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CHAPTER

28

Forage Breeding

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Plant breeding is human-directed evolution. This process developed all major crops and their respective races, strains, or cultivars. Although humans have successfully manipulated the genetic resources of plants for several thousand years, the science of genetics and breeding was not developed until the 20th century. Breeding work on a few forage crops began in the early part of the 20th century (Wilkins and Humphreys, 2003) and was focused mainly on developing strains that had improved establishment, persistence, forage yields, and improved insect and disease resistance. These remain essential attributes of cultivated forages (Burton, 1986). In the last 40 yr, objectives have expanded to include improving forage digestibility and removing or reducing antiquality factors.

A pasture or hay field consists of a population of plants. The characteristics of individual plants vary widely within cross-pollinated forage species but generally vary less for self-pollinated, vegetatively propagated, and apomictic species. The phenotype of an individual plant growing in a field or breeding nursery is expressed in a specific environment. Each phenotype (*P*) results from genetic expression of a genotype (*G*) as affected by its environment (*E*) and can be described by the equation:

$$P = G + E + G \times E \text{ (Interaction Effect)}$$

Genetically identical plants such as those of vegetatively propagated cultivars of bermudagrass may differ in size and other characteristics when grown in different environments. Plant breeders use genetic manipulation or breeding to change the genetic characteristics of plant populations so the bred plants represent improvement over the original population (Fig. 28.1).

Changing plant populations by breeding is a multistep process that includes assembling and evaluating germplasm sources, selecting plants with the desired phenotypes, mating the selected plants, and evaluating the progeny in small plots, hay fields, pastures, and seed production fields (Table 28.1). Each phase can take 5 yr or more for perennial species. Often the process of selection and mating needs to be repeated generation after generation (Fig. 28.1; Table 28.1, Phase 2) because gains per generation are often small for complex traits. New cultivars achieved by breeding are released as cumulative, stepwise genetic gains in economic value.

Identification of Production System Problems

Forage breeding can improve the value of forage to livestock producers and solve specific production system problems. Production system problems can include inadequate forage quantity or low-quality forage during specific periods of the year, lack of persistence, and losses in yield and quality due to insects and diseases. It is important to identify and characterize specific production problems before initiating a forage-breeding program. In some cases, it may be easier to solve these problems by incorporating additional or new species into a production system than by breeding to improve multiple deficiencies in an existing species. For example, if forage quality appears to be low, it is necessary to first determine if the problem is due to antiquality factors, such as alkaloids, to high concentrations or low digestibility of cell walls, or to some other factor (Vogel and Sleper, 1994). Breeding is most successful when the goal is clearly defined and good methods are available to differentiate among phenotypes for the specific traits under selection.
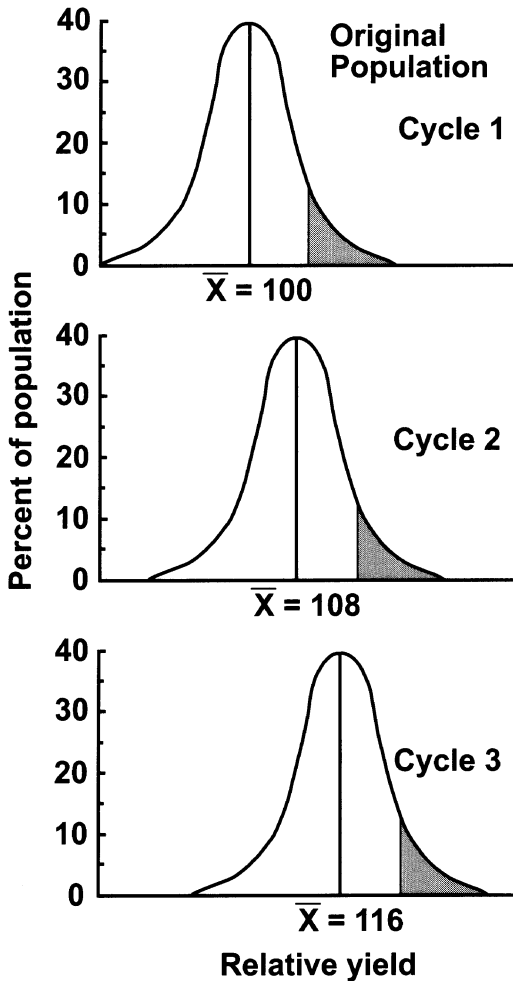
FIG. 28.1. The theoretical effects on forage yield from three cycles of restricted, recurrent selection. Response to selection for other traits, such as seed yield, forage quality, and disease resistance, would be similar in a carefully planned and implemented breeding program.

Breeding Objectives

Forage breeders attempt to modify plants for traits with economic value such as forage yield, forage quality, resistance or tolerance to abiotic and biotic stresses, and improved establishment and persistence. Breeders have improved establishment capability by breeding for increased seedling vigor, a complex trait affected by seed size, seed quality, germination rate, emergence rate, relative growth rate, and other physiological processes (McKell, 1972). Substantial genetic gains have been made in some species,

whereas in others establishment has been enhanced by improving seed quality and agronomic practices, including the use of pesticides for weed and insect control (Vogel et al., 1989).

Persistence

Persistence is an economically important trait for perennial forages because the cost of establishment (including associated loss of production) is amortized over the number of years the stand persists. Breeders have selected and bred for persistence using germplasm adapted to the climatic conditions of the target region and by breeding for resistance or tolerance to biotic and abiotic stresses (Hanson and Carnahan, 1956; Vogel et al., 1989). Adapted germplasm can be obtained by using germplasm accessions that are native to the intended region of use or introduced from an area with similar climate and soils. Improving adaptation to abiotic stresses such as drought, heat, wet soils, and other stresses is most effective through breeding that incorporates germplasm adapted to those environmental conditions.

Insect and Disease Resistance

Diseases and insects also affect forage yield, quality, and utilization by livestock. Breeding for insect and disease resistance requires team efforts of entomologists and/or pathologists and breeders. Screening for resistance or tolerance under controlled conditions identifies genetically superior individuals. Resistant or tolerant plants are intermated, their progeny are screened, selections are made, and the process is repeated until populations with adequate levels of resistance are obtained (Fig. 28.1). This process has been used to improve resistance or tolerance to diseases and insects in many grass and legume forages (Barnes et al., 1988; Casler et al., 1996). Almost all current alfalfa cultivars have resistance to several insects and diseases (National Alfalfa Alliance, 2004).

Forage Yield

Forage yield has been and continues to be a main objective of forage breeders, and significant improvements have been made in most species (Barnes et al., 1988; Vogel et al., 1989; Casler et al., 1996; Wilkins and Humphreys, 2003). In general, however, gains from breeding for yield in forages have been less than those achieved for grain yield in cereals. A significant portion of the genetic gains for grain yield has been achieved by increasing the percentage of the total biomass that is grain, that is, the harvest index. With forages, the physiological processes that result in increased aboveground biomass must be genetically improved. Furthermore, this genetic increase in forage yield must be achieved while maintaining forage quality and its acceptability by livestock (Casler et al., 1996; Casler and Vogel, 1999; Vogel and Jung, 2001).

Table 28.1. Research phases and timetable for a perennial forage breeding program

Phase	Year 1	Year 2	Year 3	Year 4	Year 5
Phase 1: Germplasm acquisition and evaluation	Establish germplasm evaluation nurseries	Evaluate forage yields, quality, and other traits	Second year of evaluation	Identify superior plants and move to crossing blocks, initial seed harvest	Harvest seed. Use seed in Phase 2. Synthetic populations can be randommated several generations
Phase 2: Recurrent selection breeding program	Establish selection nurseries using seed from selected germplasm sources	Evaluate forage yields, quality, and other traits	Second year of evaluation	Identify superior plants and move to crossing blocks, initial seed harvest	Harvest seed, repeat cycle in breeding program. Use seed to plant regional trials
Phase 3: Regional small-plot trials	Plant trials	Harvest trials	Harvest trials	Summarize data, begin seed increase of best strains for pasture trials or field-scale trials	Harvest seed from increase nurseries
Phase 4: Grazing trials or field-scale trials of advanced lines	Plant pastures or field trials	Grazing trial or field-scale harvests	Grazing trial or field-scale harvests	Increase best strain for release	Release seed to seed growers

Seed Production

Although seed is not the principal use of forage plants, cultivars must have adequate seed production to be commercially viable. Significant improvements have been made in seed production in many species, particularly if a specific problem such as shattering can be overcome (Vogel et al., 1989). Increased seed yield should be a breeding objective if low seed yields adversely affect the economic availability of seed.

Forage Utilization and Quality

Quality of forages can significantly affect both milk and meat production. Two main breeding objectives have been to reduce antiquality factors and to increase forage digestibility (Vogel et al., 1989; Casler et al., 1996; Casler and Vogel, 1999; Vogel and Jung, 2001). For example, breeding to reduce levels of undesirable alkaloids in reed canarygrass or to eliminate endophytic fungi associated with undesirable alkaloids in tall fescue has significantly improved grazing animal performance (Vogel et al., 1989; Vogel and Sleper, 1994).

Breeding for improved digestibility has significantly

improved productivity of animals grazing improved cultivars (Vogel and Sleper, 1994; Casler and Vogel, 1999; Vogel and Jung, 2001; Wilkins and Humphreys, 2003). Increased quality can increase net return by increasing body weight gain or milk production per day without requiring additional investment for more livestock (Vogel and Sleper, 1994; Casler and Vogel, 1999). Higher yield can increase net return, but the producer may need more livestock to use the additional forage.

Mode of Reproduction

The breeding system used to improve a species is determined by its mode of reproduction (Allard, 1999; Fehr, 1987). The mode of reproduction also limits the types of cultivars that can be produced. Some forage plants are propagated vegetatively and some are propagated by seed produced through sexual or asexual (apomixis) mechanisms. Sexual species can be completely self- or cross-pollinated, or something between the two. Pollen of cross-pollinated species can be transferred either by wind, especially in grasses, or by insects. Fortunately, the reproductive biology is already known for many important

Table 28.2. Modes of pollination, life cycle, ploidy level, and pollinators of some forage legumes

Forage legumes	Life cycle	Ploidy level[1]	Pollination system	Primary pollinator
Alfalfa	Perennial	4x, 2x	Cross	Leafcutter bees[2] Honey bees[2]
Alsike clover	Perennial—short lived	2x	Cross	Honey bees
Arrowleaf clover	Winter annual	2x	Cross	Honey bees
Berseem clover	Annual	2x	Cross	Honey bees
Birdsfoot trefoil	Perennial	2x, 4x	Cross	Honey bees Bumble bees[2]
Cicer milkvetch	Perennial	8x	Cross	Bumble bees
Common vetch	Winter annual	2x	Self	—
Crimson clover	Winter annual	2x	Cross	Honey bees
Kura clover	Perennial	2x, 4x, 6x	Cross	Honey bees
Lespedeza	Annual	2x	Self	—
Medics	Annual	2x	Self	—
Red clover	Perennial—short lived	2x	Cross	Bumble bees
Subterranean clover	Winter annual	2x	Self	—
Sweetclover	Biennial	2x	Cross	Honey bees
White clover	Perennial—short lived	4x	Cross	Honey bees Bumble bees

[1]Ploidy level, 2x = diploid, 4x = tetraploid, 6x = hexaploid, 8x = octaploid.
[2]Leafcutter bees (*Megachile rotundata*), honey bees (*Apis mellifera*), bumble bees (*Bombus* spp.).

species (Tables 28.2 and 28.3) (Hanson and Carnahan, 1956; Fehr and Hadley, 1980).

Inflorescence structure and physiology can determine whether a species is self- or cross-pollinated (Allard, 1999). Dioecious species such as buffalograss have staminate and pistillate flowers on different plants and, of necessity, are cross-pollinated. Monoecious species such as eastern gamagrass are also cross-pollinated because they have staminate and pistillate flowers borne in separate locations on the same plant. Differences in time of pollen and pistil maturity also can result in cross-pollination or outcrossing. Restrictions on outcrossing, which enhance inbreeding, usually involve cleistogamy, that is, fertilization before the bud opens. In grasses, cleistogamy occurs while the inflorescence is still enclosed in the upper leaf sheath, that is, at boot stage.

Self-incompatibility or self-sterility mechanisms enforce cross-pollination in plants with perfect flowers. Incompatibility is the inability of functional male and female gametes to produce normal seed following pollination (Brewbaker, 1957; de Nettancourt, 1977). The genotype of a pollen grain or its gametes is recognized as compatible or incompatible by the female flower (Dodds et al., 1997). If the genetic relationship between the pollen grain, or male gamete, and the stigma or style of the female flower is incompatible, the pollen grain will be rejected and fail to effect fertilization. Self-incompatibility

systems in plants are analogous to recognition systems like antibody–antigen systems in animals. Self-incompatibility occurs in both legumes and grasses.

When no information is available, some basic tests can be conducted to determine the mode of reproduction (Allard, 1999). The species is probably cross-pollinated and self-incompatible if covering the inflorescences with a bag prior to pollination or physically isolating plants reduces or eliminates seed set. If seed are produced and the progeny are phenotypically very similar, the plants are either self-pollinated or apomictic. If some seed are produced and progeny are phenotypically variable, the parents likely are heterozygous plants of a primarily cross-pollinated species with some self-fertility. Plants believed to be self-pollinated can be emasculated and intermated with other unrelated plants of the same species.

Plants gradually become more homozygous during several generations of self-pollination. Crossing two such plants produces F_1 plants that are genetically and phenotypically similar. Selfing the F_1 plants in subsequent generations will result in genetic segregation and offspring that differ genetically and in phenotypic appearance. However, apomictic plants, when emasculated and crossed to other genotypes, produce progeny that are uniform and identical to the maternal genotype. More comprehensive testing is needed to determine the type of apomixis (Hanna and Bashaw, 1987). Other tests can be

Table 28.3. Modes of pollination, life cycle, and chromosome number of some forage grasses

Common name	Life cycle	Chromosome number	Pollination system
Crested wheatgrass	Perennial	28	Cross by wind
Smooth bromegrass	Perennial	28, 56	Cross by wind
Tall fescue	Perennial	42	Cross by wind
Perennial ryegrass	Perennial	14	Cross by wind
Reed canarygrass	Perennial	14, 28	Cross by wind
Orchardgrass	Perennial	28	Cross by wind
Bermudagrass	Perennial	30, 36	Cross by wind
Switchgrass	Perennial	36, 72	Cross by wind
Big bluestem	Perennial	60 (6x)	Cross by wind
Buffalograss	Perennial	20, 40, 50, 60	Cross by wind
Weeping lovegrass	Perennial	40	Self (< 5% cross)
Bahiagrass	Perennial	20, 40	Cross by wind or apomictic
Dallisgrass	Perennial	40, 50, 60	Apomictic
Buffelgrass	Perennial	26, 32, 40, 54	Apomictic
Pearlmillet	Annual	14	Cross by wind, CMS[1]
Sorghum	Annual	20	Self; cross with CMS
Maize	Annual	20	Cross by wind

[1]CMS = cytoplasmic male sterility.

used to determine the extent of out-crossing and selfing in sexual species.

Many forage species are cytologically complex due to a wide range of chromosome numbers and ploidy levels among and within species (Hanson and Carnahan, 1956; Cleveland, 1985; McCoy and Bingham, 1988). The chromosome number and meiotic chromosome behavior of a species must be known before a breeding program is initiated (Vogel and Pedersen, 1993). A trait that may be simply inherited in a diploid such as perennial ryegrass may be inherited in a quantitative manner in a hexaploid such as tall fescue due to the larger number of segregating genes. Polyploids such as alfalfa have the potential to have quadrivalent (four at a time) or higher levels of chromosome pairing at meiosis, each of which can affect the traits of interest. Plants of the same species with different ploidy levels are often not cross-compatible. If crosses can be made, the progeny are not genetically stable.

The two main components of the breeding process are selection and hybridization or mating (Allard, 1999; Fehr, 1987). Forage species that reproduce through self-pollination are primarily cool-season grasses or annual legumes (Hanson and Carnahan, 1956). To make a controlled mating, flowers of self-pollinated species need to be emasculated (anthers removed) prior to pollen shed. Each emasculated plant, inflorescence, or flower needs to be bagged or isolated to prevent unintentional crossing. Pollen from the selected male plant is transferred to the stigma of the emasculated flower when it is receptive, usually when the flower is fully open. Monoecious or dioecious cross-pollinated plants must be physically isolated or their flowers must be bagged for controlled matings. Pollen must be transferred by hand.

Cross-pollinated species with perfect flowers, that is, containing both anthers and pistils, often have varying degrees of self-incompatibility (Knox et al., 1986; Vogel and Burson, 2004). Plants of completely self-incompatible species can be intermated without contamination by mutual bagging of the parents or placing the plants close together. The same process is usually used with cross-pollinated plants having some self-compatibility because few seed are produced as a result of self-pollination. Detailed mating procedures for the major forage grasses and legumes have been developed (Fehr and Hadley, 1980; Cope and Taylor, 1985; Viands et al., 1988)

Seed of advanced populations of legumes can be produced using insect pollinators in isolated plots in the field or in cages in the field or greenhouse (see Chap. 30). Controlled cross-pollination by hand is used to produce seed of selected alfalfa parents for production of synthetic populations (Viands et al., 1988). Seed of wind-pollinated species can be produced in isolated nurseries or fields. Isolation distances and procedures to restrict foreign pollen differ among species but have been determined for most forages (Fehr and Hadley, 1980).

Germplasm

Genes available for plant breeders to use in conventional breeding methods are those accumulated by a species during its evolutionary history. A germplasm accession is

a distinct genetic entity, often seed or plants collected at a specific site. Genetic variation (or variation among plants of a species for specific alleles and their frequency) exists among germplasm accessions collected from different regions (ecotype variation), among accessions of an ecotype (population variation), and among plants of a population collected from a specific site (within-population variation) (Vogel and Pedersen, 1993). Plant breeders select plants from these natural sources to use as parents in breeding programs (Asay, 1991; Rumbaugh, 1991). There is sufficient genetic variation in most forage species to allow genetic improvement in desired traits (Vogel et al., 1989; Vogel, 2000; Wilkins and Humphreys, 2003).

Germplasm resources can be from ex situ or in situ sources. Ex situ sources are seed banks such as those in the USDA National Plant Germplasm system (USDA-ARS, 2004) and from other breeding programs. In situ sources are from regions or sites where the species is growing and reproducing naturally in either private or public ownership. Ex situ germplasm sources are easily accessed, whereas in situ sites require collection trips or expeditions at the proper times for seed collection.

The germplasm base must be adapted to latitude and climatic conditions where the cultivar products of the breeding program will be used. Latitude determines natural daylength of a site during the growing season. Daylength or photoperiod regulates physiological processes such as flowering and fall dormancy of temperate forage species (see Chaps. 3 and 6).

For forage species with little or no previous breeding effort, direct selection of a superior accession or "ecotype selection" can lead to the rapid development and release of excellent cultivars (Vogel and Pedersen, 1993). Ecotype selection is initiated by collecting an array of accessions for the specified region. For native species, this method is most effective if the germplasm is collected from the intended region of use. For introduced species, germplasm is collected and assembled from areas of the world with climates similar to the target area. Both native and introduced accessions can be obtained from in situ collections or ex situ collections stored in germplasm banks.

Collected or acquired germplasm is first evaluated in replicated trials. Seed supplies of germplasms can be limited, and seed collected from native stands is often of low quality due to environmental conditions during seed production. Seed germination and seedling survival can be maximized by starting seedlings in a greenhouse and then transplanting them into space-planted plots in evaluation nurseries.

Multiple locations are preferred for germplasm evaluation, and the parameters measured will vary with species and objectives. Data from evaluation nurseries are used to select the best local ecotypes or accessions and, in some instances, the superior plants within the best accessions. Selected plants of many perennial grasses can be moved to polycross or multiple-plant-crossing nurseries simply by transplanting clonal pieces or ramets. An outstanding accession can be increased for testing and release as a cultivar without additional breeding work. Examples of cultivars developed by direct increase of germplasm accessions are 'Kentucky 31' tall fescue and 'Lincoln' smooth bromegrass (Alderson and Sharp, 1994). When accessions are increased for release without additional selection, only the genetic variation among accessions is used. Genetic variation within and among accessions is used if selection is made within accessions and the best plants are intermated in a polycross nursery to produce a new population. Strains produced by polycrossing require several years of testing before release.

The above system also is used to develop elite populations for use in breeding systems. Plants intermated in a polycross nursery produce Syn 1 (synthesis generation 1) seed. The Syn 1 should be advanced by one or more generations of random mating in a polycross or seed-increase nursery. This ensures the population is approximately at random-mating equilibrium (Falconer, 1981) so that observed phenotypic differences among plants are due to additive genetic effects rather than heterosis (Vogel and Pedersen, 1993).

Breeding Systems

A major objective of a breeding system is to reduce or identify the environmental effects on the phenotype so that true genetic differences among plants and families can be determined or estimated. Another objective is to intermate selected parents to achieve maximum genetic gains. The theoretical and practical efficiencies of an array of breeding systems available to forage breeders have been reviewed (Sleper, 1987; Vogel and Pedersen, 1993).

Self-pollinated Species

Breeding systems for self-pollinated forages are adapted from self-pollinated crops such as wheat. Depending on the degree of self-pollination, germplasms of these species usually consist of a mixture of highly inbred genotypes. Initially, parent lines are tested extensively before a few are selected and mated to produce F_1 (filial generation 1) seed. F_1 seed is used to plant the next generation, which self-pollinates naturally to produce F_2 seed and so on. In a cross between two homozygous parents, the F_2 is the first segregating generation. Two principal breeding systems, bulk or pedigree, are used for self-pollinated species (Allard, 1999; Fehr, 1987). They differ in how the segregating generations are handled in the F_2 and subsequent generations.

In the bulk breeding method, individuals of the F_2 are harvested and seed is bulked to produce the F_3 and so on (Allard, 1999; Fehr, 1987) (Table 28.4). No selection is

Table 28.4.   Comparison of pedigree and bulk breeding system for self-pollinated crops

Generation	Pedigree system	Bulk system
0	Cross made between two homozygous plants; F_1 seed produced	Cross made between two homozygous plants, F_1 seed produced
1	F_1 plants produce F_2 seed.	F_1 plants produce F_2 seed.
2	Individual F_2 plants grown. Best plants selected and seed harvested on individual-plant basis	F_2 plants grown and seed harvested as a bulk
3	F_2 family rows of F_3 plants grown. Best plants in best rows selected and harvested on individual-plant basis	F_3 plants grown and seed harvested as a bulk
4	F_3 family rows of F_4 plants grown. Best plants in best rows selected and harvested on individual-plant basis	F_4 plants grown as a bulk. Individual inflorescences (or heads) harvested, threshed, and packaged on a single-head basis
5	F_5 family rows grown. Seed harvested on a family-row basis. Selected lines given a number and advanced for testing	F_5 rows planted from single-head seed packets. Selections made on a single-head row basis. Selected rows given a line number and advanced for testing
6	Advanced testing and increase on a numbered-line basis	Advanced testing and increase on a numbered-line basis

Source: Adapted from Fehr, 1987.

made during these segregating generations. In the F_4 or later generations when, due to self-pollination, the plants in the bulk population are more than 80% homozygous, seed from selected plants are harvested individually and designated as a line. After testing, the superior lines are advanced in generation for additional testing and subsequent release as a cultivar.

In the pedigree method (Table 28.4), each F_2 plant is identified by a number, and its progeny are subsequently tracked separately during the segregating generations (Allard, 1999; Fehr, 1987). Selection occurs at each generation until the lines are almost completely homozygous (F_5 or F_6 generation), after which testing and release proceeds as in the bulk breeding method. The pedigree method enables the breeder to test the segregating lines after each generation and discard undesirable lines, but it requires significantly more labor and land area than the bulk breeding method for the same number of crosses.

Cross-pollinated Species

The most effective breeding systems for cross-pollinated forage grasses minimize hand emasculation or crossing, take advantage of their perennial nature and ability to be vegetatively propagated, and use additive genetic variation. These breeding systems are based on population genetics and use recurrent selection or repeated generations of breeding (Fig. 28.1). Objectives are to change population means for specific traits by increasing the frequency of desirable genes for those traits. Improved populations are released as synthetic cultivars.

Restricted, recurrent phenotypic selection (RRPS) and between- and within-family selection are two popular, recurrent selection breeding systems (Vogel and Pedersen, 1993). RRPS is an efficient form of mass selection (Burton, 1974, 1982). In RRPS a space-planted evaluation nursery with 1000 or more plants is established (Fig. 28.2) and then subdivided into smaller selection units of 20–50 plants each to reduce the effect of within-field environmental variation on selection decisions. Plants are evaluated for 1 yr or more for desired traits before selecting a fixed number from each selection unit, typically 5%–10%. Clonal pieces of all selected plants are transplanted to a common isolated polycross nursery either in the field or greenhouse to intermate naturally (Fehr, 1987) (Fig. 28.3).

Polycrossing the selected plants doubles the expected genetic gain from selection as compared with traditional mass selection where only the female parents are selected (Vogel and Pedersen, 1993). An equal amount of seed from each plant (genotype) in the polycross is bulked and is used to start the next cycle of selection. The polycross nursery also is used to produce seed for yield tests and serves as a source of breeder seed. Advantages of RRPS are that it is an easy breeding system to use, requires minimum time intervals per cycle, uses all the additive genetic variation, and, because a large number of plants are intermated, minimizes the potential for inbreeding depression. Disadvantages are that it is not possible to determine the actual rate of inbreeding since pedigree records of individual genotypes and their progenies are not main-

FIG. 28.2. A space-transplanted selection nursery of switchgrass located in eastern Nebraska. Note differences in maturity among the plants in the nursery.

FIG. 28.3. A polycross nursery of smooth bromegrass in Nebraska. The field nursery is surrounded by grain crop fields to isolate it from other smooth bromegrass plants. Roadside grasses may include smooth bromegrass and have been mowed to prevent pollen shed.

tained, and there is no information on the breeding value of individual genotypes.

Another breeding system uses both among- and within-family genetic variation (Vogel and Pedersen, 1993). The system is usually initiated with a single cycle of RRPS. Seeds are harvested from each plant in the cycle-1 polycross nursery and bulked by female genotypes. All seed from a single plant have the same maternal parent, but the male parents include all other plants in the polycross nursery; hence, it is half-sib seed. The seed lots are used to establish a replicated evaluation nursery of space-planted half-sib progeny at one or more lo-

cations. Replicated field plots of a half-sib family can be either single or multiple rows of 5–10 spaced plants.

After 2 yr or more of evaluation, the best families are identified. Individual plants within the best families are evaluated the following year. The best plants from the best families are then selected for polycrossing. About 5%–10% of the total plants in the nursery are polycrossed, and the process is repeated the next generation. This breeding method has advantages over RRPS for traits such as forage yield that are highly influenced by environment effects. Since family records are maintained, the rate of inbreeding can be monitored.

The above breeding methods capitalize on additive genetic variation by accumulation of desirable genes. In general, perennial forage breeders have not exploited nonadditive genetic variation, that is, heterosis, even though substantial heterosis for traits such as forage yield exists in many species. Hybrids for commercial use have not been developed for most perennial forages because of the inability to effectively emasculate large numbers of plants in seed production fields. An exception is bermudagrass, for which hybrids have been very successful because the F_1 hybrids can be propagated vegetatively by stolons as clonal cultivars.

Forage Hybrids

Methods to produce hybrids of forages propagated by seed include first-generation chance hybrids, self-incompatibile hybrids, cytoplasmic male-sterile hybrids, apomictic hybrids, and hybrids produced by the use of male gametocides (Burton, 1986; Vogel et al., 1989). First-generation chance hybrids, self-incompatible hybrids, and apomictic hybrids have been produced for a limited number of grasses. Hybrid cultivars of forage sorghum and alfalfa are currently being produced using cytoplasmic male sterility (Velde et al., 2002; Sun et al., 2003). Breeding procedures similar to those used to produce hybrid maize could be used to produce hybrid forage cultivars if pollination could be controlled effectively on a field scale. Breeders of perennial forage species have an advantage over maize breeders because parent plants can be maintained indefinitely through vegetative propagation.

Apomixis

Apomixis is an asexual form of reproduction where a seed develops without the union of a female and male gamete (Hanna and Bashaw, 1987; Bashaw and Hanna, 1990). Apomixis mimics sexual reproduction in that a female "gametophyte," that is, an embryo sac, is usually formed in an ovule. However, the apomictic embryo sac develops from a vegetative or somatic cell in the ovule, so the nuclei in the sac do not have a reduced chromosome number and all the chromosomes are from the maternal plant. The "egg cell" in an apomictic embryo sac can initiate

mitosis directly and develop into an embryo without being fertilized. Consequently, seed and progeny that develop from this embryo are exact replicas of the female parent unless a mutation occurs or an unreduced egg cell is fertilized. Frequency of these events is usually very low.

Apomixis is nature's way of cloning plants by seed, similar to propagating plants with buds, stolons, or rhizomes. Except for kentucky bluegrass, most grasses that reproduce apomictically originated in tropical or subtropical regions. Breeding systems for improving apomictic forages are unique and, in general, are not useful for improving sexual species (Hanson and Carnahan, 1956; Bashaw, 1980; Bashaw and Funk, 1987).

In nature, most apomictic species also produce some sexual offspring, so they are known as facultative apomicts. Apomixis can be either an impediment or a valuable tool to genetic improvement depending on whether a large number of different polymorphic genotypes occur naturally within the apomictic species and if sexual plants exist within the species to which crosses can be made to produce genetic variants.

In breeding programs, superior, naturally occurring apomictic ecotypes are identified using the ecotype evaluation and selection procedure (Bashaw and Funk, 1987; Hanna and Bashaw, 1987). The most vigorous and productive ecotypes are selected, increased, tested, and released as new cultivars. Additional cultivars have been developed by selecting specific genotypes among the ecotypes. The success of ecotype selection for apomictic species is improved if there is a large amount of genetic variation between and within ecotypes.

Some apomictic cultivars have been developed through chance sexual recombination in facultative apomictic species such as kentucky bluegrass and buffelgrass. Apomictic cultivars of Old World bluestems and *Brachiaria* species are grown on millions of hectares in North and South America, respectively.

Heritability and Molecular Techniques

Heritability, which can be estimated statistically, is the proportion of total phenotypic variation among plants that is due to genetic differences. For important traits such as yield or digestibility that are controlled by many genes, heritability is usually 0.30 or lower, which indicates only 30% of the total phenotypic variation is due to genetic differences among individuals (Vogel and Sleper, 1994; Vogel, 2000). The efficiency of plant breeding could be greatly enhanced if breeders could directly measure true genetic differences or identify genotypes.

Molecular markers can be used to identify desired alleles or quantitative trait loci (Brummer, 1998; see Chap. 29). They are used in major grain crops, and initial work has been done in alfalfa, perennial ryegrass, and tall fescue (Brummer, 1998). The rapidly developing field of genomics includes the use of molecular markers and in-

volves an array of sophisticated and expensive technologies (Liu, 1998).

Comparative gene maps and mapping information from other species should be very useful in developing markers for marker-assisted selection in forages. Research to date indicates genes and their structural organization within genomes have a high degree of similarity among species, including legumes and grasses (Stuber et al., 1999). Significant advances are being made in understanding the genetic control of cell wall synthesis and other traits in model species such as arabidopsis [*Arabidopsis thaliana* (L.) Heynh.] and *Medicago truncatula* Gaertn., which should enable specific genes to be targeted using marker-assisted selection in forages.

Until the last decade, genes available to use in conventional breeding programs were those already in the germplasm of the species or its close relatives. Genes could be moved between plants of closely related species using conventional mating with varying degrees of difficulty, whereas moving genes between unrelated species was not possible. New molecular genetic approaches have made it possible to clone genes from virtually any living organism and insert the cloned gene into another organism, including forage plants. The transformed plants express the cloned genes and produce the appropriate gene products. Stable, transgenic plants of perennial forages such as alfalfa, tall fescue, and switchgrass have been produced.

Conger (1998) and others have pointed out that release of transgenic forage plants could have undesirable environmental consequences if the species has wild relatives. There is currently considerable debate over the desirability and safety of transgenic plants, and the issue is complicated because the arguments are based on economic, political, and religious grounds in addition to science (Duvick, 1999). As pointed out by Duvick (1999), the primary scientific issue is safety. Laws governing the use of transgenic plants vary with the country, and breeders need to follow all rules, regulations, and laws governing the creation, testing, and deployment of transgenic organisms. These regulations and laws require that transformed forage plants be safe for domestic or wild animals and for the environment before they can be used in production systems.

Selection and Testing Procedures

Early on, most evaluation was done using visual scores, but potential progress was limited because it was impossible to visually score forage quality and other traits. Now, biological assays and technologies are used routinely to quantify the main traits. For example, use of near infrared reflectance spectroscopy enables breeders to rapidly obtain estimates of several forage-quality traits from the same sample (Vogel and Jung, 2001). Breeders often work in teams with entomologists, pathologists, rumi-

nant nutritionists, and plant physiologists to develop techniques to achieve specific breeding objectives.

Each forage breeding product, that is, strain or experimental cultivar, needs to be thoroughly tested in the target environments under management conditions for which it will be used. This requires field-plot research and pasture trials (Table 28.1). Grass breeders have relied extensively on evaluations using small-plot trials that have been managed for hay production even though most forage grasses are used in pastures. More grazing trials need to be conducted in the future to ensure that improved cultivars are adapted to the grazing environment (Casler and Vogel, 1999).

Cultivar Types

Forage cultivars released for production agriculture include clonal cultivars, line cultivars, open-pollinated cultivars of cross-pollinated species, synthetic cultivars, hybrid cultivars, composite cultivars, and apomictic cultivars. The types vary because of differences in the reproductive systems of forage species and the different breeding methods used to develop improved cultivars (Fehr, 1987).

Clonal cultivars consist of a single clone or a few very similar clones that are propagated by vegetative propagules. 'Coastal' bermudagrass is an example of a clonal cultivar (Alderson and Sharp, 1994). Line cultivars are groups of plants that are very closely related and have a coefficient of parentage greater than 0.87. These cultivars are usually self-pollinated and trace to a single plant selected at the F_3 or later generation. 'Revenue' slender wheatgrass traces to seed from a single selected plant and is an example of a line cultivar (Alderson and Sharp, 1994). Open-pollinated cultivars consist of plants or populations of normally cross-pollinated species that were selected for uniformity to a standard for some traits, but retain some variation for other traits. They are produced by cross-pollination in isolation. 'Lincoln' smooth bromegrass is an example.

Synthetic cultivars of cross-pollinated species are developed by inter-mating several selected genotypes or parent clones growing in isolation. The parent lines are designated the Syn 0 generation (Allard, 1999). The Syn 1 generation is grown from seed produced by inter-mating Syn 0 plants grown in isolation. Progeny of the Syn 1 are the Syn 2 generation, etc. In practice, Syn 1 seed is usually produced by the breeder, Syn 2 seed is foundation seed, and Syn 3 or later generations are the commercial certified seed. Most conventional alfalfa cultivars are synthetics.

Single-cross hybrid cultivars are the F_1 progenies from a cross of two inbred lines. Mating two single crosses produces a double-cross hybrid. Maize silage hybrids are hybrid cultivars. Several populations, lines, or accessions can be inter-mated to produce a highly heterogeneous population that can be released as a composite cultivar.

'Cimarron' little bluestem is an example (Alderson and Sharp, 1994).

Certification and Cultivar Protection

Limited quantities of breeder seed of a cultivar are available, requiring an increase in seed quantities to meet the needs of production agriculture (see Chap. 30). Seed increase is usually done under a controlled program to maintain and assure the genetic integrity of the cultivar. The exact process differs for public and private cultivars and also differs from country to country depending on seed laws. Breeders must learn and follow the seed laws for the countries in which their cultivars will be marketed. Breeders need to establish cooperative relationships with public foundation seed agencies or with commercial companies and experienced seed producers who manage the seed-increase process (see Chap. 30).

Private forage legume breeding programs were established in the late 1950s in the United States. In the 1960s about 20% of the alfalfa cultivars released had been developed by private companies. By the mid-1980s, this had increased to about 93% (Barnes et al., 1988), largely because a great deal of public research had been completed in plant physiology, plant growth, abiotic stress, and pest resistance of alfalfa, which led to development of screening methods that were readily adapted to produce competitive and proprietary alfalfa cultivars. Nearly all new alfalfa cultivars are now developed in industry breeding programs (AOSCA, 2002). The movement from public to private breeding programs has not been as rapid or extensive in other forage legumes or grasses.

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