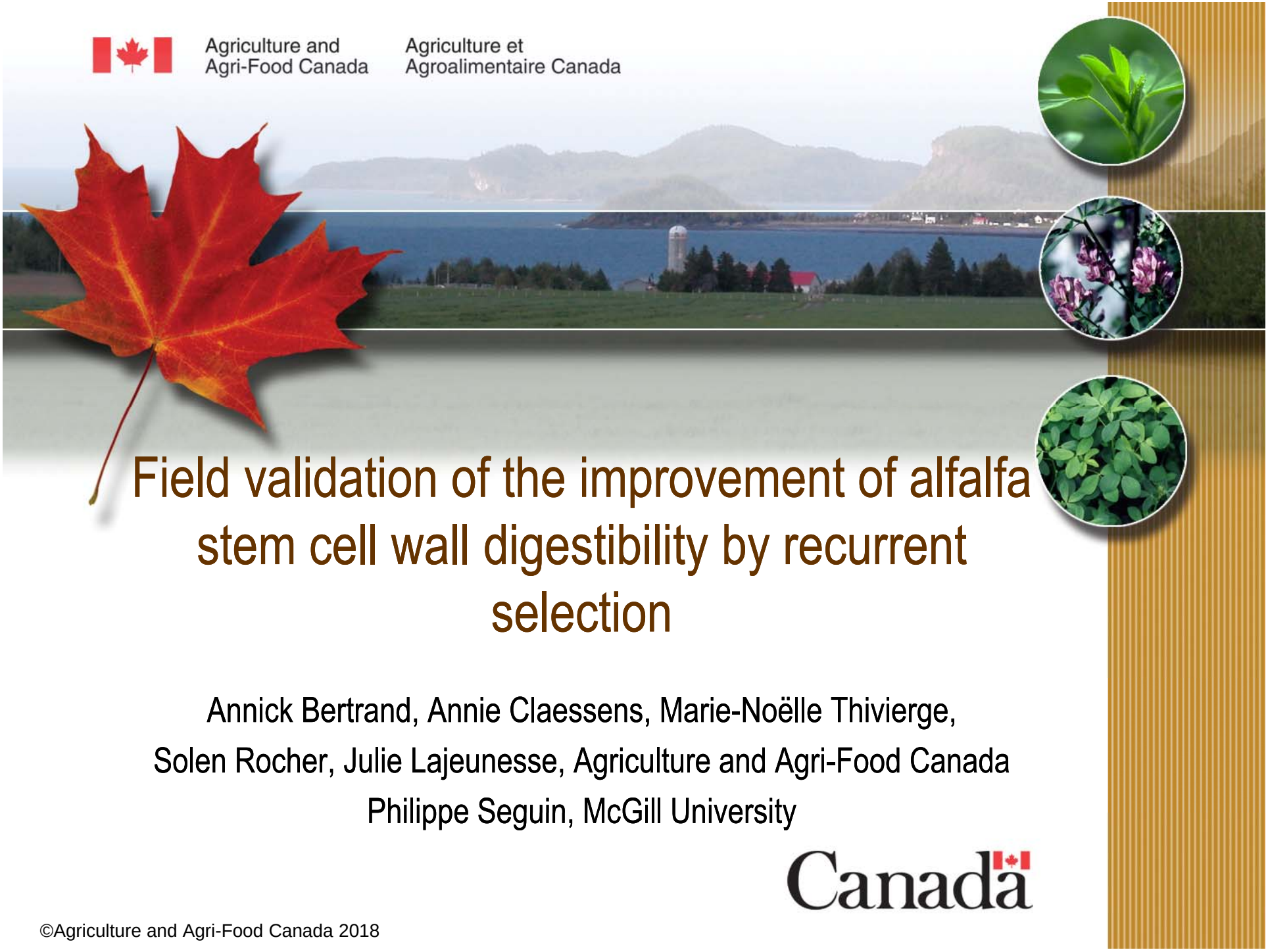


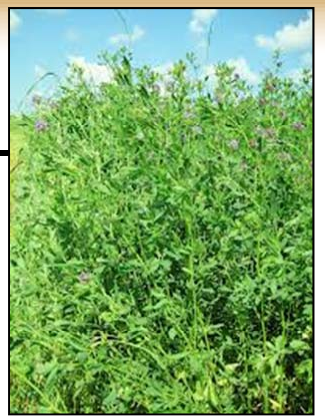
Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

Field validation of the improvement of alfalfa stem cell wall digestibility by recurrent selection

Annick Bertrand, Annie Claessens, Marie-Noëlle Thivierge,
Solen Rocher, Julie Lajeunesse, Agriculture and Agri-Food Canada
Philippe Seguin, McGill University

Canada

Introduction



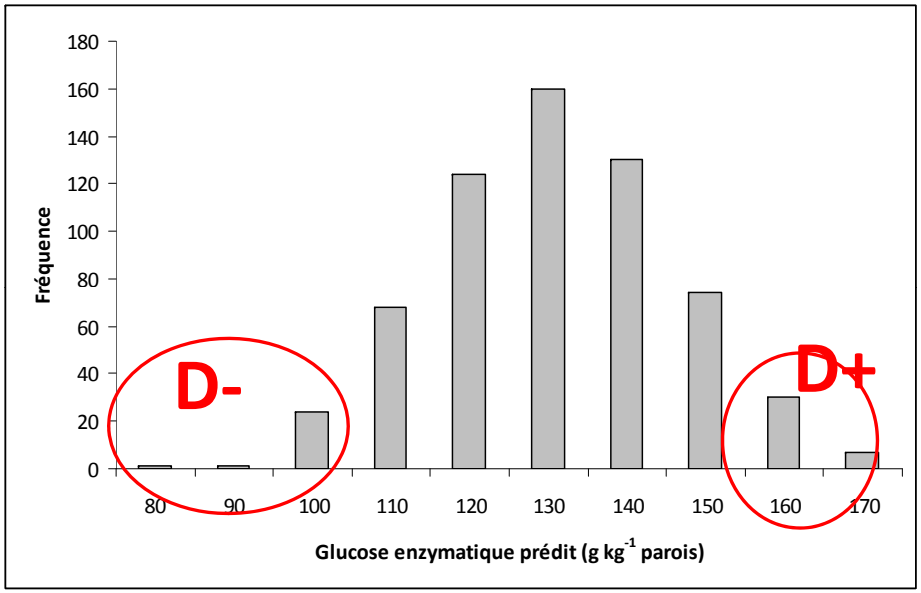
Large phenotypic variability for stem cell wall (CW) digestibility in alfalfa could be exploited to improve that trait through recurrent selection.

Little is known: - on the stability of CW digestibility in the field

- on its heritability

- on the impact of selection for stem CW digestibility on other important traits: yield, winter survival, and energy concentration

Recurrent divergent selection for stem cell wall digestibility



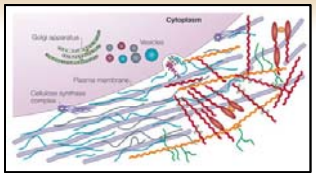
Duceppe, Bertrand *et al.* (2010)

- Selection of 20 genotypes with high (D+) and 20 with low (D-) digestibility using our screening method
- Crosses of selected genotypes
- Repeat each year
- Year 1 : D+1 and D-1; Year 2: D+2 and D-2 populations

Our screening method for stem cell wall digestibility

- Harvest stems at greenpod maturity stage
- Grind
- Purify cell walls (CW): remove starch and sugars
- Assess CW digestibility as the quantity of glucose released from cellulose after a 48h incubation with a customized enzyme cocktail (enzyme-released glucose, ERG)
- Use NIRS prediction to increase the throughput of analyses

Screening genotypes: Laboratory analyses



Stem soluble sugars and starch: HPLC

Stem CW digestibility = Enzyme-released glucose from cellulose

- CW purification
- 48h incubation at 50C with customized enzyme cocktail (Genencor and Sigma
- Glucose measured by HPLC

Near-infrared reflectance spectroscopy

- High throughput screening

Multisite Field assessment

- Ten populations: Two initial cultivars, 54V54 and Orca, and populations obtained after successive cycles of divergent selection for stem CW digestibility (D−1, D−2, D+1, and D+2)
- Three field sites: north, central and south of Québec
- Parameters measured:

  -Digestibility: enzyme-released glucose (ERG).

  -Concentration of water-soluble carbohydrates

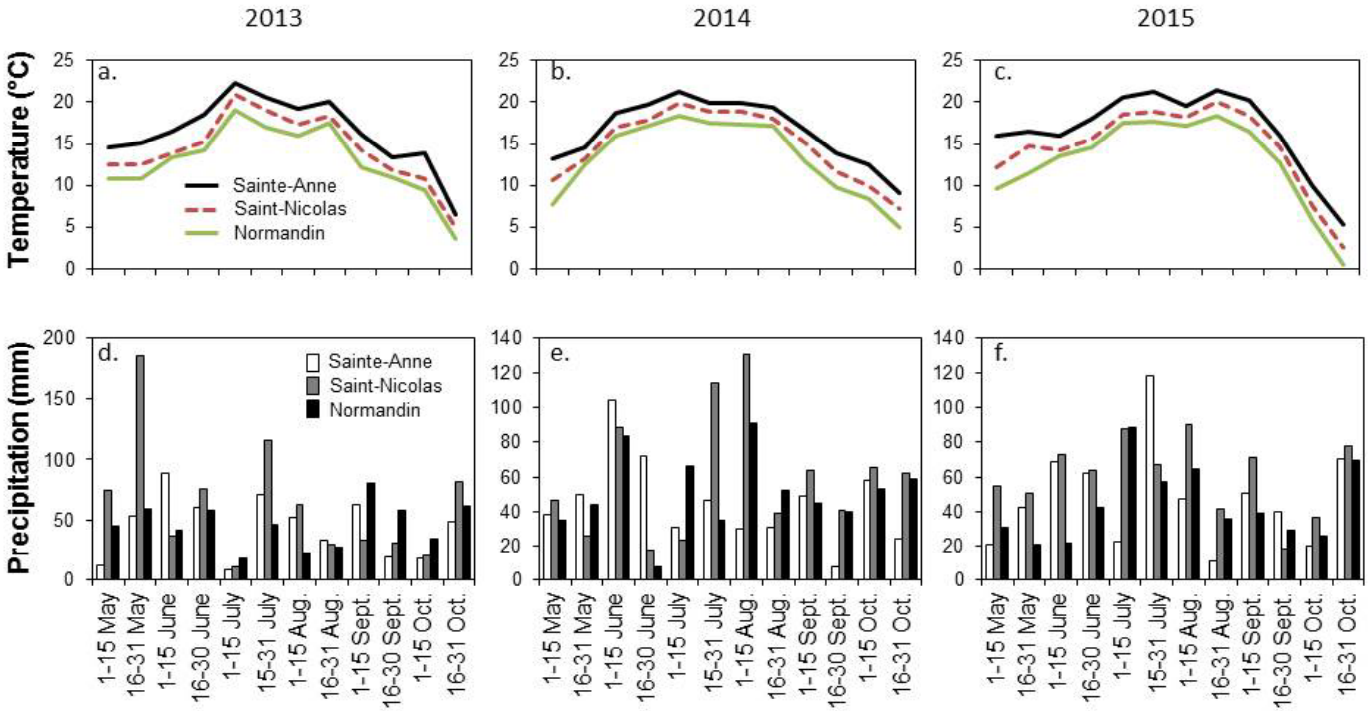
  -Yield

  -Winter survival

  -Heritability

Bertrand et al. 2018. Crop Science

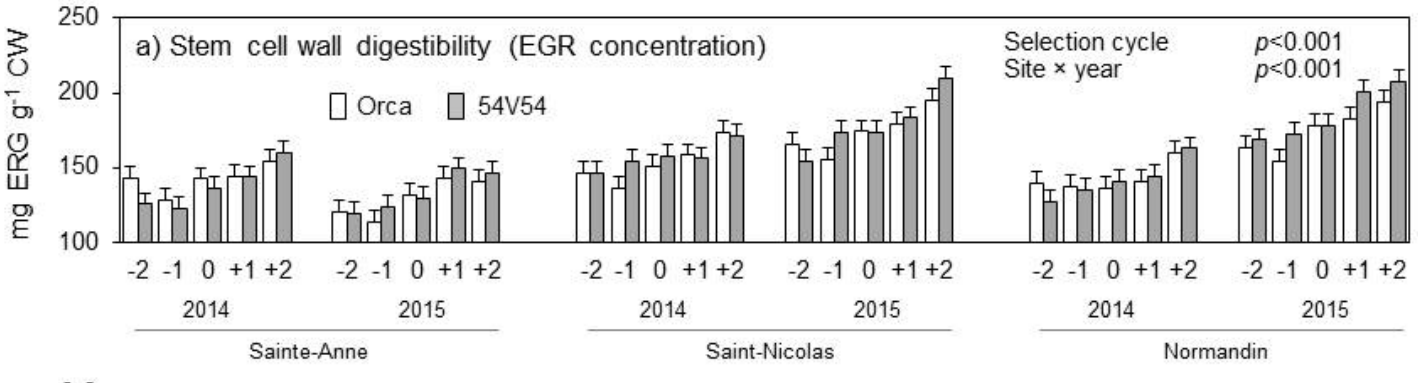
Environmental conditions at field sites



- Air temperature, precipitation, soil characteristics
- Contrasting pedo-climatic conditions between sites
- Temperature Normandin (1.7°C)<Saint Nicolas (4.4°C)<Ste-Anne (6.7°C)

Bertrand et al. 2018. Crop Science

Stem Cell Wall Digestibility (ERG)



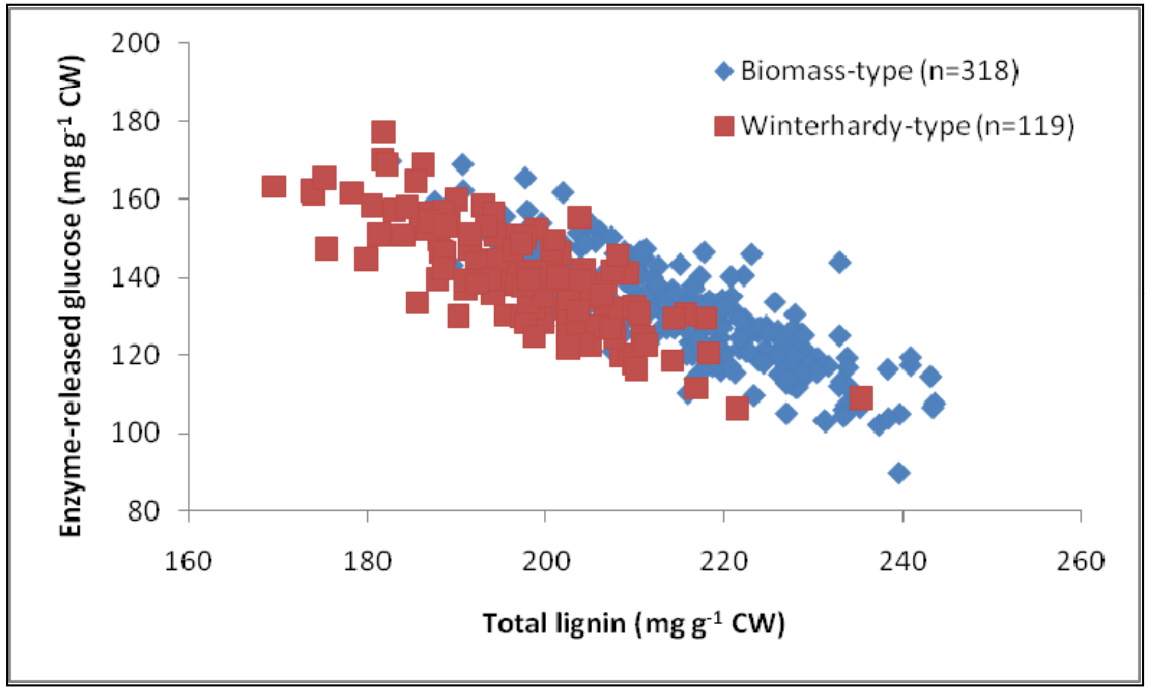
- At all sites for both years, populations obtained after two selection cycles (D+2) had significantly higher CW digestibility than initial cultivars (average of +20.7 mg ERG g^{-1} CW =13% improvement of digestibility).

- Significant positive response of stem CW digestibility to selection

- CW digestibility over years varied depending on the site where plant were grown.

…but systematically higher stem CW digestibility in populations recurrently selected (trait stability)

Relationship between ERG and IVTD, NDFD and lignin

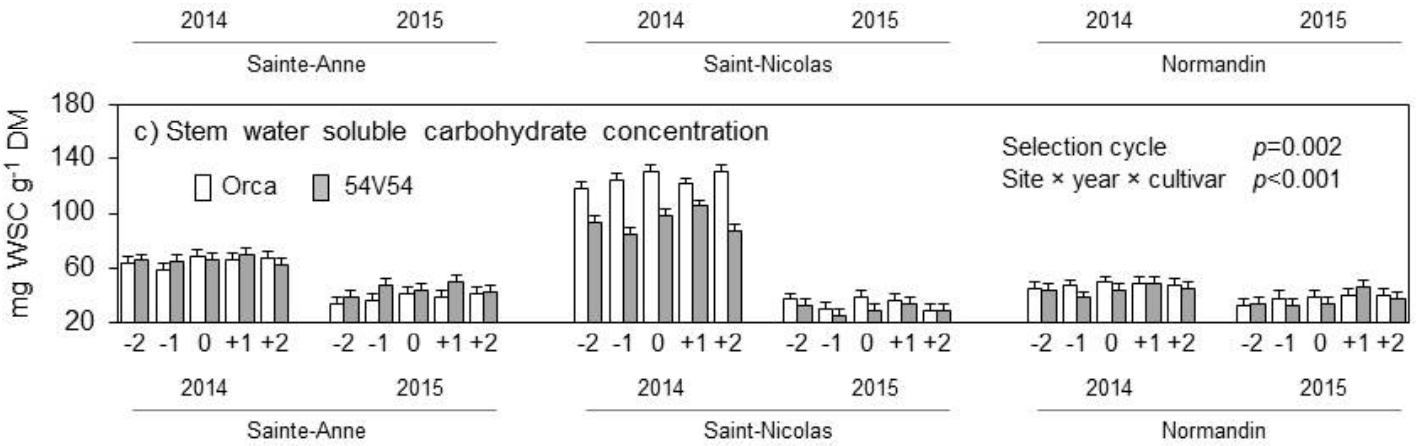


- Correlation coefficient (r) between ERG and lignin: -0.83
- Correlation coefficient (r) between ERG and IVTD: 0.72
- Correlation coefficient (r) between ERG and NDFD: 0.79

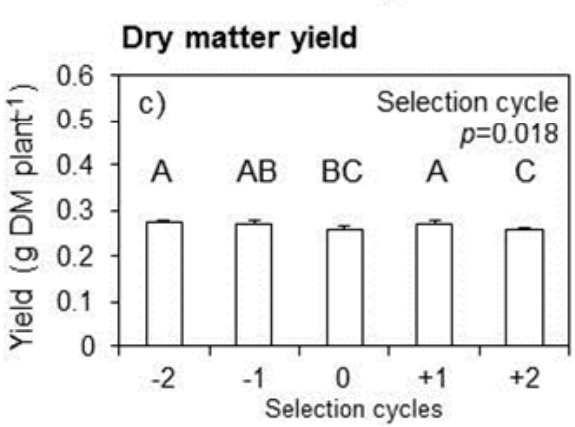
Water soluble carbohydrates

- Impact of selection for CW digestibility on WSC concentration shows that it is possible to increase digestibility without a decrease in WSC concentration

- Three way interaction between site x year x cultivar shows the large effect of environment on WSC in plants.



Dry matter yield



- Significant impact of selection on biomass yield but no correlation with selection cycles
- The D+2 populations did not differ from the initial cultivars with regard to biomass yield
- Selection targetting stems avoid the selection of genotypes with high leaf:stem ratio
- Increase in CW digestibility not achieved at the expense of DM yield

Heritability

Table 2. Variance component estimates for genotype (σ^2_P), genotype × environment (σ^2_{PL}), and error (σ^2_ε) and broad-sense heritability estimates on a plot (H^2_{Plot}) and an entry mean ($H^2_{Entry\ Mean}$) basis for two genetic backgrounds in Exp. 1

	Exp. 1	
Component	**54V54**	**Orca**
σ^2_P	74.95	43.62
σ^2_{PL}	28.16	0.00
σ^2_ε	154.72	163.17
H^2_{Plot}	0.29	0.21
$H^2_{Entry\ Mean}$	0.82	0.79

Broad-sense heritability highlights a moderate control of genetic factors over environmental factors for CW digestibility.

Conclusions

- Selection for CW digestibility is a low-throughput process that could be accelerated by our method of selection using NIRS prediction of ERG in stem cell walls

- Improvement of stem CW digestibility could increase energy available and improve ruminant performance

- The D+2 populations did not differ from the initial cultivars with regard to biomass yield, winter survival (90%), and stem water soluble-carbohydrate concentration.

Next steps

- Use of the unique genetic material generated by recurrent divergent selection for:
  - Identification of genomic regions affecting stem CW digestibility
  - Development of molecular markers to accelerate the identification of highly digestible plants

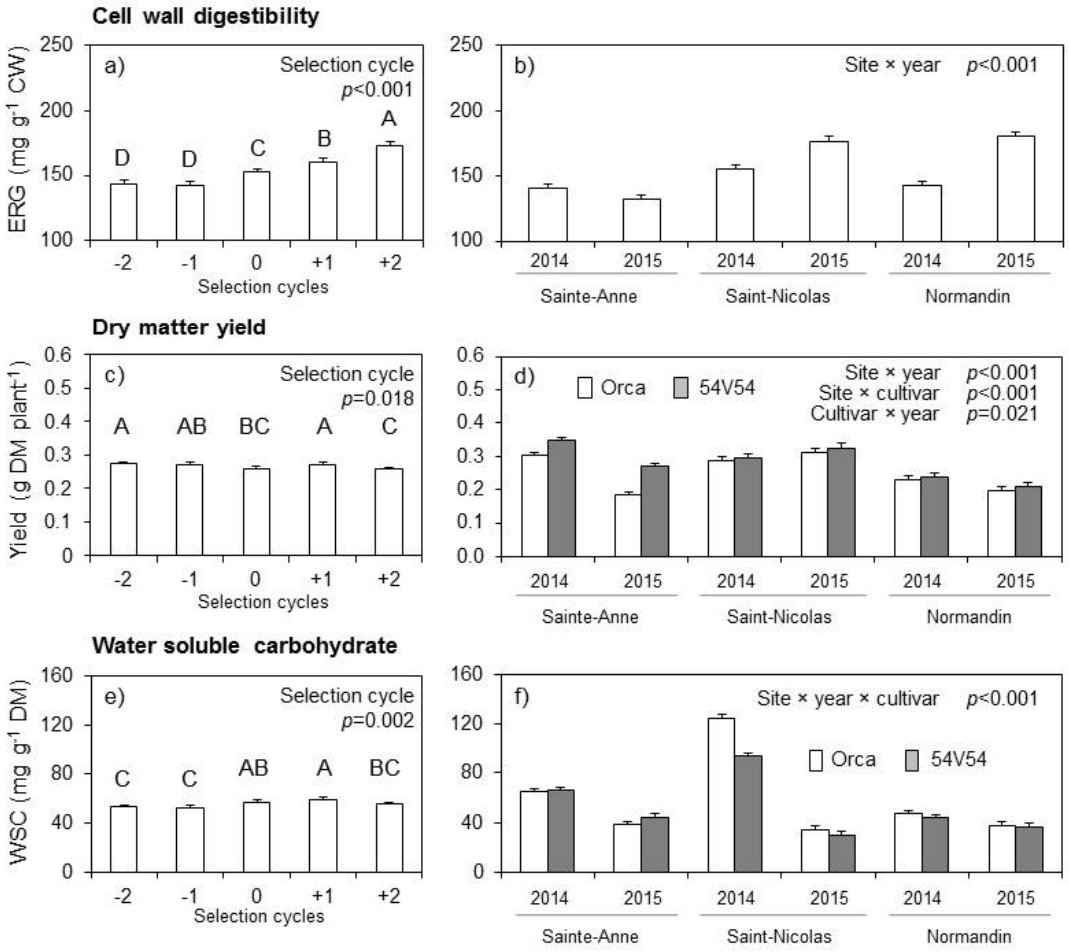
Research team

Scientists

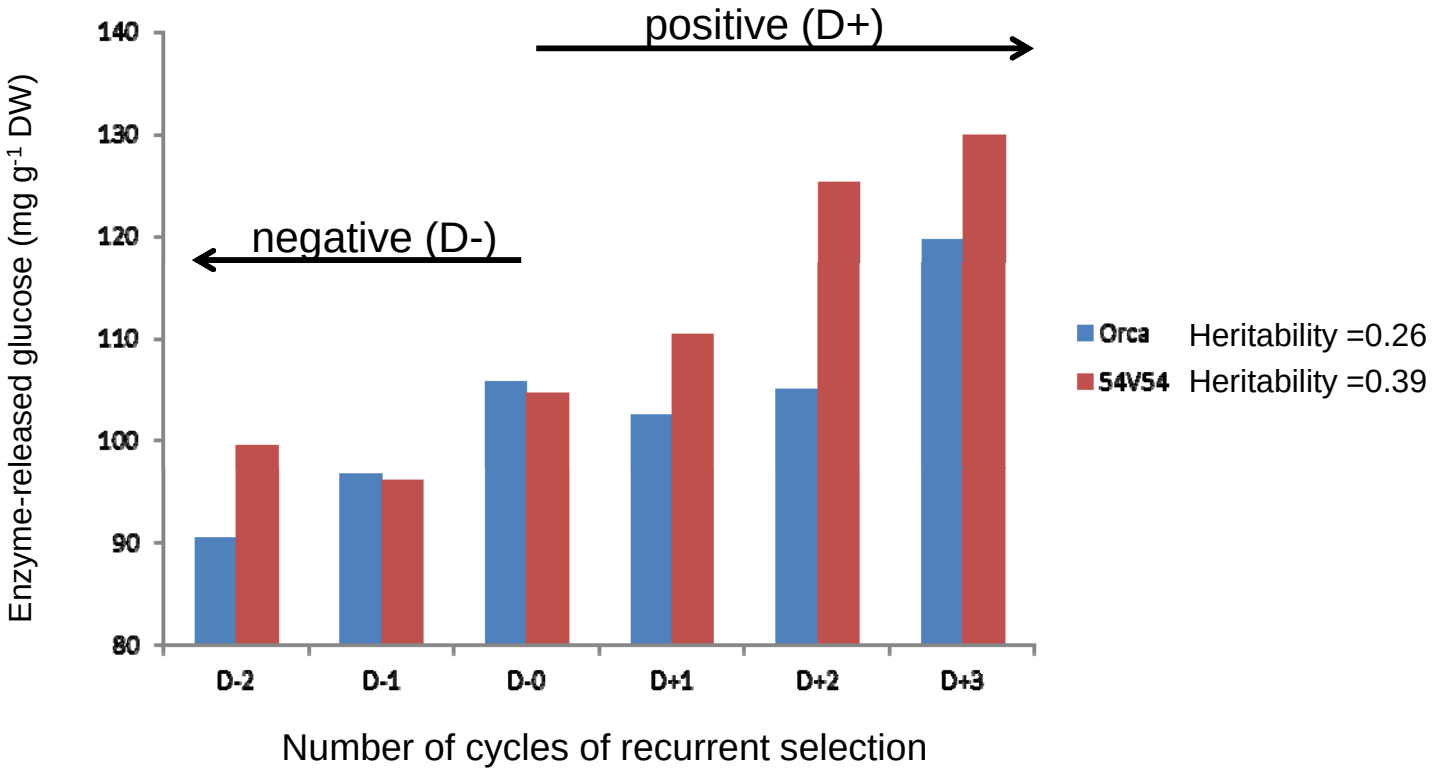
Annick Bertrand	Physiology and biochemistry
Annie Claessens	Plant breeding
Solen Rocher	Molecular genetics
Patrice Audy	Molecular biology

Research assistants

Josée Bourassa	Plant biochemistry
Sandra Delaney	Plant biochemistry
Jean Cloutier	Molecular genetics
Josée Michaud	Molecular physiology
David Gagné	Bioinformatics
Réjean Desgagnés	Molecular biology
Marie-Claude Pépin	Plant breeding
François Langevin	Germplasm development


Cell wall digestibility
a)
Selection cycle
p<0.001
ERG (mg g-1 CW)
D
D
C
B
A
Selection cycles
b)
Site × year p<0.001
2014
2015
Sainte-Anne
Saint-Nicolas
Normandin
Dry matter yield
c)
Selection cycle
p=0.018
Yield (g DM plant-1)
A
AB
BC
A
C
d)
Orca
54V54
Site × year p<0.001
Site × cultivar p<0.001
Cultivar × year p=0.021
Water soluble carbohydrate
e)
Selection cycle
p=0.002
WSC (mg g-1 DM)
C
C
AB
A
BC
f)
Site × year × cultivar p<0.001
Orca
54V54

Stem degradability is genetically inherited



- 25% increase in stem degradability
- No chemical pre-treatment
- No GMO

Next steps

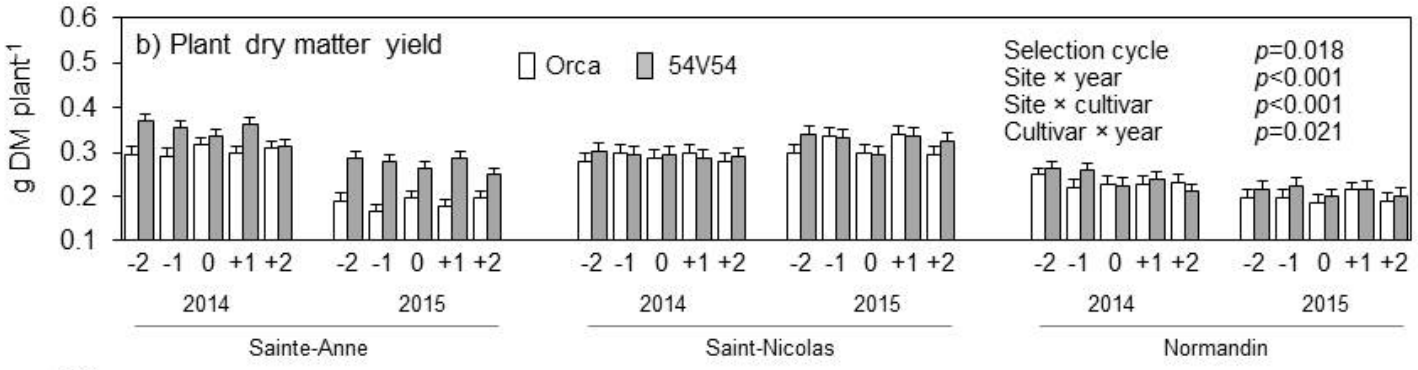
- Stem degradability assessment of progenies after 2 cycles of MAS: to confirm the link between Markers and degradability

  Impact of MAS on stem degradability

- Next generation sequencing (GBS): for genome-wide identification of regions affecting stem degradability

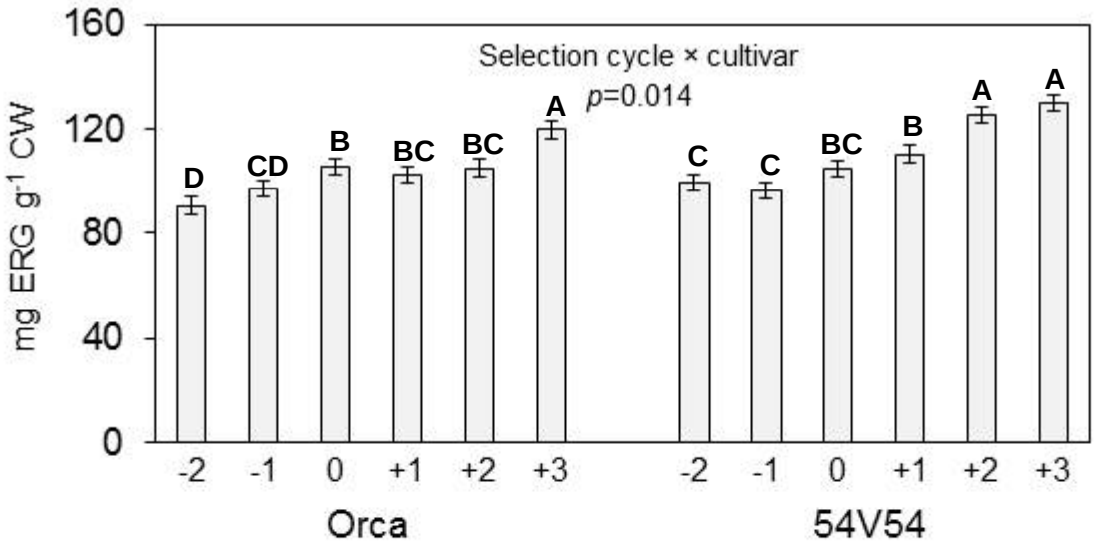
Dry matter yield

Significant impact of selection



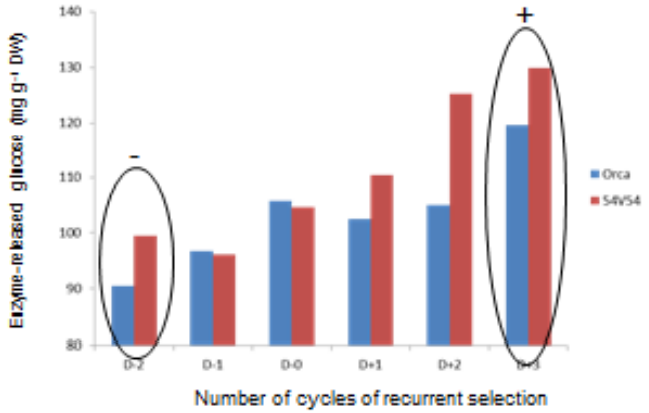
The D+2 populations did not differ from the initial cultivars with regard to biomass yield, winter survival, and stem water soluble-carbohydrate concentration.

Heritability assessment (three cycles, one site)



Next steps

Probing the genome of contrasted genetic material



Genotyping alfalfa populations seelcted for improved stem CW digestibility