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Physiological and molecular characterisation of lucerne (*Medicago sativa* L.) germplasm with improved seedling freezing tolerance

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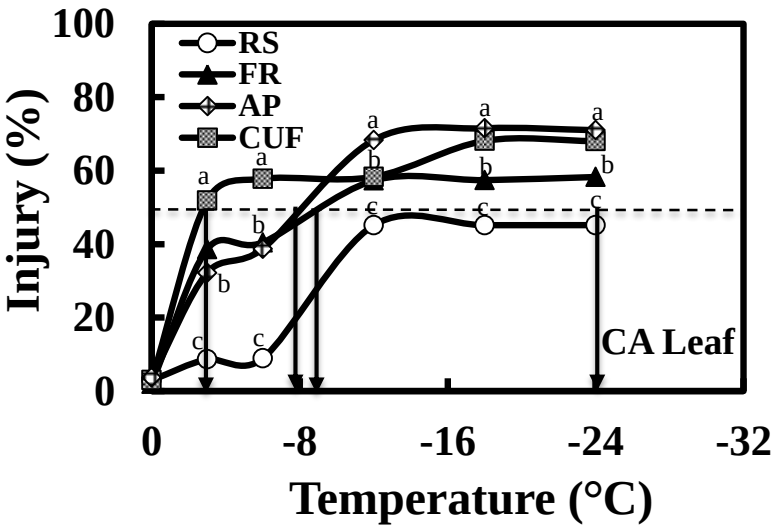

Figure S1. LT_{50} of 7-day cold-acclimated alfalfa plants in different germplasms based on leaf electrolyte leakage assay. RS, River side; FR, Foster ranch; AP, Apica; CUF, CUF-101. Data points with different letters among germplasm at the same freezing temperature are significantly different ($p < 0.01$, n = 18).

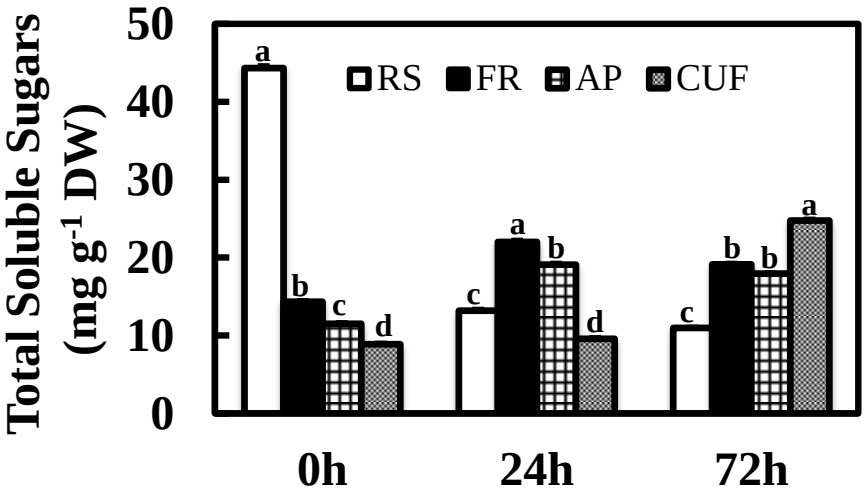

Figure S2. Total soluble sugar content in roots before or after (24 or 72 h) cold treatment. RS, River side; FR, Foster ranch; AP, Apica; CUF, CUF-101 Bars with different letters are significantly different ($p < 0.01$, n = 24) among germplasm sources at the same time point.

Table S1. Fourteen alfalfa germplasms, their abbreviated short name, ~~fall~~/autumn dormancy rating, and place of origin (sources)/accession number.

Name	Type*	Abbreviation	Full/Autumn Dormancy[1]	Sources/Accession
River side	A	RS-6	Unknown	Grand River National Grasland (GRNG) (Latitude: 45°N, Longitude: 102°W), SD, USA
Foster ranch	A	FR-5	Unknown	Grand River National Grasland (GRNG) (Latitude: 45°N, Longitude: 101°W), SD, USA
Wind river	A	WR	Unknown	Norman Bud Smith Ranch, SD, USA
Alfagraze	C	ALF	2[5]	Commercial cultivar
CHBB-04	C	CHB	Unknown	USDA-ARS, Logan, UT, USA
BCBB-04	C	BCB	Unknown	USDA-ARS, Logan, UT, USA
MT-0	C	MT	1[2]	Outlook, MT, USA
A-1991	C	A	Unknown	USDA-ARS, Logan, UT, USA
SD-201	C	SD	1[4]	South Dakota State University, SD, USA
DON	C	DON	1[2]	USDA-ARS, Logan, UT, USA
CUF-101	C	CUF	9[1]	Commercial cultivar
Ameristand	C	AM	8[7]	National Temperate Forage Legume
47 TQ				Genetic Resource Unit, Prosser, WA, USA
APICA	C	AP	3[6]	AAFC, Quebec, Canada
CARIBOU	C	CAR	2[6]	AAFC, Quebec, Canada

*A, accession; C, cultivar.

[1]Lower scores indicate that the population is more dormant on the basis of the height of autumn regrowth (Tueber et al. 1998).

[2]Peel et al. (2009) USDA-ARS, United States Department of Agriculture–Agricultural Research Service.

[3]Misar et al. (2015).

[4]National Alfalfa Alliance (2004).

[5]Hendrickson and Berdahl (2003).

[6]Schwab et al. (1996).

[7]Arris et al. (2007).

References:

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2. Peel, M.D., Asay, K.H., Waldron, B.L., Jensen, K.B., Robins, J.G., Mott, I.W., 2009. "Don", a diploid falcata alfalfa for western U.S. rangelands. Journal of Plant Registrations 3, 115–118.

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5. Hendrickson, J.R., Berdahl, J.D., 2003. Survival of 16 alfalfa populations space planted into a grassland. Journal of Range Management 56, 260–265.

6. Schwab, P., Barnes, D., and Sheaffer, C., 1996. The relationship between field winter injury and fall growth score for 251 alfalfa cultivars. Crop science 36, 418-426. (Yves Castonguay, personal communication, November 2015).

7. Ariss, J.J., and Vandemark, G.J., 2007. Assessment of genetic diversity among nondormant and semidormant alfalfa populations using sequence-related amplified polymorphisms. Crop Science 47, 2274-2284.

Table S2. Primer sequences and their corresponding melting temperature (T_m) for qPCR analysis.

Primer	Sequence	T_m (°C)
MsCBF1_5p	GTGATAGGGATGCTGTGGATATG	64.2
MsCBF1_3p	GGGAAGGAGGATTAGAACGTAAAG	63.8
MsCBF2_5p	ATGCCAGAGTTGTTGAGGAATA	62.4
MsCBF2_3p	GATGAGAAGCACTTTATGCTTGAT	62.4
MsCas15B_F	CCGGTCAGATTGGTTTGT	61.0
MsCas15B_R	GCAGCTCAATAGTAATAACTCATAC	57.0
MsTubulin_5p	ATTGGGCGAAAGGTCATTACACT	55.7
MsTubulin_3p	TTCCCATTCCAGATCCCGTTCCT	61.4

Table S3. LT_{50} of four alfalfa germplasms, RS, River side; FR, Foster ranch; CUF, CUF-101; and AP, Apica, based on leaf electrolyte leakage analysis. NA, Non-cold acclimated; CA, Cold acclimated.

Cultivars	NA_{Stem} (^0C)	NA_{Leaf} (^0C)	CA_{1d} (^0C)	CA_{3d} (^0C)	CA_{7d} (^0C)
RS	-9.8	-4.9	-11.2	-17.3	>-25
FR	-10	-4.7	-10.2	-9.6	-9.5
CUF	-4.9	-2.8	-7.8	-6.0	-3.0
AP	-5.9	-3.2	-8.3	-8.5	-9.3