Breeding Sorghum And Pearl Millet For Forage And Fuel

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Abstract

*Sorghum [*Sorghum bicolor *(L.) Moench] and pearl millet [*Pennisetum glaucum *(L.) R. Br.] are unique species in their ability to be used in many forage/livestock system roles. Such flexibility has made prioritizing breeding objectives difficult and has even contributed to contradictory opinions on appropriate forage breeding objectives. Few breeding projects identified in the USDA-ARS, USDA-CREES, or at ICRISAT had forage sorghum or forage pearl millet as their sole research assignment. In the United States, it can be argued that breeding resources committed to forage sorghum improvement are probably declining. A new forage sorghum and forage pearl millet project recently considered by INTSORMIL did not receive high enough priority to receive funding from available resources.*

This paper discusses: new technologies, including automated harvesting systems, statistical methods, and forage quality assessment methods, that allow considerable increases in the scale and efficiencies of forage sorghum and millet breeding programs; examples of genes coding for characters known to impact forage quality; the status of the ethanol industry in general; the prospect for ethanol from biomass; and production of paper from stover. Due to limited resources, forage sorghum and millet breeding programs will have to focus on narrow, high impact objectives and utilize the best available technology.

Methods of breeding sorghum and pearl millet for forage and fuel are essentially the same as for any other targeted markets. This paper will focus on the breeding objectives, problems, and opportunities facing forage sorghum and forage millet breeders. Thorough reviews of the literature on the genetics and breeding of forage sorghums and forage millets have recently been published by Andrews and Kumar (1992) and Bramel-Cox et al. (1995).

Review of Current Status

Importance of Forage Sorghum and Forage Millet

When grown primarily as forage crops, these two species are unique. They are productive warm season annuals, are readily established using conventional equipment and cropping, and have much lower water requirements than maize grown for silage. These characteristics provide considerable flexibility for for-

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age/livestock producers in managing their resources and responding to the critical forage needs of their livestock. However, such flexibility of use makes identification of breeding objectives difficult. Even the growth stage of the final sorghum or millet product varies.

Many forage/livestock systems suffer from periods of low forage productivity. Forage sorghum and pearl millet are commonly used in the vegetative stage to fill summer forage production needs through direct grazing. They also can be preserved at various maturities as hay or as silage to fill winter forage production voids. Grazing or preservation of the stalks and leaves remaining after grain harvest are common practices with grain sorghums in the United States and dual-purpose sorghums grown elsewhere. Producers also have required particular physical parameters (for example, height or seed color) to meet the definition of a "forage" in political programs. Such diversity in the use of these two species as forages has contributed to diverse and sometimes contradictory opinions on appropriate forage breeding objectives.

In the United States, only 142,000 ha of forage sorghum were harvested for silage in 1993 (U.S. Agricultural Statistics, 1994). Based on the 1985 ratio of hectares of forage sorghum harvested for silage to hectares of other forage sorghum harvested or grazed (these estimates were discontinued after 1985), approximately 24,300 additional ha of forage sorghum were harvested or grazed in 1993. This figure, however, may be a considerable underestimate. A seed industry source indicates that hectares of forage sorghum for hay may be three times greater than hectares of forage sorghum grown for silage (A. Armburst, Sharp Brothers Seed Co., 1996, personal communication). When combined with the resulting small individual target markets for each specific forage product, the diversity of breeding objectives has resulted in a limited commitment of resources to any single objective and a consequent limited impact in the marketplace.

Current Breeding Objectives

A 1988 survey of 26 public and private forage sorghum breeders (Kalton, 1988) indicated that the primary objectives of the majority of the breeders were: increased total yield, standability, disease resistance, and insect resistance. Few breeders identified improvement of forage quality as an objective.

A current search of the USDA Research (CRIS) data base (1996) using the search strings "sorghum and forage and breeding" and "millet and forage and breeding" identified a variety of current research objectives. Broad breeding objectives include:

- Evaluate introductions and wild species for genetic potential
- Efficiently utilize genes from exotic germplasm
- Create additional diversity by hybridization and tissue culture
- Develop superior genetic stocks and germplasms
- Breed better cultivars.

Specific breeding objectives include:

- Improved yield
- High yield for silage

- Improved quality
- Improved disease resistance
- Improved insect resistance
- Improved drought resistance
- Tolerance to acid soil
- Tolerance to aluminum and manganese toxicity
- Efficiency of phosphorous utilization
- Reduction of harmful substances
- Conversion of Burkina Faso pearl millet landraces to short, day-neutral pearl millet lines.

Basic research objectives include:

- Determine the constraints to superior forage sorghums
- Establish taxonomic and cytogenetic relationships
- Develop more efficient breeding methods
- Develop methods for converting and transferring genetic characters to cultivated materials
- Develop interspecific transfer methods for gene(s) controlling apomixis
- Develop RFLP methodology to enhance plant breeding effectiveness
- Clone and characterize stem elongation genes
- Determine chemical composition of sorghums carrying brown midrib genes
- Evaluate expression of lignin-associated genes for molecular understanding
- Isolate and characterize lignin-associated genes for genetic engineering
- Understand the control and inheritance of apomixis
- Understand development of genetic diversity in apomictic genotypes
- Understand development and molecular aspects of two pearl millet mutants with tendencies towards apomixis
- Map gene(s) for apomixis in interspecific hybrid.

Few projects had forage sorghum or forage millet as their sole research assignment. Most were grain projects with some commitment to forages, or forage projects that included additional species. A similar search of the ICRISAT Global Research Portfolio (ICRISAT, 1996) data base showed three pearl millet projects and five sorghum projects, but none had stated objectives of forage improvement.

Breeding Approach/Products

According to Kalton (1988), most forage sorghum breeders are developing improved hybrids. Based on the above objectives, it appears that forage sorghum and millet breeders use most of the previously discussed methods. Anticipated products from breeding programs include hybrids, parental lines, varieties, populations, genetic stocks, and cloned genes, as well as a wealth of scientific knowledge.

Current Resources

Total fiscal commitment or scientist year commitment to forage sorghum and millet breeding is difficult to determine since most such efforts represent portions of larger projects with grain or other forage crop objectives. However, in the United States, it can be argued that breeding resources committed to forage sorghum improvement are probably declining. Total USDA/ CREES/ ARS research dollars committed to grain sorghum research have declined from $3.02 million in 1986 to $2.70 million in 1996 (T. Lust, National Grain Sorghum Board, 1996, personal communication). At the same time, private sector sorghum breeding programs have been eliminated by some companies and reduced in size by others. It appears that forage sorghum breeding programs suffered similar reductions during this period, as well.

Plot harvesting equipment available to current forage sorghum and millet breeders varies, but can be as crude and labor-intensive as machetes and hanging scales. While many forage grass breeding programs include laboratory quality assessment of breeding materials and actual grazing evaluation of advanced lines, such data often is available to forage sorghum and millet breeders only on a fee basis. These limitations severely restrict the numbers of breeding lines that can be harvested and evaluated; they also restrict forage breeding objectives to characters that can be readily observed or measured.

Define State of the Art

Measuring Yield (Forage Harvest Technologies)

Harvest systems that reduce labor needs, increase capacity of programs, and provide a safe working environment for operators are essential. Such systems are in use at several public and private research locations and are based on commercially available silage harvesters. One such harvest system recently described by Pedersen and Moore (1995) has been used on both forage sorghum and pearl millet and can harvest and weigh approximately one plot/minute. Data can be collected electronically. Forage is finely chopped and easily subsampled for moisture and quality analysis. When combined with state of the art forage quality technology, quantitative yield and quality data can be obtained on greatly increased numbers of breeding lines.

Statistical Methods

The ability to increase the number of breeding lines, and the amount of data collected on each line, affects design and analysis of experiments. Non-uniformity of fields becomes more important as the ability to identify uniform blocks decreases. Unbalanced data sets become more probable as the number of lines evaluated increases. In a recent review of yield trial design and analysis, Johnson et al. (1992) point out that recent developments in computer technology have greatly enhanced our ability to utilize complex statistical models. They demonstrate that mixed linear model methodology (MLMM) can enhance analysis of combined data and/or unbalanced data, adjust for spatial variation, identify specific genotypic by environmental interactions, as well as estimate or predict genetic effects. In one example using MLMM, standard errors between entries were halved when entries and blocks were assumed to be random (the situation most plausible biologically) rather than fixed.

Nearest neighbor analysis (NNA) has been used successfully by small grain breeders (Besag and Kempton, 1988; Gleeson and Cullis, 1987) to increase experimental precision. Although Johnson et al. (1992) reported little or no benefit of

NNA in maize (*Zea mays* L.) yield trials, they concluded that NNA may be more useful in yield trials conducted in non-irrigated, stress environments. These are precisely the environments that forage sorghum and millet have traditionally occupied.

A unique problem facing forage breeders is how to interpret data from multiple harvests of the same plot throughout a growing season. Forage sorghum breeders attempting to improve sudangrass are often faced with multiple harvest data. Pedersen et al. (1991) described a concise and easily interpreted method to help breeders interpret such data. With this method, yield is regressed against an index associated with harvest dates, resulting in a single regression coefficient descriptive of cultivar response over all harvest dates. The practical value of such a technique increases as the number of lines evaluated increases.

Forage Quality Technologies

The "gold standard" for determining the quality of a forage is feeding it to cattle and measuring animal response in either weight gain (for beef cattle) or milk production (for dairy cattle). This "gold standard" has been used successfully in the final testing phases of several forage programs. However, such forage quality assessments are far too costly and require too much forage material to be practical in early generation forage breeding. Therefore, a number of laboratory assays that predict cattle performance have been developed.

One of the most (if not the most) widely accepted laboratory assays for forage quality is *in vitro* dry matter disappearance (IVDMD), originally developed by Tilley and Terry (1963). This procedure utilizes actual rumen fluid as a digestive agent with results very similar to *in vivo* digestion. However, because the procedure requires a surgically fistulated donor animal, many biological, environmental, and procedural variables can influence the final results. Marten and Barnes (1980) have summarized and presented standardization techniques for some of the many variations that have developed from the original procedure.

Shortly after the IVDMD procedure was developed, Goering and Van Soest (1970) described a forage fiber analysis system that utilizes chemical rather than biological degradation of forages. It breaks down forages into cell solubles, hemicellulose, cellulose, lignin, and ash fractions. This system is attractive because results are not so readily affected by biological, environmental, and procedural variables. Results reveal information about the structural/chemical makeup of forages, but do not imitate actual digestion by ruminants. Results also are used to predict relative feed value (RFV) of alfalfa and are used routinely for commercial alfalfa hay analysis; however, RFV would be of questionable value for forages such as sorghum and millet.

Two relatively new modifications of the *in vitro* dry matter disappearance procedure show great promise in simplifying and/or expanding the information derived from the procedure. The first is a commercially available system from ANKOM Technology Corporation (140 Turk Hill Park, Fairport, NY) that utilizes rumen fluid, sealable sample bags, and bulk digestion vials, and is currently being utilized by our lab. Initial results indicate good agreement in ranking of sorghum genotypes with traditional IVDMD results, and greatly enhanced ease of operation and sample handling. The system also is adaptable to digestion kinetics studies.

An *in vitro* procedure developed by Schofield et al. (1994) also utilizes rumen fluid, but goes one step further in providing digestion kinetics data. The rumen fluid is placed in a sealed digestion vessel, and pressure sensors measure gas production, continuously outputting data until the digestion process is stopped.

Another technology of great value is near infrared reflectance spectroscopy (NIRS). It is well described in a handbook edited by Marten et al. (1989). This technology utilizes near infrared spectral data to predict wet lab forage quality parameters. It requires collection of reference laboratory data and the development of complex prediction equations (through the use of relatively user-friendly software). Once these steps are accomplished, multiple lab values can be predicted from a single NIRS scan, which can be accomplished in approximately one minute. This technology can make measurement of forage quality data economically possible for some forage breeding projects.

Forage Quality Genes

Although most forage quality parameters appear to be quantitatively inherited (Andrews and Kumar, 1992; Bramel-Cox et al., 1995), several simply inherited qualitative characters have significant impact on forage quality. Examples include brown midrib in sorghum (Fritz et al., 1981) and pearl millet (Cherney et al., 1988) and the presence or absence of trichomes in pearl millet (Hanna et al., 1977). Other characteristics, including plant color, sweetness, juiciness, and even seed pericarp color may affect forage quality. Kalton (1988) proposes that an ideal silage sorghum would include the following traits, presumably for quality enhancement:

- Red seeded
- Yellow endosperm
- No testa layer
- Brown midrib
- Tan plant color
- Juicy stalk
- Moderate to low HCN-p
- High IVDMD
- Good protein content
- Good leafiness and green leaf retention

Genes controlling simply inherited characters that affect quality are available, more so than for most other forage grasses. The genetic knowledge base of forage sorghum and millet is high compared to other forage grasses. However, incorporating all of these, plus high yield and agronomic acceptability, into hybrids would be an ambitious effort by forage sorghum and millet breeders.

Exploring the Future

U.S. land area committed to overall sorghum production has decreased. Land area committed to forage sorghum production is relatively small and appears to have decreased as well. The number of sorghum breeders and research projects in both the private and public sector are declining. It appears that either major changes in markets available for forage sorghum and millet will have to occur and research resources committed to them increase drastically, or the "ambitious effort" needed will have to become "narrow" and "efficient" to continue breeding progress under the status quo. New technologies that may create large new markets for sorghum and millet biomass could be on the horizon. I will begin exploration of the future assuming current research and market trends.

Future Resource Allocation

Forage sorghum and millet research will continue, primarily as a portion of larger sorghum or millet projects or larger forage projects. In many cases, such research may be "bootlegged," or accomplished without any resources officially being committed to forage research. An INTSORMIL example illustrates this trend. Within INTSORMIL itself, five new projects were proposed during the past several months, including one on forages. However after funds were allocated to projects based on need and impact, as determined by an external evaluation committee, the forage project was not funded (D. Walters, INTSORMIL, 1996, personal communication). Increased resources for forage sorghum and millet cannot be expected under the status quo.

Narrow Breeding Objectives

Given very limited, or even borrowed resources, emphasis in forage breeding should be placed on objectives with the highest potential impact. Widely diverse objectives targeted at equally diverse growth stages, morphological types, and markets dilute meager resources even more. Based on land area committed, maximum impact in the U.S. appears to be probable in silage sorghum improvement. Priority should be given to enhancing silage yield and quality.

An example of the need for more focused or concentrated effort might be the incorporation of the brown midrib trait into an acceptable commercial hybrid. Brown midrib has been known in maize since at least 1926 (Eyster, 1926). Brown midrib mutants were originally induced and described in sorghum18 years ago (Porter et al., 1978) and millet in 1988 (Cherney et al.). The clear increase in forage quality (Fritz et al., 1981) resulting in increased animal performance (Lusk et al., 1984; Grant et al., 1995) has been researched and described. Yet, a commercial brown midrib hybrid was not available to growers in 1996. This will soon be accomplished (J. O'Rear, Garrison & Townsend, Inc. 1996, personal communication). Why has it taken so long?

Technology: Do More With Less

Forage sorghum and millet breeding programs will have to acquire (by some means) and utilize the best state of the art technology in order to increase impact. Few fully funded forage programs can afford these technologies alone. Because of limited resources available for forage sorghum and millet breeding, enhancing collaborations among breeders is necessary. Automated plot harvesting equipment is essential for yield testing. Enhanced NIRS capabilities, including "global" prediction equations, will become essential for quality assessment. Both technologies substantially increase the number of lines that can be evaluated, while lowering time and/or funds committed. Development and use of molecular markers for selection will become important for forage parameters as they become available and affordable.

New Technologies/Markets/Industries

Astute readers will have noted that although the title of this paper included the topic of breeding for "fuel," it has not yet been addressed. Because starch is easily converted to sugar and then fermented, a large ethanol industry has developed in the United States, utilizing grain as its primary raw substrate. Midwestern U.S. newspapers routinely report on expansion of already large industrial ethanol projects. On Thursday, Sept. 12, the *Lincoln Journal Star* reported that "High Plains Corp. has announced a $17 million contract to produce industrial-grade ethanol in its York (NE) plant" (Russo, 1996). Considerable effort has been spent developing sweet sorghum lines for ethanol production. A bibliography compiled by Duncan (1993) shows over 100 references related to this topic. At the present time, a fuel industry has failed to develop around sweet sorghum in the United States.

Recent research emphasis has begun to shift from sugar to biomass. Starch is made up of D-glucose units bound in $\alpha(1 \rightarrow 4)$ linkages (amylose) or $\alpha(1 \rightarrow 4)$ chains with $\alpha(1 \rightarrow 6)$ branch points (amylopectin). Plant cell walls are made primarily of cellulose, linear polymers of D-glucose in $\beta(1 \rightarrow 4)$ linkage. Hemicelluloses, polymers of pentoses, are also common cell wall components (Lehninger, 1977). If technologies could be developed to make the glucose in cellulose available, and to make the pentoses in hemicellulose available and fermentable, abundant biomass could become an economical raw substrate for the ethanol industry.

Advances in fermentation technology accomplished with molecular biology are making the above scenario economically possible. Research permitting efficient breakdown of cellulose and hemicellulose to simple sugars is underway (Vogel, 1996). Zhang et al. (1995) have produced recombinant bacteria that can ferment glucose and xylose and produce high (86% of theoretical) ethanol yields. Wyman (1992) indicates that the cost of producing ethanol from biomass in 1992 was $0.38 L ($1.35/gal) and predicted that it may be feasible to produce ethanol for $0.16 ($0.60/gal) by the year 2010, making ethanol from biomass equivalent to $25/barrel crude oil. If this industry develops, our challenge will be developing sorghum and pearl millet forages that can provide biomass that is competitive ecconomically, at acceptable environmental, political, and cultural costs compared to other potential biomass species.

Other developing industries also could utilize sorghum stover as raw substrate. These include industries that produce construction materials, such as fiberboard, and the paper industry. A plan to build an $89 million pulp plant in central Nebraska was recently announced (Daib, 1996). Initial estimates indicate this single plant will need 60,000 to 73,000 ha of corn stalk stover for substrate. Harvest, transportation, and storage technologies are currently being worked out. An immediate challenge is to determine if it is possible to develop sorghum or millet stover that is superior to corn. If so, narrow breeding objectives could be established to develop hybrids with this market as a target.

Conclusion

Forage sorghum and millet breeding programs exist in an era of limited resources. Unless markets for these products grow or are developed, breeders will have to become more efficient and focused to continue serving their current clientele. To close on a positive note, forage sorghum and millet breeders do have a wealth of genes and genetic knowledge to work with that is not available to breeders working with many other forage species. We may be envied by some of our forage breeding colleagues because of the availabilty of such tools in producing varieties and hybrids that meet livestock producers' needs. These tools also should enable focused and rapid response to the needs of new industrial markets as they continue to develop.

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