Genetic Selection for High Energy in Alfalfa (*Medicago Sativa* L.) Stems

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Alfalfa is highly valued for animal feed because of its high protein content. Alfalfa proteins are however highly degradable and ruminal microbes are unable to utilize all of the amino acids and ammonia released. As a result, a large proportion of proteins escapes microbial digestion and are excreted into the environment under the form of urea. To overcome these losses, a simultaneous inflow of energy (carbohydrates) must be provided to ensure an efficient use of nitrogen (N) by rumen microbes. The genetic selection of alfalfa with high energy concentration represents a sustainable approach to reduce N losses to the environment, improve animal performance, while reaping the broad benefits of cultivating alfalfa (symbiotic N fixation, soil health improvement, high quality forages, etc).

Based on the observation that stem non-fiber carbohydrate concentrations is less dependant from daily fluctuations than leaf carbohydrates and hypothesising a direct correlation between stem and whole plant carbohydrate concentrations, we developed a method of recurrent selection for high-energy in alfalfa based on stem carbohydrate concentrations. In addition, our selection approach takes into account the pectin concentration which constitutes up to 20% of alfalfa dry matter (DM) and is an important source of readily available energy. This study aims to evaluate under field conditions the direct effect of four cycles of recurrent phenotypic selection for stem non-fiber carbohydrate (NFC = sum of soluble sugars, starch and pectin) concentration on alfalfa's whole plant NFC concentration. Genotypes from four genetic backgrounds (55V48, Akori, Genoa VR and Megan) selected based on their winter survival, yield and high stem-NFC concentrations were intercrossed to produce the NFC1 population. Subsequent cycles of recurrent selection were based on plant vigor and high stem-NFC concentration and generated the NFC2, NFC3, and NFC4 populations. A control population (NFC0) was created by intercrossing alfalfa genotypes randomly selected from the four initial genetic backgrounds. The five populations produced were then evaluated in field trials established as row plantations (25 plants of a given population in each row) in summer 2020 at two locations in Quebec, Canada (Saint-Augustin-de-Desmaures and Normandin). Plots were harvested in 2021 and samples of above-ground biomass from first and second harvests were dried, ground and scanned by visible and near-infrared spectroscopy (VNIRS) to estimate nutritive attributes. Our results show that the energy concentration of the population obtain after four cycles of recurrent selection (NFC4) was significantly higher than that of NFC0, with a progressive improvement at each cycle. For instance, the concentration of non structural carbohydrate (NSC = sum of soluble sugars and starch) was 13 % higher (173 vs 153 g kg^{-1} DM) while the NFC concentration was 9% higher (237 vs 217 g kg^{-1} DM) in NFC4 than in NFC0 population. These first-year results show that our method of phenotypic selection for high stem-NFC concentration in alfalfa is an effective selection approach to improve alfalfa nutritive value. Field trials are being pursued at the two sites and an additional site was established in 2021 to validate the results for a second production year as well as under different environmental conditions.