CHAPTER SIX

Forage Grasses

David A. Sleper

The grass family (Gramineae) includes approximately 75% of the species cultivated as forage crops (Metcalf and Nelson, 1985). The family is distributed throughout the world ranging from subpolar regions to the tropics. Of these, about 150 genera and 1500 species are found growing in the United States. Examples of some cultivated perennial forage grasses in the United States are presented in Table 6-1.

Forage grasses can be grouped into two large categories: warm- and cool-season. Warm-season grasses produce most of their growth during the warmer periods of the growing season, while the opposite is true for cool-season species.

Forage grasses are utilized in many different agricultural production systems. They have their greatest value as feed for livestock. They are also useful for preventing soil erosion and maintaining soil fertility. A species may be grown alone or in mixtures with other species of grasses or legumes at high or low levels of soil fertility. They may be grazed or made into hay or silage and fed to animals.

This discussion is limited to perennial forage grasses that reproduce by sexual seed production or by vegetative propagation. Additional information on the breeding of perennial forage grass cultivars can be found in reviews published by Crowder and Chheda (1982), Hanson and Carnahan (1956) and Poehlman (1979). Development of apomictic cultivars of forage grasses is discussed in Chapter 3.
Table 6-1  Common and Botanical Names of Some Perennial Forage Grasses Cultivated in the United States

<table>
<thead>
<tr>
<th>Common Names</th>
<th>Scientific Names</th>
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<tbody>
<tr>
<td>Bahiagrass</td>
<td><em>Paspalum notatum</em> Flügge</td>
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<tr>
<td>Bermudagrass</td>
<td><em>Cynodon dactylon</em> (L.) Pers.</td>
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<tr>
<td>Big bluestem</td>
<td><em>Andropogon gerardii</em> Vitman</td>
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<tr>
<td>Buffelgrass</td>
<td><em>Cenchrus ciliaris</em> L.</td>
</tr>
<tr>
<td>Crested wheatgrass</td>
<td><em>Agropyron desertorum</em> (Fisch. ex Link) Schult</td>
</tr>
<tr>
<td>Digitgrass</td>
<td><em>Digitaria decumbens</em> Stent.</td>
</tr>
<tr>
<td>Hardinggrass</td>
<td><em>Phalaris aquatica</em> L.</td>
</tr>
<tr>
<td>Indiangrass</td>
<td><em>Sorghastrum nutans</em> (L.) Nash</td>
</tr>
<tr>
<td>Intermediate wheatgrass</td>
<td><em>Elytrigia intermedia</em> (Host) Nevski</td>
</tr>
<tr>
<td>Kentucky bluegrass</td>
<td><em>Poa pratensis</em> L.</td>
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<tr>
<td>Kikuyugrass</td>
<td><em>Pennisetum clandestinum</em> Hochst. ex Chiov.</td>
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<tr>
<td>Kleingrass</td>
<td><em>Panicum coloratum</em> L.</td>
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<tr>
<td>Limpograss</td>
<td><em>Hemarthria alissima</em> (Poir.) Stapf &amp; 1 Hubbard</td>
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<tr>
<td>Little bluestem</td>
<td><em>Schizachyrium scoparium</em> (Michx.) Nash.</td>
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<tr>
<td>Meadow fescue</td>
<td><em>Festuca pratensis</em> Huds.</td>
</tr>
<tr>
<td>Orchardgrass</td>
<td><em>Dactylis glomerata</em> L.</td>
</tr>
<tr>
<td>Perennial ryegrass</td>
<td><em>Lolium perenne</em> L.</td>
</tr>
<tr>
<td>Reed canarygrass</td>
<td><em>Phalaris arundinacea</em> L.</td>
</tr>
<tr>
<td>Russian wildryegrass</td>
<td><em>Psathyrostachys juncea</em> (Fisch.) Nevski</td>
</tr>
<tr>
<td>Slender wheatgrass</td>
<td><em>Elymus trachycaulus</em> (Link) Gould ex Shinnors</td>
</tr>
<tr>
<td>Smooth bromegrass</td>
<td><em>Bromus inermis</em> Leyss.</td>
</tr>
<tr>
<td>Switchgrass</td>
<td><em>Panicum virgatum</em> L.</td>
</tr>
<tr>
<td>Tall fescue</td>
<td><em>Festuca arundinacea</em> Schreb.</td>
</tr>
<tr>
<td>Tall oatgrass</td>
<td><em>Arrhenatherum elatius</em> (L.) Presl.</td>
</tr>
<tr>
<td>Timothy</td>
<td><em>Phleum pratense</em> L.</td>
</tr>
<tr>
<td>Weeping lovegrass</td>
<td><em>Eragrostis curvula</em> (Schrad.) Nees</td>
</tr>
</tbody>
</table>

**TYPES OF CULTIVARS**

**Mode of Propagation**

Most of the perennial forage grass species are cross-pollinated. Many forage grasses, particularly those that evolved from northern latitudes, develop seed heads only after exposure to the winter conditions of cold temperatures, short-day lengths, or both. Apical or axillary meristems subjected to these conditions are capable of producing inflorescences. Floral induction is a hormonal differentiation resulting from the fulfillment of certain thermophotoperiodic requirements. Induction of the meristems, as the result of the winter requirement, may be satisfied by exposure ei-
ther to cold temperatures or short-day lengths, or it may take both and the two may need to occur in a certain order.

Certain species, such as smooth bromegrass (*Bromus inermis* Leyss.), tall fescue (*Festuca arundinacea* Schreb.), reed canarygrass (*Phalaris arundinacea* L.), and perennial ryegrass (*Lolium perenne* L.), need to be exposed to winter conditions to develop floral shoots. However, many of the native range species of the United States, such as big bluestem (*Andropogon gerardii* Vitm.), switchgrass (*Panicum virgatum* L.) and sideoats grama (*Bouteloua curtipendula*) (Michx.) Torr. do not have a winter requirement. Even though the range grasses do not have an apparent winter requirement, winter conditions may accelerate floral induction.

Floral initiation is the morphological transformation of an induced growing point from a vegetative to a floral primordium, which occurs as a response to a favorable photoperiod. The photoperiodic requirements for floral initiation vary among species, but are largely related to latitude. Most species from the temperate latitudes are long-day plants. However, local strains and ecotypes within a species vary in their response. Forage grass species native to the northern Great Plains are largely long-day plants. Strains from the southern Great Plains are largely short-day plants, while those from the central United States show a gradation between the two extremes.

Valuable germplasm of many cross-pollinated species can be maintained by selfing or by vegetative propagation during the breeding process. Selfing may not be possible in some species because of high levels of self-sterility. In some species, selfed seed production is low. Because of its simplicity, and sometimes out of necessity, vegetative propagation is preferred to inbreeding because of poor seed production.

Vegetative propagation of cool-season grasses is usually most successful in the spring or fall. However, many warm-season grasses may be vegetatively propagated successfully in early or mid-summer, provided that moisture is not limiting. Plant fragments consisting of vegetative tillers, rhizomes, or stolons are used. When a vegetative tiller is used, the old leaf sheaths are removed and roots are trimmed. If old sheaths and roots are left on the tillers, root rot may develop. The vegetative plant parts may be planted directly into field plots or may be established in pots in the greenhouse and later transplanted to the field.

Seed may be sown directly into research plots in the field. When the seed supply is low, it may be germinated on blotting paper, sterilized sand, vermiculite, or soil. After seedlings have reached a desirable size, they can be transplanted to the field.

Seed dormancy exists to some extent in most forage grass species and can pose problems for the breeder. Dormancy can be successfully over-
come by subjecting the seed to cold temperatures. The type of cold treatment necessary to break dormancy varies according to the species.

Past and Current Cultivar Types

Historically, the cultivars of perennial forage grasses were heterogeneous ecotypes, defined as ecological races with genotypes adapted to a particular habitat as a result of natural selection. Vegetatively propagated forage grasses came from individual plant selections. Ecotypes are still used on a limited scale, but are not desirable because of their poor agronomic performance and lack of uniformity. Modern farming practices dictate that uniformity be present for such traits as maturity, forage quality, and seed production.

Today, perennial forage grass cultivars may be apomictic, synthetics, or hybrids. Single plant selections are used for vegetatively propagated species. See Chapter 3 for a discussion on apomictic cultivars.

Most perennial forage grass cultivars are synthetics. A synthetic cultivar is one in which the parents have been selected for good general combining ability and mated in all possible combinations. The cultivar is maintained through open-pollination. Because, the parents are largely cross-pollinated, the synthetic cultivar is highly heterozygous. An example of a synthetic cultivar is 'Kenhy' tall fescue (Buckner et al., 1977). It contains 11 parents that were selected for good general combining ability.

There has been little success in developing hybrid cultivars of open pollinated perennial forage grasses. The reasons are that reliable genetic male-sterile systems are lacking and the cost of producing F1 seed has been prohibitive.

The highest degree of success in developing F1 hybrids has occurred in vegetatively propagated perennial forage grass species. Many hectares in the southeastern part of the United States are vegetatively propagated with F1 hybrid bermudagrasses [Cynodon dactylon (L.) Pers.], and example of which is 'Coastcross-I' (Burton, 1972). These vegetatively propagated hybrid cultivars are homogeneous because they are derived from one plant resulting from a cross between two heterozygous parents.

EXTENT AND NATURE OF BREEDING PROGRAMS IN NORTH AMERICA

Most of the forage grass breeding is conducted by public institutions, including state agricultural experiment stations, land grant universities, and the United States Department of Agriculture, Agricultural Research Service (USDA-ARS). Most forage grasses, because of their perennial
growth habit, have limited commercial demands for seed or vegetative propagules. As a result, there is less interest by the private sector in cultivar development for perennial forage grasses than there is for many other crops. Breeding of perennial forage grasses does occur in the private sector, but is limited mostly to the seed producing perennial forage grasses, such as tall fescue and orchardgrass. The level of activity in the private sector has increased somewhat since the passage of the U.S. Plant Variety Protection Act in 1970.

**BREEDING OBJECTIVES FOR CULTIVAR DEVELOPMENT**

If forage grass breeders are going to be successful in developing a superior cultivar, they must know what characters have value. Because forage grass species differ markedly in mode of reproduction, growth habit, and growth cycle, the list of breeding objectives can be quite varied and depends on the species that is under improvement. For example, a breeder working with a species that has vegetatively propagated cultivars need not spend time or effort breeding for improved seed yield. However, much time needs to be devoted toward improving seed yield of those species that are commercially propagated by seed.

The probability of achieving the objectives of forage grass breeding should be considered. This may influence the choice of species that the breeder ultimately works on. Most forage grass breeders have more than one choice of species for their given locale. The best of several adapted but unimproved perennial forage grass species may be better than the poorest adapted species can be after genetic improvement. When choosing a species for a breeding program, the advice of persons who have had previous experience with the crop should be obtained, such as animal scientists and agronomists. Experiments designed to test the adaptability of alternative forage grass species to a given set of environmental conditions may be an important first step in establishing an extensive breeding program.

**Herbage and Seed Yield**

Developing forage grass cultivars with high herbage yield of superior quality is a goal of most breeders. Herbage yield is under the control of many genes, and the heritability for the trait is low. Progress in breeding for improved yield does occur with our current plant breeding procedures. However, in many instances, progress is slow. Progress in improving yield is often associated with breeding for insect and disease resistance and responsiveness to fertilization and management. In some
species, herbage yield can be enhanced by capitalizing on the heterosis obtained in hybrids.

Because forages are consumed by animals, breeders need to be concerned with supplying forage that has a high nutritive value. If the forage is to be harvested by a grazing animal, a cultivar should have a uniform growth rate over an extended period of time because grazing animals eat about the same amount of forage each day. If the forage is to be machine harvested for hay or silage, rapid seasonal growth that gives high herbage yield and quality may be preferred.

Components of herbage yield have been researched to identify factors that have a major impact on genetic improvement. Most of these components involve physiological traits. Sleper (1985) reviewed the relationship on physiological traits as they relate particularly to herbage yield in tall fescue and to a lesser extent for several other cool-season grasses. Physiological traits, such as carbon exchange rate, leaf area expansion rate, and dark-respiration rate have great potential for improving herbage yield of forage grasses. However, no forage grass cultivar has been released to date as the result of selecting for individual components of herbage yield. Comments on future possibilities are presented in the last section of this chapter.

When developing a cultivar of a forage grass species that is commercially propagated by seed, selection for adequate seed yield cannot be neglected. The failure of new forage grass cultivars is often due to inadequate seed production at a competitive price. Unfortunately, in many instances, there is a negative correlation between high seed yield and high herbage yield. For a new cultivar to be successful, care must be taken to keep both characters at a satisfactory level. The availability of adequate supplies of forage grass seed is essential to the maintenance of a strong forage grass agriculture in many regions of the world. Efficiency of seed production is also of prime importance during the seed multiplication phase of releasing new cultivars for commercial use.

When breeding for improved seed yield, it is useful to know the extent and nature of genetic variation, genotype × environment interactions, and heritability. The genotype × environment interactions are particularly important because selection of seed yield often takes place in a different location than where the seed ultimately will be produced (Hanson and Carnahan, 1956).

Breeding for satisfactory levels of seed production may involve selection for early maturity to avoid droughts, adaptation to a certain day length, resistance to frost, seed size, reproductive tiller height, and lodging. Understanding the correlations among these characters is useful to determine both favorable and unfavorable correlated responses to selection. In tall fescue, narrow-sense heritabilities estimated on an individual-plant basis were high for maturity score, number of panicles, panicle
length, and seed yield (Sleper, 1985). These same estimates were moderate for 1000-seed weight and seed weight per panicle. Heritability estimates were low for plant height, lodging score, reproductive herbage yield, and number of seeds per panicle. Predicted genetic gains per cycle for phenotypic selection were 33 (maturity score), 45 (number of panicles), 27 (panicle length), 34 (seed yield), and 14% (100-seed weight) of the population mean. Correlation analyses using half-sib family data showed that early-maturing plants had more panicles, shorter panicles, and higher seed yields. Long-term breeding experience with tall fescue indicates that favorable correlated responses with seed yield and size occurs when selecting plants for early maturity.

Quality

Breeding forage grasses for improved quality is an important objective that involves making changes within the plant to prevent animal disorders, enhance their productivity when consuming the herbage, or both. Forage quality may be defined as the degree of excellence of a forage when fed to livestock (Burton, 1978). Forage quality is a difficult parameter to characterize because genotype × environment interactions are important. Forage quality is ever changing because it declines as the age of the forage grass advances. To accurately characterize forage quality, the management regime under which the forage grass is growing must be defined. For reviews on breeding forage grasses for improved quality, the reader is referred to Burton (1978), Hacker (1981), and Sleper (1985).

Improving digestibility. Improving the digestibility of forage grasses has been a goal of many forage grass breeding programs. Digestibility is the preparation of feed for absorption. The development of in vitro rumen fermentation techniques has led to great advances in improving the digestibility of perennial forage grasses and, hence, animal performance.

The in vitro rumen fermentation techniques are based on incubation of forage tissues with portions of rumen contents (Hacker, 1981). The basic components of the in vitro techniques include the ground herbage substrate, an “artificial saliva” or nutrient-buffer solution, and the rumen inoculum.

An example of an in vitro rumen fermentation used by forage grass breeders is one proposed by Tilley and Terry (1963). The oven-dried, ground herbage sample is incubated for 48 hours at 38°C with a mixture of a buffer solution and strained rumen fluid. The fermentation occurs in glass centrifuge tubes sealed with a stopper to maintain anaerobic conditions. At the end of the first stage, bacterial activity is stopped by the addi-
tion of mercuric chloride (HgCl₂), followed by centrifugation. The supernatant is discarded and a pepsin solution is added to the residue, which is incubated at 38°C for 48 hours. Anaerobic conditions are not necessary during the second stage. At the end of the second stage, the insoluble residues are washed and dried to constant weight to determine the dry weight of the residue. This dry weight is subtracted from the dry weight of residue found in the blank tubes, which contain indigested feed particles and microorganisms derived from the rumen fluid. This is then the weight of digested sample.

The inheritance of in vitro dry matter disappearance (IVDMD) in forage grasses is not fully understood because heritability estimates for many species are lacking. Considerable research on inheritance of IVDMD in tall fescue has been reported (Sleper, 1985). When existing cultivars of tall fescue are evaluated for IVDMD at comparable stages of maturity, the differences usually are not significant. In broad-based breeding populations, the inheritance of IVDMD differs according to the season of the year. Some studies report no significant genetic variation within a population for the entire season, while some have reported poor genetic variation in the fall and higher values for the spring and summer (Sleper, 1985).

Vogel and Gabrielsen (1985) reported on results of selecting switchgrass for differential IVDMD levels using a rumen fermentation procedure. The high and low IVDMD populations derived from open-pollination of selected clones were used to establish replicated pastures, and the performance of animals that grazed the pastures was evaluated for 2 years. The high IVDMD population had a digestibility value of 48.9%, while the low selection was 44.1%. Average daily gains of animals grazing the high IVDMD population was 0.73 kg/day compared with 0.59 kg/day for the low IVDMD population. The high IVDMD population was released as the cultivar ‘Trailblazer.’

Other methods of assaying the digestibility of the herbage from grasses are available. The newest technology involves evaluating herbage quality with near-infrared reflectance spectroscopy (NIRS). The technique involves application of radiant energy in the near-infrared portion of the spectrum to dry, ground forage samples. The reflected energy is empirically related to quality parameters, such as protein content, digestibility, mineral content, and several other forage quality components. Research is in progress to determine the usefulness of NIRS as a tool in evaluating forage quality. The use of NIRS has the potential to allow the breeder to assay many more samples than with conventional in vitro rumen fermentation procedures.

Another recent breeding tool to assess herbage digestibility is to digest ground forage samples in a cellulase solution. The breeder can buy commercial preparations of cellulase or it can easily be produced as the result
of culturing the fungus *Trichoderma reesei* Simmons. The fungus is
grown on a mineral medium that is conducive for producing large amounts
of the cellulase solution with sufficient activity to digest dried, ground herbage
samples. The procedure involves extracting the dry, ground herbage
with a neutral detergent solution and then subjecting it to exhaustive
hydrolysis by the standard cellulase solution. Correlations between the
fungal enzyme digestion procedure and in vivo dry matter digestibility
usually are greater than 0.95. The cellulase procedure provides breeders
the opportunity to obtain estimates of IVDMD inexpensively when
animals cannot be raised as a source of rumen fluid.

*Changing Maturity.* As the forage grass plant advances to later stages of
maturity, the IVDMD decreases. The IVDMD often will be lowest at
the time of seed maturation. Forage grass plants should never be har-
vested by animals or made into hay or silage at advanced stages of matur-
ity. The best quality forage is realized when plants are harvested at imma-
ture stages, even though it may result in sacrificing some herbage dry-mat-
ter yield. The stage of maturity at which the maximum yield of digestible
nutrients can be harvested will vary for different forage grass species and
will depend on the environment in which the forage is produced. Selecting
forage grasses that mature later in the growing season can improve quality
by making highly digestible forage available for a longer period of time.

*Disease Resistance.* Most of the research related to breeding for resis-
tance to diseases has been directed toward increasing herbage yield and
quality and seed yield. There is strong evidence that fungal infection is di-
rectly related to reduced forage quality and animal performance. Di-
ferent diseases have different effects on herbage quality, ranging from
zero to a loss of more than 1 digestibility unit for every 10% increase in
diseased area.

Edwards et al. (1981) histologically compared healthy orchardgrass
leaf blades with those infected by the stem rust *Puccinia graminis* Pers. f.
*sp. dactylidis* Guyot et Massinot after various periods of *in vitro* digestion
by bovine rumen fluid. Sections from diseased leaves showed no apparent
digestion of tissues under uredia and only partial digestion of adjacent tis-
sues. Stem rust of orchardgrass reduces not only herbage yield but also
forage quality.

Two experimental orchardgrass synthetics were developed at the
University of Missouri that had high levels of resistances to *P. graminis*
*f. sp. dactylidis*. The two experimental synthetics were compared with
three check cultivars for 3 years in replicated grazing trials. Stem rust
was present in only 1 of those 3 years. For that particular year, the
animals grazing the two experimental synthetics had significantly higher
average daily gains. The improved resistance to *P. graminis* for the ex-
perimental synthetics compared with the check cultivars led to improved animal performance as the result of providing more disease-free herbage with higher digestibility. Orchardgrass cultivars have been released with improved levels of resistance to the stem rust pathogen. However, these releases have occurred without conducting animal evaluation trials.

*Removing Antiquity Components.* Antiquity components in forage grasses can severely reduce animal performance. Alkaloids in reed canarygrass have contributed various disorders in animals, including diarrhea, weight loss, and lack of appetite. Alkaloid concentration is highly heritable and under the control of two genes (Marum et al., 1979). Alkaloids have been identified in several other forage grasses, but little is known about the inheritance. In many other instances, the toxic components in the herbage have not been identified and, therefore, the forage grass breeder cannot make desirable modification in the plant.

Two of the more recently identified toxicity problems in forage grasses are the summer syndrome caused by tall fescue and the ryegrass staghers caused by perennial ryegrass (Siegel et al., 1985). Summer syndrome in cattle is characterized by rough hair coats, reduced rate of gain or milk production, increased rates of respiration, increased body temperature, a preference for shade, and standing in water during hot weather. Toxic tall fescue pastures have been shown to be highly infected with an endophytic fungus identified as *Acremonium coenophialum* Morgan-Jones and W. Gams Tul. Ryegrass staghers is reported to be caused by a similar fungus and causes similar symptoms in sheep when they graze infected perennial ryegrass. Steers grazing on infected tall fescue near Auburn University, Alabama, had average daily gains (ADG) of 0.50 kg/day while those on uninfected tall fescue (less than 5%) had ADG of 0.83 kg/day. Several researchers have shown a positive correlation between pyrrolizidine alkaloid content and summer syndrome. At present, it is not entirely clear what role, if any, alkaloids play in summer syndrome.

Both the tall fescue and ryegrass endophytes are seed-transmitted. Others mechanisms of transmission may be possible, but have yet to be proved. The level of infection in a particular field is closely related to the percentage of the viable fungus in the seed used to plant the field. Fields established with fungal-free seed remain free of the endophyte, if all sources of contaminated seeds are prevented from entering the field and germinating.

Breeders wishing to eliminate the viable endophyte from their seed stocks can do so after proper aging of the seed. Both the seeds and the endophyte continue to respire while in storage. The endophyte-infected seed has higher rates of respiration than endophyte-free seed. The respiration rate of the fungus is greater than that of the seed and the endophyte
dies before the seed. The viability of the fungus in the seed is affected by the length and conditions of storage. Higher temperatures and humidity are more damaging to the viability of the fungus than are cooler and dryer conditions. Storage studies of tall fescue seed conducted on farms in Alabama indicated that the percentage of seeds with viable endophyte was reduced to zero after 12 months. Eradication of the viable endophyte from seeds is also possible after a hot-water treatment or through the use of fungicides.

Several tall fescue cultivars are released and certified as having little or no endophyte. These include ‘Kenhy,’ ‘Missouri-96,’ ‘Triumph,’ ‘Forager,’ ‘Johnstone,’ ‘Mozark,’ and ‘Martin.’

High levels of oxalate in forage grasses have been associated with malformation of bone in nonruminants and with kidney damage in ruminants. Oxalate accumulation is observed most often in the panicoid grasses rather than the festucoid and is common in the genera *Pennisetum, Cenchrus, Setaria*, and *Panicum*. Broad-sense heritability values are low and range from 0 to 35%.

*Increasing Nutrient Content.* Inadequate levels of minerals in forage grasses often limit animal performance. In tropical grasses, nitrogen, phosphorus, copper, cobalt, and sodium are often deficient.

Grass tetany or hypomagnesemia is a metabolic disorder of ruminants that has been related to inadequate levels of utilizable magnesium. Magnesium may not be available to ruminants because concentrations are low in forage grass herbage, or because other substances interacting within the animal lead to lower blood serum magnesium levels. Grass tetany has been observed most frequently in ruminants grazing lush, cool-season forage grasses. Increasing the intake of magnesium by the ruminant will generally prevent grass tetany. It is also necessary to have a proper balance of potassium and calcium in association with magnesium.

Inheritance of the minerals associated with grass tetany are generally adequate to make progress in selecting for proper mineral balance in cool-season grasses, such as tall fescue, perennial ryegrass and orchard grass. Heritability estimates are frequently high, but the values reported depend on the genetic characteristics of the small number of plants that usually are studied.

Breeders have shown interest in improving the percentage of water-soluble carbohydrates (WSC). An environment that is cool and has high light intensity is conducive to high levels of WSC. As expected, ecotypes from more temperate climates have the highest concentration of WSC. Care should be taken in selecting for high concentrations of WSC because they generally are negatively correlated with protein concentration, although sufficient genotypic independence exists to allow simulta-
neous selection for improvement of both traits. Cultivar differences in concentration of WSC of almost twofold were recorded in perennial ryegrass and orchardgrass (Hacker, 1981).

Animal Performance

When quality components are improved in the herbage, the forage grass breeder hopes that this will be translated into improved animal performance. The only way to determine this is to conduct animal feeding trials. It is important, therefore, that forage grass breeders cooperate with animal scientists in cultivar evaluation.

Important aspects in breeding for improved animal performance include improving palatability and intake. Intake cannot be measured on small samples directly; therefore, the forage grass breeder must rely on correlated characters. Examples of such characters are leafiness and animal preference.

Most animals prefer to graze leaves as opposed to stems. Breeders have assumed that intake is best from leafy selections compared with more stemmy ones. Some animal studies have shown that the intake of leaf material is higher than stem material when they are fed at comparable digestibility levels. However, other studies have shown that leafiness is not related to intake in certain cool-season grasses. The relative importance of leaves and stems as it relates to intake should be determined for each species.

Cafeteria trials have been conducted with variable success using animals to select more palatable plants. The cafeteria trial defined in this instance is one where animals are given the opportunity to select plant genotypes within a species of equal availability and accessibility.

Certain cultivars of reed canarygrass are unpalatable when animals are given a choice. Unpalatability in this species is related to high alkaloid content. In weeping lovegrass [Eragrostis curvula (Schrad.) Nees.], selection for palatability gave rise to the cultivar ‘Morpa.’ Grazing intensity on a large number of tall fescue clones was observed and used to develop the cultivar ‘Kenwell.’ Later grazing trials, however, showed no advantage of ‘Kenwell’ over ‘Kentucky-31’.

Persistence

Because many of the forage grasses are perennial, persistence of swards is important. Ability of stands to persist depends on both the genetic characteristics of the cultivar and management factors. Reduced persistence may result from excessive heat and cold temperatures, drought stress,
poor fertility, excessive defoliation by machines and animals, and infestations from insects and diseases. The forage grass breeder needs to eliminate or minimize these factors to develop successful cultivars that persist.

**Disease Resistance**

Forage grasses are attacked by many pathogens, which results in lowered forage yield and persistence. Some of the more common pathogens are those that cause leaf diseases, including stem rust, leaf rust, and crown rust. The types of pathogens and the economic importance of the diseases vary among grass species, the environment in which they are produced, and the manner in which they are utilized. Many forage grasses are susceptible to root and crown rots. It is estimated that pre-emergence rots destroy an estimated 25% of all forage grass seed planted. Other diseases are caused by nematodes and, in certain years, ergot can cause serious reductions in seed yields.

**Insect Resistance**

Insects can be very damaging to forage grasses by reducing herbage yield and quality. If insects feed on floral structures, seed yields will be reduced. Insects may be vectors for disease pathogens and viruses. Insect damage to plants may provide avenues of entrance for many unwanted pathogens.

When breeding for resistance to insects, the forage grass breeder needs to consider the genetics of the pest and the host because complex interactions exist between insects and plants which may be anatomic, biochemical, or physiological in nature. Plant resistance may be obtained through nonpreference (suppression of feeding or oviposition), antibiosis (adverse effects on normal growth or survival of insects), and tolerance (ability of the plant to survive when insects are present).

**STEPS IN CULTIVAR DEVELOPMENT**

The steps involved in breeding forage grass cultivars will differ among species depending on the type of cultivar that is grown commercially. Most forage grasses are produced as synthetic cultivars. Breeding synthetic cultivars of forage grasses includes six steps: establishment of a source nursery, phenotypic selection, progeny evaluation, formation of experimental synthetics, animal performance trials, and release. Breeding vegetatively propagated forage grass species includes establishment of a
source nursery, phenotypic selection, animal performance trials, and release.

**Source Nursery**

Collection of germplasm is an important responsibility of the forage grass breeder. A broad-based collection is necessary if significant progress is to be realized through selection. Materials for inclusion into a source nursery may come from plant introduction stations located in the United States and other countries, natural ecotypes or landraces, germplasm pools created to produce unique gene combinations, old cultivars, and specific crosses among selected parents. The material collected for inclusion in the source nursery likely will be obtained from areas of similar environmental conditions to those where the new cultivars will be utilized. The type of material collected will depend on the breeding objectives. For example, if the objective is to breed for improved persistence under higher temperatures, collections should be obtained from areas of higher temperatures so that the plant breeder can take advantage of natural selection that may have occurred for this trait.

**Phenotypic Selection**

Before embarking on a selection program, the plant breeder should determine the mode of inheritance for the particular trait(s) under selection. Is the trait under the control of one or a few genes (qualitatively inherited) or is it controlled by many genes (quantitatively inherited)? If the trait is qualitatively controlled, the progeny from crosses between selected parents will permit the breeder to determine the mode of inheritance and suggest how to incorporate the desirable traits into an improved cultivar.

Forage grass breeders generally select for traits that are quantitatively inherited, such as herbage or seed yield. Quantitative genetic information regarding the source populations is needed to plan effective selection programs. It is necessary to obtain information on the extent and nature of genetic variation, heritability, genotype × environment interactions, correlations, and prediction of genetic advance by selection.

Many selection schemes involve recording data and selecting on an individual clonal basis. Most forages will be grown as swards in many different environments, and there may be little relationship between clonal performance and performance when the forage is grown as a sward. To overcome the problem of predicting clonal performance under different environments and relating it to solid seedings, the best-performing clones may be removed from the source nursery and subjected to varying
degrees of clonal evaluation, either in conjunction with the production of seed for progeny tests or in special clonal tests.

Progeny Evaluation

After selection of desirable clones from the source nursery, it is necessary to determine which clones would combine well to form an experimental synthetic cultivar for further testing. This is accomplished by evaluating the progeny from selected clones. The topcross, polycross, and open-pollinated progeny tests are examples of procedures used to evaluate general combining ability of potential parents for making experimental synthetic cultivars.

Experimental Synthetics

Clones with good general combining ability, as determined from progeny tests, are used as parents. The number of clones used should be sufficient so that when the seed is advanced for several generations, inbreeding is kept to a minimum. The number of clones needed can only be determined after having experience with a species. Most synthetic cultivars of perennial forage grasses have between 4 to 25 parental clones. The number of clones used may be related to the ploidy level of the species, with higher ploidy species requiring less numbers of parents than those with lower ploidy.

Seed production of an experimental synthetic begins with the establishment of an isolated polycross nursery. Because it is important to produce seed under conditions of random mating, clones will be replicated. The number of replications depends on the amount of clonal material available. The breeder should strive for at least 10 replications because it is important to multiply seed supplies rapidly to meet expected demand for the new cultivar. Care should be taken to have a similar amount of pollen shed from all plants. This can be accomplished by having roughly the same number of flowering culms per plant. Those plants possessing many flowering culms will have some removed to equal those on plants containing fewer flowering culms. It is important to have plants flower at the same time. This should not be a major problem if maturity of the plants was recorded accurately.

The parent clones of the experimental synthetic are designated as the Syn 0 generation. Equal amounts of pure viable seed is bulked from all replicates for each entry in the polycross nursery to obtain Syn 1 seed. This may also serve as breeder seed.

Forage grass breeders may choose to produce the Syn 1 seed in the
greenhouse, and make all possible single crosses by hand to improve random mating. However, seed yields are usually less in the greenhouse than in the field.

Syn 1 seed needs to be advanced one or more generations to have adequate seed stocks for further evaluation. Syn 1 seed is planted in isolation in rows to allow for hand removal of possible off-type plants and to aid in cultivation to control weeds. Seed from Syn 1 plants is harvested in bulk by machine or hand. The Syn 1 seed is planted in isolation block either in rows or swards and harvested in bulk. This process is repeated until seed supplies are adequate for testing or release. It usually is desirable to increase seed at least to the Syn 3 generation before testing. Early-generation synthetics may have a high degree of heterosis, and the performance may not be representative of the Syn 4 or later generations that the farmer would obtain.

The experimental synthetics and check cultivars are tested in small replicated plots. Plots are established at several locations and evaluated for 3 to 5 years. The types of data collected will depend on the objectives established before selection. For example, if selection was practiced for improved IVDMo, this trait will be evaluated for all entries in the test. The forage breeder is always interested in herbage yield, persistence of perennials, ability to recover after clipping, and other traits.

Separate plots are needed to evaluate seed yield of the forage grasses that are grown commercially from seed. Seed production of many forage grasses is produced in areas far from where they ultimately will be used. For example, seeds of many cool-season grasses are produced in the northwestern part of the United States, while the primary usage of the forage occurs in the Midwest and in the northeastern parts of the country. It is necessary to conduct seed yield trials in areas where the seed is going to be produced.

Animal Performance Trials

Because most forage grasses are primarily used as feed for animals, experimental synthetics should be evaluated for animal performance before release. Techniques used in evaluation of forage grasses by animals should in some way be related to output per animal or output of animal product per hectare, if the synthetics are to be useful to the plant-animal production system.

The best-performing experimental synthetics in the small-plot tests, along with selected checks, will be used in this phase of cultivar development. The number of experimental entries used in this phase may only be one to three because of the high labor requirements for animal trials.
Release

The development of a forage grass cultivar can take 10 years from the time the plants are placed in the original source nursery until the selection and testing procedures are completed. After completion of the breeding and testing procedures, the desirable selection chosen for release must be increased so that it can be available on a commercial scale. In the case of forage grasses, increases can occur from seed (sexually and apomictically) and through vegetative propagation, depending on mode of reproduction. Production of breeder seed or vegetative stocks is generally under the supervision of the plant breeder. The foundation class of seed is produced by the institution or company that developed the cultivar. The registered and certified classes are produced by selected growers, who in turn sell to commercial producers.

When releasing a cross-pollinated forage grass cultivar, a high degree of uniformity is difficult to obtain. Although a high level of uniformity may not be a necessary requirement, the new cultivar must breed true for the traits for which it was developed. As a result, great care is taken in developing the Syn 1 generation.

SOURCES OF GENETIC VARIABILITY

Types of Parents and Populations

Most cool-season or temperate forage grasses used in the United States evolved largely in Europe and western Asia (Borrill, 1976). Examples of some forage grasses evolving in this area are perennial ryegrass, annual ryegrass (*Lolium multiflorum* Lam.), timothy (*Phleum pratense* L.), orchardgrass, tall fescue, and smooth bromegrass.

Many of the forage grass species are polyploids. Both autopolyploids and allopolyploids occur, and many species are intermediate to these two categories. The polyploid nature makes it difficult to study the inheritance of important characteristics and provides a challenge in selecting desirable parental stocks. The difficulty that polyploidy can pose to breeders can be illustrated with orchardgrass. Several researchers have suggested that orchardgrass is an autopolyploid (2n = 4x = 28). Limited data available from the segregation of chlorophyll-deficient seedlings indicates tetrasomic inheritance. It also has been suggested that two closely related diploid species have contributed to the ancestry of orchardgrass. Recent meiotic data collected for 26 full-sib genotypes of orchardgrass that had given nontetrasomic segregation for reaction to the stem rust fungus (*Puccinia graminis* Pers. f. sp. *dactylidis* Guyot et Massenot) showed an
appreciable excess of bivalents compared with the number expected for independent association of long and short chromosome arms (Lentz et al., 1982). The most probable cause of this excess of bivalents was preferential pairing due to slight genomic differentiation in an autotetraploid background. Excess bivalents may have arisen also from close proximity of the two ends of each chromosome upon initiation of pairing. Inequality of chiasma formation between long and short chromosome arms was a minor contributor of excess bivalents. These observations can be reconciled if orchardgrass is not considered as a true autotetraploid, but rather a segmental allopolyploid. As a result, genetic ratios may range from being disomic to tetrasomic. Situations of this type also may exist in other forage grass species.

Another example of a polyploid species is tall fescue. Its allopolyploid nature provides a challenge in the breeding of improved cultivars (Sleper, 1985). Allopolyploidy causes difficulties in genetic analysis because allelic variation, genome constitution, and ploidy level influence phenotypic expression. The allopolyploid series and its genomic constitution are presented in Table 6-2. Tall fescue contains cultivated hexaploid and wild tetraploid, octoploid, and decaploid botanical varieties, all founded on a basic chromosome number of $x = 7$. The diploid species *Festuca pratensis* Huds. morphologically resembles *Festuca arundinacea* Schreb. and has contributed a genome to the cultivated hexaploid. The phylogenetic relationships among tall fescue and its relatives are not completely understood. Some *Lolium* species also cross readily with certain members of the *Festuca* genera.

Fescues have few diploids, resulting in difficulty in analyzing the parentage of the polyploid forms. Low crossability and hybrid infertility

<table>
<thead>
<tr>
<th>Table 6-2 Proposed Genomic Formula of <em>Festuca</em> Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
</tr>
<tr>
<td>----------------------------------------------------------</td>
</tr>
<tr>
<td><em>F. pratensis</em> Huds.</td>
</tr>
<tr>
<td><em>F. arundinacea</em> var. <em>glaucescens</em> Boiss</td>
</tr>
<tr>
<td><em>F. mairei</em> St. Yves</td>
</tr>
<tr>
<td><em>F. arundinacea</em> var. <em>genuina</em> Schreb.</td>
</tr>
<tr>
<td><em>F. arundinacea</em> var. <em>atlantigena</em> St. Yves</td>
</tr>
<tr>
<td><em>F. arundinacea</em> var. <em>letourneuxiana</em> and <em>citensis</em></td>
</tr>
</tbody>
</table>

From Sleper (1985)
occur in matings between the ploidy levels, and between geographic races having the same chromosome number.

Because forage grass breeders are heavily involved with species that are polyploid, many breeding programs work in direct cooperation with a cytogeneticist. Many forage breeders also conduct research themselves on the cytogenetics of forage grasses. Cytogenetics as related to forage grass breeding has two purposes: to elucidate the taxonomy and phylogeny of the Gramineae and to contribute fundamental information to improvement of forage grasses through breeding. Cytogenetic investigations help the breeder by contributing fundamental information on chromosome numbers, the nature and level of polyploidy, development of apomictic embryo sacs and the existence of aneuploids and chromosome series that are useful in breeding. The phylogenetic information obtained by cytogenetic investigation is useful, for example, in making decisions on what crosses will succeed for the successful incorporation of desirable traits from related species. This information also may be useful in helping the breeder to decide the direction in which certain crosses should be attempted.

Certain warm-season grasses utilized in the United States are of African origin (Harlan, 1976). Examples of these include bermudagrass, [Cynodon dactylon (L.) Pers.], pangolagrass or digitigrass (Digitaria decumbens Stent.) and buffelgrass (Cenchrus ciliaris L.). Bermudagrass also traces its origins to Asia and Europe. It is a species that appears in early history. The Hindus considered it sacred because it was the main component of the diet of cattle.

Some warm-season grasses are native to North America (Riley and Vogel, 1982). These include switchgrass, indiangrass (Sorghastrum nutans L.), the tall bluestem complex of big bluestem (Andropogon gerardii Vitman.) and sand bluestem. These grasses can be found in native rangelands and as components in pastures and conservation plantings.

Most forage grasses are cross-fertilized and largely self-sterile. As a result, individuals are highly heterozygous, which contributes to highly heterogeneous populations. Breeders have largely relied on the existing genetic variability within a given species as source material to make progress through selection. Other sources of genetic variation include planned crosses among selected clones, commercial cultivars, and populations improved by recurrent selection. Gene transfer for the purpose of improving the existing genetic variability through intergeneric or interspecific crosses also has occurred frequently. Examples of gene transfer through wide crosses has been reported in Cynodon, Cenchrus, Paspalum, Panicum, Festuca, Elymus, Dactylis, Phleum, Poa, and Bromus. The variability generated through wide crosses generally has not been utilized to its full potential in cultivar development. The tremendous diversity that occurs naturally and that is artificially induced is under the care of the
plant breeder. The forage grass breeder is responsible for collecting and preserving this variability and ensuring that it is used wisely for the benefit of humankind.

There continues to be a great need to collect additional germ plasm for most of the warm- and cool-season forage grass species to ensure that adequate genetic diversity will exist for future generations. Forage breeders need to be concerned that centers of origin of many forage grasses may not contain the wealth of diversity they once had because many natural habitats have been destroyed or altered by human encroachment.

Plant breeders wishing to improve the genetic diversity by plant collection need to know the center of origin for their species. In addition, information is needed on the latitude and altitude, at which the species grows in the center of origin and the ploidy level of potential plant collections. Information, such as previous use of the plant material, latitude, grazing pressure, moisture regime, and soil type, will affect photoperiodic requirements of germplasm from diverse origins.

Population Development by Hybridization

Procedures for Artificial Hybridization. Most warm- and cool-season grasses have perfect flowers (Hoven, 1980; Burson, 1980). There are exceptions, including buffalograss \([\text{Buchloe dactyloides} \text{ (Nutt.) Engelm.}]\), which is dioecious, and Eastern gamagrass \([\text{Tripsacum dactyloides} \text{ (L.) L.}]\), which is monoecious.

The basic floral unit of forage grasses is the spikelet. Groups or clusters of spikelets make up the inflorescence. The spikelet is in the axil of two leaf-like bracts called glumes. The floret consists of the pistil, stamens, and the lemma and palea (Fig. 6-1).

Pollen shedding occurs at different times for the various species (Table 6-3). For example, orchardgrass, timothy, and bluegrass shed pollen in the morning, whereas smooth bromegrass and Rhodesgrass \([\text{Chloris gayana} \text{ Kunth.}]\) shed pollen in the afternoon. The time of day for pollen shedding can vary according to the environmental conditions, such as soil and air moisture, temperature, light, and soil fertility.

Emasculation of forage grasses may be necessary in making hand crosses. Hovin (1980), Burson (1980), and Hanson and Carnahan (1956) have discussed preparation of the female parent by emasculation. Emasculation is unnecessary, however, if plants to be used as females can be isolated that are either completely male-sterile or completely self-incompatible.

If the forage grass breeder is interested in producing large amounts of crossed seed from completely male-sterile or self-incompatible plants, desirable individuals can be spatially isolated from other plants of the
Figure 6-1 Grass flower. Typical floret at time of blooming. (A) The lemma and palea have been forced apart and the stigma and stamens have been exposed. (B) Parts of the grass flower with lemma and palea removed (Poehlman, 1979).

species. This may be done in the greenhouse or the field. Distance is the principal means of isolation. However, differences in maturity of the parents to be mated also may be used effectively. It is difficult, however, to obtain sufficient isolation for a large number of plants simultaneously.

The emasculation of forage grass florets is not an easy task because florets are generally very small. Emasculation is tedious and slow and is generally used only if the breeder is working with a limited number of panicles. If inflorescences are compact, it is necessary to do some thining of the florets before emasculation is started. This will reduce the time required for emasculation and reduce the possibility of overlooking florets. The inflorescences should be emasculated in a systematic manner,
<table>
<thead>
<tr>
<th>Species</th>
<th>Scientific Name</th>
<th>Common Name</th>
<th>Mode of Pollination†</th>
<th>Average Pollen Dispersal Period per Inflorescence</th>
<th>Time of Day of Anthesis and Pollen Shedding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>days</td>
<td>hours</td>
<td></td>
</tr>
<tr>
<td>Cool-season grasses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agropyron cristatum (L.) Gaertn</td>
<td>Fairway wheatgrass</td>
<td>1</td>
<td>8</td>
<td>1400–1800</td>
<td></td>
</tr>
<tr>
<td>A. elongatum (Host) Beauv.</td>
<td>Tall wheatgrass</td>
<td>1</td>
<td>7</td>
<td>1400–1800</td>
<td></td>
</tr>
<tr>
<td>Elytrigia intermedia (Host) Nevski</td>
<td>Intermediate wheatgrass</td>
<td>1</td>
<td>10</td>
<td>1400–1800</td>
<td></td>
</tr>
<tr>
<td>A. smithii Rydh</td>
<td>Western wheatgrass</td>
<td>1</td>
<td>7</td>
<td>1400–1800</td>
<td></td>
</tr>
<tr>
<td>Arrhenatherum elatius (L.) Presl.</td>
<td>Tall oatgrass</td>
<td>1</td>
<td>7</td>
<td>1500–1900</td>
<td></td>
</tr>
<tr>
<td>Bromus inermis Leyss.</td>
<td>Smooth bromegrass</td>
<td>1</td>
<td>7–10</td>
<td>1400–1900</td>
<td></td>
</tr>
<tr>
<td>Dactylis glomerata L.</td>
<td>Orchardgrass</td>
<td>1</td>
<td>6–7</td>
<td>1700–2100</td>
<td></td>
</tr>
<tr>
<td>Elymus junceus Fisch.</td>
<td>Russian wildrye</td>
<td>1</td>
<td>7</td>
<td>1500–1900</td>
<td></td>
</tr>
<tr>
<td>Festuca arundinacea Schreb.</td>
<td>Tall fescue</td>
<td>1</td>
<td>9</td>
<td>1300–1800</td>
<td></td>
</tr>
<tr>
<td>F. ovina L.</td>
<td>Sheep fescue</td>
<td>1</td>
<td>6</td>
<td>1600–2000</td>
<td></td>
</tr>
<tr>
<td>F. pratensis Huds.</td>
<td>Meadow fescue</td>
<td>1</td>
<td>7</td>
<td>1500–2000</td>
<td></td>
</tr>
<tr>
<td>Lolium Perenne L.</td>
<td>Perennial ryegrass</td>
<td>1</td>
<td>7–10</td>
<td>1000–1300</td>
<td></td>
</tr>
<tr>
<td>Phalaris arundinacea L.</td>
<td>Reed canarygrass</td>
<td>1</td>
<td>5</td>
<td>0600–1100</td>
<td></td>
</tr>
<tr>
<td>Phleum pratense L.</td>
<td>Timothy</td>
<td>1</td>
<td>10</td>
<td>0400–0900</td>
<td></td>
</tr>
<tr>
<td>Poa pratensis L.</td>
<td>Kentucky bluegrass</td>
<td>1,3</td>
<td>8</td>
<td>0300–0800</td>
<td></td>
</tr>
<tr>
<td>Warm-season grasses</td>
<td>Species</td>
<td>Frequency</td>
<td>Growth Form</td>
<td>Flowering</td>
<td></td>
</tr>
<tr>
<td>---------------------------------------------</td>
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<td></td>
</tr>
<tr>
<td><em>Bouteloua curtipendula</em> (Mich.) Torr.</td>
<td>Sideoats grama</td>
<td>1, 3</td>
<td>8</td>
<td>0400 – 0900</td>
<td></td>
</tr>
<tr>
<td><em>B. gracilis</em> (H.B.K.) Lag ex Steud.</td>
<td>Blue grama</td>
<td>1</td>
<td>6</td>
<td>0300 – 0900</td>
<td></td>
</tr>
<tr>
<td><em>Buchloe dactyloides</em> (Nutt.) Engelm.</td>
<td>Buffalograss</td>
<td>4</td>
<td>Intermediate</td>
<td>0600 – 1100</td>
<td></td>
</tr>
<tr>
<td><em>Cynodon dactylon</em> (L.) Pers.</td>
<td>Bermudagrass</td>
<td>1</td>
<td>4</td>
<td>0700 – 0900</td>
<td></td>
</tr>
<tr>
<td><em>Chloris gayana</em> Kunth.</td>
<td>Rhodesgrass</td>
<td>2</td>
<td>8</td>
<td>1245 – 1400</td>
<td></td>
</tr>
<tr>
<td><em>Eragrostis curvula</em> (Schrad.) Nees.</td>
<td>Weeping lovegrass</td>
<td>2</td>
<td>10.5</td>
<td>2400 – 0800</td>
<td></td>
</tr>
<tr>
<td><em>E. trichoides</em> (Nutt.) Wood</td>
<td>Sand lovegrass</td>
<td>2</td>
<td>7</td>
<td>0700 – 1100</td>
<td></td>
</tr>
<tr>
<td><em>Panicum maximum</em> Jacq.</td>
<td>Guineagrass</td>
<td>1, 3</td>
<td>—</td>
<td>0600 – 0800</td>
<td></td>
</tr>
<tr>
<td><em>P. purpurascens</em> Raddi.</td>
<td>Paragrass</td>
<td>1, 3</td>
<td>—</td>
<td>0700 – 1200</td>
<td></td>
</tr>
<tr>
<td><em>P. virgatum</em> L.</td>
<td>Switchgrass</td>
<td>1</td>
<td>12</td>
<td>1000 – 1400</td>
<td></td>
</tr>
<tr>
<td><em>Paspalum dilatatum</em> Poir.</td>
<td>Dallisgrass</td>
<td>3</td>
<td>9</td>
<td>0430 – 0830</td>
<td></td>
</tr>
</tbody>
</table>

*Data from Hanson and Carnhan, 1956; Hovin, 1980; Burson, 1980.
†1 = largely cross-pollinated; 2 = cross-pollinated usually less than 5%, but in some species greater than that under certain conditions; 3 = apomictic; and 4 = largely dioecious.
usually starting at the top or bottom of the inflorescence and working downward or upward to reduce unnecessary handling of the florets and to prevent their injury. Anthers are removed from the florets by inserting forceps between the lemma and the palea. Moisture in the form of a mist may be used to prevent anthers from dehiscing during emasculation. Certain species may have a natural delay in dehiscing so that the anthers can be removed successfully after they have been released from the floret. Anthers are easily punctured; therefore, they must be carefully removed with the forceps. When florets of a species are extremely small, emasculation may take place under a dissecting microscope or with the aid of magnifying glasses.

Emasculation may be done successfully with a hot-water treatment if there is a temperature at which pollen is destroyed but the ovary remains viable. It is especially useful when large numbers of plants or panicles are needed for crossing. Research on smooth bromegrass shows that it can successfully be emasculated by applying hot water at 47°C for 3 minutes or 48°C for 1 minute. The temperature and time differs for most species and genotypic differences may exist within a species. Before attempting hot-water emasculation on a large scale, preliminary trials should be conducted to determine the importance of genotypic variation for sensitivity to temperature. The equipment needed for hot-water emasculation is simple and inexpensive: a wide-mouthed thermos jug, a thermometer, and a wire loop to stir the hot water in the jug. A means needs to be available for heating the water and maintaining it at the desired temperature.

Emasculation also may be conducted with chemical gametocides, but they generally are not used by forage grass breeders. Gametocides were used to emasculate weeping lovegrass, but the success of crossing was not significantly different than with the hot-water treatment (Burson, 1980).

After the inflorescence is emasculated, it is covered with a bag. Pertinent information is recorded on the bag or on a plastic label that is placed in the soil next to the plant. Information on the label may include genus, species, date, and other relevant information.

Pollen must be collected and brought to the emasculated plants for fertilization. Several pollen collection methods are used. A piece of paper with a smooth surface can be placed inside a petri dish. The dish is held slightly below the anthers and the inflorescence is gently tapped. Another method makes use of a small vacuum pump and a small glass container. The pollen is removed from the plant by suction and stored in the glass container until needed. A third method encloses the inflorescence of the male parent in a bag. After dehiscence, the bag is gently shaken to collect the pollen.

After pollen is collected, it is applied to stigmas of emasculated florets. Stigmas should not receive pollen until they have extruded from the floret. The manner in which pollen is to be applied to the stigmas depends
on how it was originally collected. If pollen was collected on paper within a petri dish or in a glass container, the female flowers can be bent and placed in direct contact with the pollen or pollen can be gently applied to the stigmas with an artist’s brush. Soft brushes are recommended so that the pollen will not be violently scattered into the atmosphere during pollination. If pollen was collected into a bag, the female florets are inserted in the bag and the bag is gently shaken.

Instead of collecting pollen, male inflorescences may be moved in close proximity of the emasculated plant and a bag placed over both sexes for pollination. The male inflorescence may be attached to the plant or it may be detached and placed in a vial of water to maintain viability.

The pollinated inflorescence is enclosed within a bag, which is securely fastened about the inflorescence until seed harvest. An identification tag with the necessary information, such as parentage and date of pollination, is attached to the culm or pollination bag.

The most widely used form of artificial hybridization is the mutual pollination technique (Fig.6-2). It is the most rapid and simple technique for crossing forage grasses. It may be used in the field or in the greenhouse. It takes advantage of the fact that most forage grasses are highly self-incompatible or that very little selfed seed is set on highly self-

**Figure 6-2** Mutual pollination of orchard grass in the field. For the two clones to be mated, several culms ready to flower are isolated inside a bag and tied to a stake. Many pollinations can be made in a small area.
compatible plants, if an adequate supply of foreign pollen is available. Reciprocal progenies resulting from mutual pollination are often homogeneous.

To accomplish mutual pollination, inflorescences at approximately the same stage of development are bagged together before anthesis. Care should be taken to ensure that no florets have already bloomed before bagging. Bags may be made of cloth or different types of paper. When making mutual pollinations in the field, care should be taken to use waterproof glue so that the bag will remain intact after a rain. Bags containing the florets need to be gently tapped daily during anthesis. Methods of pollination vary widely and depend on the preference of the breeder and the species of forage grass.

Procedure for Natural Hybridization. Natural hybridization plays an important role in the breeding of perennial forage grasses. Examples of breeding procedures where natural hybridization is important are the polycross, topcross, and open-pollinated progeny tests. Distance is the principal means of isolation and ensuring purity. The proper isolation distance depends on the species, maturity differences among strains, and on the number of plants in each isolation block. Some breeders isolate crossing blocks by planting crops such as rye (Secale cereale L.) or other cereals between crossing blocks of perennial forage grasses to ensure isolation.

**BREEDING PROCEDURES**

Establishment of the source nursery is the starting point for many forage grass breeding programs. Seedlings or cuttings may be started in the greenhouse and, after reaching a desirable size, are transplanted to a source nursery in the field. Each plant is placed at 0.5- to 3.0-m equidistant intervals to permit plant observation and to aid in the control of weeds with mechanical cultivation equipment. Smaller equidistant spacings may be convenient for a bunchgrass, such as orchardgrass; larger spacings may be required for those species that spread extensively with rhizomes, stolons, or both, such as bermudagrass. The total number of plants to be included and the number of locations chosen will depend on the breeding objectives and resources of the breeder. It is not unusual for a source nursery to contain 10,000 plants or more. Entries within the nursery may or may not be replicated. Entries should be replicated at least twice if the source nursery is grown at only one location. Culture of plants in the source nursery should be in accordance with the breeding objectives.

During selection from a source nursery or any other nursery, it is im-
important to take notes on individual plants for the purpose of identifying superior genotypes. Most characters, such as leaf area, spreading ability, winter hardiness, and recovery after clipping, are recorded as numerical ratings or as percentages. Actual measurements may be taken on such characters as culm diameter, plant height, leaf length, and leaf width. Numerical ratings may be made on individual plants on a scale from 1 to 9. For example, recovery after clipping may be rated as:

1 = rapid recovery
5 = intermediate recovery
9 = slow recovery

Verbal equivalents of the numerical ratings with regard to any plant character might be

1 = excellent
3 = good
5 = medium
7 = fair
9 = poor.

It may not be necessary to use the whole scale of 1 through 9 to characterize a particular trait. How individual notes are used and interpreted depends on the individual forage grass breeder. Uniformity of notes among breeders is not necessary, but it is useful when making comparisons. Most systems of taking notes on plant disease data are based on predetermined scales. For example, the following scale might be used for classification of types of infection for crown rust:

1. Immune: no macroscopic evidence of infection, highly resistant, no uredia or a few, small uredia always in necrotic areas; necrotic areas often produced without uredia
2. Moderately susceptible: uredia fairly abundant, small to mid-size, always in necrotic or chlorotic areas
3. Susceptible: uredia abundant, mid-size to large, with or without necrosis or chlorosis immediately surrounding the uredia.

Usually such comparisons are detailed and time consuming and are made once during a season at the apparent peak of an epidemic. It may be better to take many readings, particularly if general resistance (slow rusting) is the objective. Taking readings during the entire time that the disease is present should indicate recognizably less disease on one genotype than another. Such data, when plotted against time, may show a difference among plants in rate of disease development.

Most systems of taking plant disease data are difficult to use in rating single plants because they do not directly compare amount of disease on
different plants. Instead, they make the comparisons via a mental standard, and usually are too time consuming to use in scoring many times in a season.

To avoid these difficulties, a system of taking notes was developed by Loegering et al (1976). The method was developed while screening for general resistance to the pathogen *Puccinia graminis* Pers. f. sp. *dactylidis* Guyot et Massinot, which causes stem rust on orchardgrass. The method can be used on many other host-pathogen associations. The experimental plot area is examined for a short period of time to develop a mental image of the maximum amount of disease on individual plants. This image is designated as the disease standard for the day. Notes can be taken on a tape recorder or on a data recording device that will allow data to be transferred directly to a computer for each plant. A plant is rated as equal to (S) or less than (L) the standard. The L class may range from slightly less disease than the standard to complete absence of the disease.

Notes may be taken at 1- to 3-week intervals. Each time notes are taken, the criteria for S and L change. The amount of disease designated S at one time may be L at another. The basic principle is to evaluate the relative amount of disease on individual plants at a given time, not the absolute amount of disease. At the end of the season, each plant is characterized by the number of times it was rated S. Thus, for example, if notes were taken seven times, a plant designated as 7 means it was rated as S each time, and 0 means it was never rated S. This number represents the disease index. For seven dates of notes, an index of 0 or 1 may indicate useful general resistance, 2 to 5 questionable general resistance, and 6 or 7 poor general resistance. This classification is based on the assumption that a plant always rated L has less rust, and therefore, better general resistance than a plant which is sometimes rated L and sometimes S.

The relative maturity of plants can be classified easily by using a scale. It is necessary to know the relative maturity because maturity has an influence on forage quality and may be directly related to escape of insects and diseases. The plant breeder must know the relative maturity in establishing crossing blocks so that plants of similar maturity are included to aid in random pollination. The following scale may be used:

1 = early boot  
2 = late boot  
3 = early heading (panicles emerged from the boot but not spread out)  
4 = late heading (panicle branches completely spread)  
5 = anthesis (50% of the florets have anthers protruding)

The plant breeder rates the plants two to four times during maturation rather than record the exact date that a stage occurred. After flowering is
complete, the values are totaled for each plant. Plants having lower values would be later in maturity than those having higher values. The scale used and the number of readings taken will vary among plant breeders. Use of the rating scale instead of recording exact dates for each genotype is rapid and has enough precision for most occasions. In certain instances, it may be necessary to record the exact date for heading or anthesis. For some forage grass species, it may be necessary to record the data when seed is mature. In several species, plants can be considered mature when all of the florets are straw colored, or when 25% of the heads and more than half the florets on at least an additional 50% of the heads are straw colored.

Because seed production may be of paramount importance during the selection process and during multiplication of seed stocks for commercial production of cultivars, the self- and cross-fertility is often evaluated. Good indices are obtained by collecting data on number of seeds per panicle, number of seeds per 100 spikelets, and average number of seeds per floret. A fertility index also can be obtained by dividing the total seed weight by the total length of a given number of panicles or by obtaining germination counts of seed from individual panicles.

During selection, the forage grass breeder identifies individual plants. The identification scheme used varies among plant breeders and should be simple, systematic, and flexible. One method is to use numbers that identify the year of planting, row number, and position of the selected plant within the row. For example, 85-10-2 would signify that the plant was planted in the nursery in 1985 in row 10 and was the second plant within the row. A breeder also may want to include codes that signify the source of the plants. For example, the prefix PI may be used if plants resulted from plant introduction, or L may be used to signify germplasm from local collections.

Codes may be useful to signify different types of progenies. For example, OP (open-pollinated seed with no restrictions on source of pollen), PC (polycross seed collected in a polycross nursery), S1 (first generation of selfed seed), Syn 1 (first generation of a synthetic population), TC (topcrossed seed), C1 (first generation plants after treating with colchicine), and F1 (a single cross resulting from mating two clones under bags or in isolation). The letter F is used by forage grass breeders for a single cross produced without emasculation, even though some selfing may occur.

**Recurrent Phenotypic Selection**

Recurrent phenotypic selection is a breeding method used to increase the frequency of favorable alleles in a population. The genetic improvement of a heterogeneous population of forage grasses is accomplished by
selection based on the phenotype of individual plants. A type of recurrent phenotypic selection procedure, often referred to as mass selection, involves selecting desirable plants after open-pollination has occurred, harvesting the open-pollinated seed, and bulking it to obtain the population for the next cycle of selection. Selection is based on the maternal parent only, because both selected and unselected individuals participate in pollination.

The purpose of mass selection is to increase the proportion of superior genotypes in the random mating population. The efficiency with which this is accomplished under a system of random mating with selection depends primarily on the number of genes controlling the trait under selection and the heritability of the trait under selection. Mass selection has been most effective in increasing gene frequencies for highly heritable characters that can be evaluated visually or that can be measured easily. Examples of characters that fit into these categories would be insect and disease resistance.

Mass selection has been particularly useful in the early phases of a forage grass breeding program when little or no previous selection has been done. It allows cultivars to be developed quickly with minimal resources and provides improved populations upon which more refined breeding procedures could be used at a later date.

The method involves growing 5000 or more plants in a spaced-planted source nursery that is maintained in isolation under optimal conditions for growth of the particular forage grass. If resources permit, the plant breeder may have the space-planted source nursery growing at more than one location to permit evaluation of clones in more than one environment. The breeder may observe the plants for desirable characteristics for more than 1 year, because most forage grasses are perennials and persistence under frequent defoliation is a goal of many forage improvement programs. Natural selection in the nursery may eliminate plants that lack adequate winter hardiness, insect resistance, disease resistance, and drought tolerance. Before anthesis, the breeder may remove undesirable plants so that they do not participate in pollination with the more desirable genotypes. Seed is harvested from desirable maternal plants and composited to form a new population. The population is space-planted in a new nursery, and selection is repeated for additional cycles until the desired level of performance is reached.

‘Tioga’ deer-tongue (Panicum clandestinum L.) is an example of a cultivar that was produced using mass selection (Dronen et al., 1980). It was developed by compositing seed of 20 accessions selected for seedling vigor, as expressed in seedling emergence and rapidity of seedling development, general plant vigor, and freedom from any serious disease and insect damage. Equal amounts of viable open-pollinated seed from each ac-
cession were blended and used to establish a nursery for production of breeder seed.

In the past, forage grass breeders generally have felt that mass selection was ineffective for improving traits with low heritability, such as yield. Some breeders felt that its lack of effectiveness was due to the inability to identify superior genotypes from the phenotype of spaced plants in the nursery. Failure to control pollination was another possible factor, because selected plants are pollinated by both desirable and undesirable individuals. Other factors that could be involved include the lack of a progeny test to determine genotypic differences, and too strict of a selection pressure, which perhaps may lead to a reduced population size and inbreeding depression.

Despite the lack of widespread acceptance of mass selection by forage breeders, recurrent selection based on the phenotype of individual plants has been used successfully for increasing herbage yields of Pensacola bahiagrass (*Paspalum notatum* var. *saurae* Parodi). Burton (1982) reported that the herbage yield of Pensacola bahia grass was increased 131% over the initial population after eight cycles of recurrent phenotypic selection. This represented an average rate of gain of 16.4% per cycle. Linear regression accounted for 99% of the variation among cycles, which indicated that the improvement over eight cycles of selection was largely linear. After eight cycles of selection, the coefficient of variation (ratio of the standard deviation to the mean) decreased from 41.9% only to 32.6%. This indicated that ample genetic variation was still present to make progress through additional cycles of selection.

The recurrent selection procedure used by Burton (1982) involved the following steps. A cycle began with seed from each of 200 selected clones that had been intermated in isolation. The 200 selected clones were maintained in the field in a space-planted nursery for evaluation of winter survival and spring vigor.

*Step 1.* In December, 125 seeds of each of 200 clones were planted in the greenhouse in 5- × 50-cm rows in flats of steam-sterilized soil. The seven largest seedlings from each row were visually selected and each was transplanted into a 5-cm pot containing fertilized, steam-sterilized soil.

*Step 2.* In April, the 200 clones were rated for winter survival and spring vigor and the 35 poorest clones were discarded. Also considered in discarding clones was their actual herbage yield measured during July and October of the previous season. Accession numbers were assigned from 1 to 165 for the remaining clones. Six of the seven seedling progeny from each clone were taken to the field, removed from the 5-cm pot, and transplanted in a random arrangement. The accession number of the clone
from which the seedling originated was recorded on a field plan. The field in which the seedlings were planted had been uniformly cropped the previous season. Before planting, it was fumigated with methyl bromide to control weeds.

**Step 3.** In July, the field was subdivided into 40 grids of 25 plants each. The five plants that were judged visually to have the most herbage in each 40-plant grid were recorded on the field map. The plot number for each selection was recorded on an identification tag.

**Step 4.** From each selected plant, three culms ready to flower and with a small amount of stolon attached were harvested, fastened together with their identification tag, and placed in a 3-L container of water. A paper tent was placed over the culms to ensure that the 200 selections were isolated during pollination (Fig. 6-3). Each day at the time of viable pollen shedding, the culms were shaken to enhance pollen dispersal and random pollination.

**Step 5.** Mature seed was harvested separately from each of the 200 selections. The seed was used to initiate the next cycle of selection.

**Step 6.** Actual herbage yield of the 200 selections was determined in August and October. The data were used to discard 35 of the 200 selections, as described in step 2. After the herbage yield was recorded in August, open-pollinated seed was removed from culms of the 200 selections, bulked, and saved to represent the cycle of selection in future performance trials.

The principles of the procedure used by Burton (1982) can be adapted to other forage grasses. Sleper (1985) used recurrent phenotypic selection to improve leaf area expansion rate in tall fescue. Leaf area expansion rate is highly associated with post reproductive herbage yield. Progress in selection for high leaf area expansion rate was primarily linear and approximately 10% for each of five cycles.

Many forage grass cultivars have been produced using recurrent phenotypic selection. 'Joseph' and 'Nezpurs' Idaho fescue (*Festuca idahoensis* Elmer) were developed using three cycles of selection. The initial population was obtained by intercrossing seed collections of 89 native ecotypes from northwestern United States and southwest Canada (Ensign, 1984). The clones for each cultivar were selected on the basis of improved seed set, large seed size, superior germination and plant type. 'Joseph' is a 13-clone synthetic that has 18% better seed set, 37% larger seeds, and 14% better germination than the original population. 'Nezpurs'
Figure 6-3 Random mating of Pensacola bahiagrass in isolation indoors. 
A, Labeled culms are placed in plastic jugs of water. B, Pollination of 
selected culms occurs under a paper tent next to a northern window ex-
posure.

is a 90-clone synthetic that has produced 30% more seed set, 29% larger 
seed size, and 11% better germination than the original population. More 
plant variability exists in ‘Nezpurs’ than in ‘Joseph’ because ‘Nezpurs’ con-
tains many more parental clones.

Topcross Test

Desirable clones selected from a source nursery may be evaluated for 
general combining ability by crossing them to a broad-based tester. The 
broad-based tester usually is a well-adapted cultivar. For example, tall 
fescue breeders may use ‘Kentucky-31’ as the broad-based tester because 
of its wide adaptability, and orchardgrass breeders might choose a well-
adapted cultivar, such as ‘Hallmark.’ A broad-based population also may 
be used, such as one developed by recurrent phenotypic selection.

Seed produced from the topcross mating is harvested for each entry 
separately. This seed is used to plant a replicated trial. After 3 to 4 years
of small-plot evaluation using two to four replications each year, the best clones are identified. The superior clones may be used to form an experimental synthetic.

**Polycross Test**

The polycross test was designed as a substitute for the topcross test. This breeding procedure usually is conducted after previous phenotypic selection has occurred. The method consists of developing a replicated polycross nursery at one or more locations where random mating occurs among the entries, harvesting the open-pollinated polycross seed of each entry separately, and using the seed to evaluate each entry in a replicated polycross test (Fig. 6-4).

In forage grasses, the first step is to establish a population of previously selected clones. These clones may be from the source nursery or may be ones that the breeder has worked with previously because they expressed a desirable trait. Clones are space-planted at random in as small an area as possible so that environmental variables, such as soil heterogeneity, can be minimized. Several hundred clones may be evaluated for traits such as winter hardiness, herbage yield, seed yield, insects, diseases, maturity, or other traits that the breeder considers important.

Selected clones from the original group are replicated in an isolated polycross nursery. The goal is to obtain seed produced under random-mating conditions. Clones of similar maturity are placed in the polycross nursery to ensure synchronization of flowering. Other factors necessary to ensure random mating are uniform volume of pollen shed, uniform cross-compatibilities, no selfing, similar plant height at anthesis, and no lodging. Clones are asexually propagated to obtain the desired number of replications. Usually 4 to 10 replications of 25 to 50 selected clones are used. Selection may be improved at this stage of the polycross procedure by clipping developing seed heads or removing undesirable plants before anthesis.

Mature seed is harvested separately from each plant in the polycross nursery. For each entry, an equal amount of viable seed is bulked from all replicates. This seed will be used to plant the polycross progeny test. The polycross progeny test is grown at one or more locations for 2 to 4 years with 2 to 4 replications. High-performing check cultivars are included in the test to evaluate the potential genetic improvement that may be obtained by use of the clones in a synthetic cultivar. Polycross progenies with the best performance for such traits as yield and persistence are identified.

Superior clones identified by the polycross progeny test are used to form experimental synthetics that may be useful as new cultivars. The
Step 1

100 to 300 selected clones

The phenotype of 100 to 300 clones is evaluated for 1 to 2 years. The best 25 to 50 are selected for a polycross test.

Step 2

<table>
<thead>
<tr>
<th>Rep</th>
<th>50 clones</th>
<th>50 clones</th>
<th>50 clones</th>
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<td>I</td>
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<td>VI</td>
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</table>

The 50 selected clones are planted in isolation in 6 replicates of a polycross nursery. Seed is produced under conditions of random mating. Polycross seed from all replicates of each clone is composited separately.

Step 3

<table>
<thead>
<tr>
<th>Rep</th>
<th>50 open-pollinated entries plus checks</th>
<th>50 open-pollinated entries plus checks</th>
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</thead>
<tbody>
<tr>
<td>I</td>
<td></td>
<td></td>
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<tr>
<td>II</td>
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The polycross seed of each clone is included with one or more check cultivars in a replicated polycross progeny test at one or more locations. Superior polycrosses are assumed to be entries 4, 10, 15, 30, 31, and 40.

Step 4

<table>
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<tr>
<th>Replication</th>
<th>I</th>
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<th>III</th>
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<td>4</td>
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<td>40</td>
<td>4</td>
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Clones numbered 4, 10, 15, 30, 31, and 40 are established in isolation similar to step 2 and replicated 6 times to produce Syn 1 seed. Seed from all replicates of each clone is harvested separately and later bulked with equal amounts of viable seed from each.

Step 5

The Syn 1 seed is planted and the open-pollinated Syn 2 seed is harvested in bulk.

Figure 6-4 Steps conducted to develop a synthetic cultivar using the polycross procedure. The synthetic cultivar is evaluated as Syn 2 or Syn 3 in comparison with other cultivars.

general combining ability of selected clones may not be the same with the smaller number of selected clones in the synthetic as it was with the larger number of entries in the polycross nursery. The general combining ability of the clones does not change, instead, the synthetic evaluates the genetic interaction of the specific clones.
Open-Pollinated Progeny Test

The open-pollinated progeny test is one of the simplest and least costly procedures for genotypic evaluation. Seed is collected from desirable plants growing in a source nursery where the pollen sources are unrestricted. The selected plants most likely will be pollinated by nearby plants with similar anthesis dates. In this procedure, the plant breeder can only apply maternal selection because the pollen parents represent both selected and unselected individuals. The breeder takes detailed notes on all plants in the nursery for the purpose of identifying superior individual plants. Plants will be evaluated for such traits as seed production, forage quality, ability to spread, ability to recover after clipping, winter injury, maturity, insect resistance, and disease resistance.

The number of plants used in this procedure varies widely among forage grass breeders, but the most common number of plants would be 5000 to 10,000. The source nursery usually will be planted at only one location. If adequate resources are available, the use of more than one location is advantageous to evaluate clones under different environmental conditions. For perennial forage species, additional environments can be sampled by evaluating plants for more than 1 year at a given location and bulking open-pollinated seed over years for a selected clone. Collecting seed for more than 1 year from desirable plants also increases the supply of seed for testing.

The relative combining ability of the maternal parents are identified after evaluating the open-pollinated progeny. The progeny test is conducted in a similar manner to the polycross progeny test with a similar number of replications, locations, and years.

After the small-plot trials are completed, clones of similar maturity and with good general combining ability are selected based on the performance of the progenies. The selected clones are removed from the original source nurseries and used to produce experimental synthetics. The synthetics are evaluated as described previously in the section on experimental synthetics.

Hybridization

*Vegetatively Propagated F₁ Hybrids.* Bermudagrass is an example of a forage grass species that is propagated vegetatively as an F₁ hybrid for commercial production. The procedure used to develop 'Coastal' and 'Coastcross-1' bermudagrasses illustrates the development of F₁ hybrids in vegetatively propagated forage grass species (Burton, 1954). Coastal bermudagrass was obtained by crossing 'Tift' bermudagrass with an in-
Introduction from Africa. Five thousand individual $F_1$ hybrid spaced plants were evaluated and 147 were vegetatively propagated and evaluated in replicated small-plot trials. One of these was eventually released as 'Coastal' in 1943. Today, 'Coastal' bermudagrass is performing as well as it did over 40 years ago and is growing on over 4 million ha in the southeastern parts of the United States.

Because 'Coastal' carries many desirable genes necessary for the improvement of bermudagrass, it serves as an excellent parent for future breeding efforts. Hybrids were made between 'Coastal' and a cold-susceptible selection of *Cynodon nlemfuensis* (Burton, 1972). Nearly 400 $F_1$ hybrids were evaluated as spaced plants. Later, a smaller number were evaluated in small plots. One of these was released as the cultivar 'Coastcross-1'. 'Coastcross-1' is 12% more digestible and gives 30 to 40% better average daily gains and liveweight gains per hectare compared with 'Coastal'. The cultivar is sterile and reproduces only by stolons. Nevertheless, it has been propagated asexually on many hectares in Florida, Cuba, and the tropics. Cold-susceptible genes from *Cynodon nlemfuensis* limits its distribution into cooler areas.

*Hybrids from Self-sterility.* Burton (1984) has tried to develop hybrid seed of Pensacola bahiagrass because this species is largely self-sterile and cross fertile. Two clones whose $F_1$ hybrids yielded more dry matter than check cultivars were established in a pilot seed production field. Seed of two hybrids, 'Tifhi 11' and 'Tifhi 2' were released, but were not used for commercial production of the crop. One reason for the lack of success was that cold injury to one of the parents reduced hybrid seed production. Another reason was the cost of establishing the hybrid seed production fields by asexual propagation. If inexpensive methods could be used in establishing hybrid seed production fields, this breeding procedure might receive wide use.

*Wide Crosses.* Many forage grasses lend themselves to improvement through intergeneric and interspecific hybridization. The goal in most instances is not to capitalize on hybrid vigor, but rather to incorporate desirable traits into a given cultivar from alien sources. This procedure may be useful in forage grasses propagated by seeds or vegetatively. To make the maximum use of this technique, information is needed on the phylogenetic relationships of the plants involved.

Hybridization programs involving *Lolium* and *Festuca* are taking place both in Europe and the United States (Asay et al., 1979). The European breeders want to incorporate genes from *Lolium* sp. into tall fescue to improve winter hardiness, seedling vigor, and early spring growth. Desirable forage quality traits from annual ryegrass (*Lolium multiflorum*
Lam.), perennial ryegrass, and giant fescue (Festuca gigantea L.) are being incorporated with the excellent agronomic qualities of tall fescue for the purpose of developing superior tall fescue cultivars (Buckner, 1985).

The tall fescue cultivar 'Kenhy' was derived from an annual ryegrass (2n = 2x = 14) crossed with tall fescue (2n = 6x = 42) (Buckner et al., 1977). The initial cross was made between annual ryegrass and tall fescue to produce F₁ progeny that contained 2n = 4x = 28 chromosomes. The F₁ plants were sterile. The fertility was increased by doubling the chromosome number with colchicine to obtain amphiploids (2n = 8x = 56) that were vigorous, dark green plants with soft, lax leaves. The amphiploids were meiotically unstable and, as a result, the fertility was low. The 56-chromosome amphiploids were used as male parents and crossed with the original annual ryegrass × tall fescue hybrids (2n = 4x = 28). A high percentage of progeny from these plants had somatic chromosome numbers of 56. These 56-chromosome progenies were male-fertile, showed a wide range in seed set, and were morphologically similar to the 56-chromosome male parent. The population of 56-chromosome progenies was advanced by open-pollination for four generations. After four generations, the chromosome number stabilized at 42. Evidently, the ryegrass chromosomes were eliminated. However, ample opportunity for crossing over between the annual ryegrass and tall fescue chromosomes was possible. The plants in the fourth generation were vigorous, had good seed set, and contained the cytoplasm from annual ryegrass. Eleven clones from this population were selected for high moisture content during summer drought stress and for soft, lax leaves. These meiotically stable 42-chromosome plants were isolated in a polycross nursery to develop an experimental synthetic. After extensive small plot and animal evaluation, the synthetic was released as the cultivar 'Kenhy'.

FIELD-PILOT TECHNIQUES FOR GENOTYPE EVALUATION

Evaluation of experimental materials and cultivars is an important aspect of the total breeding effort. Small plots are used extensively to evaluate herbage yield, quality, persistence, and other characters. In many grass breeding projects, replicated field-plot tests are conducted at more than one stage of the program. For example, animal trials may be conducted in addition to conventional small-plot yield trials. Many forage grass breeders conduct replicated pasture trials in evaluation of new species, cultivars, and experimental strains.

When evaluating many entries, such as in progeny testing, lattice designs should be considered. Lattice designs are more difficult to use than the randomized complete-block design, but usually provide increased precision in analyzing data. Lattice designs are likely to be more
efficient than randomized block designs, particularly when soil heterogeneity is a problem. Because forage grasses often are grown and evaluated on land not suitable for row crops, soil heterogeneity must be taken into consideration.

The randomized complete-block design is used for most cultivar evaluation trials that contain from 50 to 75 entries. Most breeders use four to six replications planted at three to five locations for several years. For perennial forage grasses, it is necessary to obtain several years data from the same plots. The analysis of data from experimental designs, such as the lattice and randomized block, may be complicated by loss of some entries in later years.

The size of plot depends on what attribute is to be evaluated. When evaluating seed yield, row plots are commonly used. Each plot may be from 3 to 12 m long with 1 to 1.5 m between plots. The area between rows is kept free of weeds. Row plots also may be useful in obtaining visual information on strains and progenies. When evaluating herbage yield, row plantings are usually inferior to solid seedings.

Plot sizes for herbage yield trials are commonly 1.5 to 2 m wide and 3 to 6 m long. Stands will be established within plots of this size by broadcasting seed by hand or, more commonly, with the use of a small-plot seeder (Fig.6-5). When planting with a small-plot seeder, seed will be placed in rows 12 to 18 cm apart. Stands of vegetatively propagated forage grasses are planted by hand with stolons and rhizomes.

Harvesting small plots may be accomplished by using a sickle-bar mower or a flail-type small plot harvester (Fig. 6-6). An approximate 1-m center of each plot is harvested, which provides for a border between adjacent plots. Border effects can be very pronounced, and it is recommended that at least a 0.3-m border occur at the ends and sides of all plots in the test. Borders at the ends of plots usually are removed a few days before harvesting. This permits easy access to plots for taking notes. Borders separating plots are mowed immediately after the plots are harvested.

After harvesting a plot, the green weight is determined with a stationary scale or an electronic balance on the harvesting machine. Immediately after the green weight has been recorded, a random sample is taken for determining dry weight. The weighed sample is transferred to the dryer in a porous bag that is properly labeled. This subsample of approximately 50 g also may be used for chemical determinations, such as in vivo dry-matter digestibility, protein content, and mineral content. Herbage yields are expressed on a dry weight basis because factors such as growth habit, maturity, and disease, may have a strong influence on the moisture percentage in green herbage.

Forage grasses are utilized in many different ways. They may be grazed, cut for hay, used as silage, or grown in mixtures with legumes.
Figure 6-5  A mechanical plot seeder. A cone divider located at the top provides uniform seed distribution to the seed tubes leading to the press wheels. Press wheels make a firm seed bed. (Courtesy of Carter Mfg. Co., Inc., Brookston, Indiana.)

This multiplicity of uses of forage grasses presents a challenge in field evaluations. In many instances, it is desirable to design yield trial evaluations with different frequencies of cutting. Although multiple defoliations do not simulate grazing, they do give the breeder valuable insight on persistence of stands when a variable harvest schedule is implemented.

Grasses seeded in mixtures with legumes need to be evaluated for their contributions to the sward. To accomplish this, it is necessary to
conduct a botanical analysis after each harvest. Both qualitative and quantitative procedures are available to determine botanical composition of swards. Quantitative determinations of botanical composition are made on a dry-weight basis. Samples or entire plots are hand separated into the component species, dried, and weighed. Qualitative estimates may be made by visually estimating the sward before harvesting. If the qualitative approach is taken, the breeder should conduct periodic hand-separated tests as a check. With many samples, it may not be possible to make hand separations immediately after harvesting. In these instances, provisions should be made for holding samples in cold storage until time is available for hand separation.

Animal trials may be conducted to determine both the plant’s response to the grazing animal and the animal’s productivity when feeding on a cultivar. Animal trials may be conducted in feeding lots or pastures. Most forage grass breeders do not become directly involved with animal trials conducted in feeding lots when the forage is harvested and brought direct-

Figure 6-6 Forage harvester. Herbage is cut by a flail located in the front. Cut herbage is blown into basket suspended by load cell. Green weight of plot is recorded from digital readout, dial, or digital readout printer. After weighing, herbage is dropped into trailing cart, which removes herbage from plot area. (Courtesy of Carter Mfg. Co., Inc., Brookston, Indiana.)
ly to the animal. These trials usually are conducted by animal scientists. Forage breeders often participate more directly with animal trials conducted in the field.

Studies designed to evaluate the plant's response to the grazing animal may include spaced plants or swards. Clones may be asexually propagated and replicated in spaced plantings. Spaced plants may be evaluated with animals early in the selection phase of a breeding program. Data collected may include ability to recover after grazing, persistence to trampling, and animal preference. If the plant's response to grazing occurs later in the breeding program, it will probably include evaluation of replicated experimental cultivars planted in swards when adequate seed or vegetative stocks are available. Characters evaluated with swards may or may not be similar to those considered for spaced plants.

It is unfortunate that animal performance data cannot be easily obtained in early phases of cultivar development. The problem centers around the lack of enough herbage from desirable selections to conduct meaningful feeding trials. Small laboratory animals have been used to evaluate selections early in the plant breeding procedure to predict the response of large farm animals. However, to date, the use of small animals to predict quality parameters has been largely unsuccessful. To obtain estimates of forage quality early in the breeding procedure, the breeder still must rely heavily on laboratory procedures. Today, meaningful animal trials with large farm animals is usually not possible until the late phases of cultivar development when herbage stocks are adequate.

Techniques for using small pastures to evaluate animal response when grazing experimental cultivars have been discussed by Matches et al. (1983). Entries are seeded in 0.47-ha pastures replicated four times. The experimental design is a randomized complete block with four replications. Replication number 4 serves as a reserve pasture where animals are conditioned on a given selection before going on test, and where animals are maintained between grazing periods. Cattle are selected that weigh between 200 and 250 kg live weight at the start of the grazing period. When choosing animals to go on test, it is necessary to select those that originate from as few sires as possible to reduce genetic variation among animals. Grazing periods are from 35 to 68 days during separate periods in the spring, summer, and fall. Three animals generally graze each pasture in the spring and autumn and two graze in summer when less forage is available. Cattle are weighed biweekly following 16 hours of confinement without feed or water.

Equal grazing pressure (same amount of feed per animal) is maintained by adjusting the area grazed with forward or back fences. Animals are allotted a daily amount of herbage on a dry-weight basis equivalent to approximately 2.5% of their live weight and are moved to a fresh strip each week. Pasture strips are sampled weekly to estimate the amount of herb-
age available 1 day before grazing and the amount of residue remaining after grazing, with the difference approximating intake.

PROCEDURES FOR SEED PRODUCTION

Historically, forage seed was produced at the locale in which it was to be used and was a by-product from surplus forage harvested from hay or pasture fields. Forage grass seed production now is a nationwide industry in which improved cultivars are grown for seed in specialized areas and shipped to distant markets (Youngberg and Beker, 1985).

Methods for Producing and Maintaining Breeder Seed

Producing breeder seed is the responsibility of the breeder. Clonal material is replicated in an isolated crossing block and seed is harvested and maintained, as described in the section on experimental synthetics. It is the responsibility of the breeder to maintain the parental clones of a synthetic so that breeder seed (Syn 1) can be produced when needed during the life of the cultivar.

Commercial Seed Production and Marketing

State and federal laws have been established to produce and maintain pure forage grass seed. The Federal Seed Act in combination with state seed laws require that a label be attached to or printed on each sack of seed sold. The label includes the name of the crop, percentage of pure seed, inert matter, other crop seed, weed seed, and germination. The Federal Seed Act applies to all seed marketed in interstate commerce. It is a violation of federal seed laws to move seed into a state if it does not comply with the state seed laws.

Seed testing laboratories located in individual states conduct purity tests and provide information for the labels attached to sacks of seed that are sold. Seed control officials sample seed being sold, and check their analysis against the information printed on the label. Seed lots found in violation may be withdrawn from sale and relabeled, and legal action may be taken against the seed seller.

The genetic purity of forage grass seeds is assured by the seed certification process (Fig. 6-7). Seed certification monitors the seed increase process to assure genetic purity. The procedure is part of the Federal Seed Act, but is carried out by individual state agencies, usually state departments of agriculture or crop improvement associations. These
agencies are coordinated through the Association of Official Seed Certifying Agencies.

Under the seed certification procedure, genetic contamination is limited by taking into account the history of the seed production field and adhering strictly to isolation requirements. Certification is granted only after field inspections and seed tests are conducted. Seed certification rules dictate minimum genetic purity, but most states also have minimum standards for mechanical purity and germination.

Forage grass seed is still marketed to some extent as 'Common.' 'Common' is uncertified and, in some instances, represents local ecotypes. Much of this type of marketing occurs among farmers themselves. When producers buy 'Common' forage grass seed, they cannot be assured of its performance.

Most forage grass seed is marketed through retail stores. If the cultivar was developed by industry, it may be retailed by that particular firm, or the retailer may be an independent distributor.

FUTURE PROSPECTS FOR CULTIVAR DEVELOPMENT

The need for improved cultivars in the future is expected to be high. There is an increasing emphasis on the conservation of the vast soil resources in the United States and throughout the world. Some of the land that is presently under cultivation with row crops will need to be seeded to forage grasses if progress is to be made in minimizing soil erosion.

As economic conditions improve, the demand for animal products increases. Forage grasses comprise an important part of the diet of livestock. It is expected that improved forage grass cultivars will be needed to meet the increasing demand for animal products.

One of the biggest challenges facing the forage grass breeder in the fu-
ture will be to improve forage quality. Large advances have already been made in this important area. It is well recognized that higher quality forage grasses are needed to improve animal performance. It seems, however, that developing higher quality forage grasses involves more than simply selecting for higher in vitro dry-matter digestibility. More research needs to be conducted to identify those quality factors that will improve animal performance and to identify them early in the breeding program. The new technology infrared reflectance spectroscopy holds promise as a tool to identify these important quality parameters and to facilitate selection.

Breeding for improved herbage yield will continue to be a challenge. Research presently is being conducted to determine if components of herbage yield, such as carbon exchange rate (CER), leaf area expansion rate (LAER), and dark respiration (RD), can be used in the future as selection tools to develop high-yielding cultivars.

Under sward conditions in the field, significant genetic variation for CER among tall fescue clones and their progeny has been observed on both a specific leaf weight and a leaf area basis. Heritability estimates ranged from 57 to 83% in the broad sense and from 22 to 44% in the narrow sense (Sleper, 1985). Five cycles of recurrent phenotypic selection have been completed in a broad-based hexaploid tall fescue population. The population from the fifth cycle of selection for low CER had a mean CER value of 23.4 mg CO₂ fixed per dm²/hr, while the population selected for high CER had a mean value of 35.5 mg CO₂ fixed per dm²/hr. The plants in the high CER population were darker green and had shorter and thicker leaf blades than those of the low CER population. Similar findings have been reported in other cool-season forage grasses such as perennial ryegrass (Wilson and Cooper, 1970). It seems that mesophyll cell size decreases with increasing CER. It could be inferred from this that selection for high CER per se, with its associated small cell size, would be fruitless for increasing herbage yield. However, the correlation between herbage yield and CER is low.

Forage yield may be improved if the increased photosynthate from high CER can be accompanied or directed to an active sink, such as that which occurs for plants having a high LAER. LAER is highly correlated with postreproductive herbage yield in tall fescue (Sleper, 1985). The determination of LAER (mm²/day) is estimated by taking the product of leaf expansion rate (mm/day) and leaf width to identify high-yielding plants. Research is underway to combine high LAER to high rates of CER. The hypothesis is that high LAER would provide the active sink necessary to utilize the increased photosynthate (source).

Selecting for low rates of RD may be another important physiological parameter that can be used to select for improved dry-matter yields of forage grasses. Significant genetic variation is present in perennial
ryegrass for RD. Broad-sense heritabilities ranged from 31 to 62% (Wilson, 1982). Populations selected for slow RD generally had superior yields when compared with the original unselected population and with populations selected for rapid RD. Selecting for slow RD resulted in higher herbage yields and plants with 25% more tillers per unit area. Significant genetic variation for rates of RD in tall fescue is present as well (Sleper, 1985). Narrow-sense heritability estimates range from 60 to 90%. It seems that selection in tall fescue for RD is possible. Significant reciprocal effects have not been observed in tall fescue for RD.

The forage grass breeder is expected to have more tools available as genetic engineering concepts are developed for forage grasses. Many forage grass breeders already have participated in this exciting new area. However, most forage grasses at present do not readily lend themselves to in vitro techniques, and there may be a tendency to overestimate the value of such techniques. As the future of cultivar development proceeds with forage grasses, the potential is high that these genetic engineering techniques will take their place as yet another supplement to conventional plant breeding approaches.

REFERENCES


