 0.0014 + 0.0010 \times G_{i,UDP96-008} + 0.0014 \times G_{i,CFF11} + 0.0011 \times G_{i,CFM8} + 0.003 \times G_{i,PPCT025_CFF11}$$

where the genetic QTL scores $G_{i,n}$ took the values 0 or 1 depending on the allele of i at the corresponding loci.

The combined model remained accurate for most of the output variables. Goodness-of-fit of the combined model was high for flesh dry matter content, total sugar concentration, and stone fresh mass, since mean RRMSE values over the population were low (Table 4). For dry and fresh fruit masses, mean RRMSE were higher, but remained satisfactory. Evaluating that the model efficiently ranked the genotypes for fruit and stone masses, predictions of the combined model were well correlated with the observations. By contrast, predictions were less reliable for dry matter content and total sugar concentration of flesh, although it is worth noting that a few genotypes were badly represented by the combined model.

Discussion

Contributions of the approach

An innovative approach has been applied consisting of analysing the parameters involved in the development of traits, instead of considering these traits directly. The analysis of the stability between years of the parameter and quality trait values revealed better correlations between 2001 and 2002 values for the genotypic parameters than for the quality traits. Consequently, the detection of QTLs for such parameters was expected to be more successful than for quality traits (Yin *et al*., 1999). QTLs were detected for all the genotypic parameters and a number of them were common to both years of experimentation. The sum of QTL effects for each genotypic key parameter was included in the ecophysiological model. Thus parameter values could be predicted for each genotype. Finally, the quality of the combined model turned out to be moderately suitable.

Following a similar approach to that presented here, Yin *et al*. (2000) encountered difficulties with the initial accuracy of the ecophysiological model they used. Reymond *et al*. (2003) applied this method with success to a simple ecophysiological model, with only three parameters, restricted to the description of leaf elongation rate of maize. Such a method was also tested by Buck-Sorlin and Bachmann (2000) integrating additive gene effects into a morphological model. In this context, this study represents a further step towards the inclusion of genetic information into a complex ecophysiological model. The approach used here led to promising results and various potential uses of the combined model are attractive.

Perspectives of improvement of the approach

The relevance of the approach depends on the characteristics of the genotypic parameters that influence the level of the QTLs effect and the stability of the QTLs over years. Different ways lead to identifying such parameters. Reymond *et al*. (2003) have considered the parameters involved in the response curves of leaf elongation rate to environmental conditions. Response curves were based on experimental relationships valid over a large range of environmental conditions for a given genotype. Therefore parameters were considered as a stable characteristic of a genotype. In this study, some parameters (w^{matu}_{stone}, df^{1}_{stone}, r_{su}, ρ, s_1) were likewise estimated from response curves of a phenotypic trait to a measured plant signal in different environmental conditions. Other parameters (W^{321}_{fruit}, W^{ini}_{fruit}, GR^{early}_{fruit}, RGR^{ini}_{flesh}, k_{sugar}, and dd_{max}) were estimated under potential growth conditions. In this case, parameter values should reflect the intrinsic value of the genotype. However, some QTLs detected for the parameters were not common to both years and the fraction of total variation of each trait explained by the QTLs was generally low. The fraction of total variation of each trait explained by the QTLs was generally lower in 2001 than in 2002. This may be due to the fact that non-limiting fruit growth conditions were hardly met in 2001 for all genotypes. Trees were young and fruit growth may have undergone competition

Table 4. *Evaluation of the combined model (QTL and ecophysiological models combined) at maturity*

Mean values of relative mean squared error (RRMSE) over the population and Spearman correlation coefficients (COR) between observed and predicted values are presented for each output variable.

	Fruit dry mass (g)	Fruit fresh mass (g)	Stone fresh mass (g)	Flesh dry matter content (g g^{-1})	Total flesh sugar concentration g (100 g_{FM})$^{-1}$
RRMSE	0.31	0.33	0.18	0.11	0.17
COR	0.55	0.51	0.67	0.16	0.27

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with vegetative and root system growths. A further experiment under maximum fruit growth conditions is required to overcome insufficient year of testing and to check the QTL stability.

Besides the characteristics of the genotypic parameters, the detection of QTLs also depends on the saturation of the genetic map. Correlation between years for a trait provides an order of magnitude of its heritability. Accordingly, if most QTLs for a trait were detected, the total variation explained by these QTLs was expected to be approximatively equal to the corresponding correlation between 2001 and 2002 observations for this trait. In most cases, it was much lower. The most obvious case was the growth duration (dd_{max}) for which the R^2 was 0.39 and 0.54 in 2001 and 2002, respectively, whereas the correlation between years was much higher (0.96). For this reason, it was hypothesized that not all the polymorphism arising from the S and Z genomes with respect to the growth duration, and, perhaps, other parameters had been detected; this, in turn, may have reduced the power of detecting P1908 alleles affecting those traits. To cope with these limitations, it is necessary to integrate new markers for the S and Z genomes.

Further understanding of quality build-up

This approach can provide a basis for the understanding of physiological and genetical phenomena, via the dissection of the quality traits into elementary processes. Indeed, since each parameter is involved in a few identified processes, this approach helps to highlight the main processes responsible for the variations in a complex trait. Through the study of the co-locations between QTLs of parameters and traits, a physiological hypothesis could be proposed for connections between processes. Physiological mechanisms that influence a quality trait at each co-located QTL could be deduced from the function in which the parameters intervene. For example, on LG4 and 7, QTLs for fruit fresh mass are co-located with QTLs for $res.RGR^{ini}_{flesh}$: the pulp demand for dry matter growth influences the fruit fresh growth. In LG1, QTLs for fruit fresh mass are located in the same region as QTLs for parameters involved in sugar metabolism and early fruit growth. Lastly, on LG2, 4, and 7, they are co-located with QTLs for parameters involved in water fluxes in the fruit (aL, $res.aL$ and ρ). In addition, a parameter could influence different quality traits. For instance, QTLs for $res.k_{sugar}$ were located in the same region as a QTL for total sugar concentration (LG1) and a QTL for flesh dry matter content (LG3). Indeed, when k_{sugar} increases, carbon is further used for the synthesis of compounds other than sugars and total sugar concentration decreases. As a result, the osmotic potential decreases and less water enters the fruit so that flesh dry matter content increases.

Growth duration (dd_{max}) was highly correlated with four genotypic parameters (RGR^{ini}_{flesh}, aL, k_{sugar}, and r_{su}) and QTLs for dd_{max} were co-located with QTLs for these parameters. The sensitivity analysis of the model to the parameter variations revealed that quality traits were influenced by variations of dd_{max} (Quilot *et al*., 2005). However, no correlation was found between dd_{max} and quality traits. Further studies are necessary to understand these observations and the low correlations generally observed between parameters and quality traits.

This approach also highlighted the lack of knowledge regarding fruit quality development and the need for ecophysiological models dealing with genotypic variation in quality traits. Indeed, the ecophysiological model used only considered fruit growth during the phase of cell enlargement. Effects of early fruit growth and harvest time were taken into account through W^{ini}_{fruit} and dd_{max}. These two parameters appeared highly variable between genotypes and highly influential concerning quality traits at maturity. Describing the early growth stage via a model of cell division, taking into account limitations of assimilate supply, should make it possible to predict better the fruit mass at the end of the cell division stage and the sink potential of the fruit. Since maturity date appeared to be influenced by tree fruit load (Johnson and Handley, 1989), ecophysiological models should describe the underlying mechanisms involved in the maturation stage before harvest in order to predict maturity date whatever the year and the fruit load.

Potential contributions to crop improvement

The combined models may be used for practical purposes, such as predicting the genotypic variations of a plant response to environmental conditions. Yin *et al*. (2003) supported the idea that such models may help to solve genotype×environment interactions. Tardieu (2003) stated that they theoretically make it possible to predict the behaviour of plants with any combination of alleles under any climatic scenario. The interactions between processes underlined here result in difficulties to improve some traits, since the enhancement of some processes appeared to be favourable to some traits of interest but undesirable to others. In a context of multi-criteria objectives, this combined model may also provide a potential tool for rationalizing the contradictions between the effects of the processes, enhancing some traits without diminishing the others too much.

Integrating the knowledge and potentialities of physiology, genetics, and modelling to enhance the understanding of plant functioning has been considered a major challenge over the past few years. Besides the implications for genetic improvement, it is essential to note that all disciplines will benefit from this multidisciplinary approach. Indeed, modellers need to integrate the latest insight into biological mechanisms and may also incorporate the action of genes in their models. In return, models can help to test hypotheses on likely mechanisms, guide research, accelerate scientific

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understanding, and lead to practical applications of quantitative genetics.

Supplementary data

One supplementary table associated with this paper (Table SP) can be found at JXB online. It provides detailed information on the putative QTLs controlling parameters.

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