Mapping of Forage Digestibility QTL in Elite Alfalfa Germplasm

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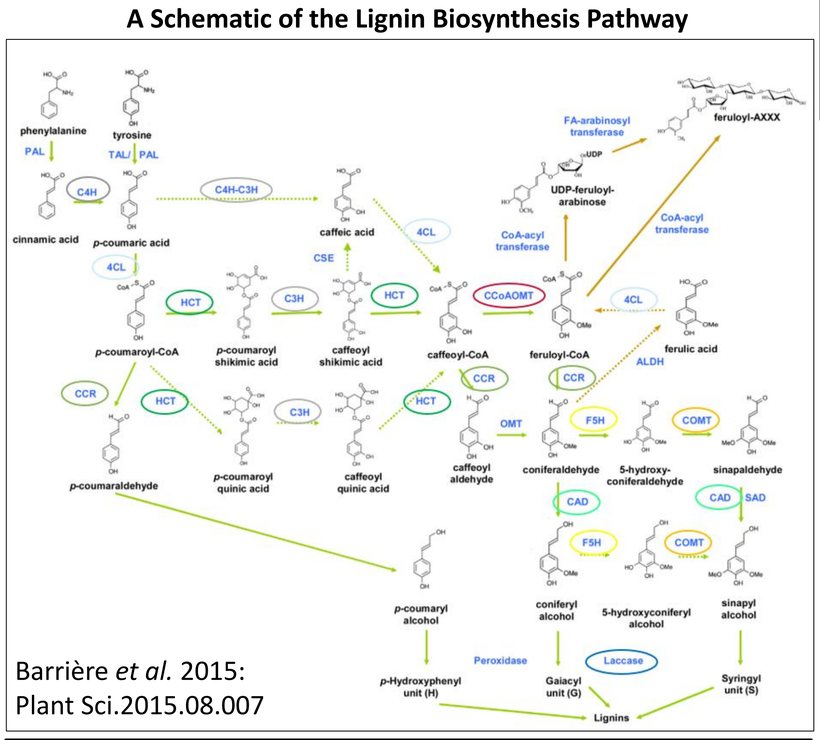
INTRODUCTION

Digestibility of alfalfa *(Medicago sativa*) for ruminant livestock is of key economic importance for meat and dairy production in many regions of the world. While the factors affecting digestibility, such as lignin content, can be well-managed by on farm practices, the genetics of the crop also plays a role. Genetic diversity for digestibility traits of multiple alfalfa varieties can be measured when such varieties are all managed on the same cutting schedule. Calculated trait indices such as Relative Forage Quality (RFQ) and Milk Per Acre (MPA) are useful as they account for both lower lignin and higher protein. Alfalfa varieties with superior genetics for digestibility can thereby be identified and shown to provide economic benefits to the farmer.

Scientists at DLF in collaboration with Corteva Agriscience have investigated genes involved in the lignin biosynthesis pathway using two separate approaches. The first approach was to investigate the well-known CCoAOMT gene by sequencing elite germplasm in search of useful mutations. The second approach was a QTL study in diverse mapping populations to discover additional regions associated with lignin. Candidate genes within such regions could be among other known genes which encode enzymes in the lignin biosynthesis pathway such as COMT, CAD, or CCR.

A Schematic of the Lignin Biosynthesis Pathway



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METHODS

Sequencing Project

CCoAOMT gene sequencing

- The CCoAOMT gene was sequenced in 90 clonally propagated alfalfa plants representing diverse germplasm pools using DNA sequencing technology available at Corteva Agriscience.
- After finding an insertion mutation of interest, specific primers were designed for a qPCR marker to be utilized in a Corteva Agriscience laboratory for tracking the mutation in breeding materials.

Characterization of CCoAOMT mutation

- Two clones that were duplex for the mutation were crossed to 20 wildtype clones to create families segregating 1D:4S:1W.
- ~100 duplex plants from the 20 families were polycrossed to create a population segregating 1Q:8T:18D:8S:1W.
- 1000 plants (~200 from each genotypic class) were established in a space planted nursery and sampled for lignin content using NIR.
- Synthetic varieties with varying allele frequencies of the mutation were also developed and entered into multi-environment forage yield trials.

Mapping Project

Development of QTL mapping populations

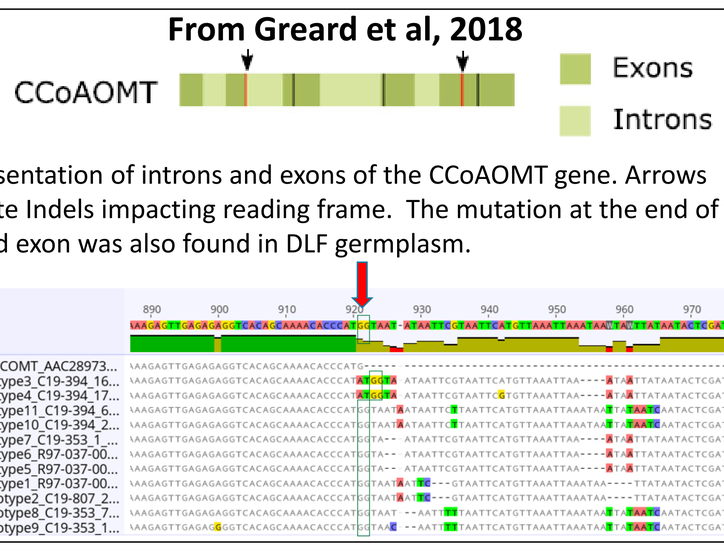
- Four clonally propagated alfalfa plants from four diverse germplasm pools were selected for digestibility extremes based on progeny testing data.
- Two biparental mapping populations were created and designated C13-0030/CE14-002 and A133087/C20-176.
- 234 plants of C13-0030/CE14-002 and 104 plants of A133087/C20-176 were established in space planted nurseries and sampled for lignin content using NIR.
- Plants of both populations were also genotyped using a custom designed Infinium array (Illumina) of ~8000 publicly available SNP's created by Corteva Agriscience.

RESULTS

Sequencing Project

2-bp insertion mutation on second exon of CCoAOMT gene

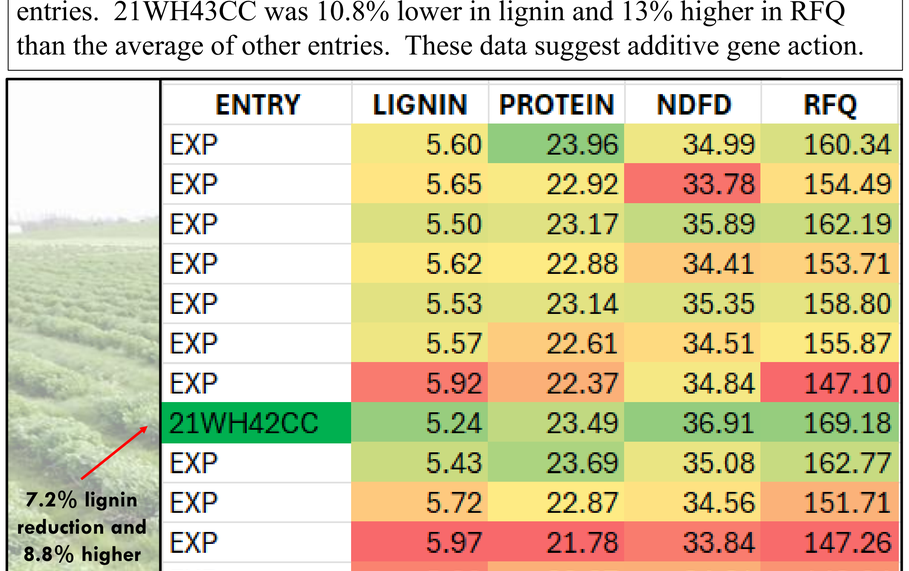
A 2-bp (AT) insertion causes a frameshift mutation and knockout allele of CCoAOMT. Genotyping of 90 plants initially and hundreds more since the inception of the project has led to the conclusion that the mutation is common in DLF elite germplasm pools, being found in ~25% of genotyped plants. Development of a qPCR marker has enabled the allele frequency of the mutation to be increased in breeding materials through marker assisted selection for several years.



Representation of introns and exons of the CCoAOMT gene. Arrows indicate Indels impacting reading frame. The mutation at the end of the second exon was also found in DLF germplasm.

Nursery plant characterization suggests recessive gene action

Nearly 1000 individual plants representing the five genotypic classes were utilized as a validation population to characterize the mutation. The plants were sampled for lignin content analysis by NIR via repeated measures in June and July. Both samplings showed only the homozygous quadraplex mutant plants were significantly lower in lignin at alpha = 0.05 (below). The result of the first sampling was a 5.2% reduction in lignin vs. the wildtype and the result from the second sampling was a 7.8% reduction in lignin vs. the wildtype. The conclusion from this dataset is recessive gene action.



Varietal testing suggests additive gene action

Marker assisted selection of plants with varying copy numbers of the mutation has been ongoing for several years. Two varieties were entered into multi-environment forage yield trials and characterized for forage quality traits along with many other experimental varieties and commercial products. The first variety was created as a synthetic of mostly simplex and duplex plants and designated 21WH42CC. The second variety was created as a synthetic of mostly duplex and triplex plants and designated 21WH43CC. Trial plots were sampled multiple times at multiple locations and analyzed using NIR. The two marker-assisted CCoAOMT varieties both outperformed the experimental varieties and products for lignin and other calculated traits such as NDFD and RFQ (below). 21WH42CC was 7.2% lower in lignin and 8.8% higher in RFQ than the average of other entries. 21WH43CC was 10.8% lower in lignin and 13% higher in RFQ than the average of other entries. These data suggest additive gene action.

ENTRY	LIGNIN	PROTEIN	NDFD	RFQ
EXP	5.60	23.96	34.99	160.34
EXP	5.65	22.92	33.78	154.49
EXP	5.50	23.17	35.89	162.19
EXP	5.62	22.88	34.41	153.71
EXP	5.53	23.14	35.35	158.80
EXP	5.57	22.61	34.51	155.87
EXP	5.92	22.37	34.84	147.10
21WH42CC	5.24	23.49	36.91	169.18
EXP	5.43	23.69	35.08	162.77
EXP	5.72	22.87	34.56	151.71
EXP	5.97	21.78	33.84	147.26
EXP	5.90	22.37	34.21	149.84
EXP	5.89	22.25	34.17	149.58
AFX 439	5.52	23.02	35.17	158.89
EXP	5.42	24.41	36.08	167.00
EXP	5.73	22.77	34.11	152.61
EXP	5.81	22.65	34.19	149.38
EXP	5.34	23.20	36.15	164.19
AFX 589	5.47	22.85	35.09	159.06
21WH43CC	5.04	23.55	37.91	175.82
EXP	5.75	22.32	34.34	152.10
WL 349HQ	5.66	22.62	34.34	154.66
EXP	5.85	21.98	33.79	147.86
EXP	5.71	22.78	34.69	153.91
EXP	5.60	22.57	35.81	156.43
HVX Megatron	5.26	22.83	37.32	159.88
EXP	5.35	23.41	35.39	164.31
EXP	5.81	23.12	34.22	155.97
EXP	5.76	22.73	33.71	151.46
EXP	5.75	23.07	33.85	153.33

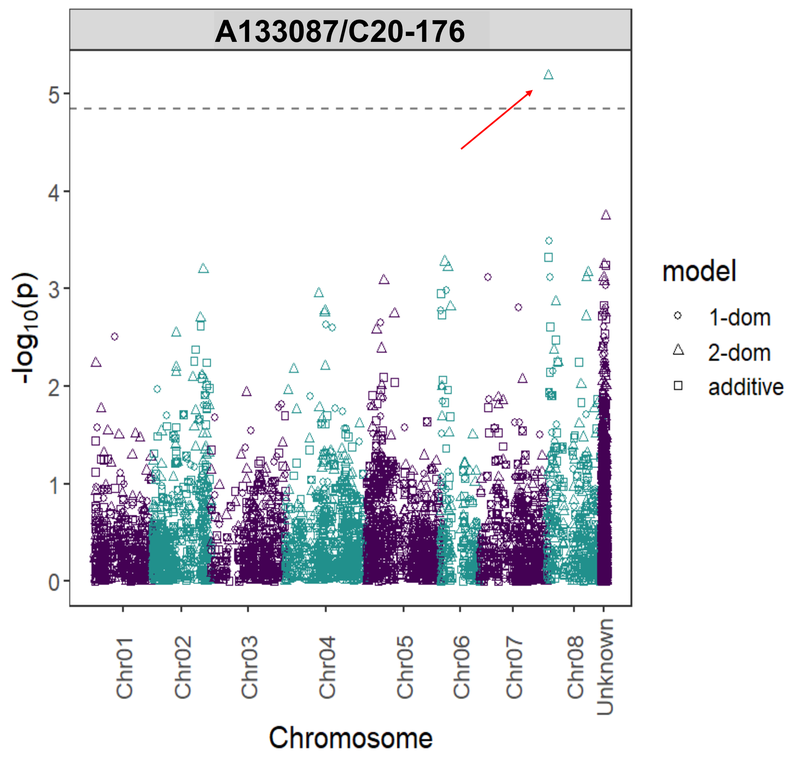
7.2% lignin reduction and 8.8% higher RFQ compared to conventional entries

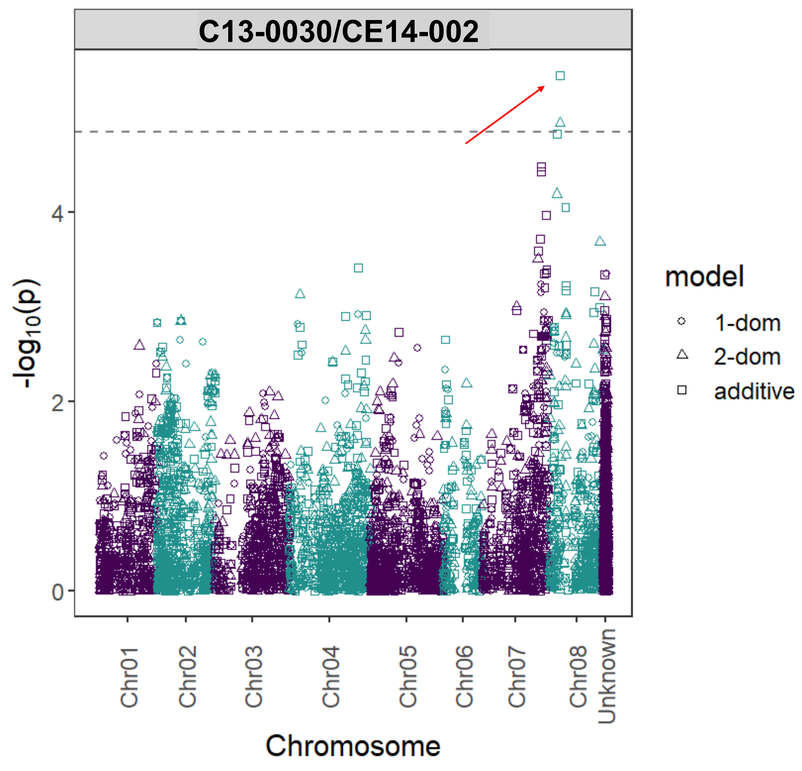
10.8% lignin reduction and 13% higher RFQ compared to conventional entries

Mapping Project

Major Lignin QTL on chromosome 8

Two biparental mapping populations with parents representing diverse germplasm pools both reveal a QTL for lignin within a 7 million bp region on chromosome 8. Because the QTL is detected among these diverse genetics, it is expected to be present in several DLF elite germplasm pools and have a robust effect across pools.





Genetic Mapping Process

The GWASpoly package in R was used to merge phenotypic and genotypic data for mapping. Key SNP markers were BLASTed on the medsa.XinJiangDaYe *Medicago sativa* genome assembly to confirm positions. A significant association with lignin content was detected on chromosome 8 at ~82 million bp in the A133087/C20-176 population. A significant association with lignin content was detected on chromosome 8 at ~71 to 75 million bp in the C13-003/CE14-002 population. It is likely that the favorable allele of a QTL in this region has been captured in DLF elite germplasm through many years of selection for improved digestibility. In the future, qPCR using molecular markers in this region can be utilized to more efficiently generate breeding materials that contain the QTL.

CONCLUSIONS

Sequencing Project

The sequencing of CCoAOMT in DLF alfalfa germplasm has revealed a useful mutation that reduces lignin. The mutation may be recessive or additive in certain genetic backgrounds. Selection for the mutation and the increase of its allele frequency in breeding populations have proven rather easy due to the common natural occurrence in ~25% of genotyped plants.

Mapping Project

The mapping of an important region contributing to lower lignin content on chromosome 8 provides insight on the source of high digestibility in DLF alfalfa germplasm. Molecular markers in this region will provide an additional tool for breeders to more efficiently select useful plants. Taken together, these innovations will lead to the next generation of Hi-Gest products available through DLF market channels.

SPECIAL THANKS

To the Corteva Agriscience High Throughput Genotyping Laboratory Johnston, IA

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