

Integrative approach of tomato fruit texture using multiblock analysis

R. Aurand ^{1,2}, M. Faurobert ², M. Hanafi ³, G. Mazerolles ⁴, R. Tisiot ⁵, L. Rosso ⁵, B. Navez ⁶, N. Bertin ¹.

¹INRA - Unité Plantes et Systèmes de cultures Horticoles, 84914 Avignon Cedex 9, France

²INRA - Unité de Génétique et Amélioration des Fruits et Légumes, 84143 Montfavet BP94, France,

³Unité de Recherche de Sensométrie et Chimométrie (USC INRA), ONIRIS, Site de la Géraudière, Rue de la Géraudière BP 82225, 44322 Nantes Cedex 3, France

⁴INRA-SPO, 2 place Viala 34060 Montpellier, France

⁵CTIFL - Centre de Balandran, BP 32 30127 Bellegarde, France

⁶CTIFL - Centre de Saint-Rémy de Provence, 13210 Saint Rémy de Provence, France

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Abstract

Tomato fruit texture is one of the most critical quality traits for both the consumer and the production chain. Texture is a complex trait for which several QTL and genes were found. However, interactions between the molecular, histological, physical and biochemical components of fruit texture have been rarely investigated. In this work an integrative approach based on multiple co-inertia analysis (MCOA) was applied to point out links among the different levels: from protein to fruit, then to identify main physiological mechanisms involved in fruit texture. Three contrasted parental lines (Cervil, Levovil, VilB) and three derived QTL-NILs harbouring texture QTL on chromosome 4 and 9 were analyzed. Measurements were performed at cell expansion stage, at red ripe stage and after 7-days post-harvest storage at 20°C. To increase texture variability, water deficit was induced by decreasing water supply by 40% from flowering of the third truss. Three blocks of data (texture, physico-chemical traits and proteome datasets) were analysed. Results showed a common multi-scale structure obtained from the three datasets with a main contribution of texture and biochemical blocks. At all levels, MCOA outlined strong genotype discrimination, indicating that the genetic factor was the main factor of variability, in contrast with water deficit. The first common component separated the genetic background and correlated with firmness and sugar traits. The second component represented QTL effect. The percentage of the variance of the protein block taken into account to build the common structure was low. Proteins which mainly contributed to the common components at the three developmental stages were implicated in carbohydrate metabolism. Multiple co-inertia analysis provides an interesting tool to characterize complex trait such as fleshy fruit texture by integrating several levels of studies.

INTRODUCTION

Tomato fruit texture is one of the most critical quality traits for both the consumer (purchase) and the production chain (transport, handling, storage) (Causse et al., 2003; Seymour et al., 2002; Sinesio et al., 2010). It is also involved in sensory perception (Causse et al., 2003). Fruit texture may vary according to different factors, such as growth conditions (Rosales et al., 2009), harvest and post-harvest conditions (Casierra-Posada and Aguilar-Avendano, 2008; Ketelaere et al., 2004; Moneruzzaman et al., 2008) and genotype (Saliba-Colombani et al., 2001). Regarding the environmental factors, water supply plays a major role, but controversial effects are reported. For example, deficit irrigation enhances fruit

firmness in apple (Leib et al., 2006; Van Hooijdonk et al., 2007), but not in apricot (Perez-Pastor et al., 2007). Texture is a complex trait, mixing several components such as firmness, meltiness, mealiness, juiciness or crunchiness, for which several QTL and genes were found (Saliba-Colombani et al., 2001). However, links between the molecular, histological, physical and biochemical components of fruit texture have been rarely investigated. In this work an integrative approach based on multiblock analysis was applied to point out links among the different levels: from protein to fruit, then to identify main physiological mechanisms involved in fruit texture.

MATERIALS AND METHODS

Three contrasted parental lines (Cervil, Levovil, VilB) and three derived QTL-NILs (NIL-L4, NIL-L9, NIL-V9) harbouring texture QTL on chromosome 4 and 9 were analyzed. Cervil is a cherry tomato with 7 g fruits, good taste, and high aroma intensity. Then QTL-NILs were developed in two genetic backgrounds: Levovil, a large fruited line with low firmness and VilB, also large fruited, but firmer and with better post-harvest performance than Levovil (Chaib *et al.*, 2007). The genotypes were grown under two water regimes: control (C) and water deficit (S) corresponding to a 40% decrease in water supply from flowering of the third truss until the end of the experiment.

Texture was assessed through two rheological tests (compression and puncture tests) applied either on whole fruits with a texturometer (Texture analyser TAplus: Ametek, Lloyd Instruments Ltd., Fareham, UK). Locule number was recorded and fruit pericarps were frozen in liquid nitrogen, ground and stored at -80°C . Organic acid, soluble sugars (sucrose, fructose, and glucose) contents were extracted and measured by HPLC (Gomez et al., 2002), then alcohol insoluble solid, vitamin C, dry matter pericarp and starch contents were obtained. From the same samples, proteins were separated by two-dimensional electrophoresis, spots were quantified and identified by mass spectrometry (nanoLC-MS/MS) (Page et al., 2010). Measurements were performed from four biological repetitions (included 5 fruits) for each genotype and condition at three stages: cell expansion stage, harvest and after 7-days post-harvest storage at 20°C .

To identify a multi-scale structure among data sets measured at the protein, tissue and fruit levels, a multiple co-inertia analysis (MCOA) was applied based on a covariance criterion, using ADE-4 software (Thioulouse et al., 1997). Simultaneous analysis was performed from several blocks to outline common patterns of the variability, if any (Chessel and Hanafi, 1996). For that purpose, three datasets named “blocks” were processed:

Block 1 contained physico-chemical data such as contents in vitamin C, soluble sugars, organic acids, alcohol insoluble solids (AIS) and dry matter (DM), locule number and fruit weight (FW).

Block 2 included mechanical measurements of fruit texture, such as firmness (CPmax, FPmax), fruit deformation (CPdef, FPdef), work (FPw) and stiffness (CPslop, FPSlop) obtained by compression and puncture tests. “p” before label means that measurement was performed on peeled fruit.

Block 3 included the quantities of 400 proteins (identified by 2D-MS),

All data were obtained from the same samples for each genotype, condition (water deficit and control) and stage of development. Cervil was discarded from the analysis to avoid bias, because of its huge difference in some traits such as fruit weight. Data were normalized and each block was subjected to a scale factor (1/inertia).

RESULTS AND DISCUSSION

Multiple co-inertia analysis (MCOA) from the three blocks reflected a common multi-scale structure. This structure was found for the three stages of development and was stable

during fruit development and ripening. The reference structure was a projection of genotypes on two main components (calculated to account for the maximum of variance of each block) so-called “compromise” (Fig. 1, 3 and 5). Impact of the genetic factor was the most striking feature. The first common component separates the two genetic backgrounds: L (Levovil, L4, L9) and B (VilB, V9), while the second component reflects QTL effects. The water deficit effect (“S” on labels) was low and evidenced only at the red ripe stage. Projection of the variables on the compromise is shown in Fig. 1, 3 and 5. Numbers represent functional class of the ten proteins which better contributed to the first and second components. Protein data had a strong inertia, therefore all proteins clustered near the origin. Compared to the Levovil background, VilB and V9 fruits were associated to a higher local firmness (puncture test), dry matter, sucrose and starch (at the expansion stage only) contents, but lower glucose, fructose, malic acid contents and locule number. QTL9 was associated to low global firmness and stiffness (compression test). Stiffness was associated to high vitamin C content at the cell expansion stage, and low calcium content in the post-harvest suggesting an effect on cell integrity.

The difference (arrow lengths) between the compromise (point) and each block (end of arrow) was represented for each stage of development (Fig. 2, 4 and 6). These low differences were confirmed by links between block score and global score (vectorial correlation > 0.8) (Table 2). The first two components of the compromise reflected more than 50% of the variance of texture and biochemical blocks, but only 20% of the variance of the proteome block (Table 1). The protein block hardly influenced the compromise. We first, examined the ten proteins which better contributed to the first and second axis. The first axis of the compromise separated proteins involved in carbohydrate metabolism, the second axis correlates with stress response proteins. Interestingly, at harvest stage and after 7-days post-harvest storage at 20°C, proteins implicated in cell wall metabolism mostly contributed to the first axis. Deeper analyses of protein data are in progress, to point out clear functional links between biochemical data and proteins.

CONCLUSIONS

Multiple co-inertia analysis allowed us to demonstrate a common multi-scale structure among molecular, histological, physical and biochemical components of tomato fruit texture.

However for this method, which applies to large datasets, a scale factor was applied to consider the weight of each block. The way this factor is calculated may influence the final results. In this study, in order to equilibrate the weight of each block, the scale factor was the inverse of the inertia of each block. The “omic” level, highly complex and exhibiting a strong inertia with 400 proteins, hardly influenced the multi-scale structure.

A selection or a sub-division of data based on functional analysis may improve their integration. In this way, a method to separate and to analyze genetic and environmental factors could be used to deepen low effects (Mazerolles et al., 2011).

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Tables

Table 1. Percentage of the variance of each block used to build the compromise (squared covariance). More than 50% of the variances of texture and biochemical blocks were explained with the first two components of the compromise.

Table	Cell expansion stage		At harvest (red ripe)		After 7-days post-harvest storage at 20°C	
	Component	Component	Component	Component	Component	Component
	1	2	1	2	1	2
Bioch	0,42	0,13	0,43	0,07	0,45	0,12
Tex	0,47	0,24	0,60	0,26	0,57	0,27
Prot	0,11	0,08	0,16	0,08	0,16	0,07

Table 2. Links between block score and compromise score (vectorial correlation), Blocks were all well correlated to the compromise for the three stages of development.

Table	Cell expansion stage		At harvest (red ripe)		After 7-days post-harvest storage at 20°C	
	Component	Component	Component	Component	Component	Component
	1	2	1	2	1	2
Bioch	0,91	0,84	0,96	0,81	0,93	0,80
Tex	0,92	0,94	0,97	0,97	0,94	0,95
Prot	0,93	0,89	0,91	0,90	0,90	0,90

Figures

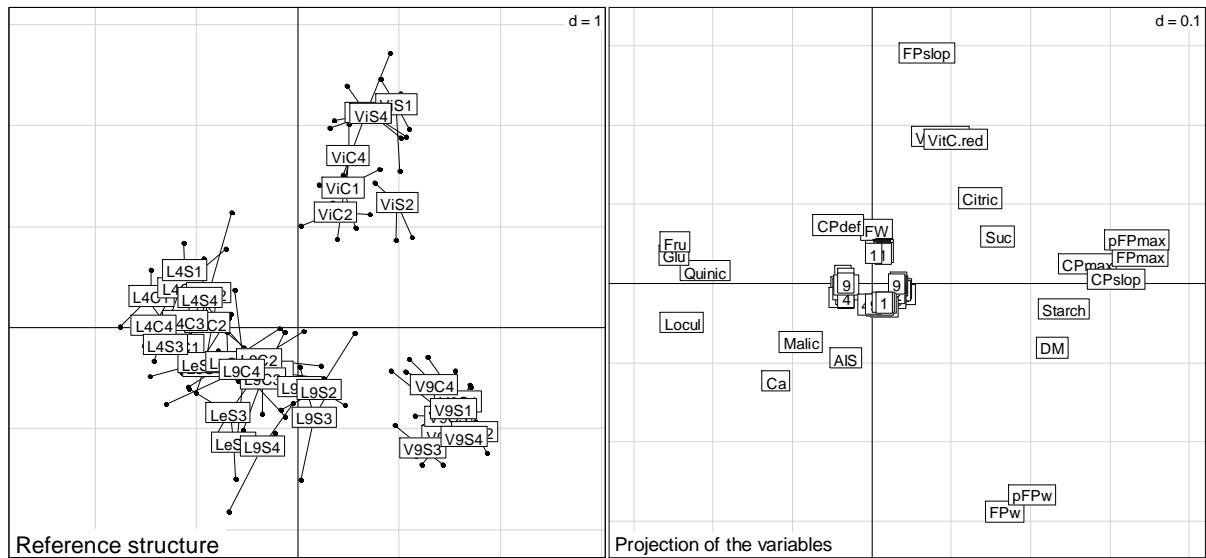


Fig. 1. At cell expansion stage, the reference structure derived from the three data blocks: labels for each genotype (Le: Levovil, L4, L9, Vi : Vi1B and V9) and treatment (control: C and water deficit: S) represent barycenters of each individual block (points), while the length of the segments indicates the similarity among blocks (the shorter, the more similar). On projection of the variables projection, numbers represent functional class of protein (1: stress response, 2: carbohydrate metabolism, 3: energy, 4: cell wall, 5: proteolysis, 6: secondary metabolism, 7: signaling, 8: ripening, 9: other, 10: regulation of protein activity, 11: amino acid metabolism).

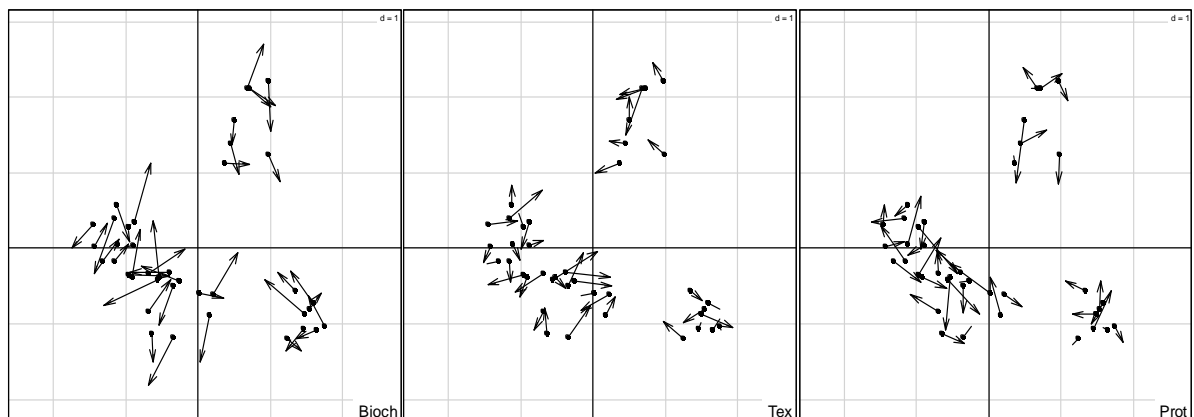


Fig. 2. At cell expansion stage, the 3 figures show the difference (arrow lengths) between the compromise (point) and each block (the end of arrow), “Bioch”: block 1, “Tex”: block 2 and “Prot”: block 3.

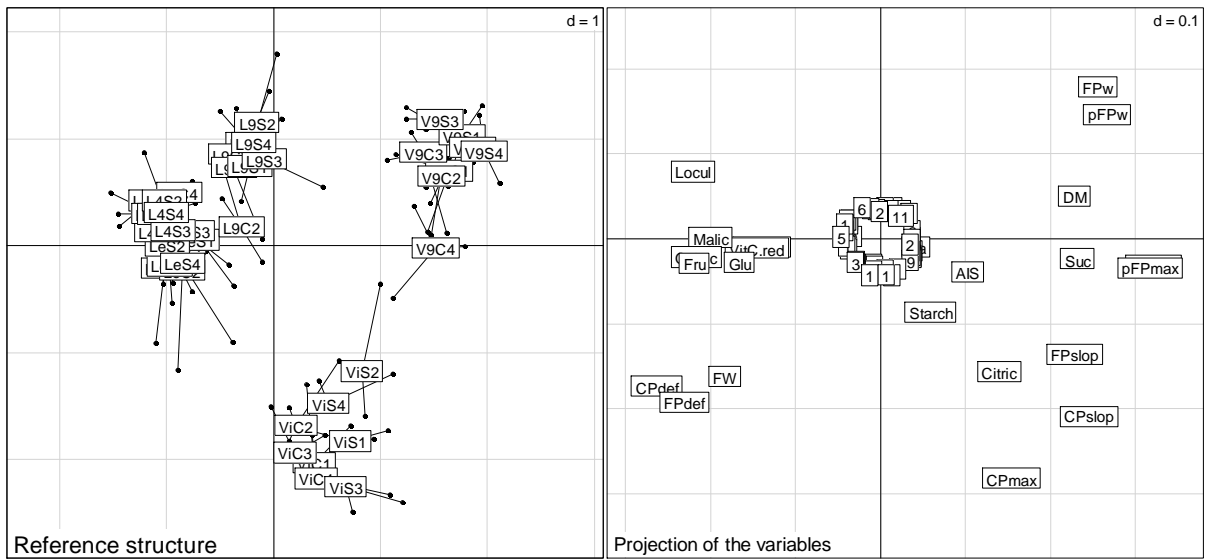


Fig. 3. At harvest (red ripe), the reference structure derived from the three data blocks. On the right: projection of the variables used to the reference structure.

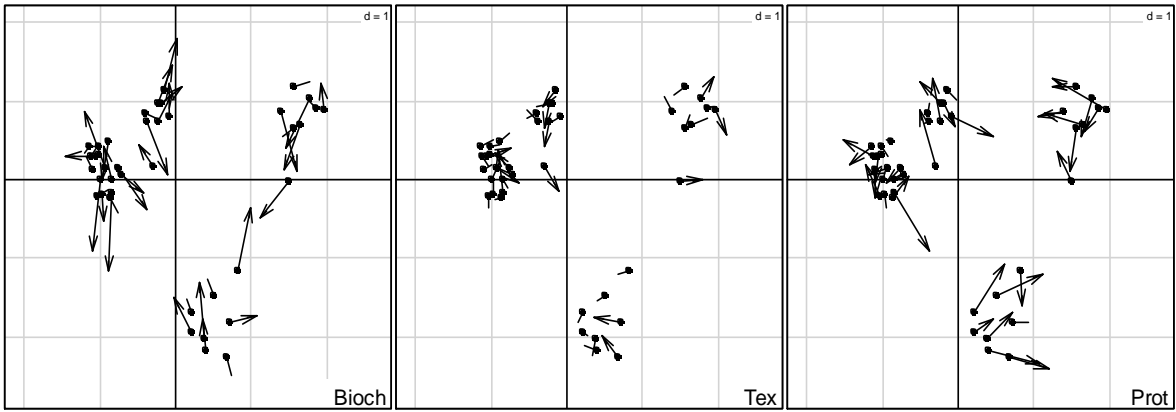


Fig. 4. At harvest (red ripe), the 3 figures show the difference (arrow lengths) between the compromise (point) and each block (the end of arrow), “Bioch”: block 1, “Tex”: block 2 and “Prot”: block 3.



Fig. 5. After 7-days post-harvest storage at 20°C, the reference structure derived from the three data blocks. On the right: projection of the variables used to the reference structure.

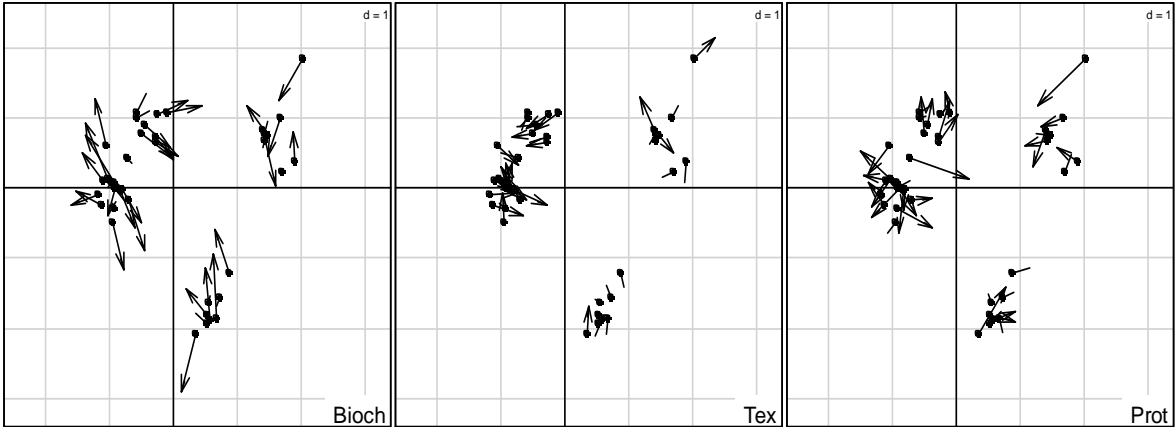


Fig. 6. After 7-days post-harvest storage at 20°C, the 3 figures show the difference (arrow lengths) between the compromise (point) and each block (the end of arrow), “Bioch”: block 1, “Tex”: block 2 and “Prot”: block 3.