RESEARCH

Production of oil in vegetative tissues to increase the nutritive value of forage legumes

IMPROVING THE ENERGY CONTENT OF LEGUMES

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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%.

Objectives: The objectives of this study are to:

1. Gain insight into the variability in oil content of vegetation from existing lines of alfalfa and sainfoin.
2. Establish proof of concept for increasing total oil content in alfalfa and sainfoin vegetation using chemical mutagenesis.
3. Characterize promising lines for oil content and sequence those lines to reveal the genes altered by the mutagenesis that are responsible for improved oil production, and to ensure that no other agronomic characteristics are impaired.

What they did: AAC Mountainview sainfoin and AC Blue J alfalfa seeds were exposed to ethyl methanesulfonate (EMS) at varying concentrations to induce mutations. This is considered a conventional plant breeding technique and the resulting product is not considered a genetically modified organism (GMO). Once the optimum EMS concentration was determined, seeds were incubated for 24 hours at room temperature, rinsed, and then planted in trays in a greenhouse. Plants were allowed to grow for 6-8 weeks, until about 10% of the plants had flowered, when they were cut (to simulate grazing) and allowed to regrow for another 6-8 weeks and cut again. The total leaf oil content was analyzed using near infrared spectroscopy (NIRS) and chromatography.

Concurrently, two genomic approaches were also explored. Virus-induced gene silencing (VIGS) was used to selectively "turn off" genes known to influence oil content in the leaves of other plant species. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas 9, was explored to selectively introduce small insertions or deletions into two target genes in order to improve oil content.

What they learned: The conventional mutation approach yielded alfalfa and sainfoin plants that repeatedly increased the leaf oil content from essentially zero to about 5% in both species. No other significant visual changes were observed in these selected plants. Turning off or silencing any of three specific genes with VIGS resulted in a significant increase in oil content in the sainfoin and alfalfa leaves. The original CRISPR/Cas 9 approach had low efficiency, so the team is working to optimize this approach.

What it means: This is just the first step of many in improving the shoot oil content; and therefore energy content, of these forage species through specific plant breeding techniques. The next steps involve further rounds of selection with the germplasm developed by this project to ensure the mutations are stable, as well as field testing and further agronomic and quality analysis to ensure that other important traits are not affected. This is particularly important when dealing with plants produced by inducing mutations with EMS, as you can't guarantee the mutations you want will be the only ones you get. The genomic based approaches are much more specific, however they come with particular regulatory and social challenges attached to the "GMO" moniker.

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