Crop management impacts the efficiency of quantitative trait loci (QTL) detection and use: case study of fruit load×QTL interactions

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Abstract

Mapping studies using populations with introgressed marker-defined genomic regions are continuously increasing knowledge about quantitative trait loci (QTL) that correlate with variation in important crop traits. This knowledge is useful for plant breeding, although combining desired traits in one genotype might be complicated by the mode of inheritance and co-localization of QTL with antagonistic effects, and by physiological trade-offs, and feed-back or feed-forward mechanisms. Therefore, integrating advances at the genetic level with insight into influences of environment and crop management on crop performance remains difficult. Whereas mapping studies can pinpoint correlations between QTL and phenotypic traits for specific conditions, ignoring or overlooking the importance of environment or crop management can jeopardize the relevance of such assessments. Here, we focus on fruit load (a measure determining competition among fruits on one plant) and its strong modulation of QTL effects on fruit size and composition. Following an integral approach, we show which fruit traits are affected by fruit load, to which underlying processes these traits can be linked, and which processes at lower and higher integration levels are affected by fruit load (and subsequently influence fruit traits). This opinion paper (i) argues that a mechanistic framework to interpret interactions between fruit load and QTL effects is needed, (ii) pleads for consideration of the context of agronomic management when detecting QTL, (iii) makes a case for incorporating interacting factors in the experimental set-up of QTL mapping studies, and (iv) provides recommendations to improve efficiency in QTL detection and use, with particular focus on model-based marker-assisted breeding.

Key words: Breeding, gene expression, genotype×environment interactions, fruit load, fruit quality, QTL×environment interaction.

Introduction

Crop science has seen great increases in quantities of genetic data through modern genotyping technologies (Ragoussis, 2009) and of phenotypic data through novel high-throughput phenotyping platforms (Montes et al., 2007). These rapid developments require up-to-date evaluation methods through which the power of these large data...
sets can be exploited to the maximum (van Eeuwijk et al., 2010).

Many scientists have pointed to a major weakness in this revolutionary increase in information. Although we are now able to create a myriad of genetic information, to pile up huge quantities of phenotype data, and to create biophysical crop growth models enlarging the power of the statistical approaches in quantitative trait loci (QTL) mapping or other types of genetic analysis, crop physiologists and breeders are always faced with the genotype-to-phenotype gap due to the strong interactions between QTL and environment (Hammer et al., 2006), i.e. the expression, detection, and use of QTL always depend on the environment in which the phenotyping takes place. In practice, this implies that which QTL are detected, how many QTL are detected, and the variation in the phenotype accounted for by these QTL all depend on the environment in which the phenotyping is carried out.

QTL×environment interactions should not be ignored, but unravelling them requires intelligent approaches. Phenotyping should therefore be carried out under relevant and realistic circumstances, both in terms of physical conditions (e.g. evaluating crop stands in the field rather than single plants in a phytotron) and in terms of crop management (e.g. mimicking commercial crop management with respect to fruit or leaf pruning) (Yin & Struik, 2008, 2010). We surmise that any aspect of crop management that has a strong influence on crop physiology should reflect common practice or should be varied as a co-factor to obtain a reliable and relevant phenotyping data set. This probably holds for all crops, including permanent grassland, perennial crops, arable crops, field-grown vegetables, and (annual) fruit crops and greenhouse crops. In this paper, we want to demonstrate the significance of the relationship between QTL expression and crop management using the example of fruit load management, a common practice in the cultivation of many different annual and perennial species producing fruits.

Fruit load: an essential determinant of fruit characteristics

The genetic components of phenotypic traits have been studied extensively for fruit-bearing crop species. Fruits are important sink organs. Knowledge and understanding of molecular physiology and QTL underlying fruit traits are rapidly progressing. The correlations between these QTL and phenotypic traits are in theory largely determined by the strength of the interactions of the associated gene expression with crop phenological stages, environmental factors, and crop management. However, these interactions often tend to be overlooked or ignored in studies investigating the genetic components of phenotypic traits, or merely empirically assessed through multi-site experiments. The interaction between QTL effects and fruit load in the establishment of fruit size and composition is particularly relevant because:

1. Fruit load strongly influences QTL effects on fruit size and composition.
2. Fruit pruning is a routine element in crop management, which may create significant fruit load differences compared with plants used for QTL detection.
3. Fruit load, when uncontrolled, can vary considerably and leads to high temporal and spatial fruit-to-fruit variability in size and composition.

Ignoring effects of fruit load on gene expression during QTL mapping may impede successful detection of QTL in at least two ways. First, QTL identified in unpruned plants may not correlate (as strongly) with the desired trait in pruned plants, as the pruning strategy may interfere directly with the associated gene expression or its influence on the phenotypic trait. Secondly, in pruned plants the phenotypic trait may be under the control of different genes compared with the unpruned plant, in which case the associated QTL under pruned conditions will be left undetected when QTL mapping is done based on phenotypes of unpruned plants.

Early removal of a fraction of the flowers and young fruits leaves the remaining flowers and fruits as well as the vegetative organs to develop and grow with less competition for resources. The developmental stage at which flowers or fruits are removed is essential, as the timing will determine which processes are affected most and thus will influence the final phenotype. As a result, altered hormone signalling, sugar–hormone cross-talk, or alternative signalling modes trigger a readjustment between photosynthetic assimilate supply and metabolism in sink organs, which can be observed at different organizational levels. Fruit pruning has traditionally been used by plant physiologists to study competition effects at plant and organ level. In addition, a growing number of systems biology studies have recently applied fruit load modulations combined with observations of transcriptomic, proteomic, and metabolomic changes, as well as tissue-specific cell number and cellular morphology (Bertin et al., 2003; Baldet et al., 2006; Prudent et al., 2009, 2010). These studies have
significantly improved our understanding of the mechanisms behind responses to variations in fruit load. In this review, findings from fruit load studies are synthesized, by separating effects expressed at different spatial and temporal scales, and showing the functional connections between responses at cell, tissue, fruit, and plant levels (Fig. 1).

In the following paragraphs, we will first assess fruit load influences on fruit size and composition. These findings at the organ level are subsequently linked to processes at lower (tissue, cell) and higher (plant) levels of spatial integration. In the final paragraph, we will outline recommendations to improve QTL detection and use of QTL in breeding of fruit crops.

What fruit traits are affected by fruit load?

One of the most clearly observable effects of high numbers of competing fruits per plant is the reduction in average fruit size. The limited availability of assimilates and other nutrients for growth generally leads to a decrease in the accumulation of dry as well as fresh weight in fruits of tomato (Bertin, 2005; Guichard et al., 2005; Massot et al., 2010; Fanwoua et al., 2012), apple (Dal Cin et al., 2007; Naor et al., 2008, 2013), citrus (Guardiola & García-Luis, 2000; Poiroux-Gonord et al., 2013), kiwifruit (Boyde & Barnett, 2011), mango (Léchaudel et al., 2005), peach (Quilot et al., 2002), and grape (Dai et al., 2009, 2011). Fruit development can be affected by the prevailing concentration of hexose and sucrose, showing a more pronounced and earlier transition to ripening when sugar is plentiful (e.g. Dal Cin et al., 2007; Pastore et al., 2011, 2013). The negative correlation between fruit load and fruit size can thus be counterbalanced by a lengthening effect of fruit load on fruit developmental duration.

Fruit composition is also heavily influenced by fruit load. Fruits grown under different fruit load regimes tend to exhibit differences in dry matter concentration and sugar and organic acid concentrations. Fruit load influences on fruit compositional traits are determined primarily by water and solute accumulation in the fruit, metabolic inter-conversions, and the incorporation of solute into structural material; moreover, these influences are also subject to significant interactions with environmental factors (Bertin, 2005; Gautier et al., 2005; Massot et al., 2010). Therefore, the sign and strength of the individual correlations between each compositional trait and fruit load are not necessarily easily predictable and are often species specific. Dry matter and sugar content expressed on a fresh-weight
basis decline with high fruit load in some species (Iglesias et al., 2002; Dai et al., 2009; Boyd & Barnett, 2011), although water and sugar accumulation usually decrease proportionally in fruits like tomato (e.g. Massot et al., 2010). Organic acid and sugar content show opposite patterns during development, and are often also oppositely affected by fruit load (e.g. Bertin et al., 2000; Fanasca et al., 2007; Poiroux-Gonord et al., 2013). As the sugar/organic acid ratio is an important determinant of taste, as well as the composition of the hexose pool, taste can also be strongly affected by fruit load. Finally, levels of several secondary metabolites and vitamins can also be significantly altered by fruit load, among which are various health-promoting substances like carotenoids and vitamin C (Gautier et al., 2005; Telef et al., 2006; Do et al., 2010; Massot et al., 2010; Poiroux-Gonord et al., 2013).

Fruit load influences on fruit size and composition are not reflected in the various studies that seek to determine QTL for the same traits. Whereas substantial effort is put into the repetition of QTL mapping analyses over a number of seasons and growing sites, the interactions of fruit load, as well as other crop management practices, with gene expression tend to be overlooked or ignored. Fruit load is not varied during QTL mapping studies, and plants used for mapping are either left unpruned or pruned according to prevailing agronomic standards (e.g. Devoghaeraë et al., 2012). During the last decade, a number of papers were published in which QTL were identified for tomato fruit traits on a limited number of inbred lines (Bertin et al., 2003; Prudent et al., 2009, 2011; Do et al., 2010) using two contrasting fruit load treatments. The results are summarised in Table 1, and clearly show a strong influence of fruit load on QTL detection. For instance, a total of 40 different QTL were identified for fruit size and sugar content (Prudent et al., 2009), but only 16 were identified under both fruit load treatments. These results indicate

### Table 1. Fruit load (FL) influences QTL detection for a wide range of traits in tomato

Comparison between two NILs in Bertin et al. (2003) is also included, for which detected QTL effects are denoted with an ‘x’. NA, not applicable.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Total detected QTL effects</th>
<th>QTL effect only found under high FL</th>
<th>QTL effect only found under low FL</th>
<th>QTL effect found for both FLs</th>
<th>FL-stable QTL effect (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Quality traits</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh weight</td>
<td>14</td>
<td>0</td>
<td>7</td>
<td>7</td>
<td>50</td>
<td>Prudent et al. (2009)</td>
</tr>
<tr>
<td>Dry matter content</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td>Bertin et al. (2003)</td>
</tr>
<tr>
<td>Sugar concentration</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>6</td>
<td>60</td>
<td>Prudent et al. (2009)</td>
</tr>
<tr>
<td>Sugar concentration (based on pericarp dry weight)</td>
<td>11</td>
<td>3</td>
<td>7</td>
<td>1</td>
<td>9</td>
<td>Prudent et al. (2009)</td>
</tr>
<tr>
<td>Sugar concentration (based on pericarp fresh weight)</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>40</td>
<td>Prudent et al. (2009)</td>
</tr>
<tr>
<td><strong>Process traits</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugar import into fruit</td>
<td>7</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>43</td>
<td>Prudent et al. (2011)</td>
</tr>
<tr>
<td>Sugar metabolism (incorporation into other components)</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>6</td>
<td>60</td>
<td>Prudent et al. (2011)</td>
</tr>
<tr>
<td>Sugar dilution</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>71</td>
<td>Prudent et al. (2011)</td>
</tr>
<tr>
<td><strong>Physiological traits</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of fruit development</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>60</td>
<td>Prudent et al. (2009)</td>
</tr>
<tr>
<td>Seed number</td>
<td>8</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>50</td>
<td>Prudent et al. (2009)</td>
</tr>
<tr>
<td>Pericarp cell number</td>
<td>8</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>13</td>
<td>Prudent et al. (2009)</td>
</tr>
<tr>
<td>Pericarp cell size</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>Prudent et al. (2009)</td>
</tr>
<tr>
<td>Cuticular conductance</td>
<td>20</td>
<td>3</td>
<td>8</td>
<td>9</td>
<td>45</td>
<td>Prudent et al. (2009)</td>
</tr>
<tr>
<td>Fruit cracking</td>
<td>6</td>
<td>0</td>
<td>5</td>
<td>1</td>
<td>17</td>
<td>Prudent et al. (2009)</td>
</tr>
<tr>
<td>Pericarp cell number x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Berlin et al. (2003)</td>
</tr>
<tr>
<td>Pericarp thickness x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Berlin et al. (2003)</td>
</tr>
<tr>
<td>Cell area x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Berlin et al. (2003)</td>
</tr>
<tr>
<td>No. cell layers in pericarp</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td>Berlin et al. (2003)</td>
</tr>
<tr>
<td><strong>Whole-plant traits</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height of the 4th truss</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>60</td>
<td>Prudent et al. (2009)</td>
</tr>
<tr>
<td>Leaf number</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Prudent et al. (2009)</td>
</tr>
<tr>
<td>Specific leaf weight</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>33</td>
<td>Prudent et al. (2009)</td>
</tr>
<tr>
<td>Total leaf area</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>33</td>
<td>Prudent et al. (2009)</td>
</tr>
<tr>
<td><strong>Metabolic traits</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Several metabolic QTLs</td>
<td>324</td>
<td>196</td>
<td>84</td>
<td>44</td>
<td>14</td>
<td>Do et al. (2010)</td>
</tr>
<tr>
<td>Glucose</td>
<td>x</td>
<td>x</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td>Berlin et al. (2003)</td>
</tr>
<tr>
<td>Fructose</td>
<td>x</td>
<td>x</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td>Berlin et al. (2003)</td>
</tr>
</tbody>
</table>

[a] Only detectable at anthesis.
[b] Only detectable in distal fruits.
that the previously mentioned pruning strategies during QTL mapping are critical for the specific QTL that will be identified, and also that neither approach will be very efficient in determining the full genetic potential for each trait.

**Cellular processes underlying fruit load effects on fruit traits**

Fruit development is characterized by distinct phases of tissue-specific cell proliferation and cell expansion. The resulting fruit growth might be sigmoidal (e.g. tomato) or double sigmoidal (e.g. peach, grape), depending on fruit type. Cell number or average cell size in the enlarged fleshy components of carpel or floral tissues is often found to be a target of QTL affecting fruit size. For example, a number of major tomato fruit size QTL in a comparison of near-isogenic lines (NILs) were found to be associated with the number of cells in the pericarp (Bertin et al., 2009). Similarly, genotypic variation in fruit size was to a large extent explained by differing cell numbers in many other crops, including peach (Sorotza et al., 1991), melon (Higashi et al., 1999), apple (Harada et al., 2005; Dash et al., 2013), pear (Zhang et al., 2006), blueberry (Johnson et al., 2011), and grape (Houel et al., 2013). These differences in cell number are usually established very early during flower bud development and may be severely affected by fruit load (Goffinet et al., 1995; Bertin et al., 2002, 2003; Wünsche & Ferguson, 2005; Baldet et al., 2006; Prudent et al., 2009, 2010; Dash & Malladi, 2012; Fanwoua et al., 2012). Baldet et al. (2006) showed that low fruit load increases transcript abundance of proliferation-promoting cell-cycle regulators in tomato flowers, and consequently increases pre-anthesis ovary cell numbers. Dash & Malladi (2012) observed an increase in apple cortex cell number due to low fruit load, which could be linked to the increased transcription of various cyclins and cyclin-dependent kinases as well as cyclin-dependent kinase inhibitors, mediated by fruit-load-sensitive expression of two apple orthologues of the Arabidopsis AINTEGUMENTA gene, a putative transcription factor involved in regulation of organ growth via cell proliferation as well as expansion. Dash et al. (2013) indicated that increased carbohydrate availability associated with early low apple fruit load had a strong influence on key transcription factors and cell proliferation genes, thus enhancing early fruit growth. Several QTL were found for pericarp cell number in tomato (Prudent et al., 2009), but most of these QTL effects disappeared when early pruning was applied (Table 1).

Final cell size might also be affected by fruit load (Marcelis, 1993; Link, 2000; Dash & Malladi, 2012; Fanwoua et al., 2012). Cell expansion is driven by a difference in hydrostatic pressure potential between the subcellular vacuole and its surroundings and primarily constrained by the elastic and plastic properties of the cell wall (Lockhart, 1965). This driving force allows control over cell expansion by metabolic inter-conversion between hexose, sucrose or starch, sorbitol, and organic acids like citrate and malate, as well as less abundant metabolites with contrasting osmotic molarities. Additionally, cell-wall properties might also be subject to sugar-dependent modification; for instance, several genes involved in cell-wall modification in tomato fruit were differentially expressed between different fruit loads (Prudent et al., 2010). Water fluxes to and from the fruit may also exert control over cell expansion and are often found to be influenced by fruit load (e.g. Gautier et al., 2001). Guichard et al. (2005) reported increased water influx and efflux as a result of low fruit load. In contrast, Prudent et al. (2010) showed that low fruit load in tomato reduced expression of two aquaporin genes, potentially reducing the water-conducting properties of the cell wall and vacuolar membrane. A number of QTL were identified for tomato fruit cuticular conductance (Prudent et al., 2009). Interestingly, most of these QTL were found at both high and low fruit load treatments, but fruit load reversed the sign of the correlation between cuticular conductance and all identified QTL (Prudent et al., 2009).

Endoreduplication, a truncated cell cycle leading to DNA multiplication in the absence of cell division, is associated with cell expansion in many fruits (reviewed by Bourdon et al., 2010). Malladi & Hirst (2010) found cell expansion related to endoreduplication to be an important factor in fruit size differences between the apple cultivar ‘Gala’ and a spontaneous large-fruited mutant, ‘Grand Gala’. In particular, tomato pericarp cells are known to exhibit extraordinarily high ploidy levels due to repeated endoreduplication (Chevalier et al., 2011), and the resulting polyploidy has often been suggested to raise the upper limit for cell and corresponding tissue and organ size (Chenilet et al., 2005). On the other hand, it is not very likely that effects of fruit load on cell expansion are associated with endoreduplication, as in tomato it was shown that competition between fruits can significantly affect cell size without affecting ploidy (Bertin et al., 2003; Bertin, 2005). These conflicting results need further testing and analysis.

Cell-cycle progression as well as cell expansion and differentiation are subject to a diverse range of influencing factors. Auxin (Devoghalaere et al., 2012; Mounet et al., 2012) as well as other phytohormones and various sugar signals are strongly involved in proliferation, expansion, and ripening. Their interplay assures coordinated fruit growth and development in response to assimilate supply (see recent reviews by Hartig & Beck, 2006; Perrot-Rechenmann, 2010; Wang & Ruan, 2013). Sugar supply for growth is derived from source leaves, relocation from storage organs or organelles, or local production via fruit photosynthesis. Fruit photosynthesis may exceed respiratory CO₂ release during distinct developmental phases (e.g. Hiratsuka et al., 2012; Breia et al., 2013), and thus might contribute (marginally) to fruit growth (Marcelis & Baan Hofman-Eijer, 1995; Obiadalla-Ali et al., 2004) additional to more complex roles in fruit developmental processes (Lytvochenko et al., 2011). Photosynthetic assimilate production in leaves and fruit is carefully tuned to local metabolite concentrations, and it is therefore not surprising to find fruit photosynthesis-related gene expression to be affected by fruit load (Table 2).

Fruit load also affects fruit metabolism. Determinations of metabolite content QTL in tomato fruit at two fruit load treatments by Do et al. (2010) indicated a greater influence of development and crop management than genotype on fruit
composition. A total of 324 metabolic QTL were identified, but only 14% of the loci gave significant correlations at both fruit load treatments (Table 1). Fruit load effects on contents of specific metabolites are difficult to generalize due to strong interactions with environmental factors like temperature and water availability (Bertin, 2005; Gautier et al., 2005; Dai et al., 2011). Analysis of metabolic content determinations might be further complicated by the chosen sampling strategy. When fruit grown at different levels of competition are either sampled all at the same time or at a specified developmental stage (Dai et al., 2011), inconsistent results arise due to interaction between fruit load and duration of fruit development (Prudent et al., 2009) and ripening (Génard & Gouble, 2005; Dal Cin et al., 2007).

Fruit load affects processes at integration levels surpassing fruit scale

Development of reproductive structures only occurs when the vegetative stages during early plant development have passed. The first floral induction may already take place soon after sowing in herbaceous indeterminate crops such as greenhouse cultivars of tomato, sweet pepper, and cucumber, but occurs in many fruit trees (e.g. apple, pear, citrus, and peach) only years after planting. Although the exact mechanism remains unclear (Bangerth, 2009) in fruit trees, heavy fruit load negatively affects vegetative growth and next season’s flowering, by—as we surmise—influencing expression of genes controlling floral induction and meristem identity such as FLOWERING LOCUS T (FT), TERMINAL FLOWER (TFL), LEAFY (LFY), SUPPRESSOR OF OVEREXPRESSION OF CONSTANS (SOC), and APETALA (AP) (Kittikorn et al., 2011; Muñoz-Fambuena et al., 2011, 2012; Shalom et al., 2012). As a consequence, a repetitive 2-year cycle of subsequent heavy and low fruit load is observed at tree level, termed ‘alternate bearing’ (Samach & Smith, 2013; Smith & Samach, 2013), and these are known as ON/OFF trees. These cyclic yield oscillations are mirrored by cyclic yield flushes in indeterminate species like tomato and sweet pepper, where heavy fruit load may impair flower and seed set and promote abortion of flowers and young fruits, followed by a phase where new flowers and fruits will be less impeded by competition from earlier fruits (e.g. Bertin, 1995; Marcelis et al., 2004; reviewed by Ruan et al., 2012).

Fruit growth may decline as a result of competition with vegetative sinks, although (after initial developmental stages) fruits usually are considerably stronger sinks than vegetative organs. During sympodial growth, tomato meristems follow a developmental programme to form a number of leaves and a final inflorescence. As a result, fruit load effects on growth of the meristem are reflected in the initial size of the subsequent inflorescence and influence fruit size as a function of position on the plant and within the truss (Bertin et al., 2002). Positional differences also arise as a result of different flowering times, for instance between the proximal to distal end of an inflorescence (Bohner & Bangerth, 1988). These positional differences are generally more strongly expressed at high fruit load, and may even disappear altogether at low fruit load (Bertin et al., 2003; Prudent et al., 2009).

The importance of vascular structures for solute transport as well as long-distance signal transduction is considerable. Sufficient vascular development is required to facilitate bulk flow of substrate via the xylem (remobilization from storage organs) or phloem (transport from source leaves) to growing tissues. Auxin (Bünger-Kibler & Bangerth, 1983; Wang et al., 2005) and gibberellins (Zhang et al., 2005) play important roles in early vascular differentiation, which is critical for early fruit and seed development (Ruan et al., 2012). Auxin efflux from sink organs and subsequent transport via the vascular network is also an important signal to establish various modes of positional dominance between competing sinks (Samach & Smith, 2013). Fruit solute import may be limited by fruit sink metabolism, via metabolic conversion, synthesis of cell wall or storage product, subcellular compartmentation, or respiratory flux (Marcelis, 1996; Zhang et al., 2005). Invertases have been highlighted in a number of reviews (e.g. Koch, 2004; Bihmidine et al., 2013) as important response factors in organ sink metabolism, because, additional to their role in sucrose metabolism and hexose signalling, transcription of invertase genes is sugar responsive, which allows feedback as well as feed-forward adjustment of sink metabolism based on assimilate availability. When phloem unloading is limited, carbohydrate accumulation in upstream leaves may on the one hand reduce phloem loading of photosynthetic

Table 2. Fruit load influences expression of genes, which are specifically linked to major processes in plant growth and development

<table>
<thead>
<tr>
<th>Process</th>
<th>Crop</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photosynthesis (fruit)</td>
<td>Tomato, grape</td>
<td>Prudent et al. (2010); Pastore et al. (2011)</td>
</tr>
<tr>
<td>Carbon metabolism</td>
<td>Citrus, grape, tomato</td>
<td>Prudent et al. (2010); Nebauer et al. (2011); Pastore et al. (2011);</td>
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<td>Shalom et al. (2012); Pastore et al. (2013)</td>
</tr>
<tr>
<td>Hormonal responses</td>
<td>Apple, citrus, grape, tomato</td>
<td>Dal Cin et al. (2007); Prudent et al. (2010); Pastore et al. (2011);</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shalom et al. (2012)</td>
</tr>
<tr>
<td>Cell expansion</td>
<td>Citrus, tomato</td>
<td>Prudent et al. (2010); Shalom et al. (2012)</td>
</tr>
<tr>
<td>Cell division</td>
<td>Apple, tomato</td>
<td>Baldet et al. (2006); Dash &amp; Malladi (2012); Fanwoua et al. (2012)</td>
</tr>
<tr>
<td>Flower induction</td>
<td>Apple, citrus, mandarin</td>
<td>Kittikorn et al. (2011); Muñoz-Fambuena et al. (2011, 2012); Shalom et al. (2012)</td>
</tr>
<tr>
<td>Water transport</td>
<td>Tomato</td>
<td>Prudent et al. (2010)</td>
</tr>
</tbody>
</table>

*Fruit load effect was observed by comparing natural occurrence of ON versus OFF trees.*
assimilates and, by means of feed-back inhibition, reduce leaf and canopy photosynthetic gas exchange, stomatal conductance, and transpiration, but on the other hand increase build-up of storage compounds (Valantin et al., 1998; Iglesias et al., 2002, 2003; Syvertsen et al., 2003; Léchaudel et al., 2005; Wünsche et al., 2005; Nebauer et al., 2011; Poiroux-Gonord et al., 2013; reviewed by Ainsworth & Bush, 2011). Thus, fruit load may affect source activity itself, which underlines the need for experimental control of such processes in QTL mapping studies.

Towards more efficient QTL detection and breeding of fruit-bearing crops

Fruit size, fruit shape, and fruit composition are complex, quantitatively inherited traits, which are controlled by several interacting genes, expression of which is subject to environment and crop management. Tanksley (2004) reported that about 30 QTL account for the variation in fruit size and shape of tomato. Of these 30 QTL, fewer than 10 major ones account for most of the changes in fruit size and shape of tomato that have occurred during the domestication of this crop. Six key loci controlling fruit size are \( fw1.1, fw2.2, fw3.1, fw4.1 \), fasciated, and locule-number (Tanksley, 2004). Locus \( fw2.2 \) is known to regulate early cell division.

As a result of the quantitative inheritance, QTL effects usually involve only small fractions of the observed genotypic variation and are often sensitive to genetic background, environment, and crop management. In the previous paragraphs, we have illustrated this by focusing on interactions of QTL effects with fruit load. The consensus on whether to control fruit load during QTL mapping seems to vary among crops with differing growth strategies. Generally, fruit pruning during mapping is either applied following standard crop management practices (most tree species) or not applied to leave the fruit load subject to undisturbed fruit set (e.g. tomato). The second option may also substantially interfere with the detection of QTL or genes involved in fruit traits. This was shown by Schauer et al. (2006), who identified harvest index in unpruned tomato plants as a central pleiotropic hub in a network reconstruction of various metabolic and crop morphological traits. It is therefore vital to carefully consider crop management (and sampling) strategy during mapping studies. Impractical it may be, but in many cases fruit pruning will significantly reduce sample variance and thus increase statistical power.

However, it should be realized that the approach and design of QTL mapping studies depends on their objectives. A breeder needs to define and characterize the target environments for which (s)he wants to breed, including the type of management that could be considered as part of that target production system (cf. Tuberosa, 2012). The QTL studies aiming at marker-assisted selection should then be carried out on the basis of phenotypic information from correctly managed plants grown under relevant conditions (e.g. pruned tomato plants in a modern greenhouse). A crop physiologist might be interested in creating a QTL-based model to predict the interactions between genotype and stress factors affecting the regulation of fruit size. (S)he then needs to create a model system in which the detection of the QTL is most likely but also needs to carry out phenotyping studies in an agronomically representative situation with crops grown under variable field conditions in different (stressful and stress-free) environments with the crop management that is prevailing in those field conditions. The answer to the question ‘To prune or not to prune?’ is then given on the basis of possible interactions between the stress factor and carbon competition. Usually the answer will be ‘to prune’, but a crop physiologist will not prune if (s)he wants to investigate the impact of a stress on fruit setting or wants to identify QTL for fruit number.

Traits to be assessed can be classified as constitutive traits and responsive traits (Tuberosa, 2012). In our example of fruit load effects on QTL expression, constitutive traits are expressed in both unpruned and pruned plants, although the level of expression may vary between the two treatments, whereas responsive traits are specifically expressed in pruned or unpruned plants.

The most straightforward recommendation is to consider inclusion of fruit-load variations as a co-factor in the experimental design of QTL mapping studies. However, to extensively evaluate fruit load (or other influential environment or crop management) effects within a mapping study might be laborious and expensive. Alternative methods have been proposed that hold promise to (at least partially) alleviate the need for environmental or crop management treatments within the mapping study and more efficiently use information from genotype-phenotype observations (e.g. Wang et al., 2012). In particular, the benefits of combining physiological modelling with knowledge about underlying genetic variation or QTL effects have been stressed in a number of reviews (e.g. Tardieu 2003; Yin et al., 2004; Struik et al., 2005, 2007; Yin & Struik, 2008, 2010; Bertin et al., 2010; Baldazzi et al., 2012; Keurentjes et al., 2013).

In this opinion paper, we have chosen to elaborate on the way fruit load interacts with QTL detection. Other relevant co-factors include leaf:fruit ratio, truss position, plant density, and environmental conditions (including light conditions, temperature, and \( \text{CO}_2 \) concentration), water and nutrient availability in the root environment, and others. As so many co-factors might be involved, there may also be many QTL involved in influencing the final fruit load if not controlled. Yet this number might perhaps be lower than expected on the basis of the number of co-factors, as different co-factors might have similar target traits. Fruit pruning intends to reduce the influence of other co-factors. Therefore, in the following paragraph we will describe a modelling approach that allows post-hoc identification of the QTL with the largest impact on the relevant trait(s).

Complex traits can be separated into various interacting components. These interactions may be simulated by bottom-up (based on mechanistic knowledge of underlying processes), top-down (statistical regression to establish links between data and phenotype), or middle-out (combination of bottom-up and top-down) modelling approaches (Bertin et al., 2010; Yin & Struik, 2008, 2010; Keurentjes et al.,
In these models, so-called component traits are characterized in terms of model parameters (Fig. 2) which, instead of the complex trait itself, are subsequently being linked to underlying genetic variations. This linkage often yields stronger correlations as these component traits are less influenced by environment or are expressed as a function of environment. In order to obtain sufficient statistical power, stringent use of the parsimony principle is required. Models should contain a relatively small set of biologically relevant model parameters, which directly relate to easily measurable physiological traits and are sufficiently able to capture the observed variation in the trait of interest. This concept was successfully applied to analyse fruit size and composition in peach (Quilot et al., 2005a,b; Bertin et al., 2010) and tomato (Prudent et al., 2011; see Table 1). If the component traits are significantly less complex than the original
trait, correlations with underlying genetic variation should improve. Additionally, use of network analysis, and especially incorporation of mechanistic knowledge on the nature of the interactions between the component traits usually improves predictive power significantly, and, furthermore, allows the design of virtual ideotypes (Quilot-Turion et al., 2012).

Current literature (e.g. Quilot et al., 2005a, b; Bertin et al., 2010; Prudent et al., 2011) suggests that the first steps made to analyse genotype×environment interactions in fruit growth are very promising. However, we also identified a number of issues holding back wider implementation. First of all, existing models for several complex fruit traits are not complete, as pointed out by Fanwoua et al. (2013), or are not subtle or versatile enough to predict such interactions. Moreover, the quality of the prediction is usually drastically reduced if the model is applied to a population of genotypes for which the model was not calibrated, in particular when parents with a different genetic background were used. Widening environmental variation also reduces the quality of the predictions. However, despite these issues, the suggested model-based approach still has clear benefits over traditional QTL mapping. Namely, more QTL are usually identified, the identified QTL tend to be more stable, and the importance of identified QTL can be ranked for conditions differing from the environment during the mapping study. One example of our approach could be the inclusion of genetic variation in basic parameters related to leaf photosynthesis (e.g. genetic variation in maximum carboxylation rate or in maximum electron transport rate over photosystem II) and transpiration (e.g. genetic variation in stomatal or membrane conductance) in models predicting fruit size and quality of tomato plants under various pruning regimes. This would allow manipulation of the possible genetic linkage between photosynthesis and transpiration at the leaf level and fruit size under various fruit loads.

Conclusions

As clarified in the introductory parts of this opinion paper, the interaction between fruit load and QTL expression is an economically and scientifically highly relevant example of the much more common phenomenon that we can only detect and use meaningful QTL when phenotyping is done in a range of commonly occurring conditions and practices of crop management. When interacting factors have very strong influences on the correlation between QTL and agronomic traits, like the example of fruit load in this paper, we recommend including these as co-factors in the experimental design and using modelling to identify component traits for mapping, rather than the trait itself. When component traits are sufficiently less complex than the trait itself, correlations with the associated QTL should be less influenced by interacting factors. An additional advantage of this approach is that QTL from different mapping studies including the mentioned co-factors can more easily be combined into one mechanistic framework. Our outlined approach to make maximum use of the power of large genotyping and phenotyping data sets will therefore—obviously with the necessary adjustments to cope with crop-specific mechanisms—be instrumental in bridging the gap between genotype and phenotype.

References


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