

Supplemental tables and figures

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Improved Forage Quality in Alfalfa (*Medicago sativa* L.) via Selection for Increased Stem Fiber Digestibility

Zhanyou Xu ^{*,†}, Deborah J. Heuschele [†], JoAnn F. S. Lamb, Hans-Joachim G. Jung and Deborah A. Samac

USDA, Agricultural Research Service, Plant Science Research Unit, 1991 Upper Buford Circle,
St. Paul, MN 55108, USA
* Correspondence: zhanyou.xu@usda.gov
† These authors contributed equally to this work.

Table S1. Calibration statistics for near-infrared reflectance spectroscopy (NIRS) prediction equations for in vitro neutral detergent fiber digestibility (IVNDFD), detergent fiber components, and cell wall traits of alfalfa stems.

| Component | No samples | Mean | Minimum | Maximum | SEC | R ² |
|-----------------------------------|------------|------|---------|---------|-----|----------------|
| 16-h IVNDFD (g kg ⁻¹) | 138 | 21 | 15 | 36 | 2 | 0.68 |
| 96-h IVNDFD (g kg ⁻¹) | 138 | 47 | 34 | 72 | 2 | 0.81 |
| NDF (g kg ⁻¹ DM) | 138 | 64 | 44 | 77 | 1 | 0.96 |
| ADL (g kg ⁻¹ NDF) | 138 | 10 | 4 | 15 | 9 | 0.86 |

SEC, standard error of calibration; DM, dry matter weight.

Table S2. *p*-values of the 5-way analysis of variance (ANOVA) for 16-h IVNDFD (NDFD16H), 96-h IVNDFD (NDFD96H), acid detergent lignin (ADL), neutral detergent fiber (NDF), stem dry weight (SDW), and percent stems (Per_stem) among the five variables: year (Y), location (L), germplasm (G), maturity (M), and harvest (H).

| Term | NDFD16 | NDFD96 | ADL | NDF | SDW | Per_stem |
|-----------|------------------|-----------|-----------|-----------|-----------|-----------|
| Y | 5.60E-150 | 4.50E-23 | 2.30E-25 | 9.60E-49 | 4.40E-02 | 1.20E-14 |
| M | 0.00E+00 | 2.70E-226 | 1.70E-188 | 0.00E+00 | 1.70E-120 | 1.10E-244 |
| L | 8.10E-36 | 1.00E-96 | 1.80E-214 | 1.10E-09 | 4.60E-05 | 1.80E-10 |
| H | 3.10E-145 | 2.80E-62 | 3.40E-152 | 2.00E-100 | 3.60E-219 | 0.00E+00 |
| G | 4.30E-128 | 2.20E-212 | 2.20E-227 | 2.70E-138 | 2.10E-22 | 2.80E-14 |
| H:G | 5.50E-06 | 5.50E-05 | 6.30E-01 | 8.20E-07 | 3.30E-02 | 4.50E-01 |
| L:G | 2.20E-01 | 2.00E-02 | 8.30E-04 | 2.30E-02 | 1.20E-02 | 8.10E-01 |
| M:G | 4.70E-03 | 1.10E-10 | 1.10E-03 | 2.50E-04 | 1.90E-07 | 1.20E-01 |
| Y:G | 1.50E-01 | 5.90E-03 | 5.50E-02 | 5.50E-02 | 8.60E-01 | 6.80E-01 |
| L:H:G | 5.30E-01 | 7.30E-01 | 9.00E-01 | 5.60E-01 | 9.90E-01 | 4.00E-01 |
| L:M:G | 4.20E-03 | 3.10E-03 | 7.50E-02 | 9.90E-02 | 9.50E-11 | 4.70E-02 |
| M:H:G | 4.40E-01 | 9.40E-01 | 9.90E-01 | 3.10E-01 | 9.80E-01 | 4.40E-01 |
| Y:H:G | 1.20E-01 | 8.60E-01 | 5.90E-01 | 2.90E-01 | 1.00E+00 | 9.10E-01 |
| Y:L:G | 2.00E-02 | 1.20E-04 | 3.00E-05 | 1.30E-01 | 6.60E-01 | 8.30E-01 |
| Y:M:G | 9.60E-01 | 5.80E-01 | 5.20E-02 | 7.40E-01 | 3.10E-01 | 8.10E-01 |
| L:M:H:G | 9.50E-01 | 8.90E-01 | 9.20E-01 | 8.40E-01 | 9.30E-02 | 6.30E-01 |
| Y:L:H:G | 8.70E-01 | 9.90E-01 | 9.80E-01 | 7.90E-01 | 1.00E+00 | 8.60E-01 |
| Y:L:M:G | 5.20E-01 | 2.80E-01 | 3.60E-01 | 3.00E-01 | 5.70E-01 | 1.10E-01 |
| Y:M:H:G | 6.70E-01 | 8.40E-01 | 9.90E-01 | 9.40E-01 | 9.80E-01 | 5.00E-01 |
| Y:L:M:H:G | 9.70E-01 | 1.00E+00 | 1.00E+00 | 9.70E-01 | 9.90E-01 | 9.70E-01 |
| L:H | 2.10E-34 | 5.50E-08 | 3.20E-03 | 5.60E-50 | 2.40E-16 | 3.30E-36 |
| L:M | 4.30E-11 | 1.00E-11 | 4.00E-13 | 9.20E-14 | 2.70E-02 | 1.60E-11 |
| M:H | 2.50E-162 | 2.40E-84 | 6.80E-37 | 4.10E-206 | 1.10E-16 | 9.80E-76 |
| Y:H | 2.20E-101 | 3.50E-96 | 3.30E-32 | 1.20E-30 | 2.70E-05 | 1.60E-17 |
| Y:L | 6.20E-01 | 1.50E-02 | 4.90E-12 | 7.00E-05 | 5.20E-01 | 2.50E-08 |
| Y:M | 1.60E-26 | 4.70E-02 | 1.80E-47 | 1.10E-82 | 6.60E-06 | 3.20E-05 |
| L:M:H | 4.00E-06 | 3.20E-13 | 3.20E-16 | 8.90E-08 | 1.50E-02 | 3.00E-06 |
| Y:L:H | 4.50E-02 | 1.00E-04 | 9.70E-08 | 6.70E-02 | 1.40E-08 | 2.50E-14 |
| Y:L:M | 7.90E-25 | 7.10E-11 | 3.20E-08 | 2.50E-24 | 6.10E-01 | 2.80E-22 |
| Y:M:H | 2.80E-208 | 1.20E-102 | 2.20E-43 | 1.60E-215 | 1.20E-25 | 6.30E-54 |
| Y:L:M:H | 1.20E-21 | 1.60E-05 | 5.20E-03 | 2.70E-09 | 6.00E-02 | 1.10E-14 |

Table S3. Summary of 16-h IVNDFD (NDFD16H), 96-h IVNDFD (NDFD96H), acid detergent lignin (ADL), neutral detergent fiber (NDF), stem dry weight (SDW), and percent stems (Per_stem) of alfalfa populations across years, locations, harvests, and maturities. C0, unselected population; C1, cycle 1 population; C2, cycle 2 population; H \times H, H16 \times H96, intermating of plants with high 16-h IVDFD and high 96-h IVNDFD; L \times L, L16 \times L96, intermating of plants with low 16-h IVDFD and low 96-h IVNDFD. NDF is on a dry matter (DM) basis. ADL is on an NDF basis.

| Traits | C0 | C1 H \times H | C2 H \times H | C1 L \times L | C2 L \times L |
|-------------------------------|-----|-----------------|-----------------|-----------------|-----------------|
| NDFD16H (g kg ⁻¹) | 189 | 193 | 198 | 186 | 182 |
| NDFD96H (g kg ⁻¹) | 432 | 439 | 451 | 414 | 400 |
| ADL (g kg ⁻¹ NDF) | 166 | 163 | 159 | 170 | 174 |
| NDF (g kg ⁻¹ DM) | 601 | 593 | 582 | 609 | 617 |
| SDW (g/plant) | 25 | 25 | 19 | 20 | 27 |
| Per_stems (%) | 52 | 52 | 51 | 54 | 53 |

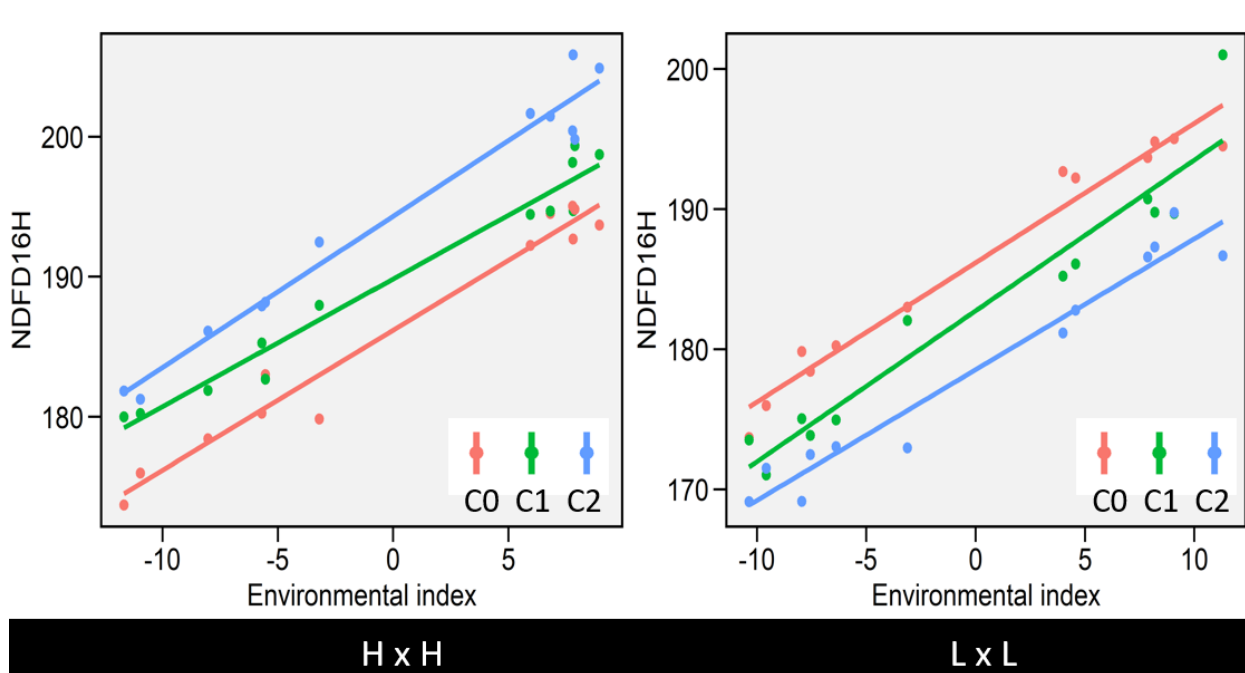


Figure S1. Environmental stability of 16-h IVNDFD with selection cycles across the 12 harvest environments. **(Left)** H \times H, intermating of plants with high 16-h IVDFD and high 96-h IVNDFD. **(Right)** L \times L, intermating of plants with low 16-h IVDFD and low 96-h IVNDFD.

Table S4. Change in ADL in the H16 × H96 populations with cycle of selection at the early bud (EB), late flowering (LF), and green pod (GP) maturity stages over three harvests. LSD is Fisher's least significant difference test with different letters within a maturity stage indicating a significant difference; %/cycle is the percent change from C0 to C1 and C1 to C2.

| Maturity | Harvest | Cycles | ADL | LSD (ADL) | %/cycle |
|-----------------|----------------|---------------|------------|------------------|----------------|
| EB | 1 | C0 | 152.02 | a | |
| EB | 1 | C1 | 150.07 | a | -1.30 |
| EB | 1 | C2 | 146.35 | a | -2.54 |
| EB | 2 | C0 | 160.85 | a | |
| EB | 2 | C1 | 156.95 | a | -2.48 |
| EB | 2 | C2 | 156.43 | a | -0.34 |
| EB | 3 | C0 | 167.96 | a | |
| EB | 3 | C1 | 166.54 | a | -0.86 |
| EB | 3 | C2 | 164.34 | a | -1.34 |
| | | | | | |
| LF | 1 | C0 | 166.25 | a | |
| LF | 1 | C1 | 160.74 | b | -3.43 |
| LF | 1 | C2 | 156.84 | b | -2.48 |
| LF | 2 | C0 | 169.08 | a | |
| LF | 2 | C1 | 162.87 | b | -3.81 |
| LF | 2 | C2 | 158.28 | b | -2.90 |
| LF | 3 | C0 | 175.12 | a | |
| LF | 3 | C1 | 171.84 | ab | -1.91 |
| LF | 3 | C2 | 167.17 | b | -2.80 |
| | | | | | |
| GP | 1 | C0 | 171.99 | a | |
| GP | 1 | C1 | 171.15 | ab | -0.49 |
| GP | 1 | C2 | 166.33 | b | -2.90 |
| GP | 2 | C0 | 171.25 | a | |
| GP | 2 | C1 | 167.22 | a | -2.41 |
| GP | 2 | C2 | 162.54 | b | -2.88 |
| GP | 3 | C0 | 176.52 | a | |
| GP | 3 | C1 | 171.27 | b | -3.06 |
| GP | 3 | C2 | 167.16 | b | -2.46 |

Table S5. Summary of stem dry weight (SDW) and herbage dry weight (HDW) changes among maturities, harvests, and selection cycles. LSD is Fisher's least significant difference (LSD) test with different letters within a maturity stage indicating a significant difference; %/cycle is the percent change from C0 to C1 and C1 to C2.

| Maturity Group | Harvest time | Cycle No | SDW mean | LSD (SDW) | %/cycle SDW | HDW mean | LSD (HDW) | %/cycle HDW |
|-----------------------|---------------------|-----------------|-----------------|------------------|--------------------|-----------------|------------------|--------------------|
| EB | 1 | C0 | 30.46 | a | | 53.11 | a | |
| EB | 1 | C1 | 28.53 | a | -6.32 | 50.49 | a | -4.93 |
| EB | 1 | C2 | 26.48 | a | -7.19 | 48.42 | a | -4.11 |
| EB | 2 | C0 | 11.50 | a | | 25.86 | a | |
| EB | 2 | C1 | 11.32 | a | -1.58 | 24.66 | a | -4.64 |
| EB | 2 | C2 | 10.44 | a | -7.79 | 22.88 | a | -7.22 |
| EB | 3 | C0 | 13.44 | a | | 28.19 | a | |
| EB | 3 | C1 | 13.00 | a | -3.26 | 27.88 | a | -1.11 |
| EB | 3 | C2 | 11.69 | a | -10.10 | 24.19 | a | -13.23 |
| LF | 1 | C0 | 54.25 | a | | 81.25 | a | |
| LF | 1 | C1 | 48.67 | a | -10.29 | 76.34 | a | -6.04 |
| LF | 1 | C2 | 40.88 | a | -16.01 | 64.94 | a | -14.94 |
| LF | 2 | C0 | 23.64 | a | | 45.08 | a | |
| LF | 2 | C1 | 21.44 | a | -9.31 | 41.00 | a | -9.04 |
| LF | 2 | C2 | 18.34 | a | -14.43 | 37.34 | a | -8.92 |
| LF | 3 | C0 | 15.69 | a | | 34.63 | a | |
| LF | 3 | C1 | 14.73 | ab | -6.08 | 33.00 | a | -4.69 |
| LF | 3 | C2 | 11.56 | b | -21.52 | 27.75 | a | -15.91 |
| GP | 1 | C0 | 65.56 | a | | 96.18 | a | |
| GP | 1 | C1 | 54.44 | a | -16.97 | 79.38 | a | -17.47 |
| GP | 1 | C2 | 36.00 | b | -33.87 | 51.83 | b | -34.70 |
| GP | 2 | C0 | 25.06 | a | | 46.13 | a | |
| GP | 2 | C1 | 24.88 | a | -0.75 | 46.13 | a | 0.00 |
| GP | 2 | C2 | 16.33 | b | -34.34 | 30.33 | b | -34.24 |
| GP | 3 | C0 | 17.50 | a | | 37.38 | a | |
| GP | 3 | C1 | 16.63 | a | -5.00 | 36.38 | a | -2.68 |
| GP | 3 | C2 | 10.00 | b | -39.85 | 21.56 | b | -40.74 |