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Near-infrared reflectance spectroscopy prediction of enzyme-released glucose in alfalfa stems

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Context

To increase the competitivity of alfalfa as feedstock for ethanol production, there is a need for the development of new genetic resources with:

- Highly degradable cell walls (high content in fermentable carbohydrates)
- High biomass yield under harsh winter conditions specific to Canada

Harvest fractionation of alfalfa

Aerial part

Stems

Energy production

Leaves

Feed protein co-product

- High cellulose content (ethanol)

- High biomass yield (45%)
- High protein content (30%)
- Co-products (flavonoids, biopharming)

Approach

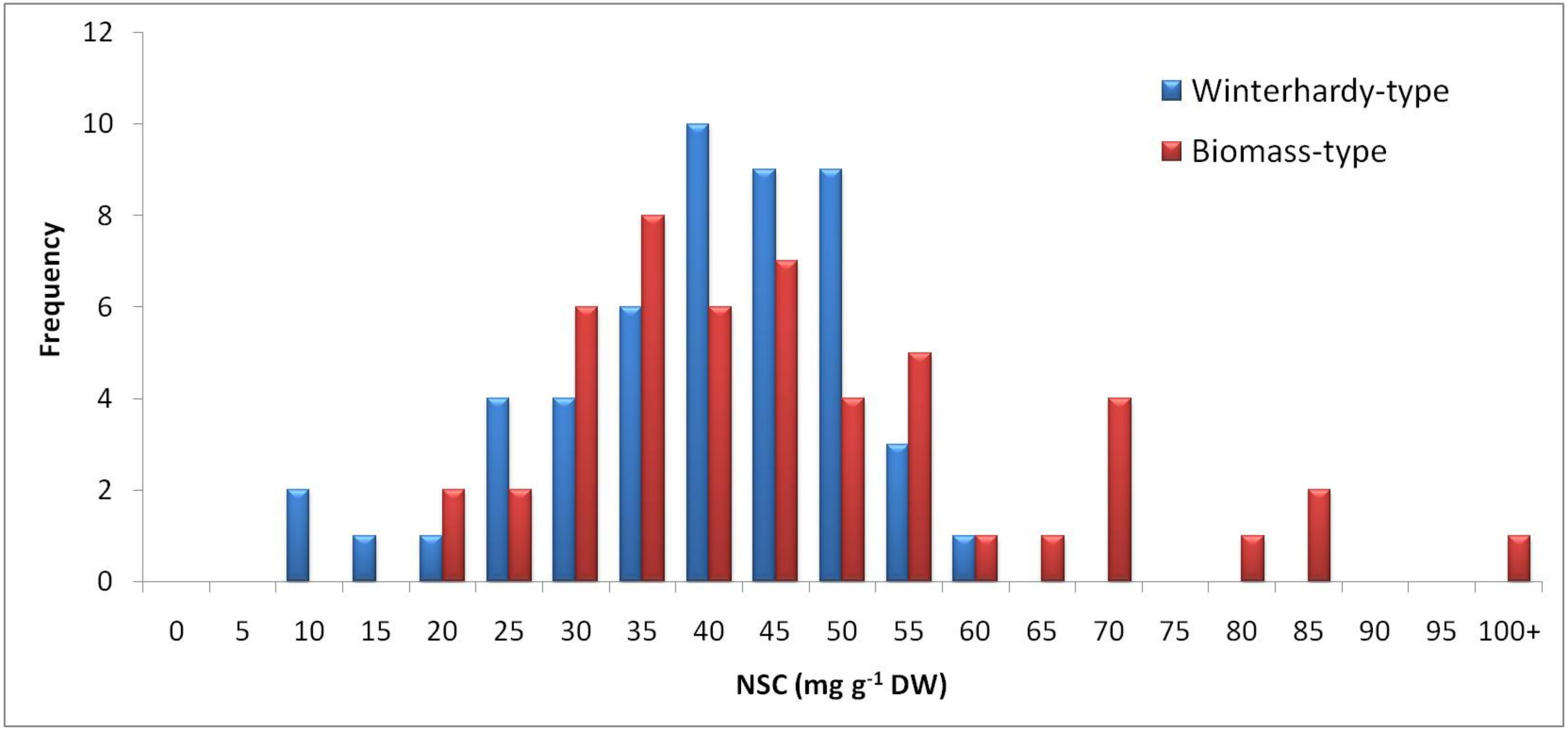
1. Field pre-selection of genotypes with high biomass yield and persistance (Biomass-type and Winterhardy-type) – (3000 genotypes seeded – 600 selected)

2. Assessment of genetic variability for parameters linked with production of cellulosic ethanol :
    a) Non structural carbohydrate content (NSC)
    b) Structural carbohydrate content (SC, cell walls)
    c) Cell wall degradability (enzymatic saccharification)

3. Intercross selected genotypes

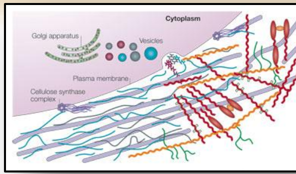
4. Search for molecular markers

Non-structural sugars in lignified stems



- Easily extracted and stable source of readily fermentable sugars
- Large genetic variability for NSC content (1 to 10% of lignified stem biomass)
- Differs according to cultivars

Structural sugars in lignified stems



Alfalfa stem composition at 25% flowering

Component	Sugars	mg/g CW
Cellulose	Glucose	436
Hemicellulose	Xylose	129
	Mannose	27
	Fucose	2
Pectin	Uronic acids	134
	Arabinose	27
	Galactose	27
Lignin		205

Jung et Lamb (2003)

Enzymatic cocktail for alfalfa stem degradation

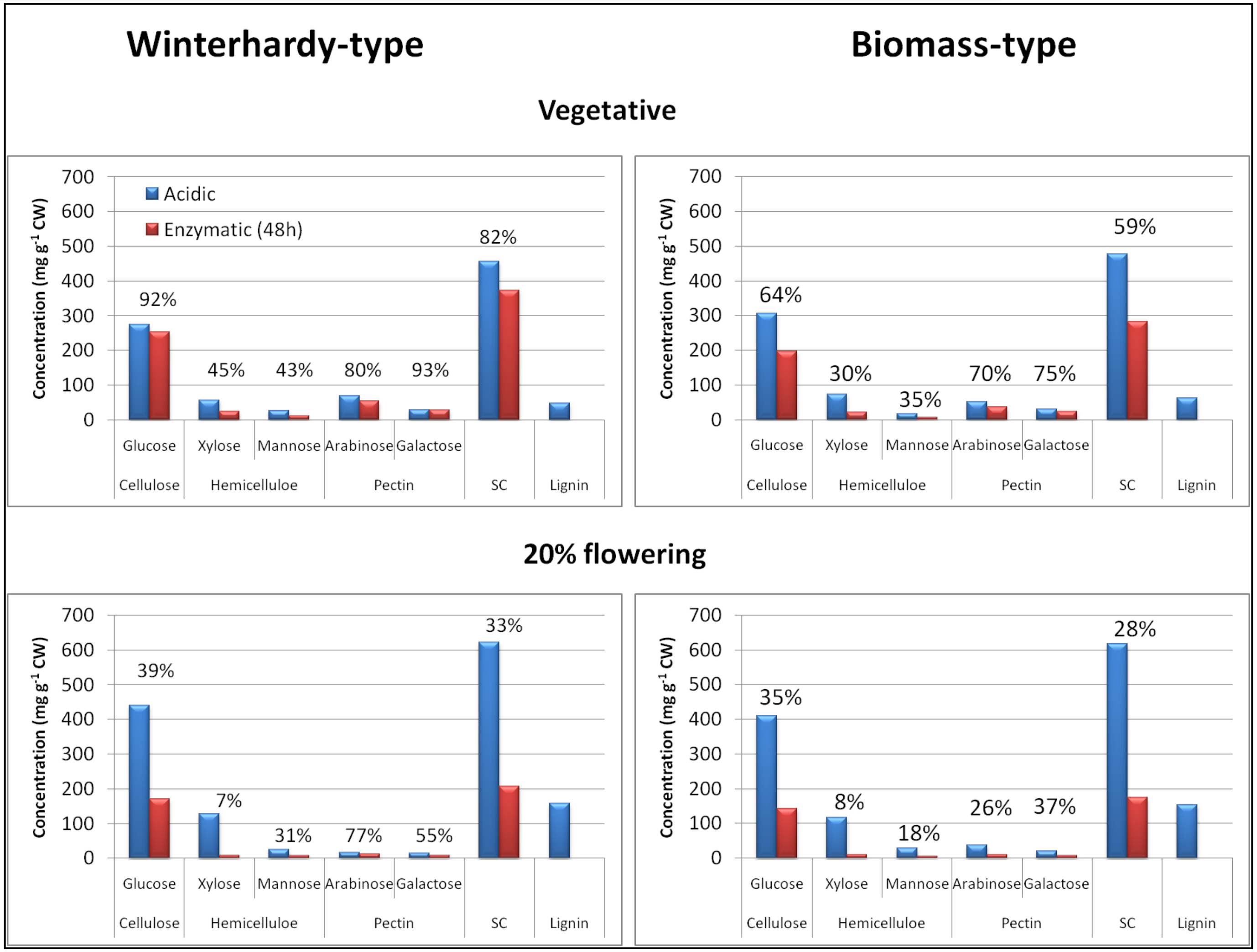
- Accellerase 1500 (**Genencor**)

  Cellulase and β-glucosidase activity
  - Xylanase and cellulase additive (XC)
  - Xylanase additive (XY)

- Pectinex 3XL (**Sigma**)

  Pectinase, cellulase et hemicellulase

Enzymatic saccharification efficiency



Test discriminates between stems with high (D+) and low (D-) degradability

Near-infrared reflectance spectroscopy

- Prediction of physicochemical parameters
- Minimal sample preparation
- High throughput screening
- Accurately predicts carbohydrate fractions in alfalfa and ethanol yield in switchgrass

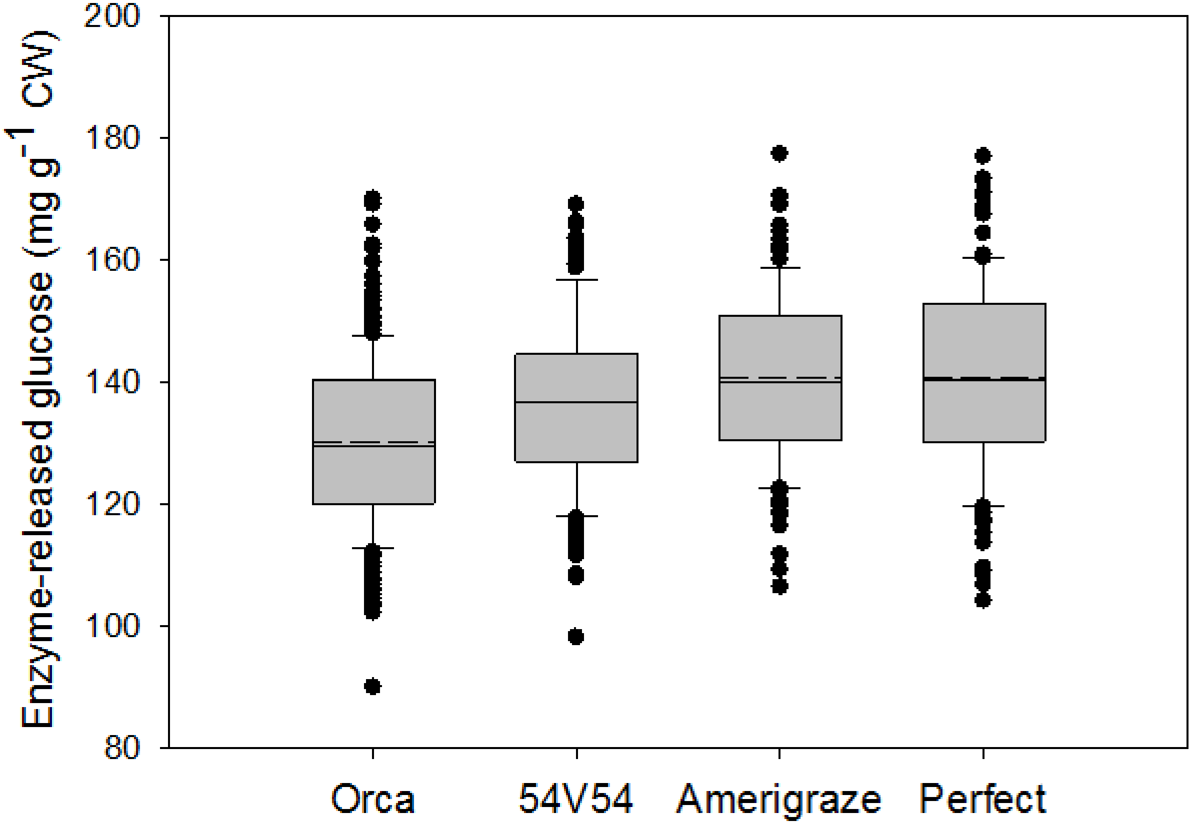
NIRS – Predictions

Parameter	R^2
A- Enzymatic-released glucose	0,94
B- Enzymatic structural carbohydrates	0,86
C- Lignin	0,64
D- Soluble sugars	0,97
E- Starch	0,78
F- Non-structural carbohydrate (D + E)	0,97
G- Fermentable carbohydrates (B + F)	0,85

- Enzyme-released glucose is accurately predicted in alfalfa by NIRS (R^2 = 0.94)

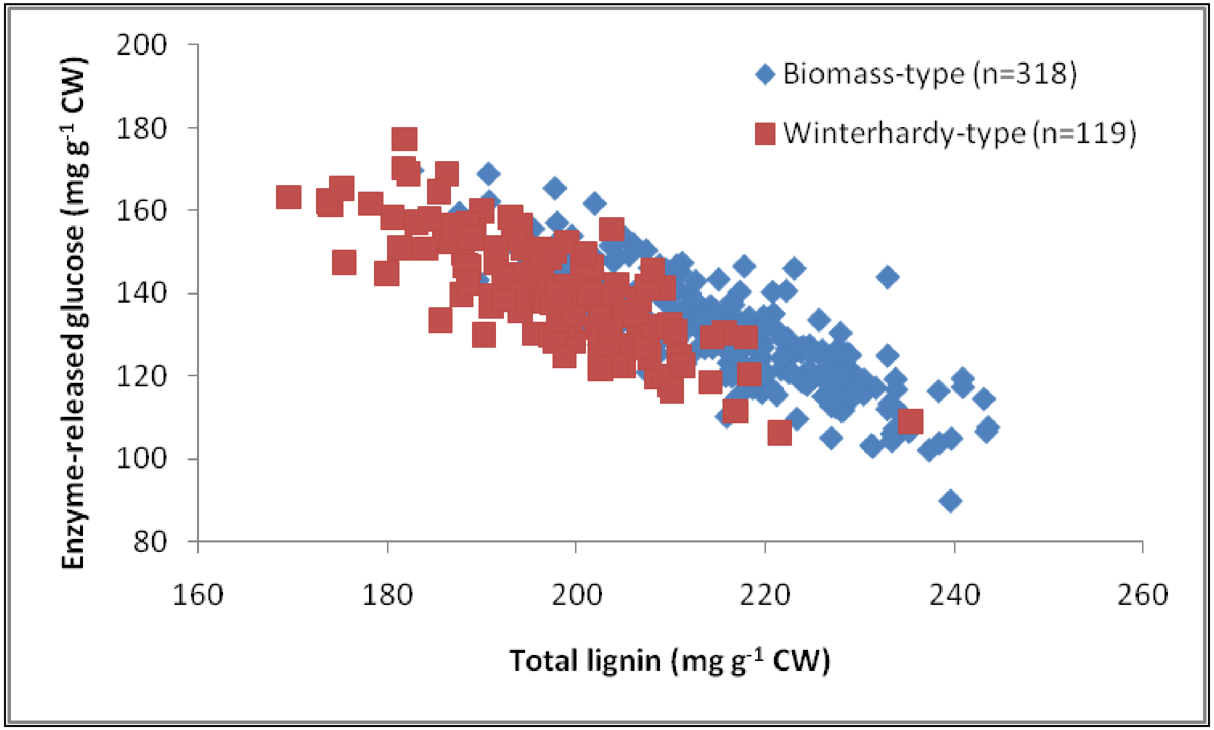
Duceppe et al. 2012 Bioenerg. Res. Online May 2012

Enzyme-released glucose in four genetic backgrounds



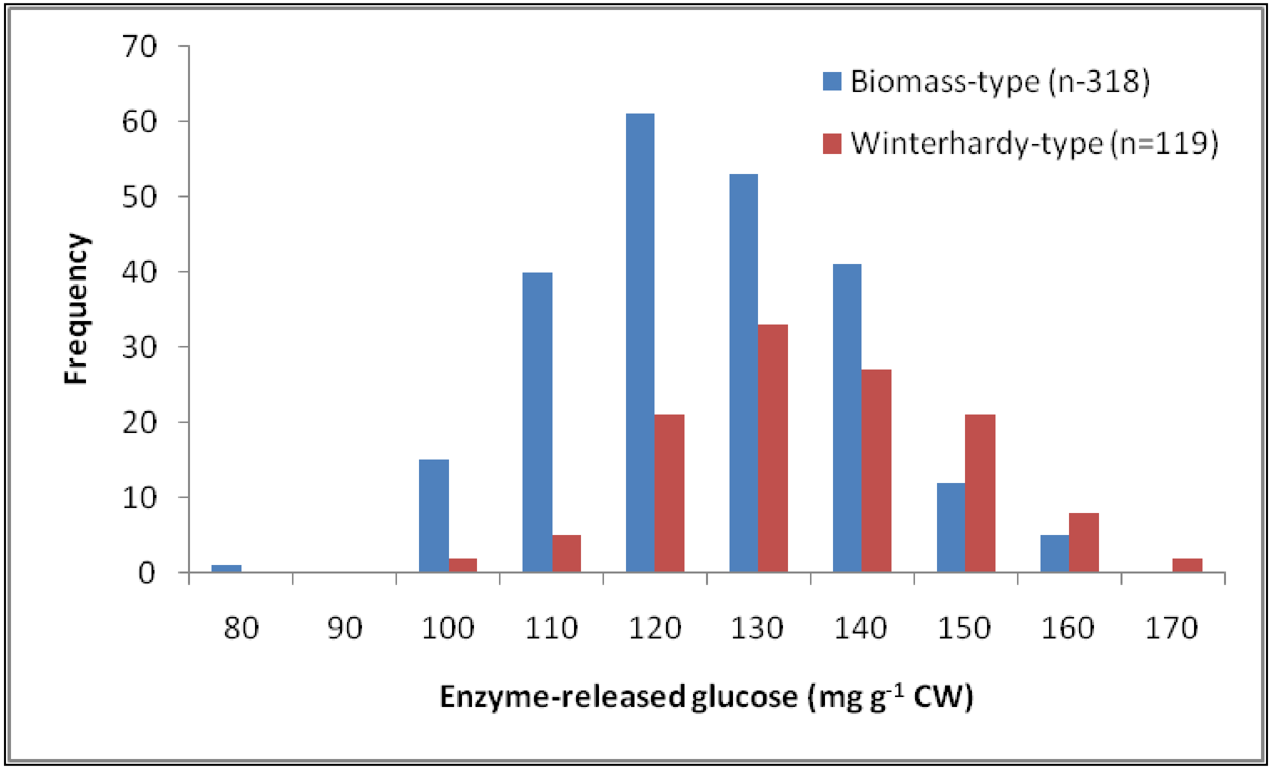
- Within each cultivars, E-R glucose varied extensively
- On average, degradability of winterhardy-type cultivars (54V54, Amerigraze, Perfect) is higher than for the biomass-type cultivar Orca

Relationship between lignin and E-R glucose



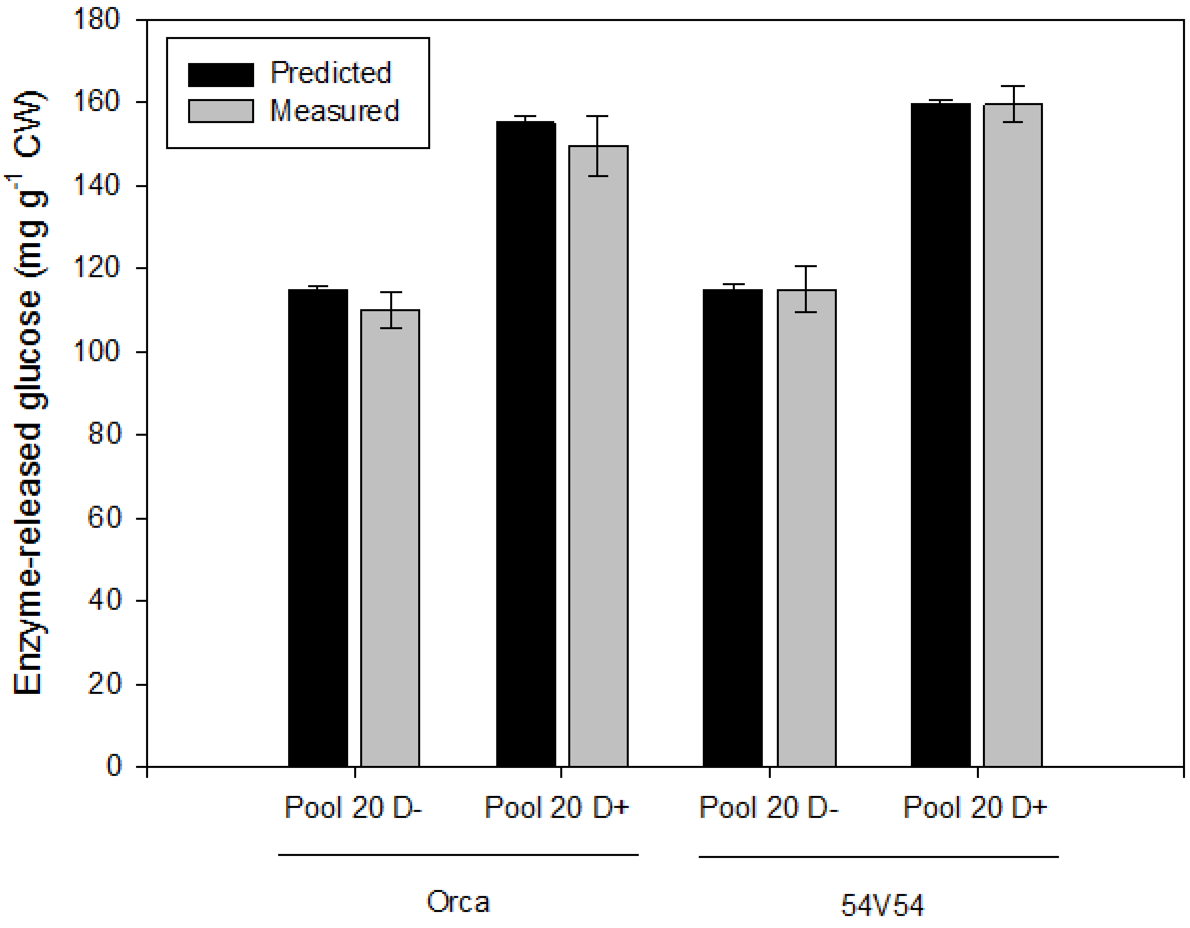
- Strong negative correlation (R = -0.83) between lignin concentration and E-R glucose
- Large genetic variability for lignin content

Enzyme-released glucose (NIRS)

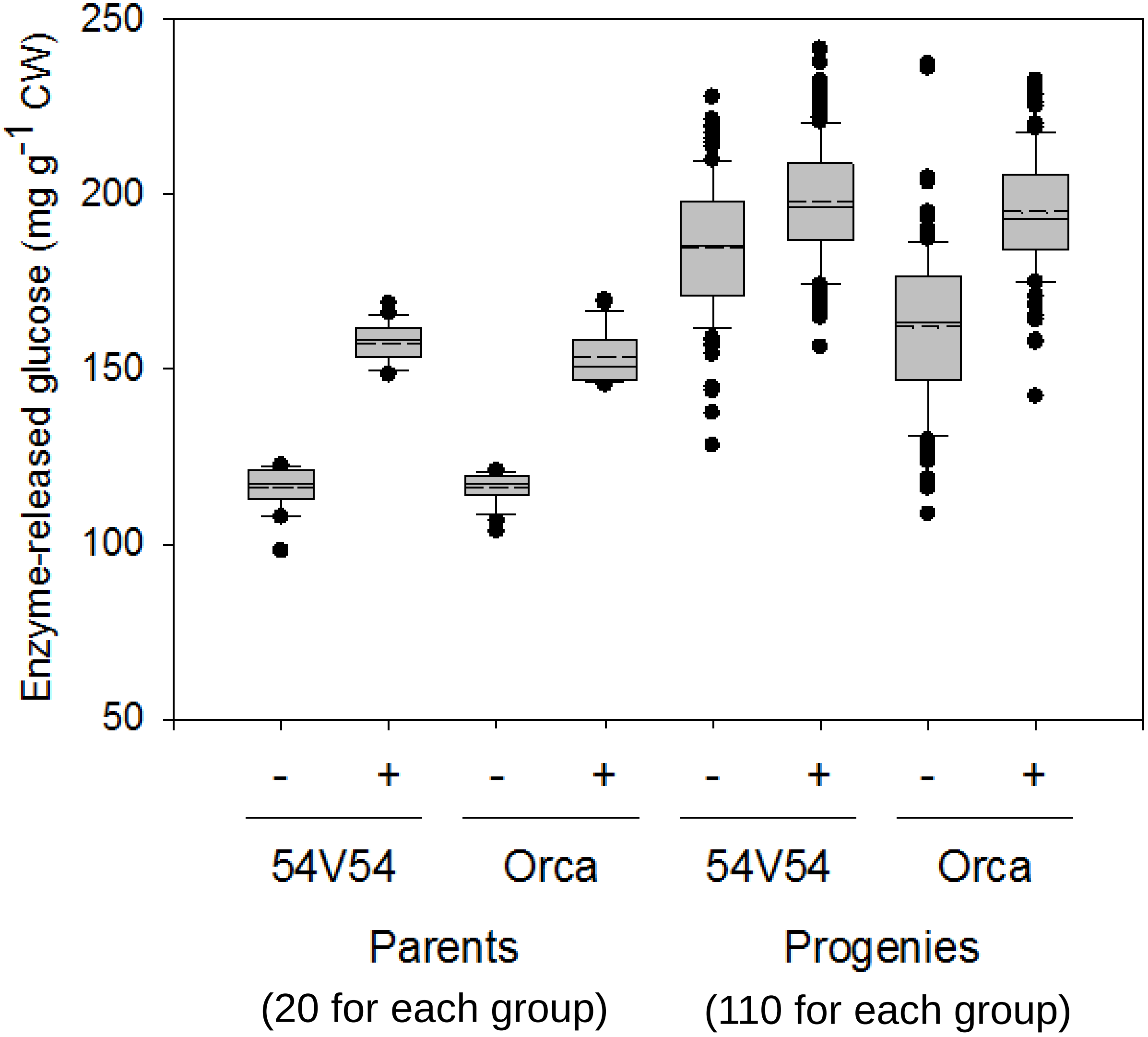


- Rapidly characterize CW degradability of hundreds of genotypes
- Selection of 20 D+ and D- genotypes by NIRS prediction

Validation of NIRS prediction



Enzymatic-released glucose is genetically inherited



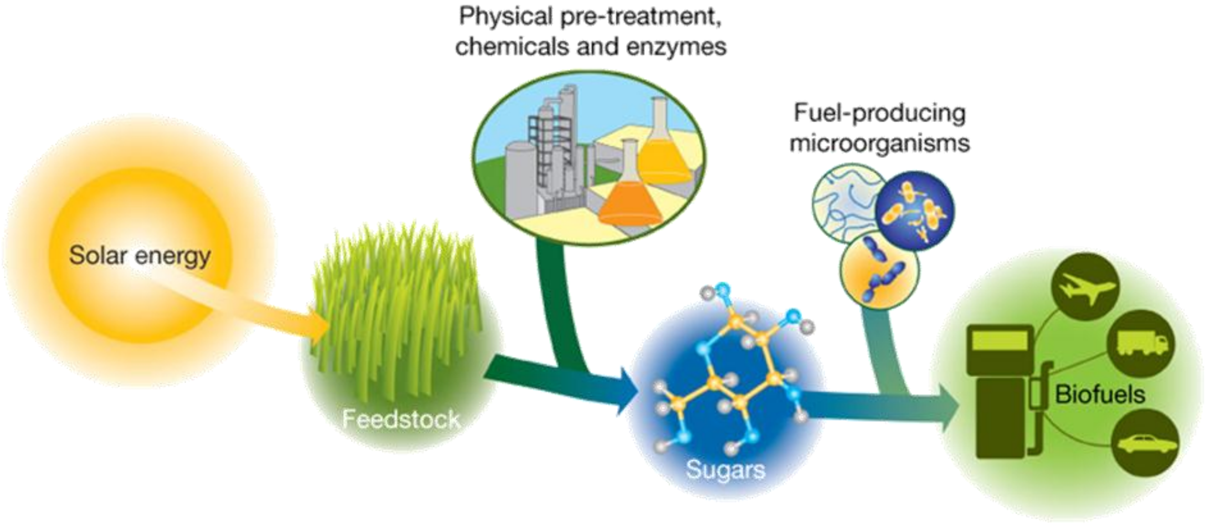
Conclusions

- NIRS efficiently predict alfalfa stem composition and degradability - High throughput screening.
- Genotypes with high (D+) and low (D-) CW degradability were identified in two genetic backgrounds.
- D+ genotypes had on average 20% less lignin than D- genotypes and were 35% more degradable.
- CW degradability was significantly higher in progenies from crosses of D+ genotypes showing heritability of that trait.
- Assessment of DNA polymorphism suggests the presence of genomic region that affect CW degradability .

Current activities

- Continue recurrent selection for CW degradability and increased NSC content in lignified stems.

- Identify DNA polymorphisms to develop molecular marker applications.



Acknowledgements

We would like to thank the Agricultural Bioproducts Innovation Program of Agriculture and Agri-Food Canada for financial support.

Thanks to:

Annie Claessens (Plant breeder)

Josée Bourassa (Research assistant in plant biochemistry)

Josée Michaud (Research assistant in molecular biology)

Marie-Claude Pépin (Research assistant in plant breeding)