Senescence-activated protease genes in the three main cultivated tobacco
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Objective

During the first phase of curing, corresponding to the so-called yellowing phase, which varies between 2 – 8 days depending on the tobacco type, leaf metabolic activities are drastically changing due to the activation of a senescence program leading notably to chlorophyll, starch and protein degradation. From curing and tobacco types it results in different leaf chemistry and properties. Only a few data have been reported yet on the activities of proteases in tobacco leaf and their resulting products. Taking advantage of our recent efforts to sequence the tobacco genome (Siervo et al., Nature com, 2014), we focused here on identifying protease genes activated during leaf curing in the three main tobacco types to categorize protease gene expression by curing or tobacco type.

Results

Figure 1. Expression of known senescence-activated genes SAG12 and CYP82E4 increased after 48h curing in the three main tobacco types A 48h time-point following the curing start was selected to screen senescence-activated protease genes based on Affymetrix data.

Table 1. The 80 senescence-activated protease genes belongs to 21 different protease families. The protease genes activated during tobacco curing belong to 21 families of different proteases. The most represented families are “Cysteine proteasines superfamilie protein” and “Eukaryotic aspartyl protease family protein”.

Figure 2. The expression of 80 senescence-activated protease genes increased in the three main tobacco types Search for tobacco proteases. A. thaliana sequences were extracted from TAIR10 with the following terms in their annotation: “protease, peptidase, protease”. Further candidates were identified by BLAST against TAIR10 (e-value=1e-80). Tobacco proteases were identified by a BLAST search of the A. thaliana proteases against the database of transcripts for the 3 varieties Burley, Virginia and Oriental (e-value cutoff 1e-80). Equivalent genes in the 3 varieties were identified by a mutual best BLAST hit search of the transcripts of the 3 varieties. Differential expression. The tissue samples were sequenced using RNA-seq; reads were mapped to the genomes of the 3 varieties using TopHat. Previously published gene models were used as the basis for the differential gene expression analysis. Expression changes during curing were calculated using the Cufflinks software based on the mapped reads. Genes were considered up-regulated if their expression levels increased significantly during the first 48h of curing, and not if the change was insignificant or decreased. The data shows the number of senescence-activated genes in the 3 cured varieties.

Summary and Conclusion

80 protease genes were found to be activated during tobacco curing. The most represented families of activated proteases are “Cysteine proteasines superfamilie protein” and “Eukaryotic aspartyl protease family protein”. In sun-cured oriental tobacco, the number of activated protease genes is limited compared to flue-cured Virginia and air-cured Oriental tobacco, possibly due to the harsher condition of curing (UV light and high temperature). Interestingly, some families of transcripts are more or less represented in each tobacco type, thereby suggesting that additional factors are involved in specific activation depending either on genetic or curing conditions. As an example, One APA1 gene is only activated in flue-cured Virginia and neither in air-cured Burley nor in sun-cured Oriental.