Production of oil in vegetative tissues to increase the nutritive value of herbaceous legume forages.

IMPROVING THE ENERGY CONTENT OF LEGUMES - NEXT STEPS

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Background: Many producers recognize the value of a high proportion of legumes in pasture stands. In addition to fixing nitrogen and reducing reliance on fertilizer, legumes tend to have high yields and quality. Some legume species, such as sainfoin, can be mixed with alfalfa to also reduce bloat.

Oil is twice as energy-dense as carbohydrates, which make up most of the leaves and stems of plants. Traditionally, seeds have been the main source of plant oils and primarily used for food (i.e. canola oil) or biofuel production, with few plants having a significant amount of oil in the leaves. Research groups in other parts of the world have been successful in improving the oil content in the leaves of certain plants. Increasing the oil content in vegetative tissues of forage legumes like alfalfa and sainfoin means those plants would contain more energy, and therefore be a more efficient and productive feedstuff for cattle. Adding oil to feedlot diets at levels that do not exceed 6% of total fat intake has also been shown to decrease methane production by 10-25%.

The previous iteration of this project created alfalfa and sainfoin mutant plants that increased the leaf oil (lipid) content from essentially zero to a maximum of about 5% in both species, without any other visual changes to the plants. This was achieved using conventional breeding techniques (non-GMO). The present project continues the plant breeding selection process in more advanced generations.

Objectives: The objectives of this study were to:

1. Determine the stability of increased oil content and fatty acid composition in subsequent generations of sainfoin and alfalfa
2. Examine the potential of applying CRISPR/Cas9 genome editing techniques to favourably alter lipid metabolism in these forage legumes

What they did: The conventional breeding technique used seeds from selected alfalfa and sainfoin plants (AC Blue J and AAC Mountainview varieties) with higher leaf oil content from the previous project. 500 plants from the second selection cycle of both species were planted. From these, ten plants of each species with greater leaf oil content were grown in the greenhouse, evaluated for protein content and agronomic traits, and provided seed to advance the next selection cycles.

The CRISPR/Cas9 genome editing technique focused on the SDP1 and PXA1 genes in alfalfa, which were previously demonstrated to affect lipid content. This work primarily

focused on “knocking out” the SDP1 gene, so that its expression would be reduced and lipid content in the alfalfa plants would be increased. The genome edits were confirmed by gene sequencing.

What they learned: Full evaluation of this selection cycle was impacted by COVID-19. However, the leaf oil content remained about 20% higher in this selection cycle compared to the first round of selection. However, there were no major differences in protein content between these plants and the controls, nor did this selection cycle reveal any major plant deformities.

Further selection cycles and analysis are currently underway.

What it means: Plant breeding is an incremental process, and more selection cycles are required before new varieties of sainfoin and alfalfa with improved leaf oil (lipid) content are available on the market. The initial selection results have been promising thus far, indicating that the initial mutations are indeed heritable and stable in subsequent generations with no notable impacts on other important agronomic traits to date.

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