Stop #1: Improvement of Bermudagrass, Kikuyugrass, and Zoysiagrass for Winter Color Retention and Drought Tolerance

Adam J. Lukaszewski and Jim Baird
Department of Botany & Plant Sciences
University of California, Riverside

Background and Justification

California is suffering from a serious drought and the future of turfgrass and other landscape plants has never been more uncertain. Use of drought tolerant plant species should be at the forefront of water conservation management plans for golf courses and other landscapes. Warm-season or C4 grasses are better adapted to warmer, drier climates and use at least 20% less water compared to cool-season grasses, yet their use in California and abroad is limited primarily due to the aesthetics of winter dormancy. Despite attempts by the turfgrass industry to develop cool-season turfgrasses with improved drought tolerance, repeated testing in Riverside, CA, a Mediterranean climate characterized by hot, dry summers with less than 200 mm of annual rainfall, has demonstrated that even the most drought tolerant cool-season cultivars do not even come close to the warm-season species in terms of drought tolerance and water use efficiency. Thus, we strive to improve winter color retention in and therefore greater acceptance of warm-season turfgrasses for regions where these grasses are adapted. In addition, drought tolerance is not created equal both among and within warm-season species. While buffalograss is considered to be among the most drought tolerant of the warm-season turfgrass species, the primary mechanism for this is drought avoidance by summer dormancy. In California, general observations are that bermudagrass retains the best quality and green color under drought or deficit irrigation, although differences within cultivars are less substantiated. Other warm-season species appear to possess “lesser” drought tolerance, but zoysiagrass and kikuyugrass are best able retain green color longer in response to cooler temperatures.

Project Objectives

1. Develop bermudagrass, kikuyugrass, and zoysiagrass turf-type genotypes with improved winter color retention and drought tolerance for Mediterranean and arid climates.
2. Utilize Diversity Arrays Technology (DArT) markers to aid in breeding efforts and marker-assisted selection.
3. Develop techniques to reduce kikuyugrass ploidy level to diploid by androgenesis in order to reduce aggressiveness and improve turf quality and playability characteristics.

Bermudagrass

Bermudagrass is commonly used throughout the southern U.S. and is considered the “go to” warm-season species for many golf courses in California. Its major disadvantage is winter dormancy. We have initiated a project to address this issue, with the primary goal of shortening winter dormancy (if it can be eliminated at all, it certainly would not be a single step process). For this purpose we established a collection of all six Cynodon species in Riverside, by requesting samples from the USDA and several other sources. At present the collection exceeds 100 accessions; all six species are represented by at least several genotypes each. We started intercrossing these species and generated a large number of interspecific hybrids. Some of these were created by controlled one x one cross hybridization (both parents are known) using the detached tiller approach; many others were created by open pollination among the collection accessions left unmowed for several months. In this case only the female parent is known. The hybrids show variation for every observable characteristic, including the onset of winter dormancy and spring green-up.

Our immediate plans are to attempt to intercross the hybrids with latest dormancy and the earliest green-up, on the assumption that the next generation hybrids may show reduced dormancy period. In the meantime, the best-looking hybrids are being tested in various environments including: the Coachella Valley Agricultural Research Station in Thermal, CA; Arizona Country Club in Scottsdale, AZ; and The Preserve Golf Club in Carmel, CA. Dramatic differences in their behavior are clearly evident. In addition, a separate area of these grasses were established at UCR and, once established, irrigation was turned off to evaluate relative drought tolerance. New sets of hybrids are also being generated, again by open pollination of selected collection accessions. Presently we are expanding these grasses for further evaluations in larger plots (for more realistic cultural care and better evaluation of quality characteristics) across several climatic zones in California.

To establish the parentage of the existing hybrids, the collection and a sample of hybrids were genotyped using the DArT technology. The results are confusing in some way, as they indicate that in some cases accession designations may be incorrect (some accessions group with species other than those listed); in several cases the accessions appear to be amphiploid, as they share markers of two (or even more, up to four) original known diploid species. This makes tracking the parentage difficult. However, in many cases we were able to infer the paternal parent in hybrids from open pollination. The method is not perfect, as we have too few accession-specific DNA markers. However, species-specific markers show quite clearly the parental contribution.

Kikuyugrass

Kikuyugrass is a warm-season species that originated from the east African Highlands and now inhabits every continent except Antarctica (Mears, 1970). It was first imported into California in the 1920s for soil erosion control on hillsides and riverbanks (Garner, 1925); however, it quickly spread to colonize much of coastal southern and central California. Today, kikuyugrass is officially considered as an invasive weed with sale and transport prohibited in several California counties. Furthermore, it is on the Federal Noxious Weed list, which restricts importation of germplasm into the country and across state boundaries (USDA, 2012). Kikuyugrass spreads aggressively by rhizomes, stolons, and seed (Youngner et al., 1971). Also found in Hawaii and scantly in Arizona, the species is well suited to Mediterranean climates like California because it can photosynthesize across a wide temperature range as evidenced by its superior winter color retention among the warm-season turfgrasses (Wilen and Holt, 1995). These characteristics have allowed kikuyugrass to invade areas including golf courses, athletic fields, and lawns, where it often becomes the dominant managed turfgrass species rather than attempts to selectively remove it (Gross, 2003).

To assess the extent of genetic variation present among available accessions of kikuyugrass, local populations were sampled from throughout California, as well as from the collections at UCR (ca. 20-25 yrs. old), Hawaii and Australia. A total of 20,000 single nucleotide polymorphism (SNP) makers were discovered using the Diversity Arrays Technology sequencing (DArTseq) platform. The hierarchical plot, gap statistics, and the principal coordinate analysis all showed that the 336 accessions in the study separated into three main clusters. Seventy-seven percent of the total genetic variation was due to within population variation, while 23% represented among population variation. The main axis of the principal coordinate analysis accounted for 33% of the total variation. Accessions from California showed the least genetic variation with all but six located in the same cluster. Accessions from Australia and Hawaii showed a much broader degree of genetic diversity and would be valuable stock for breeding should such effort become feasible and the exchange of germplasm possible. The level of variation is not impressive, but it does offer hope that progress by selection is possible, even if no germplasm can be imported. Individual accessions now must be screened for their behavior under standard turf management and under various conditions including drought and winter color retention. Some variation in appearance is evident even under cursory evaluation. Selected accessions will be intercrossed and new hybrids screened and selected.

Kikuyugrass is tetraploid (presumably autotetraploid). It is very vigorous and aggressive. Autotetraploids in general are larger and more vigorous than their diploid predecessors. We assume that ploidy reduction will automatically reduce vigor and plant size, perhaps creating turf with much finer texture, and less aggressive growth. We will attempt to generate haploids of tetraploid kikuyugrass by androgenesis and/or microspore culture. While there is no technology specifically crafted to this particular species it has been tried on some related species, and is routinely used to generate haploids and doubled haploids in many grasses, including economically important cereals. The critical steps that must be tested involve proper stress (specific factor and its intensity) to induce the switch from the gametophytic to sporophytic development of microspores, and culture conditions. Several of these are currently being tried with first clear signs that the microspores can be induced to such switch.

Our assumption in this approach is that reduction of ploidy level to diploid will reduce plant vigor and size. We cannot predict, however, if such diploids will be fertile. In Festulolium where we reduced the ploidy level from tetraploid to diploid (Kopecky et al., 2005), some diploid individuals were in fact fertile and could be intercrossed to generate viable populations. Whether this will work in kikuyugrass is an open question; much depends on the level of differentiation of the genomes in the tetraploid, of which there are no data available.

Zoysiagrass

Zoysiagrass (*Zoysia sp.*) is generally considered to have optimal winter color retention among the warm-season turfgrasses. UCR has some tradition in breeding of

Zoysiagrass. In the 1980's UCR released cv. 'El Toro', a *Z. japonica* accession developed by the late Dr. Victor B. Youngner (Gibeault 2003). El Toro had a much faster establishment rate, better late season color and more rapid spring green-up than other *Z. japonica* grasses, and less thatch production. This release was followed by two cultivars, 'De Anza' and 'Victoria' which were created by a complex hybridization 'El Toro' x hybrid (*Z. matrella* x (*Z. japonica* x *Z. tenuifolia*). De Anza is known for very good winter color retention. Unfortunately, all germplasm from those breeding efforts has disappeared and if the breeding is to be initiated again, a new germplasm collection has to be established. We propose to request sample accessions from existing germplasm collections and breeding programs to be screened under Southern California conditions for their winter color retention and other critical turf characteristics. Early on progress will be slow, given the long establishment time for zoysiagrass. However, once interesting accessions are identified and hybrids are made, progress should accelerate rapidly.

Winter Color Retention Germplasm Evaluation

In an effort to help expedite development of warm-season turfgrasses with improved winter color retention and drought tolerance, bermudagrass germplasm from Oklahoma State University and the University of Florida, zoysiagrass germplasm from Texas A&M University, and germplasm from other breeding programs will be evaluated in Riverside, CA together with bermudagrass, zoysiagrass, and kikuyugrass germplasm from UCR. Replicate space plantings will be established in August 2016 and accessions will be evaluated periodically for establishment vigor, turf quality, winter color retention, spring green-up, and tolerance to deficit irrigation. Ratings will include visual, digital image analysis, and possibly by remote sensing using the latest drone technologies.

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