Candidate genes associated with increased concentration of fermentable sugars in alfalfa.

Solen Rocher, Josée Michaud, Annie Claessens, Marie-Noëlle Thivierge, Annick Bertrand.

An efficient way to improve nutritive value of forages relies on increasing the fermentable energy, and can be achieved through a better balance between readily fermentable energy and rapidly degradable proteins. This improved balance can be obtained by increasing the concentration of fermentable sugars [Neutral Detergent Soluble Carbohydrates (NDSC) = soluble sugars + starch + pectin]. Here, we used a divergent phenotypic selection approach to develop alfalfa populations with contrasted NDSC concentrations using stem NDSC concentration as the selection criterion, and to identify candidate genes associated with stem NDSC accumulation in alfalfa.

Two groups of genotypes contrasting in their NDSC concentrations (NDSC+ and NDSC-) were identified within the initial cv. Akori. Five clones were produced from each genotypes (25 NDSC+ and 25 NDSC-) (250 plants), grown under controlled conditions and harvested at around 10% flowering. Two stem subsamples were collected in AM and PM from each plant and used as biological replicates, and the 25 genotypes of each repetition were pooled for the biochemical analysis of NDSC and for RNA extraction. A cDNA synthesis was performed using pooled samples [2 groups (NDSC+/-) x 2 (AM/PM) x 5 clones x 2 repetitions = 40 samples] and SRAP-cDNA analysis was conducted with 46 pairs of SRAP primers. We obtained 84 fragments differentially amplified between AM/PM samples and/or between NDSC+/NDSC- populations. These fragments were sequenced and 48 candidate genes were identified through homology with *M. truncatula* reference genome [(1)]. Specific primers were developed to analyse the expression of these candidate genes by RT-qPCR. This analysis confirmed the differential expression of 24 candidate genes between AM/PM and/or NSC+/NSC- samples. Among those, sixteen (16) genes showed significant expression shifts between AM and PM only, whereas eight (8) genes showed significant expression changes between both AM and PM samples and NSC+ and NSC- populations.

Taken together, our results confirmed the quantitative control of NDSC biosynthesis and accumulation in alfalfa. We observed extensive diurnal variation of candidate genes expression, sometimes linked with differences of expression level between phenotypically contrasted samples. Our study also revealed the large effect of sampling time on both NDSC concentration and candidate genes expression, and highlighted the importance to distinguish genotypic effects from genotype x environment interactions. This work confirmed that stem NDSC can be used as a selection criterion to improve alfalfa energy content, and revealed that molecular genetics approaches are promising to accelerate phenotypic gain for this trait. In that perspective, high-throughput genotyping of contrasted populations will be undertaken to identify markers linked to key genes involved in NDSC accumulation on a genome-wide scale.

(1) Tang H. et al. 2014. BMC Genomics;15(1):312.