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RESEARCH FACTS
RESEARCH & TECHNOLOGY DEVELOPMENT FOR THE CANADIAN BEEF INDUSTRY

Improving abiotic stress tolerance in alfalfa

Project Title:		Project Code:	FRG.06.17
Improving abiotic stress tolerance in alfalfa through the simultaneous down-regulation and/or genome editing-mediated knockout of multiple genes.		Completed:	*In Progress. Results expected in March 2022.*

Researchers:

Stacy Singer Ph.D. stacy.singer@canada.ca
Stacy Singer Ph.D. (Agriculture Agri-Food Canada Lacombe) and Surya Acharya Ph.D. (Agriculture Agri-Food Canada Lethbridge)

Background:

Alfalfa is susceptible to numerous stresses including drought, waterlogging, salinity, frost and/or winterkill. Sometimes stress tolerance improves when certain genes are under-expressed. Transgenic approaches to down-regulate particular genes have worked better than conventional breeding in some cases. However, transgenics also face public acceptance and regulatory hurdles. Genome editing (“fixing” genes that are already there, rather than adding new genes from a different species) may help avoid GMO concerns by down-regulating genes that are naturally present rather than by introducing new genes from a different species.

Objectives:

To assess the efficacy of previously discovered genes in enhancing alfalfa performance under abiotic stress conditions when down-regulated via RNA interference (RNAi). Researchers will attempt to make further improvements in abiotic stress tolerance, and, if technology permits, utilize CRISPR-Cas9 as an alternative approach for gene knockout.

What They Will Do:

In other plants, four genes (called CBF2, ACBP3, FAO and TAC1) have been found that allow those plants to cope with freezing, drought, flooding, and salinity better when those genes are down-regulated. Another gene (MtHB2) has a similar effect when up-regulated. These genes will be cloned into alfalfa. Seven transgenic lines will be developed and grown under non-stressful conditions. The four lines showing the most downregulation for each of these genes will be grown and exposed to salinity, drought,

freezing and flooding stress until the control plants show visible signs of stress. The plants will be allowed to recover, and survival, root and shoot growth will be measured. Lines that differ from the controls will be re-tested, and a larger range of biochemical and physiological measures will be collected. They will also follow the same procedure with all the genes in the same plant at the same time. This will identify whether or which of these genes or gene combinations allow the plant to grow normally under normal conditions but survive better in stressful conditions.

Based on this, and providing suitable technology is available, they will knock-out some of these genes in alfalfa. If so, they may be able to produce a more stress tolerant alfalfa using a non-GMO gene editing approach, faster than they could through conventional breeding.

Implications:

This project will use modern breeding tools to develop alfalfa varieties that survive and flourish in marginal lands characterized by flooding, drought, salinity and temperature extremes, where forage and cattle production often occurs.

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