

BREEDING FOR IMPROVED FORAGE QUALITY: POTENTIALS AND PROBLEMS

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ABSTRACT

Forage breeding has undergone a dramatic change since the development of *in vitro* digestibility tests in the 1960s. The emphasis of many breeding programs changed radically to focus on forage quality, while many other programs have added forage quality traits as selection criteria. Genetic variation or selection progress for forage quality traits has been documented in many forage crops. Although realized heritability is usually less than 30%, the progress made by selection is generally repeatable across diverse environments and harvest managements. Although no long-term selection experiments have been reported, progress toward increased forage quality has shown consistent and sustained levels through at least three selection cycles in three different species. In some species, forage quality and yield have been simultaneously increased, while in others, forage yield has decreased or remained constant. Despite forage yield reductions in some species, the increased forage quality of some cultivars is enough to compensate for the yield reduction, giving superior animal gains (profit) per hectare. Single-gene mutations that cause large increases in forage quality have been useful in some species, but they have required intensive efforts to improve forage yield and disease resistance. Reduced lignification and modified lignin composition, partly responsible for increased forage quality in some species, represent an erosion of some disease and insect resistance mechanisms. These discoveries may present upper bounds to the limits of genetic improvement in forage quality; at the least they complicate breeding programs. Plant transformation can be used to down-regulate lignin synthesis, creating potentially useful transformants.

KEYWORDS

Digestibility, fiber, genetic variation, heritability, lignin, protein, ruminant nutrition, selection.

THE PAST AND PRESENT

Conscious efforts to select and propagate superior existing strains of forage crops were first documented in late 18th-century Great Britain. Formal selection and breeding for the purpose of strain improvement and cultivar development began in the late 19th century in both Europe and North America. These early efforts focused almost exclusively on morphological, reproductive, and vigor-related traits, such as forage and/or seed yield, growth habit, timing of reproductive maturity, disease reaction, and persistence. Forage breeders rapidly discovered a wealth of additive genetic variation for many of these traits, leading to some dramatic improvements in the geographic distribution and range of adaptation of many forage crops. The spread of forage germplasm to ever-greater geographical limits has been one factor in the development of a relatively stable and profitable worldwide seed industry.

The first documented notion of high quality forage can be traced back to some of the original 18th century efforts at selection and propagation of existing natural strains of perennial ryegrass (Beddows, 1953). However, these initial references to identification of plants or strains possessing "high quality" are vague in that they do not define the term quality. Most likely the term referred to plants or strains relatively free from visual blemishes such as disease lesions, insect damage, or early senescence and perhaps possessing a uniformly robust appearance. Remarkably, we now know that fungal infection can reduce digestibility (Edwards et al., 1981), a modern

measure of forage quality, suggesting a possible correlation between historical and modern uses of the term "forage quality".

Forage quality can be considered as the ability of a forage to support animal functions such as maintenance, growth, reproduction, and lactation. It has three components: digestibility, intake potential, and energetic efficiency (Raymond, 1969). Digestibility and intake potential can be estimated or predicted from relatively simple laboratory procedures, whereas energetic efficiency is rather difficult to estimate or predict in the laboratory (Van Soest, 1994). Each component is a characteristic of a plant that is a function of many simpler plant traits, such as its structure, anatomy, morphology, chemistry, and stage of development. In turn, each of these simpler plant traits is under genetic control, ie. they can be manipulated either by natural selection or by human-directed selection. Paradoxically, the three components of forage quality (digestibility, intake potential, and energetic efficiency) cannot be defined in the absence of an animal and, thus, cannot be strictly defined as plant traits. It is only by arbitrarily classifying a plant as a feed that its genetic traits and their optimal values become defined in terms of animal nutrition concepts.

Based on this modern concept of forage quality it is clear that any effort at selection and breeding for forage quality cannot be based simply on plant traits alone. Embarkation on such a program demands a careful analysis and identification of plant traits that are correlated with forage quality, ie. animal performance. Because a successful plant breeding program demands screening of literally thousands of genotypes to identify the one or few superior candidates, a paradox exists: animal performance cannot be measured on thousands of genotypes.

There are isolated reports of correlations between simple-to-measure morphological traits and laboratory measures of forage quality (Lentz and Buxton, 1991), but these relationships are either too weak or not general enough to be broadly applicable in breeding programs. Changes in maturity may affect forage quality (Gately, 1984), but this may also require a change in harvest management, as either date-of-harvest or harvest-growth-stage definitions may vary as a result of selection. Increased disease resistance, documented in many forage crops (Casler and Pederson, 1996), may also lead to increased forage quality. While increased forage quality can be achieved via these and similar methods, they represent unconscious and possibly serendipitous improvements, which are not necessarily predictable. Laboratory methods represent the only method of consciously selecting and breeding for increased forage quality with some degree of predictability for the end result of selection.

Interest in using laboratory measures of forage quality for breeding purposes began in the mid-20th century with documentations of genetic variation or cultivar differences for crude protein, mineral element, or crude fiber concentrations. The first documentations of actual breeding progress first appeared in the 1960s. The single most significant event to change our methods, outlook, and commitment to breeding for increased forage quality was the development of the *in vitro* digestibility (or disappearance) test by Tilley and Terry (1963). Modifications of this screening tool have been applied in laboratory test tubes inoculated with rumen liquor or in nylon bags suspended in the rumen, both having been proven to be repeatable and capable

of measuring heritable variation. Numerous studies have demonstrated genetic variation and breeding progress for *in vitro* digestibility, as well as cultivars with improved digestibility, in a wide range of species, including warm- and cool-season species, perennials and annuals, grasses and legumes. Recent reviews provide excellent documentation of this progress (Burton, 1989; Buxton and Casler, 1993; Clark and Wilson, 1993; Hacker, 1982; Hanna, 1993; Marten, 1989; Vogel and Sleper, 1994).

The USDA bermudagrass [*Cynodon dactylon* (L.) Pers.] program at Tifton, GA, USA was the first to document progress in genetically improving forage quality (Burton et al., 1967). Four cultivars, released since the adoption of the nylon bag digestibility technique, have shown incremental gains in both digestibility and forage yield (Fig. 1). These cultivars represent dramatic gains over 'Coastal' bermudagrass and they have been planted to millions of hectares in tropical regions of the world.

The successful efforts at increasing forage quality of bermudagrass and several other forage crops, combined with recent advances in laboratory methodologies, have spawned a heightened interest in forage quality selection and breeding. The result has been increased research activity and greater emphasis on a multidisciplinary team approach for a wider range of plant species. These efforts should lead to a realization of genetically improved forage quality in more species and to a greater extent in those species for which improvements have been documented. Such gains have been achieved by a wide array of methods, including recurrent selection (Carpenter and Casler, 1990), wide hybridization (Burton et al., 1967), and mutation breeding (Cherney et al., 1991). Future progress may also result by transfer of DNA from other organisms via plant transformation.

The various approaches to breeding for increased forage quality have advantages and disadvantages which, in turn, raise several issues/questions that impact the probability of a successful cultivar release. The remainder of this paper will focus on some of these issues and questions.

ISSUES FOR THE FUTURE

The Limits to Progress. We have barely begun to utilize what appears to be a wealth of additive and non-additive genetic variation for laboratory measures of forage quality in most species. The wide-hybridization program in bermudagrass has shown an approximately 2 g kg⁻¹ yr⁻¹ increase in *in vitro* dry matter digestibility during the first 30 years following publication of the Tilley and Terry paper (Fig. 1). Because bermudagrass is clonally propagated, all forms of genetic variation can be utilized during the selection process; the end result is the single best clone in the population. Thus, the bermudagrass results appear to represent an upper bound on the rate of forage quality improvement that can be realized. Nevertheless, the rate of improvement has shown no indication of declining. For annual forage crops such as maize (*Zea mays* L.), sudangrass [*Sorghum bicolor* (L.) Moench.], and pearl millet [*Pennisetum glaucum* (L.) R. Br.], similar rates of improvement may be possible because their hybrids are genetically similar to bermudagrass clones. Indeed, trends in maize hybrid breeding between 1950 and 1990 indicate a dramatic expansion of genetic variation for silage digestibility (Barrière et al., 1992).

For most perennial species, in which a group of superior plants must be identified and intercrossed, only the additive portion of genetic variation can be utilized during selection. Realized heritabilities usually indicate that less than 30% of the parental advantage can be

captured among the progeny of selected individuals for this method (Carpenter and Casler, 1990). Nevertheless, recurrent selection experiments for *in vitro* digestibility have shown consistent progress of 5 to 10 g kg⁻¹ cycle⁻¹ (Buxton and Casler, 1993). Because most breeders can complete a selection cycle in 2 or 3 years, a 20-year effort could theoretically result in a new cultivar with an *in vitro* digestibility 35 to 100 g kg⁻¹ higher than the original population. Unfortunately, it will be a number of years before any existing programs will be sufficiently mature to allow a test of this hypothesis.

To date, only two studies have been described as approaching a long-term recurrent selection program for increased forage quality, both reporting three cycles of selection. In both switchgrass (*Panicum virgatum* L.) and smooth bromegrass (*Bromus inermis* Leyss.), selection was sustained through three cycles of selection, with little or no sign of a decline in rate of response. The fact that these two grasses represent extremely different genera (warm- vs. cool-season) and the selection criteria were different suggest a relative broad applicability of these results. Combined with what we know from long-term recurrent selection experiments for divergent oil and protein concentration in maize (Dudley and Lambert, 1992), these results suggest that long-term progress from recurrent selection for increased forage quality should be possible. While the results may not be as dramatic as for species with hybrid or clonal cultivars, feeding and grazing studies have shown that small changes in digestibility can have a significant and measureable impact on animal performance (Marten, 1989; Vogel and Sleper, 1994).

The Value of Breeding for Increased Forage Quality. First, a successful program will easily justify itself as increased revenue for forage producers. Vogel and Sleper (1994) document several examples of breeding programs that have used field selection and laboratory measures of forage quality to improve animal performance. Monetary values or economic benefits of such improvements are difficult to document, because they depend on the magnitude of the improvement (which can be quantified) and the extent to which the new cultivar is utilized (which is almost never documented). Using the switchgrass example of Vogel and Sleper (1994), first assume it cost \$500,000 to develop the improved cultivar Trailblazer over a 15-yr period, including all salaries, supplies, and overhead. With a \$100 ha⁻¹ benefit of Trailblazer over the parent cultivar Pathfinder (Vogel and Sleper, 1994), it would only require about 5000 ha be planted to Trailblazer for producers to make the breeding program pay for itself in a single year of animal production. Furthermore, improved-digestibility cultivars such as Trailblazer are generally not marketed with an increase in seed cost which might dilute some of the economic benefit.

Second, the overwhelmingly large potential effects of management practices on forage quality, compared to relatively small genetic differences, are sometimes stated as a reason to minimize funding for breeding programs. However, genetic progress from breeding for increased forage quality is nearly always highly consistent across environments and harvest managements. Numerous studies have evaluated populations selected for increased forage quality in a range of environments, including multiple sites, years, and harvests. In general, their findings have consistently indicated that genotype x environment interaction is relatively unimportant in determining the results of breeding for increased forage quality. Some examples include alfalfa (Coors et al., 1986; Sumberg et al., 1983), switchgrass (Gabrielson et al., 1990; Hopkins et al., 1993), perennial ryegrass (Humphreys, 1989), and smooth bromegrass (Casler et al., 1996). In addition, forage quality selections show similar responses under differential harvest management systems for alfalfa (Vaughn et al.,

1990) and smooth bromegrass (Ehlke et al., 1986). Finally, nearly all of the successful selection experiments reported by Buxton and Casler (1993) were based on a single sample of a plant at a single point in time. The rate of progress in these and other studies, measured over a wide range of environments further attests to the relative unimportance of genotype x environment interaction in determining forage quality. This may be a remarkable statement to some researchers, given the large potential environmental effects on forage quality traits. However, it reinforces the point that environmental effects per se and genotype x environment interaction effects are statistically independent of each other in most experiments.

Thus, an improved cultivar will usually have increased forage quality under most environmental or management conditions to which it is subjected. This makes the processes of breeding for increased forage quality and modification of management to increase forage quality act in an additive manner. Furthermore, a perennial crop cultivar with increased forage quality often represents a permanent one-time increase in forage quality, whereas a management scheme to increase forage quality must continually be re-applied each year or growing season.

Third, and more esoteric to those interested in new cultivars, is the scientific benefit of maintaining a long-term commitment to breeding for increased forage quality. The germplasm derived from such a program can be useful in answering basic questions regarding quantitative and population genetics, ruminant nutrition, physiology and genetics of cell wall development, and mechanisms of plant resistance to diseases and insects.

Potential Negative Consequences of Genetic Increases in Forage Quality. Most efforts to genetically improve forage quality use crude protein concentration, some method of digestibility determination, or the concentration of some form of fiber as a selection criterion. Very often these are inter-correlated so that selection for one trait results in predictable changes in the others (Carpenter and Casler, 1990; Clements, 1969; Coors et al., 1986). In general, high protein concentration, high digestibility, and low fiber concentration are all associated with high forage quality, i.e. they generally confer an increase in animal performance.

Prior to the recent successes of several breeding programs, a common scientific dogma was that forage yield and quality were negatively correlated. Few researchers were willing to devote monetary resources or many years toward an objective that might be self-defeating. The results of recent efforts show that the relationship between yield and quality may be dependent on species and the trait used to predict forage quality, but that simultaneous improvements are possible. In bermudagrass, both forage yield and quality have shown rapid improvements in recent years (Fig. 1). However, if the future of the program had depended on these simultaneous improvements from the beginning it might not have survived the first 30 years, witnessed by the reduced yield of Tifton 44. Accumulating favorable alleles for several important traits and breaking up negative linkages by allowing genes to recombine in new crosses requires patience, perseverance, and sometimes several cycles of crossing.

Divergent selection for IVDMD in switchgrass led to increases in forage yield in both directions through cycle 2 (Fig. 2a). The increase in forage yield in both directions is likely due to selection of only the most vigorous plants for IVDMD analysis (Hopkins et al., 1993). The dramatic reduction in forage yield during cycle 3 suggests that a threshold was reached at this point in the selection process. In making

the last incremental increase in IVDMD, a plant factor (or factors) essential for normal growth and development, but detrimental for high IVDMD, may have become limiting. Cycle 3 has also been shown to have reduced winterhardiness (Buxton et al., 1995). This may possibly be due to reduced selection pressure for winter survival or to a cause-and-effect relationship between the two traits that did not arise until cycle 3. Lignin and lignin-like compounds, which are associated with IVDMD, may be important in regulating cold tolerance (Chalker-Scott and Fuchigami, 1989), although this is not a general observation associated with genetic increases in forage quality (Clark and Wilson, 1993).

In smooth bromegrass, selection for low fiber concentration led to a concomitant reduction in forage yield (Fig. 2b). Selection for high fiber concentration led to a slight reduction in forage yield, an amount that could be attributed to the normal inbreeding that occurs during recurrent selection. Thus, part of the loss in forage yield due to selection for low fiber concentration may have been due to inbreeding. Two cycles of divergent selection for IVDMD did not affect forage yield of smooth bromegrass (Carpenter and Casler, 1990; Casler and Ehlke, 1986). Apparently, forage yield may be correlated with fiber concentration, but not with IVDMD in smooth bromegrass. Conceivably, this positive correlation might be overcome if selection is based on both forage yield and fiber concentration. However, because forage yield of spaced plants has a heritability of nearly zero, this would be very difficult in smooth bromegrass (Carpenter and Casler, 1990). Results from reed canarygrass possibly suggest more optimism for decreasing fiber concentration without decreasing forage yield (Surprenant et al., 1988).

A simultaneous decrease in fiber concentration, combined with either an increase or no change in forage yield may be theoretically difficult in forage crops. Consider that, from a practical standpoint, selection must be based on a laboratory measure of a plant's phenotype at a single point in time, i.e. the exact time of harvest or sampling. Plants with reduced cell wall concentration, roughly equivalent to total (neutral detergent) fiber concentration likely result from a reduction in the relative proportion of cells with highly developed cell walls, such as sclerenchyma, xylem, and bundle sheath cells (Ehlke and Casler, 1985). Those genotypes with reduced levels of these types of cells at the time of harvest become identified as having reduced cell wall concentration. Because these cell types function primarily as structural components of stems and leaves, their development occurs primarily later, ontogenically, than cells with less-developed cell walls, such as mesophyll cells. Thus, genotypes selected for reduced cell wall or fiber concentration are those plants which are not as structurally or ontogenically advanced. Because plant dry matter increases during the ontogeny of forage plants, low fiber plants necessarily have reduced yield.

This relationship may be regulated by other factors. For example, in the smooth bromegrass study, inbreeding in the high-fiber direction appeared to overcome the potential for a yield increase (Fig. 2b). Other factors such as growth rate of individual cells, cell size, cell wall thickness, and orientation of vascular bundles may cause this relationship to break down. Genetic variation for these traits might allow simultaneous selection for low fiber concentration and high yield, analogous to that practiced in the switchgrass study (Hopkins et al., 1993). In alfalfa (*Medicago sativa* L.), simultaneous selection for vigorous plants with low acid detergent fiber (ADF) concentration led to plants with reduced ADF concentration and no change in forage yield (Coors et al., 1986; Sumberg et al., 1983). Furthermore, a similar selection protocol for crude protein (CP) concentration led to a simultaneous increase in CP concentration and forage yield (Sumberg

et al., 1983).

The problems associated with a single sampling at a single point in time can be partly overcome by multi-stage selection in which putative extreme individuals are sampled a second, and possibly third, time to verify their status as extreme individuals (Casler et al., 1996; Godshalk et al., 1988a). This modification to the typical selection protocol may be necessary in cases where genotype x environment interaction is important. This appears to be more necessary when selection for increased forage quality is based on some measure of fiber concentration or cell wall constituents, rather than CP concentration or IVDMD, or when the population to undergo selection has considerable genetic variation for timing of reproductive maturity (Surprenant et al., 1993).

Whether increased forage quality is brought about by selection for low fiber concentration, high CP concentration, or high IVDMD, it will likely cause correlated responses for other plant traits. For example, divergent selection for CP concentration in phalaris (*Phalaris aquatica* L.) resulted in dramatic changes to plant morphology and growth rates (Clements, 1969). Selection for low fiber concentration also leads to changes in fiber composition, specifically a reduction in fiber lignification (Carpenter and Casler, 1990; Coors et al., 1986). Increased IVDMD in smooth bromegrass results almost exclusively from decreased lignin concentration in a ratio of 7 g kg⁻¹ IVDMD to 1 g kg⁻¹ lignin (Buxton and Casler, 1993). Lignin composition appears to be of secondary importance in smooth bromegrass (Jung and Casler, 1990) and primary importance in switchgrass (Gabrielson et al., 1990) in determining genetic changes in IVDMD. High IVDMD plants and populations had higher ratios of ferulic to *p*-coumaric acid, a pattern similar to that observed between normal and high-digestibility brown-midrib (*bm3*) maize (Barrière and Argillier, 1993).

These results suggest that ferulic cross-linkages between arabinoxylans and lignin core molecules may be more important in regulating genetic variation for IVDMD than the concentration per se of these constituents. Ferulic acid esterifies to arabinose subunits of arabinoxylan chains while *p*-coumaric acid esterifies to core lignin during plant development (Jung, 1989). These ferulate polysaccharide esters act as initiation sites for lignification and may regulate the degree of lignin-polysaccharide cross-linking that occurs as plants develop (Ralph et al., 1995). Because the concentrations of lignin monomers (Jung and Casler, 1990) and polysaccharide monomers (Godshalk et al., 1988b) appear to be under genetic control in forage crops, it is theoretically possible to create genotypes with an altered rate of lignin-polysaccharide cross-linkage formation during development.

A reduction in lignin concentration and/or the concentration of certain phenolic acids may lead to a loss of disease and/or insect resistance in forage crops. Rapid formation of lignin or lignin-like compounds has been implicated in the hypersensitive resistant reaction to fungal pathogens (Nicholson and Hammerschmidt, 1992). These compounds appear to form structural barriers inhibiting further penetration of fungal hyphae (Grisebach, 1981). Indeed, selection for reduced fiber concentration in smooth bromegrass led to increased leaf damage by *Cochliobolus sativus* (Ito et Kurib.) Drechs. ex Dastur, suggesting that fiber concentration or composition may be an important component of the resistance to this fungus (Han et al., 1996).

While selection for disease resistance can protect forage plants from reductions in forage quality associated with disease infection, disease resistance per se does not appear to reduce forage quality (Buxton

and Casler, 1993). There may be two potential pools of lignin and phenolic compounds in forage plants, one responsible for normal cell wall structure and support and a second capable of rapid mobilization in response to fungal pathogens. Because there are no reports of increased lodging or reduced seed production associated with increased forage quality, the resistance pool may be the one more amenable to genetic manipulation by recurrent selection for increased forage quality. Selection for increased disease resistance operates on either the substrate or the enzymes of the resistance pool, causing greater or more rapid mobilization of phenolics in response to fungal pathogens, but without affecting forage quality. Conversely, selection for increased forage quality reduces the size of the resistance pool, decreasing disease resistance without affecting the plant's structural integrity. These pools are not likely discrete entities, but are more likely represented by lignin monomers in different frequencies. Genetic lines divergently selected for one or more components of the cell wall or IVDMD should be useful in testing this hypothesis.

Lignin appears to partially regulate resistance to European corn borer (*Ostrinia nubilalis* Hübner) in maize, although the mechanism is uncertain (Buendgen et al., 1990). Four cycles of recurrent selection for increased European corn borer resistance in maize led to correlated increases in total fiber, lignin, and ash concentrations (Fig. 3). Lignin and ash, which is primarily made up of silica, had the largest proportional responses, suggesting their importance in this particular mechanism of insect resistance.

Increased water soluble carbohydrate (WSC) concentration is another mechanism by which IVDMD may be increased in forage crops (Humphreys, 1989). However, because WSC and structural carbohydrate concentrations are necessarily negatively correlated in plants, this mechanism may be similar to those discussed above. Indeed, selection to increase WSC concentration by 37% in perennial ryegrass (*Lolium perenne* L.) led to a 128% increase in the incidence of crown rust caused by *Puccinia coronata* Corda (Breese and Davies, 1970).

Forage Quality Mutants. Several genes have been discovered to cause dramatic increases in forage quality of annual forage crops. The most widely distributed of these is the group known as brown-midrib mutants, occurring in maize, sorghum [*Sorghum bicolor* (L.) Moench.], and pearl millet, and reviewed by Cherney et al. (1991). This first of these mutants was reported in 1931 and, chiefly due to their apparent pleiotropism for reduced forage yield, none have been commercialized. Vogel and Sleper (1994) recommended that efforts be focused on selecting for increased forage yield in brown-midrib populations to improve economic returns. Over 15 years of intensive selection in sudangrass and sorghum has identified some promising lines that may be commercialized from programs in Iowa, Georgia, and Wisconsin, USA. Feeding studies with near-isogenic brown-midrib and normal lines suggest that a small reduction in forage yield can be tolerated while maintaining superior animal performance of the brown-midrib line (Cherney et al., 1991). Brown-midrib mutants may also be more susceptible to fungal diseases than normal lines (Nicholson et al., 1976).

The dwarf gene of pearl millet has been commercialized in the hybrids 'Tifleaf 1' and 'Tifleaf 2' (Burton et al., 1969; Hanna et al., 1988). The dwarf phenotype produces shorter internodes, but the same number of leaves as the normal phenotype, increasing its forage quality as measured by animal gain per day. Its forage yield reduction compensates the increased forage quality, giving equal animal weight gains per hectare for the normal and dwarf lines. Therefore, its

advantage over the normal line comes not from greater economic benefit per se, but from greater ease of management due to the dwarf phenotype (W.W. Hanna, 1996, personal communication). Additional selection efforts resulted in some forage yield and disease resistance improvement of Tifleaf 2 over Tifleaf 1, suggesting that considerable potential exists to modify phenotype using background genes.

These results emphasize an aspect of mutation breeding that may not necessarily be important during recurrent selection for increased forage quality. Because genetic mutants can have a dramatic effect on forage quality by severely affecting plant growth and development, they may also cause significant, seemingly intolerable, pleiotropic reductions in forage yield and/or disease resistance. Commercialization of the first-generation strains may be possible through interdisciplinary collaboration to develop an innovative management scheme and/or the proper data and documentation to demonstrate a value for the novel phenotype. If such a value cannot be demonstrated in the first-generation strain, additional selection will be necessary to introgress desirable genes from other sources. The latter appears to be necessary for successful commercialization of the brown-midrib mutants.

Potential Molecular Genetic Contributions. Plants can be modified by the process of transformation, the insertion of a cloned gene from another organism along with a functional promoter sequence of DNA. The advantages of plant transformation include an ability to target modification of individual steps in specific metabolic pathways and to do this in relatively short time periods. Most efforts have focused on down-regulation of enzymes in the lignin biosynthesis pathway [phenylalanine ammonia-lyase (PAL), cinnamyl alcohol dehydrogenase (CAD), and various forms of *O*-methyltransferase (OMT)] using antisense RNA, virus enhancer sequences, or ribozymes.

Down-regulation of PAL in tobacco (*Nicotiana tabacum* L.) led to reductions in lignin concentration, but to severely pleiotropic effects on overall phenotype of transformants (Elkind et al., 1990). PAL may not be a good choice for down-regulation because of multiple gene sites, its position early in the lignin biosynthesis process, and its severe pleiotropic effects. Down-regulation of CAD and OMT have shown some success at reducing lignin concentration and preserving a visibly normal plant phenotype, but results have been inconsistent. Targeting caffeic acid 3-*O*-methyltransferase led to a reduction in lignin concentration but no major changes in lignin composition of alfalfa (Ni et al., 1994). Other transformation experiments with OMT and CAD have shown modified lignin composition without quantitative changes in lignin concentration (reviewed by Boudet et al., 1995). Transformation of stylo (*Stylosanthes humilis*) with an antisense OMT construct led to plants with 16% higher digestibility than control plants (Rae et al., 1996).

As it pertains to the development of novel and/or improved germplasm, molecular genetic results to date are parallel to those from mutation breeding. Brown-midrib genes in maize and sorghum appear to be those responsible for OMT or CAD synthesis, with mutant alleles causing reduced enzyme activity (Pillonel et al., 1991; Vignois et al., 1995). Gene constructs appear to have pleiotropic effects on transformed plants and may not have predictable effects on forage quality. Although many transformants visually appear to have normal growth and development, agronomic assessments and, if necessary, hybridization with adapted germplasm and selection will require years of breeding efforts to produce marketable cultivars. Inhibition of the enzymes involved in lignin biosynthesis can also lead to loss of disease resistance (Moerschbacher et al., 1990),

necessitating some of the same selection and breeding efforts required during recurrent selection and mutation breeding. Brown-midrib genes cause dramatic loss of forage yield and vigor (Miller et al., 1983), suggesting a similar possibility for antisense transformants.

CONCLUSIONS

The quality of forage crops, as measured by meat, milk, or wool production, can be dramatically improved by conventional plant breeding methods. Successful programs have been developed for warm- and cool-season grasses and for legumes and have led to the development of new cultivars with documented increases in forage quality. Because forage quality can be increased without losses in forage yield, or enough to compensate for losses in forage yield, greater profitability usually results from these breeding efforts. Cultivars with increased forage quality rapidly pay for their development expenses in terms of increased profit for growers. Genetic variation is still sufficient that plant breeders can continue to make significant progress using conventional and possibly molecular techniques. Some methods of increasing forage quality, such as reductions in lignin or fiber concentration, may have negative consequences for forage yield and pest resistance. Extra efforts focused on selecting for other agronomic traits during the initial or later stages of selection and breeding may be needed to ensure a viable product. Plant breeding has had a tremendously favorable impact on animal agriculture throughout the world, resulting both from efforts directed to improve forage quality per se and from efforts to improve other traits. The most successful programs are those that incorporate the plant breeder into a team, including but not limited to, agronomists, animal scientists, and plant pathologists.

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Figure 1

Progress in breeding bermudagrass for *in vitro* dry matter digestibility (IVDMD) and forage yield between 1947 and 1993. Cultivars were Coastal (1947), Tifton 44 (1978), Tifton 68 (1984), Tifton 78 (1988), and Tifton 85 (1993). Data taken from Hill et al. (1993).

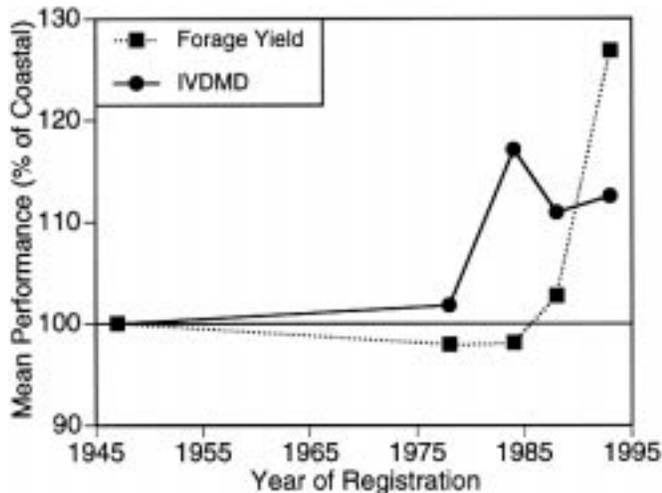


Figure 2

Responses to selection: (a) three cycles of positive selection and one cycle of negative selection for *in vitro* dry matter digestibility in switchgrass, and (b) three cycles of negative selection and one cycle of positive selection for neutral detergent fiber (NDF) concentration in smooth bromegrass. Switchgrass data taken from Hopkins et al. (1993). Smooth bromegrass data taken from Casler (1995).

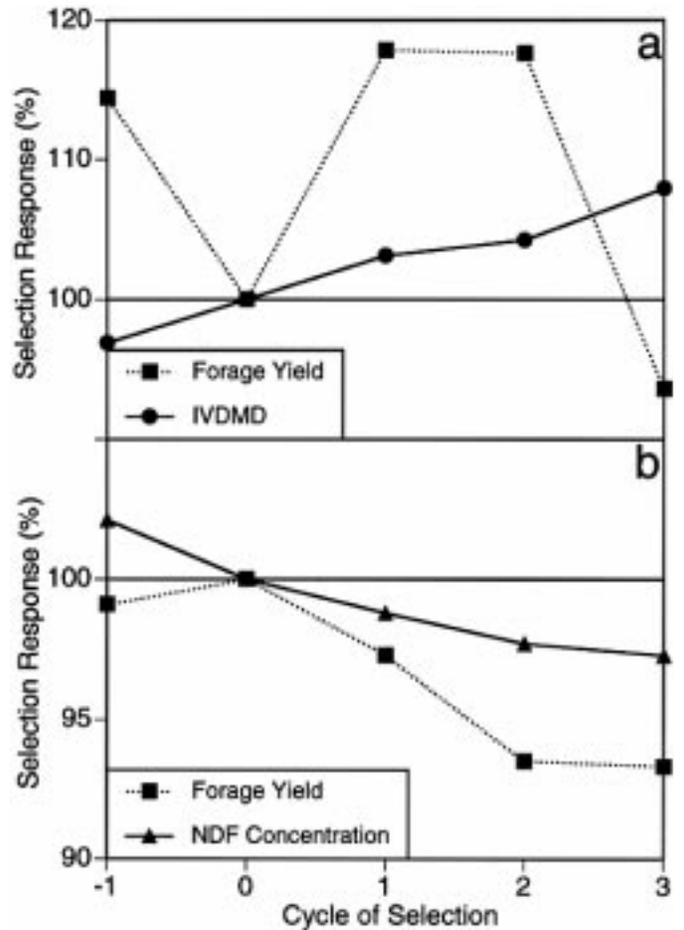


Figure 3

Responses to selection for European corn borer resistance in BS9 maize: (a) measured as number of cavities, and (b) measured as neutral detergent fiber (NDF), lignin, or ash concentration. Data taken from Buendgen et al. (1990).

