**Genetic Improvement Systems for Cool-Season Turfgrasses**

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Currently, more than a dozen **cool-season turfgrass species** are cultivated for use on **golf courses, home lawns, parks, and sports fields** around the world. The most popular species belong to the genera **Lolium**, **Poa**, **Festuca**, and **Agrostis**. Some minor genera, such as **Koeleria** and **Deschampsia**, may also be used in specific situations.

Perennial grasses like turfgrasses present **unique characteristics** compared to other annual grass crops. First, turfgrasses have **very small floral parts**, which makes artificial hybridization tedious and manual pollination control a very complex task. Therefore, these practices are not commonly used in the genetic improvement of cool-season turfgrasses. Second, many turfgrass species exhibit **strong self-incompatibility**, which maintains **genetic diversity** and reduces **inbreeding depression**, but also results in **highly heterozygous genotypes**. This heterozygosity becomes a challenge when attempting to use and develop genomic tools such as **whole-genome sequencing** and **genetic linkage maps**.

In addition, many turfgrass species are **polyploid**, adding complexity to both the development of genomic tools and the selection of genotypes with improved agronomic traits. Another unique aspect of turfgrasses is that **parental germplasm can be vegetatively propagated**. This can be advantageous, as it allows breeders to preserve parent lines for maintaining **Syn 0** generations (as in the case of Penncross bentgrass [Hein et al., 1958]) or **Syn 1 breeder seed**, and to **recombine parents** based on progeny performance.

Lastly, the **primary use** of turfgrasses is as **mown turf/forage**, not just seed production — which is typically the main selection trait in most annual monocot crops. To further complicate matters, plant evaluations in **row plots for seed production** do not necessarily reflect performance under **mowed turf conditions**. As a result, there is a need for **parallel selection —** one for **seed yield** and one for **turf performance —** or for **modifying spaced plant selection** methods to simulate turf conditions (Heineck et al., 2021). Moreover, historically, there has been a **negative correlation between seed yield and turf quality** (Johnson et al., 2003). However, recent studies have shown **no correlation** between turf quality and seed yield, suggesting that cultivars can be developed with **both high turf quality and improved seed yield** (https://turf.umn.edu/news/potential-tradeoffs-between-turfquality-and-seed-yield).

With the exception of **Poa pratensis**, most cool-season turfgrasses are **cross-pollinated species**. Conventional breeding methods for cross-pollinated plants — including **ecotype selection**, **genotypic and phenotypic recurrent selection**, **development of synthetic and composite cultivars**, and **modified backcrossing —** continue to be used in turfgrass breeding programs, with specific adaptations for turfgrasses (Funk, 1981; Funk et al., 1983; Bonos and Huff, 2013). Turfgrass breeders take advantage of positive unique traits such as **the ability to vegetatively propagate superior genotypes**, while managing challenges like **self-incompatibility limitations**.

The backbone of any reputable plant breeding program begins with the assembly of germplasm. Germplasm can be defined as the entire set of available genetic resources, which may include collections of local and exotic ecotypes (specifically from centers of origin), improved cultivars, plant breeding lines, mapping populations, whole-genome association panels, and related species or taxa that can cross with the species of interest.

It is beneficial to collect germplasm from stressful environments (drought, poor soils, etc.) to maximize the potential for finding resistance genes in the germplasm. This has proven positive in identifying **gray leaf spot tolerance** (a disease caused by *Pyricularia grisea*) in **Lolium perenne**, where 50% of the germplasm with **gray leaf spot** tolerance was identified from collections from the center of origin of **Lolium perenne** (Bonos et al., 2004).

Once the germplasm is collected, crossing and selection can begin. If the genotypes are not adapted or contain endophytes transmitted maternally, a modified backcross scheme may be used. This method is similar to the general backcross technique, but instead of using the same recurrent parent for the backcrossing, different parents are used for each backcross (Figure 1). This is necessary in cross-pollinated grasses, as **self-incompatibility** would limit continued backcrossing with related individuals. This method has been used to incorporate **gray leaf spot tolerance** in **English ryegrass** (Bonos et al., 2004) and to incorporate useful endophytes into new cultivars of grasses (Funk et al., 1993) in the **Rutgers grass breeding program**.

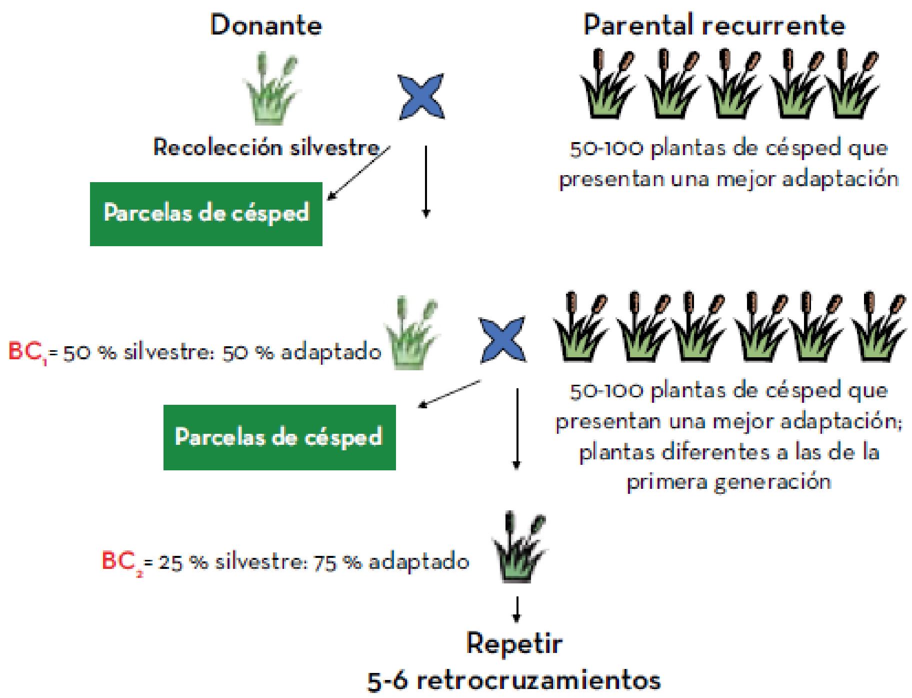



*Figure 1 – Modified backcross genetic improvement scheme*

- **Recurrent Parental Donor**
  50-100 turf plants exhibiting better adaptation
  50-100 turf plants exhibiting better adaptation; plants different from the first generation
  Turf plots
  BC1 = 50% wild: 50% adapted
  BC2 = 25% wild: 75% adapted
  Turf plots
  Wild collection
  Repeat 5-6 backcrosses

Most cross-pollinated grass cultivars are developed as **synthetic or composite cultivars**. **Synthetic cultivars** are established from 1-3 generations of random reproduction with a limited number of clones selected for their high combining ability (Poehlman and Sleper, 2006) (Figure 2). The idea is to exploit **hybrid vigor** by selecting clones with high combining ability, while limiting the number of generations to maintain **heterozygosity** and reduce the possibility of **inbreeding depression** (Poehlman and Sleper, 2006).

In the case of synthetic cultivars, the **parental clones** are preserved to reconstitute the cultivar. This plant breeding system has both positive and negative aspects. On the one hand, the parental clones must be maintained vegetatively **indefinitely** during the production of the cultivar, which requires **greenhouse space** and continuous care to maintain the plant material. On the other hand, because the parental clones are maintained, the **genetic integrity** of the cultivar remains stable and can be reproduced whenever necessary. The **Penncross** is a synthetic cultivar derived from the **polycrossing of three distinct clones** of **Agrostis stolonifera** (Hein et al., 1958).

1. **Visual evaluation and identification of clones**

   in the source nursery.

2. **Establishment of a nursery of clonal lines**

   for further evaluation of superior clones.

3. **Evaluation of the combining ability**

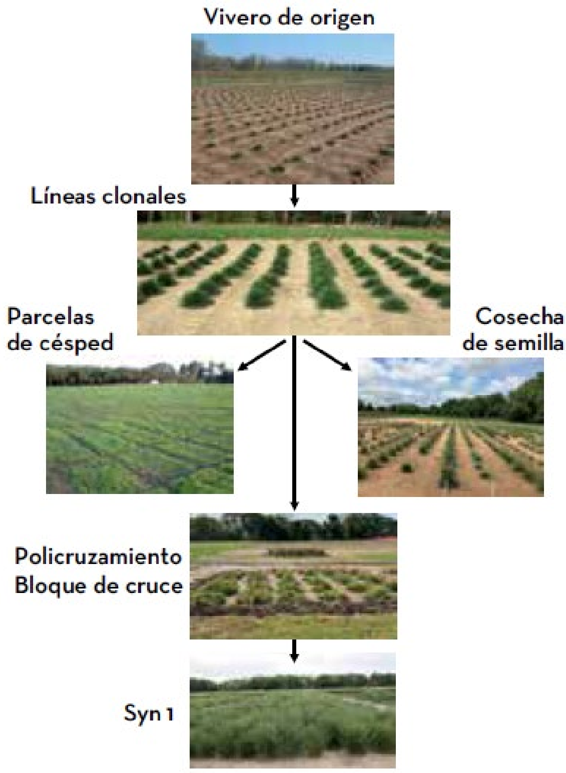
   of superior clones after isolated crossing.

4. **Synthesis of a cultivar by cross-pollinating**

   a limited number of clones with superior combining ability.

5. **Advancement of the synthetic cultivar**

   with one or two generations of random reproduction, population stabilization, and increase of seeds for commercial production.



**Source Nursery**
Several thousand plants are gathered from many sources.

**Isolated Crossing Block**
From ten to several hundred plants cultivated in isolation.

Cross-pollination is allowed. Seeds are harvested individually.

**Progeny Evaluation in Turf Plots**
Seeds from the individual plants mentioned above,

planted in individual turf plots to evaluate

their combining ability.

**Clonal Line Evaluation**
For further evaluation of superior clones,

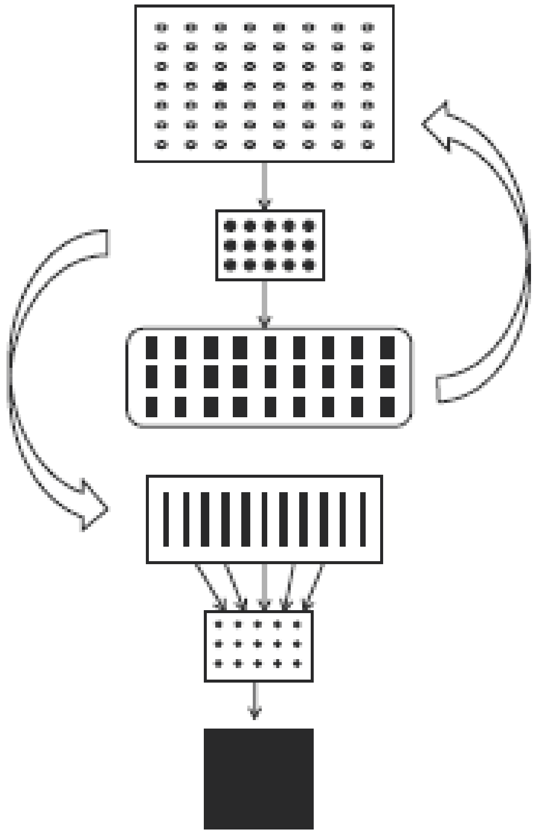
an isolated crossing block may be established.

**Poly-crossing**
Twenty-five to fifty superior clones grown in isolation.

Cross-pollination is allowed. The seed is grouped by clones for eva

**Seed Increase of a New Synthetic or Composite Cultivar**
Equal amounts of seed from each clone are harvested

and grouped to cultivate Syn 1 seed or the breeder's seed of the new cultivar.



**Stable Yield for Over 60 Years**
The development of composite cultivars is similar to that of synthetic cultivars, except that the parents are not kept to reconstitute the cultivar. With each selection cycle for seed harvest, the progeny is also studied to check its performance in turf plots under mowing. Figure 3 describes a typical selection scheme for cross-pollinated turfgrass. It should be noted that this plant breeding scheme is a general one and that modifications to these methods often occur based on the creative ideas of breeders to improve cultivar development. Additionally, as new technologies are discovered, breeders incorporate them (and will continue to do so) to improve selection efficiency. The overall success of the cross-pollination selection method is due to the concentration of alleles for selected traits through recurrent selection. Each selection cycle is based on the previous one, ensuring that each generation shows an improvement in the selected trait compared to the previous cycle (Poehlman and Sleper, 2006). This has been effectively used to darken the color of turfgrasses in the United States, decrease the growth habit of turfgrasses to reduce the number of mowing sessions required, and increase seed harvest (Stacy Bonos, personal observation; Bonos and Huff, 2013).

**Poa pratensis** reproduces through asexual reproduction known as apomixis, which requires a completely different breeding scheme compared to cross-pollinated species. However, unlike the cross-pollination breeding schemes, the methods used for apomictic *Poa pratensis* may take many more years and are unpredictable both in terms of combining traits of interest and recovering the apomictic reproductive behavior. Apomixis results in the production of seed without recombination, so all seeds of an apomictic cultivar are a clonal copy of the mother plant. Apomixis limits genetic recombination, so breeders need to find ways to create new genetic variation in the species. Fortunately, apomixis in *Poa pratensis* is facultative (Bashaw and Funk, 1987), meaning the species has different levels of apomixis ranging from 100% apomictic to 100% sexual (cross-pollination). The ideal scenario is to deactivate apomixis to create new variation in the species and then, in the next generation, restore apomixis to fix hybrid vigor and achieve stable and uniform seed production. Pepin and Funk (1971) developed a crossing technique to optimize sexual hybridization in *Poa pratensis*. This method, with modifications and improvements, is still useful today to create new *Poa pratensis* cultivars.

In *Poa pratensis* crosses, parents are chosen that differ in apomictic behavior (i.e., a female sexual plant crossed with an apomictic male plant) and that are complementary in traits (e.g., seed harvest and tolerance to summer patch caused by *Magnaporthiopsis poae*). After the initial cross, it takes about five years for an experimental cultivar from that cross to enter replicated turf performance and seed harvest trials (Figure 4), and several more years of trials before a company can commercially produce it. This breeding system requires many more resources, such as time, labor, and funding, than cross-pollination breeding. However, once a cultivar is successfully developed, apomictic seed production will ensure the cultivar remains stable indefinitely.

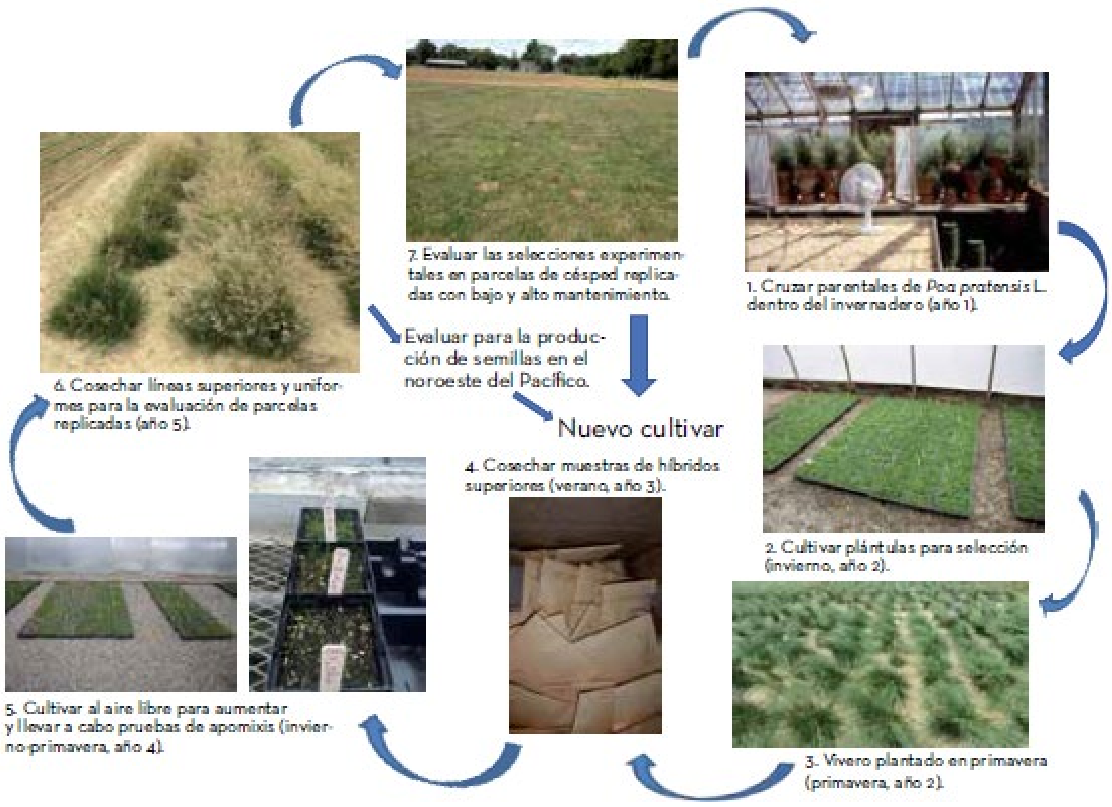



*Figure 3*

Turfgrass breeders began selecting grasses in the early 1920s (Piper and Oakley, 1923) for higher stem density, low growth habit, excellent mowing quality, dark green color, and disease tolerance. Breeders have made remarkable improvements in these traits to the point that, in some cases, they may have gone too far, as occurred with the plant height in tall fescue (*Lolium arundinaceum*). Dwarf-type tall fescues showed more susceptibility to brown patch disease (caused by *Rhizoctonia solani*) and thermal stress than semi-dwarf types, indicating that selection had gone too far for the low growth habit in tall fescue (Watkins and others). Additionally, some of these traits, such as color and plant height, are relatively easy to select for now, as much of the germplasm in breeding programs already exhibits these characteristics. Breeders are now focusing on traits that were previously difficult to select for, such as drought, heat, salinity, and/or shade, to name a few. Diseases remain a priority for most breeding programs, as pathogens continue to evolve and affect plants in different ways. These pathogens are also influenced by the changing environment. New pathogens that were once not considered a problem are now becoming major issues (e.g., gray leaf spot in tall fescue). Climate change and environmental uncertainty in the future are also concerns for breeders (Meyer and others, 2017). Breeders need to predict the traits that will be important and/or react quickly when new traits, such as disease resistance, are needed.

New genomic sequencing and high-throughput phenotyping technologies are tools that breeders can use to streamline the selection process and maximize its benefits. These technologies can also be used to quickly redirect selection procedures if new pests emerge. High-throughput phenotyping tools using drones and multispectral cameras can now be applied to help breeders more efficiently select for drought, heat, and salinity tolerance, which previously required labor-intensive physiological measurements to evaluate germplasm. As mentioned earlier, most cool-season turfgrasses are complex polyploids (except *Lolium perenne* and *Agrostis canina*), which made it difficult to use genomic tools. However, recent advances in sequencing, polyploid genetics, and polyploid data analysis (and with the help of Tools for Polyploids - NIFA USDA) have opened up new possibilities for more efficient breeding **SCRI Award # 2020-51181-32156**, turfgrass breeders are now better equipped to implement genomic resources for genetic improvement. Turfgrass breeding in the future will likely include a combination of new approaches along with traditional breeding methods in order to develop the next generation of turfgrasses with better pest tolerance and stress resistance for the changing climate and customer needs.

**Referencias**


Bashaw, E. C.; Funk, C. Reed. 1987. P. 40-82. En: *Fehr, Walter R., ed. Principles of Cultivar Development: Volume 2 Crop Species*. Nueva York, NY: Macmillan Publishing Company.

Bonos, S.A., C. Kubik, B.B. Clarke y W.A. Meyer. 2004. ≪Breeding for gray leaf spot resistance in perennial ryegrass≫. *Crop Science,* 44:575-580.

Bonos, S.A. y D.R. Huff. 2013. ≪Cool-season turfgrass breeding≫. En: J. Stier, B. Horgan y S.A. Bonos Eds. *Turfgrass Monographs 3rd Edition*. Pags. 591-660.

Funk, C. Reed. 1981. *Perspectives in turfgrass breeding and evaluation. Proceedings of the International Turfgrass Research Conference*. 4: p. 3-10.

Funk, C.R., White, R.H. y Breen, J.P., 1993. ≪Importance of Acrernonium endophytes in turfgrass breeding and management≫. *Agriculture, Ecosystems & Environment*. 44: 215-232.

Hein, M.A. (1958), *Registration of Varieties and Strains of Grasses 1*. Agron. J., 50: 399-401. https://doi-org.proxy.libraries.rutgers.edu/10.2134/agronj1958.00021962005000070015x

Heineck, G.C., N.J. Ehlke y E. Watkins. 2021. ≪Predictive ability of perennial ryegrass spaced-plant nurseries for turfgrass and seed production swards in Minnesota≫. *Crop Science* 61(5): 2861-3858.

Johnson, R. C., Johnston, W. J., & Golob, C. T. 2003. ≪Residue management, seed production, crop development, and turf quality in diverse Kentucky bluegrass germplasm≫. *Crop Science*, 43(3), 1091-1099.

Meyer, W. A., L. Hoffman y S. A. Bonos. 2017. ≪Breeding cool-season turfgrass cultivars for stress tolerance and sustainability in a changing environment≫. *International Turfgrass Society Research Journal*. 13:p. 1-8.

Pepin, G. W., C. R. Funk. 1971. ≪Intraspecific hybridization as a method of breeding Kentucky bluegrass (Poa pratensis L.) for turf≫. *Crop Science*. Mayo/junio. 11(3): p. 445-448.

Piper, C. V.; Oakley, R. A. 1921. ≪Turf experiment plots at golf courses≫. *Bulletin of the Green Section of the U.S. Golf Association*. 23 de marzo. 1(3): p. 42-43.

Poehlman, J.M. y D.A. Sleper, 2006. B*reeding Field Crops, 5th Edition*. Wiley-Blackwell. 432 p. ISBN: 978-0-813-82428-4.

Watkins, E. y W.A. Meyer. 2004. ≪Morphological characterization of turf-type tall fescue genotypes ≫. *HortScience*. June. 39(3): p. 615-619.