Published online July 30, 2007

RESEARCH

A Comparative Study of Alfalfa and *Medicago truncatula* Stem Traits: Morphology, Chemical Composition, and Ruminal Digestibility

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ABSTRACT

Alfalfa (*Medicago sativa* L.) is an agronomically important forage, but digestibility of stem cell wall material is low. Because the tetraploid genome of alfalfa complicates genetic dissection of complex pathways, the diploid *M. truncatula* Gaertn. could serve as a model for stem cell wall development in alfalfa. We compared stem morphology, chemical composition (protein, soluble carbohydrates, cell wall polysaccharides, and lignin), and in vitro ruminal cell wall polysaccharide digestibility of two alfalfa clones (Regen-SY27 and 718) and four *M. truncatula* inbred lines (A17, A20, DZA315.16, and R108) in a replicated growth chamber experiment. Stem tissue development and cell wall lignification observed by light microscopy were similar between the species. While differences in stem morphology, composition, and digestibility were observed among the germplasms, there was overlap between the alfalfa and *M. truncatula* germplasms for all traits except protein concentration, which was greater for the two alfalfa clones. Younger stem internodes (top third of the stem) of both species had a higher protein concentration and greater cell wall polysaccharide digestibility, and lower cell wall concentration than older internodes (bottom third of stem). Based on the data presented here, it appears that *M. truncatula* is a suitable model for stem development, composition, and digestibility of alfalfa.

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Abbreviations: ADL, acid detergent lignin.

Alfalfa (*Medicago sativa* L.) is a valuable forage crop for ruminant livestock in all temperate regions of the world (Barnes and Sheaffer, 1995). Alfalfa is generally regarded as one of the best forage crops for feeding ruminant animals because of its perceived high nutritive value. Alfalfa leaves are protein-rich and low in cell wall concentration and, therefore, highly digestible (Jung et al., 1997). In contrast to leaves, stems exhibit low digestibility as a result of high concentrations of cell wall polysaccharides and lignin (Buxton and Russell, 1988). A major objective of many forage breeding programs is the improvement of digestibility (Buxton and Redfearn, 1997). Poor stem digestibility results in major losses in potential livestock feeding value, particularly because the stem portion represents 50 to 70% of crop biomass (Mowat et al., 1965). Even minor improvements in alfalfa stem digestibility would have great economic impact on agriculture (Jung and Allen, 1995).

Published in Crop Sci. 47:1672–1680 (2007).
doi: 10.2135/cropsci2006.12.0762

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Many potential targets for increasing the digestibility of alfalfa stems through genetic engineering exist. One strategy is to increase the amount of readily digestible cell wall polysaccharides (Hatfield, 1992). Because pectin is able to undergo rapid and extensive digestion, increasing cell wall pectin content would enhance stem digestibility (Chesson and Monro, 1982). Recent efforts to increase pectin content in alfalfa by vascular tissue–specific expression of a key enzyme in the synthesis of pectic polysaccharide component sugars indicated that manipulation of multiple steps in carbohydrate metabolism may be necessary to alter the pectin content of alfalfa (Samac et al., 2004). A second strategy for improving stem digestibility is to alter the quantity and/or composition of lignin (Baucher et al., 1999; Guo et al., 2001). Lignification of cell walls is correlated with a concomitant decrease in cell wall digestibility and transgenic efforts are underway to decrease the extent of lignin deposition (Jung and Deetz, 1993; Boerjan et al., 2003).

Unfortunately, genetic studies of agronomically important traits in alfalfa, such as digestibility, are complicated by autotetraploidy, large genome size, and allogamy (Thoquet et al., 2002). Studying these complex pathways of cell wall metabolism would be simplified in a diploid organism with valuable genetic and genomic resources on hand. For this reason, a close relative of alfalfa, *M. truncatula* Gaertn. has emerged as a model species for the legume family (Barker et al., 1990; Cook, 1999). Features of *M. truncatula* that make it an excellent model legume include its small, diploid genome, self-fertilization, prolific seed production, and ability to be genetically transformed and the growing availability of genomics resources (Cook, 1999; VandenBosch and Stacey, 2003; Cannon et al., 2005; Young et al., 2005). *Medicago truncatula* is a particularly good model for alfalfa because a high degree of conservation in gene content and order has been documented (Julier et al., 2003; Choi et al., 2004).

The genetic relatedness of alfalfa to *M. truncatula* would seemingly make the latter an excellent model for stem cell wall modification. However, the growth habit of these two species is markedly different. Alfalfa is a perennial with erect stems, while *M. truncatula* is an annual with decumbent stems. Although numerous publications characterize stem cell wall development and digestibility of alfalfa (Nordkvist and Aman, 1986; Engels and Jung, 1998; Jung and Engels, 2002), no such studies have been done in *M. truncatula*. Our objective was to determine if *M. truncatula* stems are sufficiently similar in tissue morphology and cell wall characteristics to alfalfa such that *M. truncatula* would serve as an appropriate genetic model for manipulation of stem traits.

MATERIALS AND METHODS

Plant Material

Two alfalfa clones and four *M. truncatula* inbred lines were chosen for use in this study. The alfalfa clones Regen-SY27 and 718 were chosen for their enhanced regeneration and transformability, and previous use in cell wall development and digestibility studies, respectively (Jung and Engels, 2002; Samac and Austin-Phillips, 2006). The *M. truncatula* inbred line R108 was included for its superior in vitro regeneration and transformation properties (Hoffmann et al., 1997; Trinh et al., 1998). Genome sequencing and extensive expressed sequence tag projects are underway in *M. truncatula* cultivar Jemalong genotype A17 (hereafter referred to as A17), while inbreds DZA315.16 and A20 are parents used in genetic mapping populations (Frugoli and Harris, 2001; Thoquet et al., 2002).

Medicago truncatula seed was scarified with sandpaper and imbibed on moistened filter paper at 30°C overnight. Upon germination, seedlings were placed in small cells (6 by 4 by 5.5 cm) filled with MetroMix200 planting medium (The Scotts Company, Marysville, OH). After 3 wk, plugs were transferred to large pots of MetroMix200 (15 by 15 by 17 cm) and fertilized with OsmoCote controlled release spheres (14–20–14 plus micronutrients, The Scotts Company). Ten pots, each containing two *M. truncatula* plants, were used in each replicate growth period. Alfalfa clones were grown from stem cuttings dipped in Bontone Rooting Powder (Bonide Products, Inc., Oriskany, NY) and placed in vermiculite for 2 wk. Rooted cuttings were placed in pots (15 by 15 by 17 cm) of MetroMix200 and fertilized with OsmoCote. For each growth period replicate, four pots (two plants each) were used for each alfalfa clone. More *M. truncatula* plants were required to provide sufficient plant material for the analyses.

The experiment was conducted in a growth chamber with three replicate growth periods. Pots were randomly arranged in the growth chamber and maintained at 23°C during the day and 19°C at night under a 14:10 h (light/dark) photoperiod with a light intensity of approximately 350 μmol m^{-2} s^{-1}. Plants were watered as needed. At the beginning of each replicate growth period, the *M. truncatula* plants (42–44 d after planting) were trimmed to remove any stems that extended past the edge of the pot. At the same time, alfalfa plants were cut back to a 6- to 10-cm stubble height. Plants were allowed to regrow for 30 to 35 d before harvesting. At harvest, stems were collected from both species leaving a 6- to 10-cm stubble height. One representative stem, visually appraised to be of approximately average length, from three individual pots of each germplasm was removed for physical measurements (stem length, number of total and elongating internodes, and mean internode length) and then placed in 50% ethanol for subsequent microscopic analysis. Remaining stems were harvested and each stem was divided into approximate thirds (top, middle, bottom) to provide three maturity stages for separate analysis. Leaves, petioles, flowers, seedpods, and axillary branches were removed from stems. Stem segments were dried at 60°C and ground in a ballmill (Spex CertiPrep 8000, Metuchen, NJ). This grinding treatment results in particle size reduction such that 98% of the ground stem material passes through a 106-μm screen (Jung et al., 2000). Ground stem segments were combined for each genotype by replicate growth periods and mixed before subsampling.

Microscopic and Chemical Analysis

Stems preserved in 50% ethanol were used for microscopic analysis. Seven to 10 cross-sections (100-μm thick) were made from the middle of each stem internode, from two stems per

germplasm in each replicate growth period. These cross-sections were examined by light microscopy and degree of development was assessed for individual tissues (Jung and Engels, 2002). Additional cross-sections were stained with phloroglucinol to visualize patterns of lignification (Jensen 1962).

Nitrogen was determined by the dry combustion method (1350°C) using a LECO CN-2000 analyzer (LECO Corporation, St. Joseph, MI). Crude protein content was calculated as N × 6.25. Soluble carbohydrates, cell wall polysaccharides, and lignin were determined using a sequential procedure (Dien et al., 2006). Soluble carbohydrates were extracted with 80% (v/v) ethanol at 60°C overnight. The alcohol-insoluble residue was treated with heat-stable α-amylase and amyloglucosidase in 0.1 *M* acetate buffer, pH 5, to release glucose from starch (Theander et al., 1995). Sufficient 95% (v/v) ethanol was added to reach an alcohol concentration of 80%, after which the supernatant was removed for analysis of glucose released from starch. The 80% alcohol extracts were analyzed by high performance liquid chromatography for monosaccharides (glucose and fructose) and oligosaccharides (sucrose, stachyose, and raffinose). Total soluble carbohydrates were calculated by summation of these components.

Stem cell wall concentration and composition were determined using the Uppsala dietary fiber method (Theander et al., 1995). After removal of simple sugars and starch, the cell wall residues were hydrolyzed with sulfuric acid in a two-stage procedure. Klason lignin was measured gravimetrically as the ash-free, nonhydrolyzed residue and the neutral sugar components of the cell wall polysaccharides were determined as alditol-acetate derivatives by gas chromatography. Uronic acid polysaccharide components were measured colorimetrically in an aliquot from the first step of the acid-hydrolysis procedure using galacturonic acid as the calibration standard (Ahmed and Labavitch, 1977). Total cell wall concentration was calculated as the sum of Klason lignin, glucose, xylose, arabinose, galactose, mannose, rhamnose, fucose, and uronic acid residues. On the basis of the known general composition of alfalfa polysaccharides (Hatfield, 1991), cellulose concentration was estimated as the glucose residue content; hemicellulose as the sum of xylose, mannose, and fucose residues; and pectin as the sum of uronics, arabinose, galactose, and rhamnose residues. All data were corrected to an organic matter basis by determining 100°C dry matter content overnight and subsequent ashing at 450°C for 6 h.

Cell Wall Polysaccharide Digestibility

In vitro cell wall polysaccharide digestibility of stems by rumen microbes was determined as described by Jung et al. (2000). The donor animal was a rumen-fistulated, lactating Holstein cow fed a total mixed diet containing alfalfa hay, maize (*Zea mays* L.) silage, and a concentrate supplement. Rumen fluid was collected 12 h after feeding. Thirty milliliters of a 20% (v/v) rumen fluid–McDougall's buffer (McDougall, 1948) mixture was added to 50-mL screw-cap centrifuge tubes containing 200 mg of dried stem sample. No supplemental N source was added. During a 48-h incubation at 39°C, samples were periodically mixed. At the end of the incubation period, the contents of the tubes were frozen and subsequently lyophilized. The fermentation residues were analyzed for cell wall components using the Uppsala dietary fiber method. To correct for cell wall material contributed by the rumen fluid, empty centrifuge tubes were inoculated, incubated, and analyzed in parallel. Digestibility of total cell wall polysaccharides was calculated.

Statistical Analysis

All chemical and digestibility analyses were done in duplicate and the results averaged. The stem morphology results were subjected to an analysis of variance as a randomized complete block design with three replications and germplasm source as the treatment effect. The chemical composition and in vitro cell wall polysaccharide digestibility data were analyzed as a randomized complete block design with three replications and a split plot arrangement of treatments. Germplasm was the main plot treatment. Stem segment maturity (top, middle, and bottom thirds) and the germplasm × maturity interaction were subunits in the split-plot design. For model parameters with a significant *F*-test ($P < 0.05$), means were compared using the least significant difference test (Steel and Torrie, 1960). All statistical analyses were done using PC-SAS (SAS Institute, 1985).

RESULTS

Stem Morphology

Throughout plant growth and development, the most notable difference between alfalfa and *M. truncatula* was plant growth habit. Alfalfa had primarily erect stems whereas *M. truncatula* had decumbent stems. The *M. truncatula* inbred lines A20 and R108 had the earliest flowering and pod development. In all three replicate growing periods, flowers and green seedpods were present on the A20 and R108 plants while only flowers were present on some of the Regen-SY27 and A17 plants in the first replicate growing period. Stems of both species showed alternating leaf placement and leaves were trifoliate.

At the time of harvest, stem lengths among the six germplasms varied considerably. As shown in Table 1, *M. truncatula* inbred lines had both the shortest (DZA315.16) and longest (R108) stem lengths. Number of internodes did not differ among the six germplasms. Mean internode length varied among germplasms, with DZA315.16 having shorter internodes than all other germplasms (Table 1). As alfalfa and *M. truncatula* stem internodes matured, the internodes became rigid and more difficult to bend compared with young internodes. We identified the internode at which the stem transitioned from flexible, elongating internodes to rigid, postelongation internodes where cambial activity resulted in addition of xylem tissues. Number of elongating internodes, as indicated by the position of the internode which was in transition between these developmental stages, did not differ among germplasms (Table 1). The number of internodes in the top, middle, and bottom thirds of stems did not differ among the six germplasms; however, the three stem segments did differ for number of internodes, (5.5 ± 0.1, 3.0 ± 0.1, and 3.6 ± 0.1 internodes in the top, middle, and bottom segments, respectively).

Table 1. Stem length, number of internodes, mean internode length, and position of transition internode (shift from elongation to postelongation development) in alfalfa and *Medicago truncatula* germplasms.

Germplasm†	Stem length	Internodes	Internode length	Transition internode‡
	cm		cm	
Regen-SY27	32.6bc§	10.3	3.1ab	3.7
718	36.8b	11.9	2.9b	4.4
A17	35.4b	11.4	3.0ab	3.3
A20	34.9b	12.1	2.8b	3.7
DZA315.16	24.8c	11.5	1.9c	3.3
R108	45.9a	12.6	3.5a	3.2
SEM	2.5	0.6	0.2	0.4

†Regen-SY27 and 718, alfalfa; A17, A20, DZA315.16, and R108, *M. truncatula*.

‡Internodes were counted from the apex of the stem.

§Means in the same column not sharing a common letter differ ($P < 0.05$).

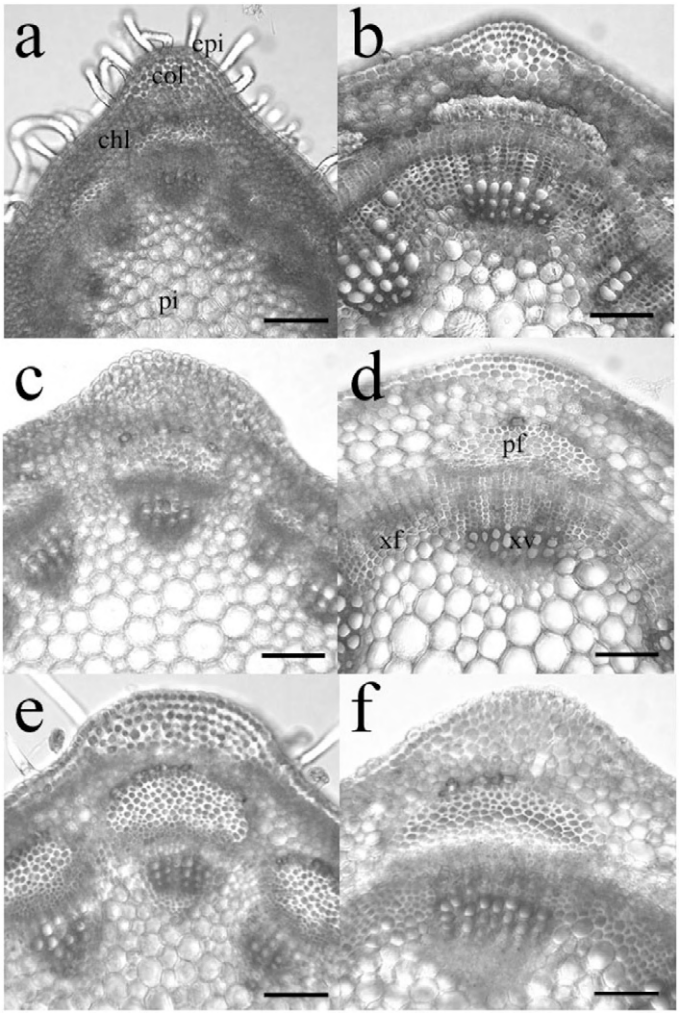

Figure 1. Morphology of 100-µm cross-sections taken from immature, elongating (a, c, and e) and mature, post-elongation (b, d, and f) stem internodes of alfalfa and *Medicago truncatula.* Thin sections were viewed by light microscopy. Panels a and b are from alfalfa clone 718, panels c and d are from *M. truncatula* inbred line A17, and panels e and f are from *M. truncatula* inbred line DZA315.16. epi, epidermis; col, collenchyma; chl, chlorenchyma; pf, phloem fibers; pi, pith; xf, xylem fibers; xv, xylem vessels. Bars represent 100 µm.

Cross-sections of alfalfa and *M. truncatula* stems showed similar patterns of stem tissue development. Stems of all germplasms had a squarish cross-sectional profile due to the presence of four collenchyma tissue bundles on the stem periphery. Figure 1 shows representative stem cross-sections from one alfalfa clone (718) and two *M. truncatula* inbred lines (A17, DZA315.16) at two stages of development (elongating and postelongation internodes). In young, elongating internodes, the cell walls of chlorenchyma, pith parenchyma, and epidermis were thin (Fig. 1a, 1c, 1e). In more mature internodes, epidermal cell walls had thickened, but the cell walls of the chlorenchyma and pith parenchyma remained thin (Fig. 1b, 1d, 1f). The primary cell walls in collenchyma thickened rapidly, as it was difficult to find internodes large enough to section which had thin-walled collenchyma (Fig. 1a, 1c, 1e). None of the previously mentioned tissues lignified during development as indicated by their lack of staining with phloroglucinol (data not shown). Cambial activity postelongation resulted in the addition of xylem fiber and vessel tissues. Phloem fiber and xylem tissues underwent extensive secondary wall formation and thickening during postelongation development. These tissues stained positive for the presence of lignin, and the intensity of staining was greatest for the xylem tissues (data not shown). Our visual appraisal of the two species suggested that the two alfalfa clones had more extensively developed xylem tissues postelongation and that the four *M. truncatula* inbred lines had larger phloem fiber bundles. However, as discussed later, these apparent differences in stem tissues were not reflected by consistent species differences in cell wall content.

Chemical Composition

Significant differences were observed for all stem chemical composition traits among the six germplasms, across stem segment maturities (Table 2), and among the three stem segment maturities, across germplasms (Fig. 2). In addition, germplasm × stem segment maturity interactions were found for soluble carbohydrate and cell wall concentrations, and also for hemicellulose and pectin concentrations in the cell wall. In the case of cell wall concentration and pectin concentration in the cell wall, the germplasm × stem segment maturity interaction was due to small changes in statistical rank among some germplasms for one or more stem segment maturities, but for all germplasms cell wall concentration increased and cell wall pectin content decreased with increasing segment maturity. Because the shifts in magnitude of differences among germplasms were small for these traits, and did not alter our conclusions, the mean values across stem segment maturity stages are shown in Table 2 for crude protein and cell wall concentration, and cell wall composition (Klason lignin, cellulose, and pectin) of the six germplasms.

The chemical component concentrations overlapped for one or both of the alfalfa clones with one or more of the *M. truncatula* inbred lines. Crude protein was the only exception to this pattern, where both alfalfa clones had greater crude protein concentrations than all four *M. truncatula* inbred lines; however, the two alfalfa clones also differed from one another. The highest and lowest cell wall concentrations among genotypes of both species were observed for *M. truncatula* inbred lines (R108 and DZA315.16, respectively). Three of the *M. truncatula* lines (A17, A20, and R108) had higher cellulose concentrations in the cell wall than both alfalfa clones and pectin concentration was also greater in three *M. truncatula* lines (A17, A20, and DZA315.16) than the alfalfa clones. In contrast, the Regen-SY27 alfalfa clone had a higher Klason lignin concentration in the cell wall than all four *M. truncatula* lines and the 718 alfalfa clone was higher in Klason lignin than all *M. truncatula* lines other than R108.

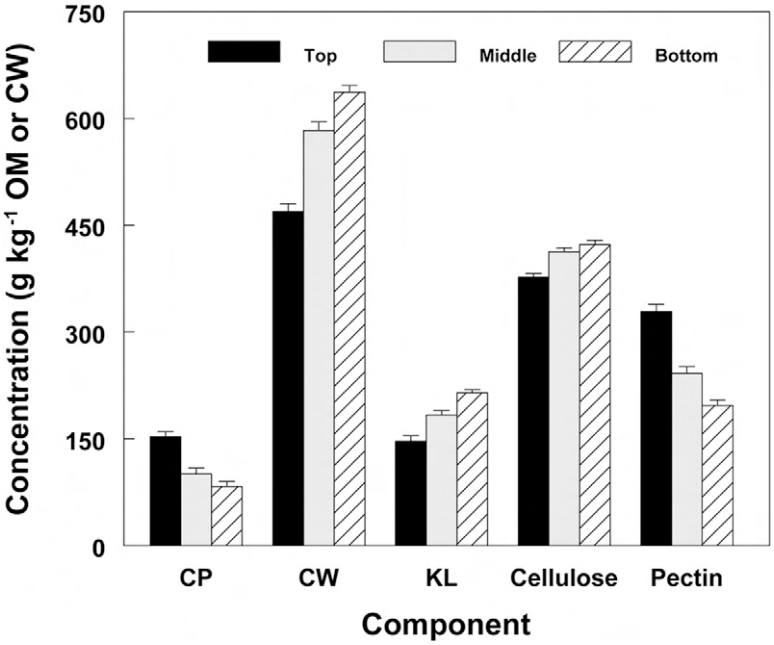

Figure 2. Composition of the top, middle, and bottom thirds of stems averaged (±1 SE) across two alfalfa clones and four *Medicago truncatula* inbred lines. Crude protein (CP) and cell wall (CW) concentrations are expressed as a proportion of organic matter (OM). Klason lignin (KL), cellulose, and pectin concentrations are shown as a proportion of total cell wall. Differences among all stem segment maturities were significant ($P < 0.05$) for all components.

The impact of stem segment maturity on chemical composition was very apparent for most traits. Crude protein concentration and cell wall pectin concentration declined from the youngest to the oldest stem segments, whereas cell wall concentration and Klason lignin and cellulose concentrations of the cell wall increased with stem segment maturity (Fig. 2). These patterns among stem segment maturities did not differ among the six germplasms.

Concentrations of soluble carbohydrates and cell wall hemicellulose exhibited more complex patterns, which accounted for their significant germplasm × stem segment maturity interactions. Four of the germplasms (718, A20, DZA315.16, and R108) did not differ in soluble carbohydrate concentration among the stem segment maturities (Fig. 3). The *M. truncatula* inbred line A17 had a greater soluble carbohydrate concentration in the top stem segment than for the bottom segment, with the middle segment not being significantly different than either of the other two stem segments. In contrast, soluble carbohydrate concentrations in alfalfa clone Regen-SY27 declined significantly between each of the stem segment maturities. Regen-SY27 also had a greater soluble carbohydrate concentration in the youngest stem segment than all other germplasms, but this difference was not consistently observed for the other stem segment maturities. The germplasm × stem segment maturity interaction for cell wall hemicellulose concentration was due to *M. truncatula* inbred line R108 not differing among stem segments whereas all other germplasms had less hemicellulose in cell walls from the top stem segment (data not shown). Hemicellulose concentration of alfalfa clone 718 increased significantly as maturity increased among the three stem segments whereas the middle and bottom stem segments of the other germplasms did not differ for hemicellulose concentration. While soluble carbohydrate and cell wall hemicellulose concentrations were more variable than other stem components, alfalfa and *M. truncatula* did not differ consistently on a species-specific basis.

In Vitro Ruminal Cell Wall Digestibility

To determine if *M. truncatula* differed from alfalfa for digestibility of stem cell wall polysaccharides, bovine rumen fluid was collected and incubated with ground stem segment samples. Statistical differences were found among germplasms, among stem segment maturities, and

Table 2. Chemical composition of stem material from alfalfa and *Medicago truncatula* germplasms.

Genotype†	Crude protein	Cell wall	Klason lignin	Cellulose	Hemicellulose	Pectin
	g kg^{-1} organic matter		g kg^{-1} cell wall			
Regen-SY27	154a‡	564b	217a	379b	179a	225d
718	140b	560b	204ab	386b	165b	245c
A17	107c	548b	152d	421a	154c	274b
A20	84d	573b	172cd	419a	147d	262b
DZA315.16	115c	507c	159d	387b	141d	314a
R108	75d	628a	190bc	428a	167b	215d
SEM	6	11	7	5	2	5

†Regen-SY27 and 718, alfalfa; A17, A20, DZA315.16, and R108, *M. truncatula*.

‡Means in the same column not sharing a common letter differ ($P < 0.05$).

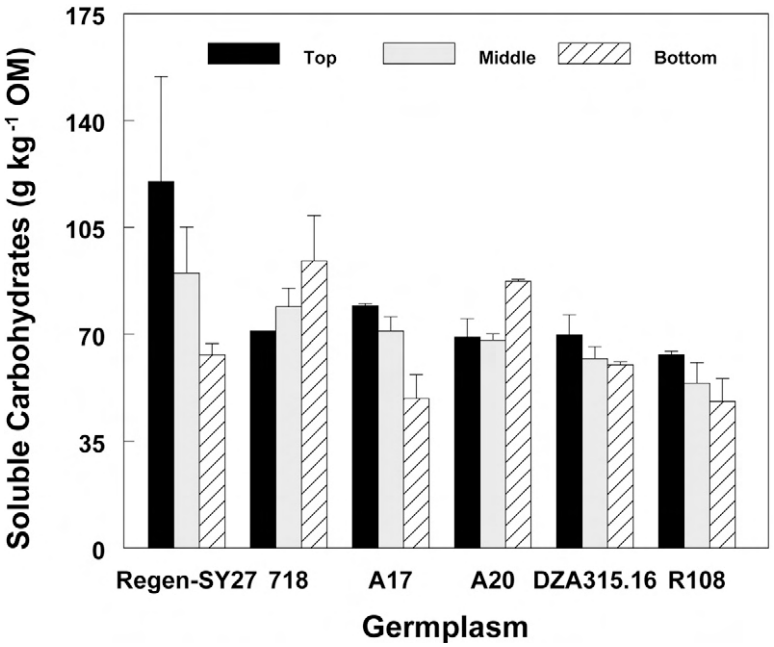

Figure 3. Soluble carbohydrate concentrations (mean ± 1 SE) of top, middle, and bottom thirds of stems from alfalfa (Regen-SY27 and 718) and *Medicago truncatula* (A17, A20, DZA315.16, and R108) germplasms.

for the germplasm × stem segment maturity interaction. As expected, the youngest stem segment in all germplasms was the most digestible, followed by a significant decline in digestibility of the middle segment, and the least digestible maturity stage was always the bottom stem segment. As seen in Fig. 4, total cell wall polysaccharide digestibility of *M. truncatula* inbred lines overlapped with the two alfalfa clones. Across stem segment maturities, DZA315.16 had the highest cell wall polysaccharide digestibility but was not significantly different from *M. truncatula* lines A17 and A20. The digestibility of alfalfa clone Regen-SY27 was lowest but was not statistically different from *M. truncatula* line R108. Alfalfa clone 718 overlapped *M. truncatula* lines A20 and R108 for total cell wall polysaccharide digestibility. These same patterns were observed for cellulose, hemicellulose, and pectin digestibilities (data not shown). As expected, pectin was consistently the most digestible polysaccharide (807 ± 10 g kg^{-1}) and hemicellulose was the least digestible (468 ± 19 g kg^{-1}), with cellulose being intermediate (696 ± 13 g kg^{-1}), across all germplasms and stem segment maturities.

DISCUSSION

To conclude that *M. truncatula* is a good model plant for research on alfalfa stem cell wall development and structure requires three pieces of evidence: (i) that the two species are sufficiently genetically related that genomic information can be transferred between the species, (ii) that alfalfa and *M. truncatula* germplasm overlap for relevant measures of stem morphology and cell wall composition and structure, and (iii) ideally, that the growth and development of alfalfa and *M. truncatula* under the growth chamber conditions of the current study are similar to observations under field conditions. Previous reports have shown that the genetic relatedness of alfalfa and *M. truncatula* is high (Julier et al., 2003; Choi et al., 2004), thereby satisfying the first criterion.

The second decision criterion has been substantially met because we have demonstrated that these two *Medicago* species are very similar for stem traits. The number and length of stem internodes and stem tissue morphology were similar for the two *Medicago* species. While the two alfalfa clones did have higher stem crude protein concentrations than the four *M. truncatula* inbred lines, cell wall concentration and composition overlapped among the germplasms of these two species. In the current study, data were collected on the composition but not structure of stem cell walls. However, because digestibility of plant cell walls by rumen microbes is a function of cell wall structure (Chesson, 1993), the lack of species differences in cell wall digestibility provides indirect support for the conclusion that alfalfa and *M. truncatula* stems are similar in cell wall structure.

The final criterion for concluding *M. truncatula* is a good model plant for stem development in alfalfa can be satisfied by a comparison of current results to previous literature reports on alfalfa. Jung and Lamb (2006) observed similar numbers of stem internodes for 13 field-grown alfalfa clones, when harvested at approximately 50% flower, compared to the two alfalfa clones in the current growth chamber experiment. However, overall stem length was less in the current experiment because mean internode length was lower for the growth chamber grown alfalfa (2.9–3.1 cm) than observed for the field experiment (4.0–4.6 cm). Number of elongating internodes at time of harvest was also similar between the field and growth chamber experiments. Proliferation of xylem tissues, development of thick secondary cell walls in phloem and xylem fiber tissues, and lignification of these tissues during stem internode maturation observed for alfalfa clones Regen-SY27 and 718 under growth chamber conditions correspond to previous observations with three alfalfa clones, including clone 718 (Engels and Jung, 1998; Jung and Engels, 2002), and for two alfalfa cultivars (Vallet et al., 1996) grown in the field. It would appear that stem development of the alfalfa clones under the growth chamber conditions of the current experiment was representative of alfalfa stem development in the field. Other than the difference in growth habit of the two species, stem morphology of alfalfa and *M. truncatula* appeared to be very similar.

Genetic variation in stem chemical composition traits of alfalfa has been demonstrated previously. Buxton et al. (1987) reported that stem crude protein concentration varied among 14 plant introduction germplasms and five vari-

eties. Forage quality components, including crude protein and neutral detergent fiber, were found to differ among nine alfalfa germplasms sources (Lenssen, 1991). While permanganate lignin concentration did not differ among these nine germplasm sources (Lenssen, 1991), selection for divergent acid detergent lignin (ADL) concentration in another alfalfa population did result in significant differences for stem ADL concentrations of the resultant lines (Kephart et al., 1990). These same low and high ADL alfalfa lines also differed for Klason lignin concentration in the cell wall, along with differences in cell wall cellulose, hemicellulose, and pectin concentrations (Jung et al., 1994). Clearly there is significant variation among alfalfa germplasms in chemical composition and we observed this for alfalfa clones Regen-SY27 and 718 in the current study. We believe this is the first demonstration of similar genetic variation in *M. truncatula* for chemical composition of stems.

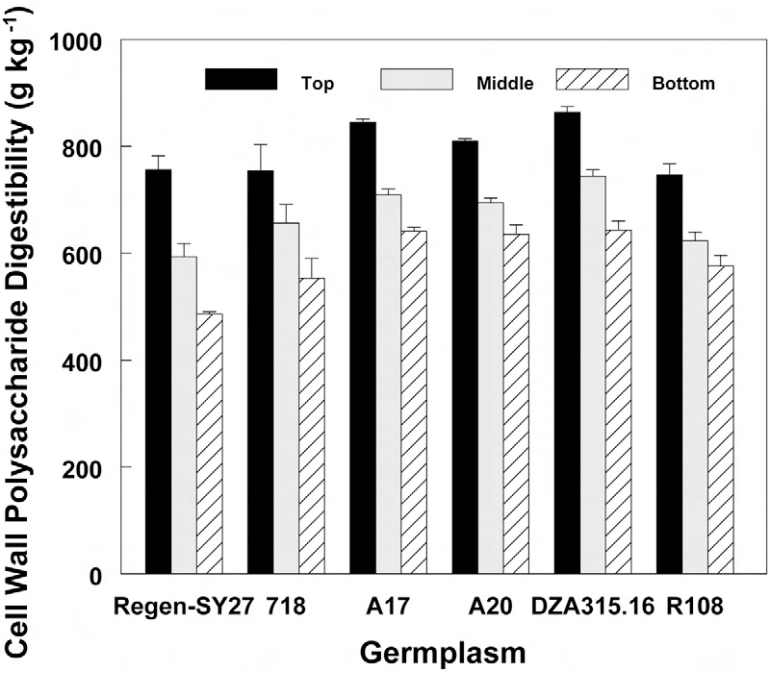

Figure 4. In vitro digestibility of total cell wall polysaccharides (mean ± 1 SE) from stem top, middle, and bottom thirds of alfalfa (Regen-SY27 and 718) and *Medicago truncatula* (A17, A20, DZA315.16, and R108) germplasms.

The data on cell wall polysaccharide digestibility in the current study were consistent with previous reports for alfalfa. Jung and Engels (2002) had also observed that pectin was the most digestible alfalfa cell wall polysaccharide while hemicellulose was the least digestible. As alfalfa stems become more mature, the digestibility of the cell wall declines (Buxton and Russell, 1988; Jung and Engels, 2002). It has been demonstrated that digestibility of alfalfa stems varies among alfalfa germplasms (Buxton et al., 1987; Lenssen, 1991; Jung et al., 1994; Jung and Engels, 2002). We are not aware of any previously published reports on digestibility of *M. truncatula* stems.

Previous comparisons of growth and development between alfalfa and *M. truncatula* were done under field conditions and involved whole herbage rather than only stems. Zhu et al. (1996) compared five commercial Australian cultivars of *M. truncatula* with the alfalfa cultivar Nitro. Whole herbage crude protein, neutral detergent fiber, and acid detergent fiber concentrations of one or more *M. truncatula* cultivars was similar to the alfalfa cultivar. In a trial comparing Nitro alfalfa to a different commercial *M. truncatula* cultivar, whole herbage concentrations of crude protein, neutral detergent fiber, and acid detergent fiber again did not differ consistently between the species (Shrestha et al., 1998). These whole herbage results support phenotypic similarity of alfalfa and *M. truncatula* for compositional traits; however, no literature reports on stem material or cell wall composition data of *M. truncatula* are available for comparison with the current study.

The difference in growth habit of alfalfa and *M. truncatula* (upright and prostrate, respectively) cannot be explained by the results presented here. Lignification of plant cell walls is generally considered to serve a structural support function (Boerjan et al., 2003). Differences in growth habit between the two *Medicago* species were somewhat reflected in their cell wall Klason lignin concentrations. Both alfalfa clones had numerically higher Klason lignin concentrations than the four *M. truncatula* lines, but alfalfa clone 718 was not significantly different from the *M. truncatula* inbred line R108. The two alfalfa clones also had the lowest numerical cellulose concentrations, but they were not significantly different from the *M. truncatula* inbred DZA315.16. Whether the apparent greater proportion of xylem tissues in mature alfalfa internodes accounts for the difference in growth habits of these species is unknown, although clearly this apparent tissue difference was not directly reflected in cell wall concentration or composition. Based on the limited germplasm sources examined for both *Medicago* species, it is not possible to recommend one *M. truncatula* inbred line over the others as the best model for alfalfa stem traits.

CONCLUSIONS

Stem morphology did not differ between alfalfa and *M. truncatula* for stem length, internode number, or number of elongating internodes. The same stem tissues were found in both species, and their developmental patterns were similar although alfalfa appeared to have more xylem tissue deposition whereas *M. truncatula* had larger phloem fiber bundles. Differences were noted among the six germplasms for soluble carbohydrate and cell wall traits; however, there was extensive overlap among the six germplasms rather than segregation by species. A higher crude protein concentration of alfalfa stems compared to *M. truncatula* stems was the only consistent difference between species detected. Our data suggest that despite the difference in growth habit, *M.*

truncatula is a suitable model for study and possible genetic manipulation of alfalfa stem development.

Acknowledgments

The authors wish to thank Ted Jeo for carrying out chemical analyses on dried stem material. Also, we are grateful to Divya Chandran and Melinda Dornbusch for their assistance with harvesting tissues.

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