Biennial bearing in apple: Proteomic profiling of developing buds

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Biennial bearing in apple: Proteomic profiling of developing buds

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1. Introduction, Knowledge, Objectives

The two major factors determining crop load in apple (*Malus domestica* Borkh.), are flower density and fruit set, respectively (Hanke *et al.* 2007). As fruit set can be adjusted to some extent by flower or fruitlet thinning, the critical factor is the number of flower buds per tree (Dennis 2003). Fruit growers aim for stable numbers of flower buds every year to reduce the risk of triggering the phenomenon of biennial bearing (Jonkers 1979). This cropping irregularity is characterized by large yields of small-sized fruit in the 'on-year' and low yields of oversized fruit in the 'off-year' (Williams and Edgerton 1981). Trees bearing fruit in the first year (on-year) can change to 'off' bearing status in the subsequent year (off-year) and vice versa. Cultivars differ in their degree of biennial bearing behavior. 'Fuji' shows a strong tendency to bear biennially, whereas 'Gala' is considered a regular bearer (Hampson and Kemp 2003).

The physiological reason for entering an 'off' year is supposedly the competitive overlap of flower bud formation for the subsequent season and fruit development during the current season (Pratt 1988; Dennis and Neilsen 1999).

Perennial fruit trees such as apple, start their reproductive cycle early in the growing season with the first phase in flower bud development, the flower induction (Buban and Faust 1982). It is assumed that the initially vegetative meristems perceive signals, which trigger various biochemical and structural changes. For apple, there is evidence that the transcript of a TFL1-encoding gene correlates with flowering (Haberman *et al.* 2016). However, it is still largely unknown whether those signals actively promote flower bud development or suppress factors that cause the meristem to remain in a vegetative state (Dolega and Link 2002). At microscopical scale, the first visible changes in the meristem appear during the second phase, the flower initiation. A pronounced doming and broadening of the apex can be recorded as the first sign of floral commitment and definite development of floral structures (Foster *et al.* 2003; Hanke *et al.* 2007). Internal regulatory mechanisms synchronize the flower bud initiation to a certain extent and bud initiation in apple is reported

to persist for three to four weeks (Dolega and Link 2002). The subsequent flower differentiation comprises the development of inflorescence primordia, followed by floral organs and ends with the onset of bud dormancy in winter (Wilkie *et al.* 2008). Formation of pollen sacs and ovules completes the bud differentiation in the following spring shortly before bud burst (Tromp 2000).

Heavy crop load is reported to have a negative effect on flower bud development and thereby reduces flower density in the following season (Monselise and Goldschmidt 1982; Wünsche and Ferguson 2010). Consequently, the experiment described here aims at inhibiting flower bud development by maintaining a high crop load, whereas completely thinned trees are assumed to promote flower bud development. The difference in the two developmental pathways will be evaluated on the bases of proteomic profiles of sampled buds. The objective of this study is to identify proteomic differences between apple buds from 'On' and 'Off' trees in a preliminary experiment.

2. Material and Methods

The field experiment was conducted during the growing season in 2015 at the Centre of Competence for Fruit Cultivation near Ravensburg, Germany (47°46'2.89"N 9°33'21.21"E, altitude 490m).

2.1. Experimental design
The strongly biennially bearing apple cultivar 'Fuji' was used for the experiment. Tree age at the time of the experiment was 8 years. Trees were grafted on M9 rootstocks and trained as vertical axis to a height of 3.5 m. Orchard management included standard pruning as well as fertilizer and pesticide applications following best practice guidelines. At full bloom on 30th of April 2015 one tree was completely deflowered by hand ('off' treatment) and one tree did not receive any flower thinning to keep the naturally high flower set ('on' tree).

2.2. Bud sampling
Buds were sampled on 22th of July 2015, 85 days after full bloom (DAFB). Sample size was 12 buds from each treatment tree, resulting in 24 buds in total. Buds were sampled only from 2-year old spur wood. Bud scales were removed and were then immediately snap frozen in liquid nitrogen.

2.3. Protein identification
Four buds from each treatment were pooled and replicated three times. The buds were ground using a cryo mill (CryoMill, RETSCH GmbH, Haan, Germany) without cooling in a buffer (150mM Tris-HCl, 2% sodium dodecyl sulfate, 20mM dithiothreitol at pH 6.8). Proteins were then precipitated using a standard methanol/chloroform series and kept in denaturation buffer (50mM Tris at pH 8.5, 6M urea) until further processing. Proteins were digested in-solution using a mixture of Trypsin (Roche Pharma AG, Basel, Switzerland) and Lys-C (Wako Chemicals GmbH, Neuss, Germany) to reduce the number of missed cleavages in the resulting peptide mixture (Saveliev *et al.* 2013). Peptide mixtures were injected through a nanoflow ultra-high performance liquid chromatography (UHPLC) system (EASY-nLC 1000, Thermo Fisher Scientific Inc., Waltham, USA) for separation into an orbitrap hybrid mass spectrometer (Q-Exactive Plus, Thermo Scientific Inc., Waltham, USA)

by electrospray ionization. Mass-spectrometry (MS) data were analyzed using the MaxQuant software package (Cox and Mann 2008) with oxidation of methionine as variable modification, carbamidomethylation of cysteine residues as fixed modification and the match-between-runs function switched on. A cut-off threshold was set to at least two unique peptides identified per protein. Statistical analysis was performed using Perseus version 1.5.6 (Tyanova *et al.* 2016).

3. Results

A total of 3011 proteins were identified across both 'On' and 'Off' treated 'Fuji' trees and all three replicates. Most of the proteins have a molecular weight between 20kDa and 80kDa as shown in Figure 1. Only 43% (1309) of those proteins were successfully mapped to a KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway, that describes the function of the protein in a specific metabolic pathway. A Student`s T-Test resulted in 181 differentially abundant proteins between treatments at a false discovery rate (FDR) of 0.01.
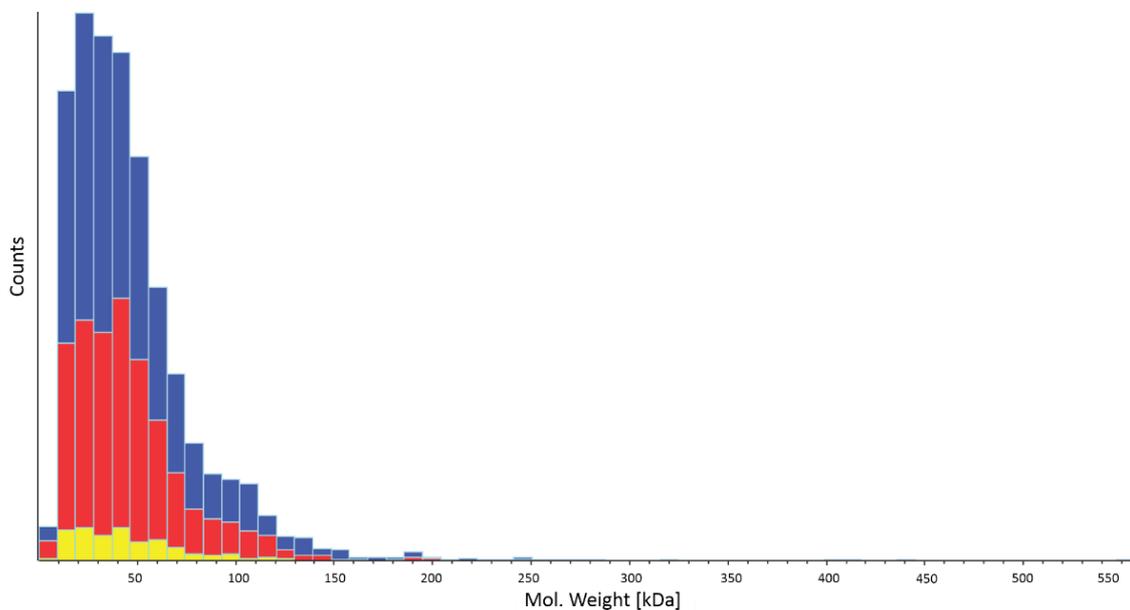

Figure 1 Histogram showing the molecular weight frequency of total proteins identified in 'Fuji' apple buds (blue), proteins mapped to KEGG pathways (red) and proteins significantly different between treatments (yellow).

The volcano plot in Figure 2 shows a clear separation of protein abundances between treatments. 81 proteins are significantly more abundant in buds from the 'Off' treatment and 100 proteins in the 'On' treatment, respectively. The relative $\log_2$ fold change contrasting 'On' against 'Off' treatments ranged from 3.17 (zinc metalloprotease ZmpB-like, gi|658025497) to -2.78 (nigrin b-like, gi|658043493).
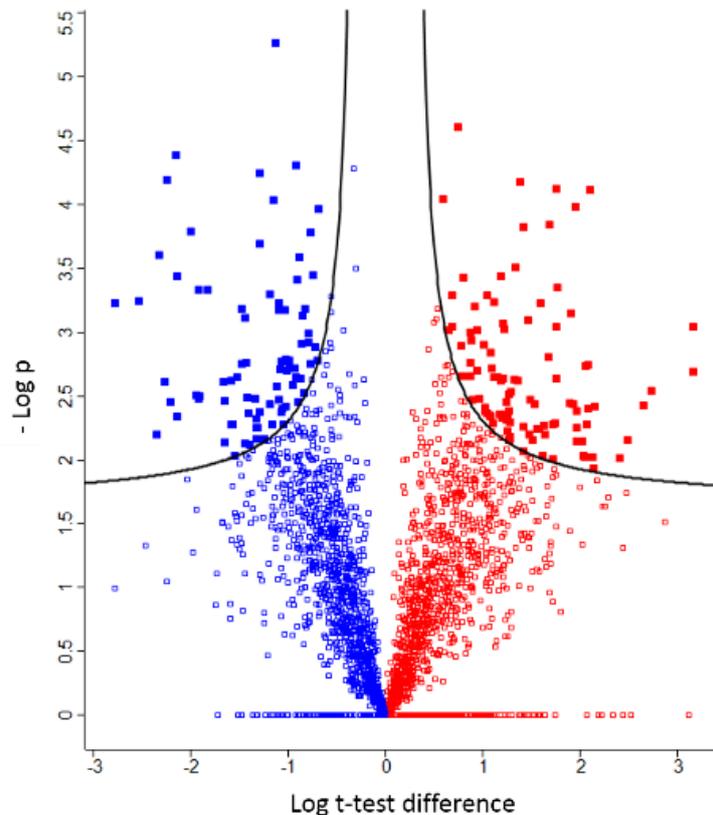
Figure 2 Volcano plot of identified proteins. FDR=0.01, S0=0.1. Red: T-test difference ON-OFF>0 (relative protein abundance higher in buds from 'On' treatment); blue: T-test difference ON-OFF<0 (relative protein abundance higher in buds from 'Off' treatment).

4. Discussion

This preliminary experiment clearly shows a distinct difference in the proteomic profile of apple buds from an 'On' and 'Off' tree at 85 dafb in 2015. It is a strong indicator for two different developmental pathways of apple buds from 'on' and 'off' trees, respectively. Buds from 'Off' trees must have been induced and switched to floral bud development, whereas buds from 'On' trees remained in a vegetative state, resulting in clear protein expression differences.

5. Conclusions

Further studies will explore individual proteins and more importantly, determine the time point where the first proteomic differences in buds between 'On' and 'Off' trees appear. Time-series analysis will depict temporal changes in specific protein expression patterns to further identify possible signals on the proteomic level that can be related to flower induction. Finally, the results of these studies are combined with RNAseq data and metabolomic profiles to draw a holistic explanation for understanding the underlying mechanisms of biennial bearing in apple.

6. Literature

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