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Integrative approach of tomato fruit texture using multi-block analysis

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Background - Objectives

Tomato fruit texture is one of the most critical quality traits for both the consumer (purchase) and the production chain (transport, handling, storage). 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 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.

Plant material and cultural conditions

Three contrasted parental lines (Cervil, Levovil, ViIB) and three derived QTL-NILs harbouring texture QTL on chromosome 4 and 9 (L4, L9 and B9) (Chaïb et al, 2007) 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. Fruit size and texture were measured on individual fruits, then composition and proteome were assessed on four pools of 5 fruits for each genotype and each condition. Measurements were performed at the cell expansion stage, at harvest (red ripe stage) and after 7-days storage at 20 C.



Statistical analysis

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, for that ADE-4 software was used (Thioulouse et al. 1997). Simultaneous analysis were performed from several blocks to outline common patterns of the variability, if any (Chessel and Hanafi, 1996). For that purpose, three data categories named "blocks" were processed:

Block 1 : contained physico-chemical data, vitamin C, soluble sugars, organic acid, alcohol insoluble solid (AIS), dry matter contents (DM), locul 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 measurement was achieved on peeled fruit.

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

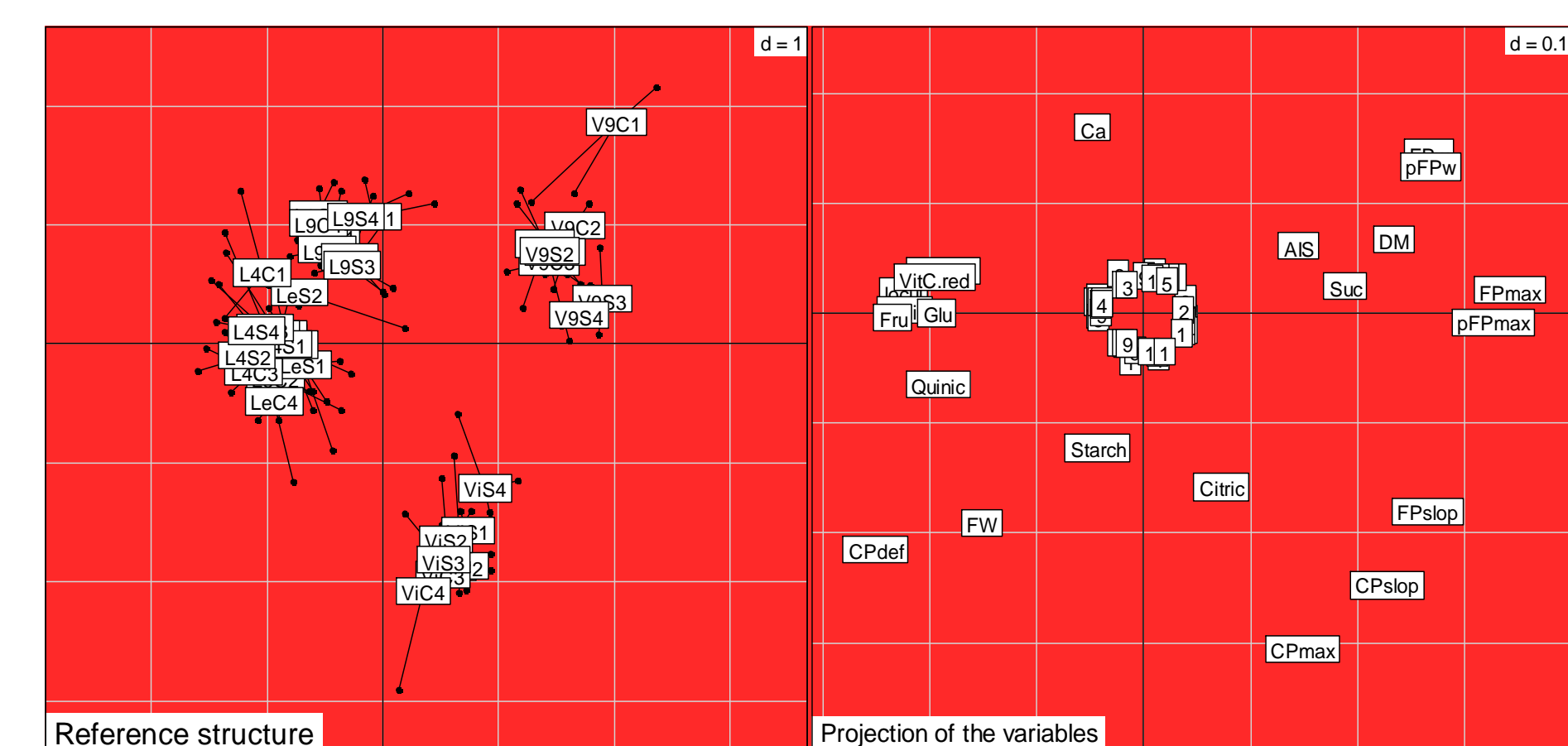
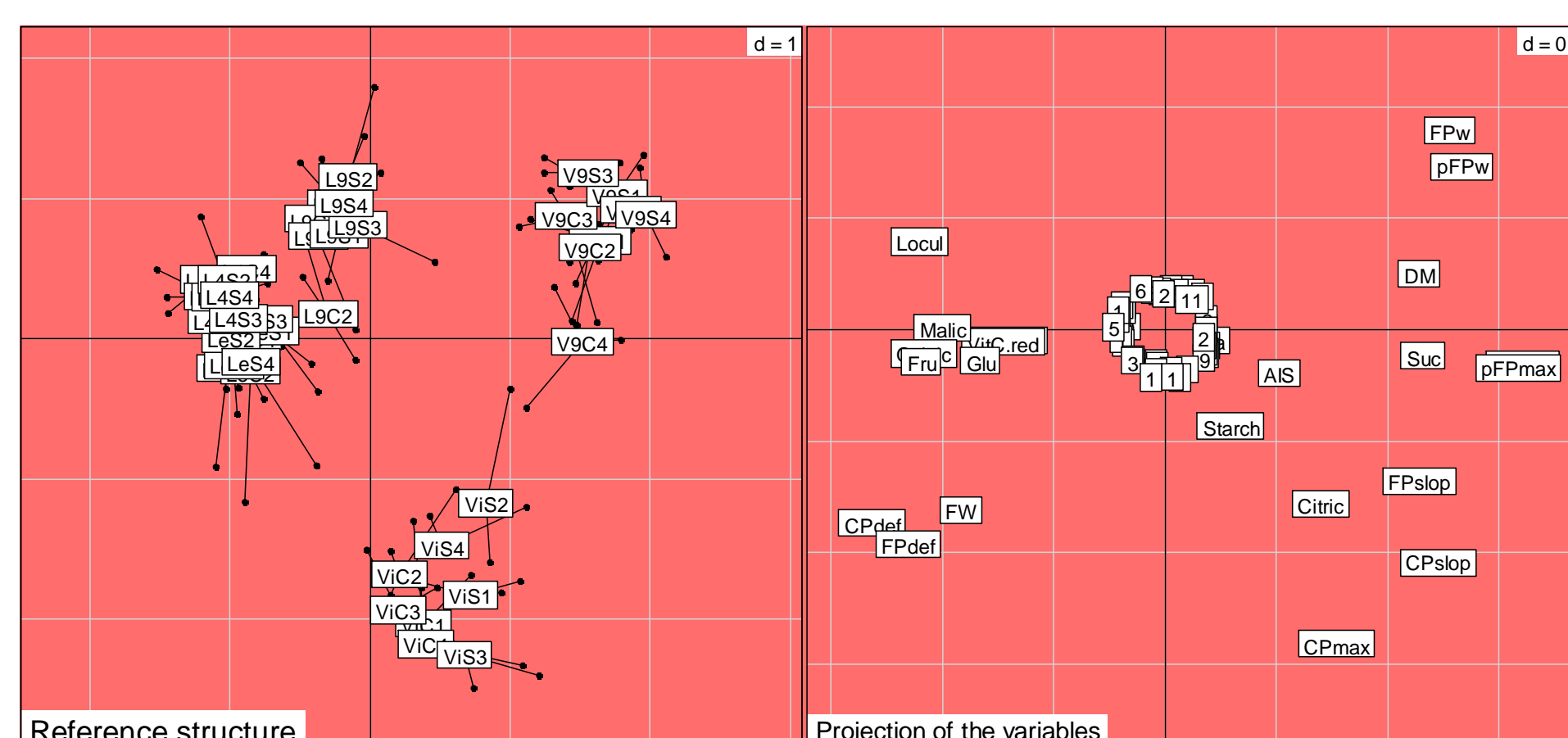
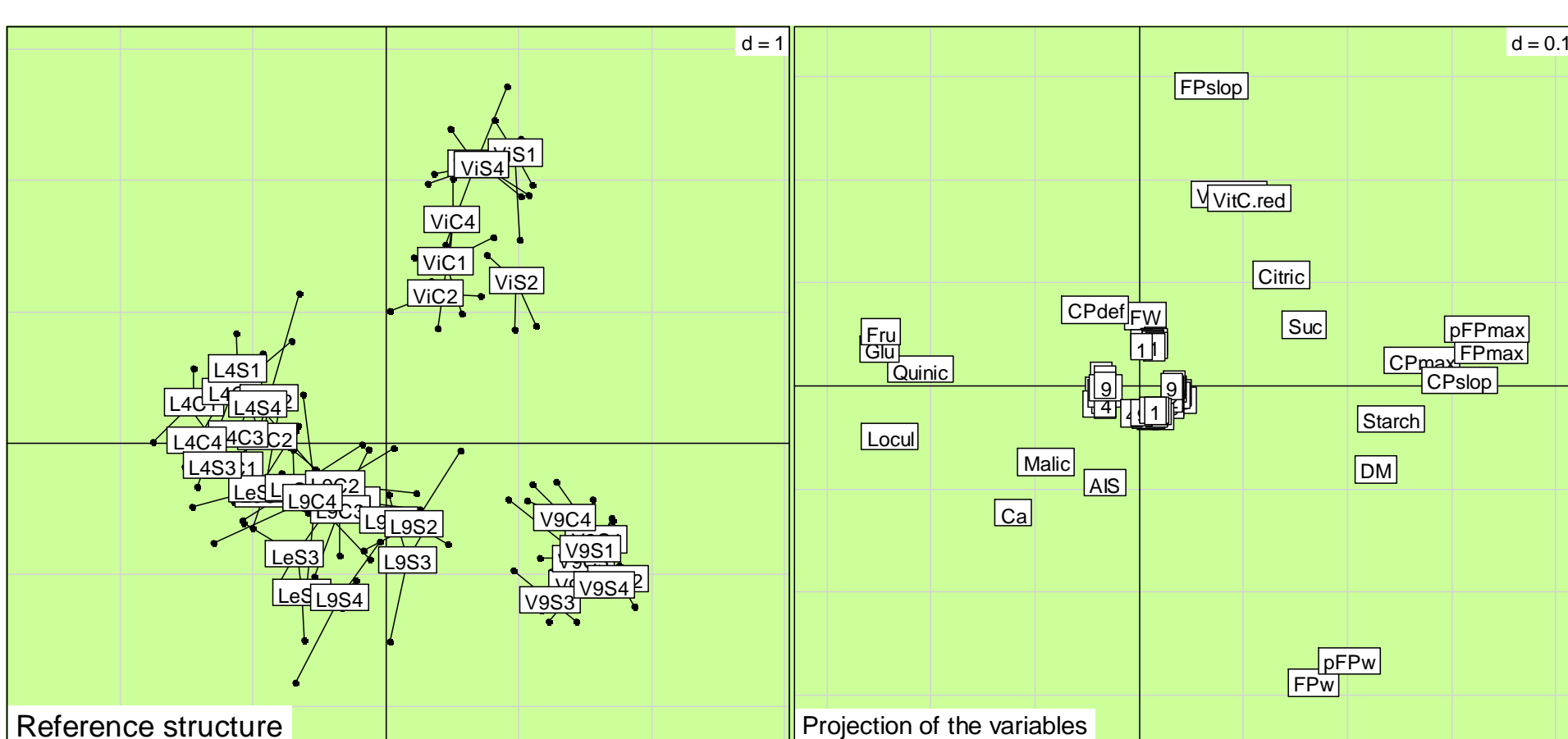
All data were obtained from the same sample for each genotype, condition (water deficit and control), and stage of development. Cervil was discarded from the analysis to avoid bias, because of its huge difference in some traits such as fruit weight. Data were normalized and each block was subjected to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

Results

Cell expansion stage

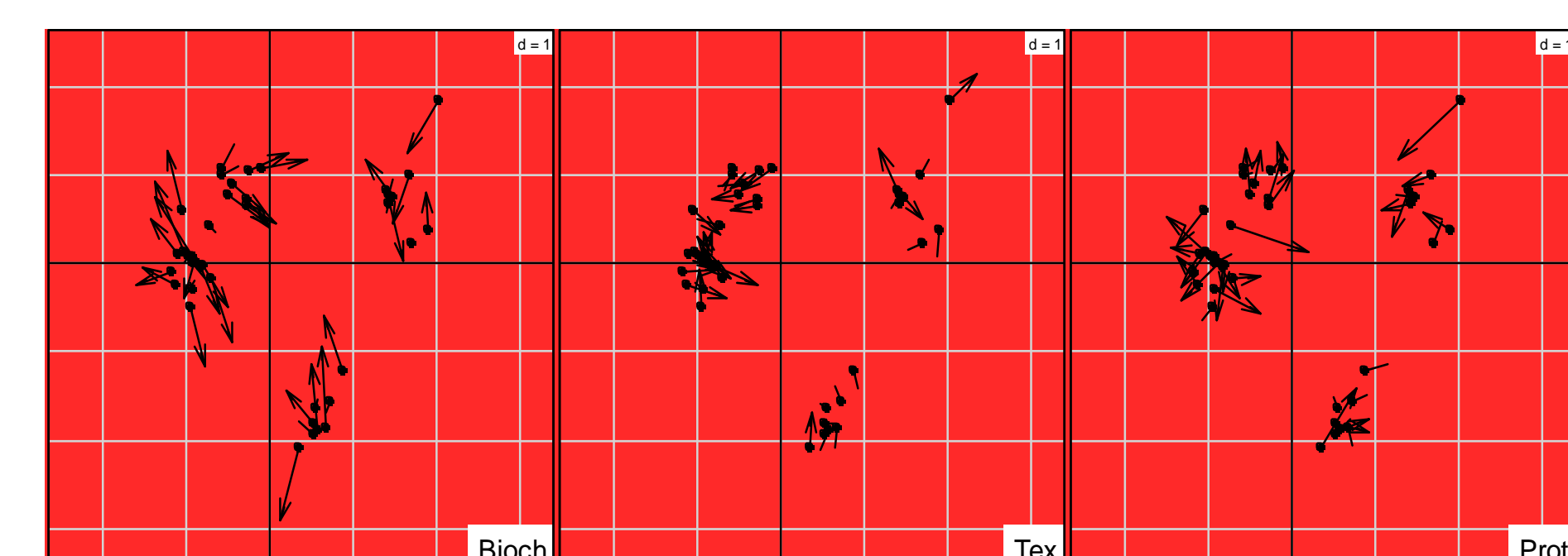
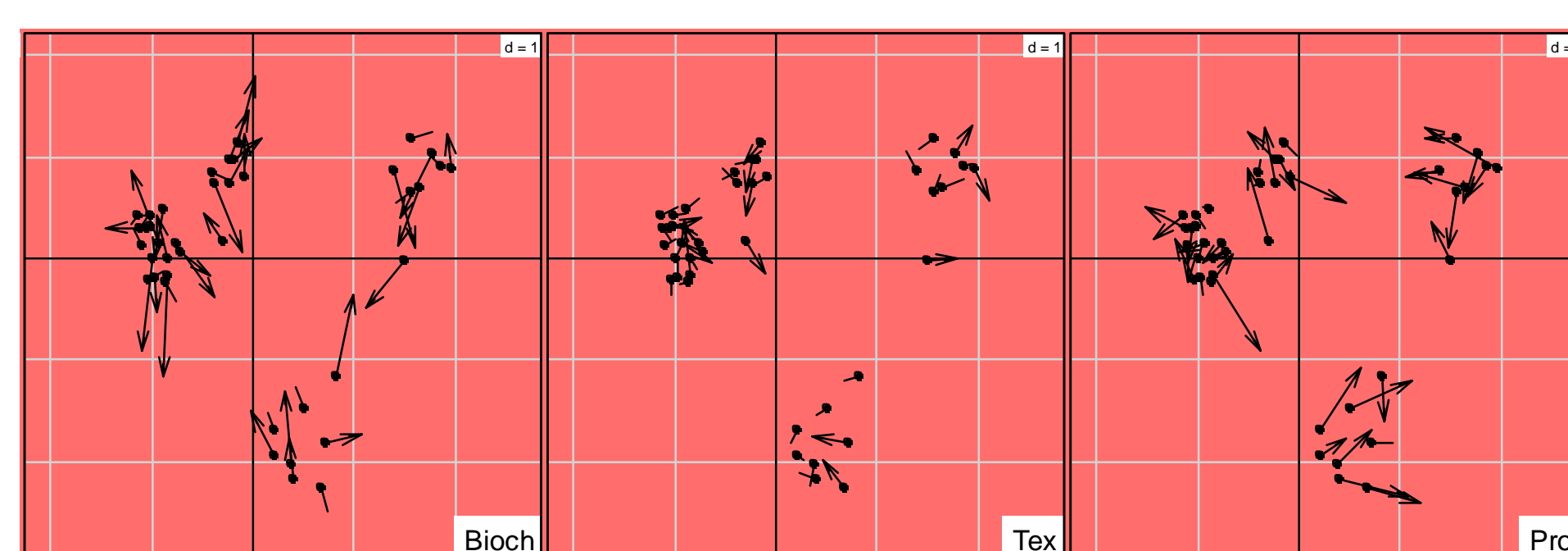
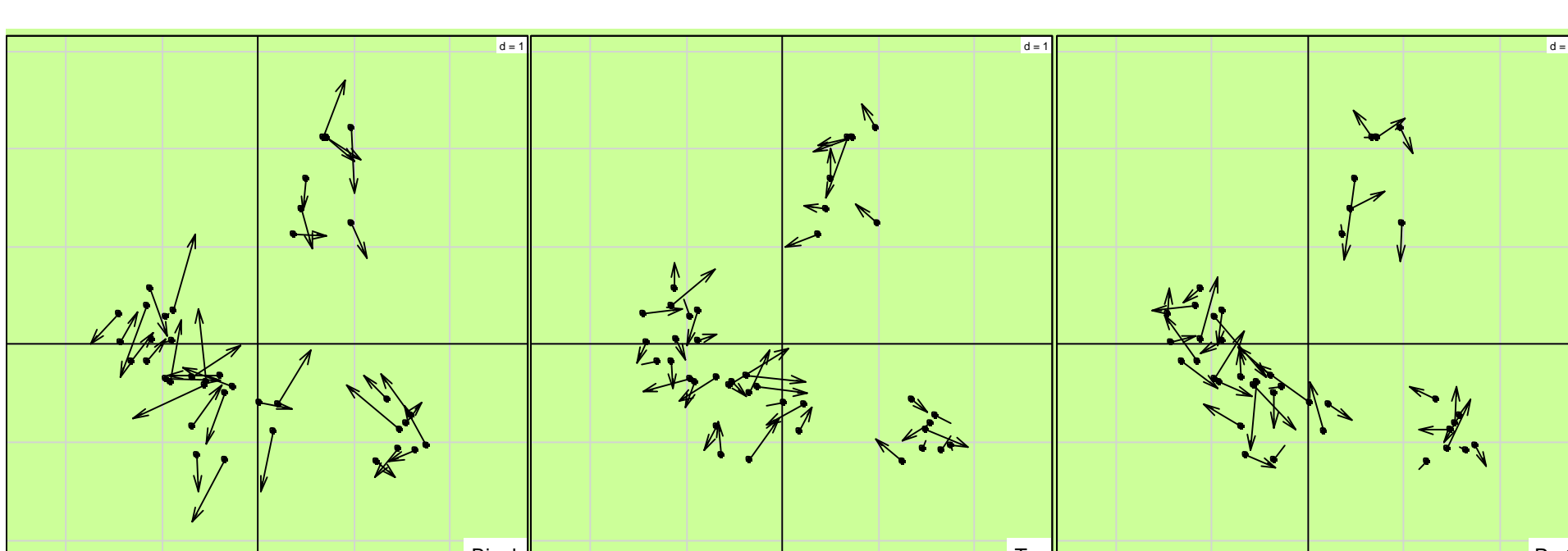
At harvest (red ripe)

After 7-days storage at 20 C



The **reference structure** (using the first two components) so-called "compromise" derived from the three data blocks: labels for each genotype and treatment represent barycenters of each individual block (points), while the length of the segments indicates the similarity among blocks (the shorter, the more similar).

On **variable projection in the compromise**, numbers represents 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). Protein data had strong inertia, all proteins clustered near the origin.



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 for each stage of development.

% of the variance of each block used to build the compromise (squared covariance)

Block	Cell expansion stage		At harvest		After 7-days storage at 20°C	
	Component 1	Component 2	Component 1	Component 2	Component 1	Component 2
Bioch	0,42	0,11	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

More than 50% of the variance of texture and biochemical blocks were explained with the first two components of the compromise. Only 20% for proteome block.

Links between block score and compromise score (vectorial correlation)

Block	Cell expansion stage		At harvest		After 7-days storage at 20°C	
	Component 1	Component 2	Component 1	Component 2	Component 1	Component 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

Blocks were all well correlated to the compromise for the three stages of development.

Results showed :

- Impact of genetic variability : a common structure between the different blocks which reveals a common multi-scale structure mostly related to the genetic variability. The first common component separates the two genetic backgrounds (L and B), while the second component reflects QTL effects.
- Compared to the Levovil background, ViIB and ViIB9 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 locular number. The 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 water deficit effect was low and evidenced only at the red stage.
- Although the protein block hardly influenced the compromise, we 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. At harvest stage and after 7-days storage at 20 C, proteins implicated in cell wall metabolism mostly contributed to the first axis. However no clear functional links between biochemical data and proteins could be up to now outlined.

Conclusion

Multiple co-inertia analysis is an interesting method to explore a common multi-scale structure and to characterize complex trait such as fleshy fruit texture by integrating several levels of studies. However for this method, the weight of each block depends on inertia of block. Therefore the "omic" level, highly complex exhibiting a strong inertia, is more difficult to exploit in the analysis. A selection or a sub-division of data based on functional analysis may improve their integration.