Gene expression analysis of the walnut oleosin variants A and B in 3 different *Juglans regia* cultivars using RNA sequencing data

Shanna Bastiaan-Net[1], Jurgen B. van Schijndel[1,2], Renata M.C. Ariëns[1], Martie C.M. Verschuren[2]

[1]Wageningen Food & Biobased research, Food, health & Consumer research, Wageningen UR, Wageningen, The Netherlands
[2]Avans University of Applied Sciences, Analysis techniques in the life sciences, Breda, The Netherlands

Background: Oleosins are a class of seed proteins associated with oil bodies. With its central hydrophobic domain and amphipathic N- and C-terminus, oleosins are believed to stabilize the phospholipid layer that encapsulate triglycerides within the oil body. Oleosins are known to cause (severe) allergic symptoms in some seed/nut allergic individuals. Two types of oleosin allergens are known in the English walnut species *Juglans regia*, namely variant A (G8H6H8) and B (G8H6H9). Using next generation sequencing (NGS) RNA sequencing, we investigated whether transcription of these two oleosin genes differed between the walnut cultivars Fra Noix de Grenoble, Fra Franquette and Californian Hartley.
Material and Methods: First, a BLAST analysis on the *de novo* assembled NGS reads in CLC Gx Bio software was performed using the oleosin sequences JN409447 and JN409448 as query, to identify oleosin bearing contigs in each cultivar database. Next, contigs per oleosin variant between each cultivar were matched to be able to compare the correct contigs with each other. At last, the total read count per contig was evaluated and compared to gain an impression of oleosin expression levels per cultivar.
Results: The oleosin expression levels varied widely between the three different cultivars investigated. Cultivars Fra Franquette and Fra Noix de Grenoble express both types of oleosins of which Fra Franquette shows the lowest oleosin expression. Between the two oleosin variants however, no significant differences in read count in these two cultivars was observed. Oleosin variant B seemed not to be present in cultivar California Hartley, while variant A was expressed twice as high in this cultivar as compared to the read count in Fra Noix de Grenoble
Conclusion: Between the cultivars tested, oleosin expression varied according to NGS data. That oleosin expression levels may differ greatly between walnut cultivars indicates that for allergy diagnostics in walnut-allergic patients, it might be informative to use protein extracts from different cultivars to perform skin prick testing or IgE-measurements, instead of using only one type of cultivar.