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

Adam J. Lukaszewski, Marta Pudzianowska, and Jim Baird
Department of Botany & Plant Sciences
University of California, Riverside, CA 92521

Project Milestones Since Field Day 2016:

- ✓ New project funded by CTLF, USGA, MWD, and WMWD.
- ✓ Dr. Marta Pudzianowska hired as postdoctoral scholar in turfgrass breeding and genetics.
- ✓ Planted ca. 1,000 bermudagrass and zoysiagrass accessions in replicate plots from University of Florida, Oklahoma State University, Texas A&M, and UCR for evaluation of winter color retention and drought tolerance in Riverside.
- ✓ Continued crossing of UCR bermudagrass accessions, with emphasis on genotypes possessing desirable winter color retention, early spring green-up, and drought tolerance.
- ✓ Conducted a second genetic analysis of existing and new UCR bermudagrass accessions using DArT technology.
- ✓ Established new replicated trials in Riverside, at West Coast Turf in Thermal (Coachella Valley), and at Meadow Club in Fairfax (Northern California) to evaluate 12 of our most promising bermudagrass hybrids or accessions in comparison to Tifway, Santa Ana, TifTuf, and Bandera cultivars.
- ✓ Established a new collection of 105 kikuyugrass genotypes representing greatest genetic diversity from California populations.
- ✓ Selecting for desirable traits among individual kikuyugrass seedlings from wild type seed stocks.
- ✓ Made second attempt to create haploid kikuyugrass plants via androgenesis. Reducing ploidy level often results in reduction of vigor (i.e., aggressiveness) and we hope general improvement in turf quality characteristics.

Background and Justification:

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. With water supplies in California uncertain the future of turfgrass and other landscapes is shaky. 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. 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.

Thanks to new or continued support from the California Turfgrass and Landscape Foundation (CTLF), United States Golf Association (USGA), Metropolitan Water District (MWD) of Southern California, and Western Municipal Water District (WMWD) we are able to continue this project with full speed ahead. Dr. Marta Pudzianowska (Ph.D., Warsaw University of Life Sciences) joined our team in spring 2017 as a postdoctoral scholar in turfgrass breeding and genetics.

<u>Project Objectives:</u>

1. Develop bermudagrass, kikuyugrass, and zoysiagrass turf-type genotypes with improved winter color retention and drought tolerance for Mediterranean and arid climates.
2. Screen a large collection of bermudagrass and zoysiagrass genotypes from the University of Florida, Oklahoma State University, Texas A&M, and UCR for winter color retention and drought tolerance in Riverside CA.
3. Develop techniques to reduce kikuyugrass ploidy level to diploid by androgenesis to reduce aggressiveness and improve turf quality and playability characteristics.
4. Utilize Diversity Arrays Technology (DArT) markers to aid in breeding efforts and marker-assisted selection.

<u>Bermudagrass:</u>

Bermudagrass is commonly used throughout the southern U.S. and is considered the “go to” warm-season species for many golf courses and athletic fields in California. Its major disadvantage is winter dormancy. Our project focuses on 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 approaches 160 accessions; all six species are represented by at least one genotype each. The collection also includes a growing number of samples collected locally, or donated to us by others. These are mostly from abandoned or heavily travelled sites, including a spot in Coachella Valley where no irrigation water was applied for at least three consecutive (and very dry) years. 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. 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. Last year a separate area of these grasses were established at UCR and, once established, irrigation was turned off to evaluate relative drought tolerance. After initial conditioning, two of our new hybrids survived the dry-down in surprisingly good shape. Because of new plantings in the area, the test could not be repeated this year and will be repeated only after a new dry-down area is established, away from any irrigation systems. New sets of hybrids are also being generated, again by open pollination of selected collection accessions. To go back to much more successful cross-pollinations from several years ago we have established a new crossing block on an exposed site with more morning winds.

To establish the parentage of the existing hybrids, the collection and a sample of hybrids were genotyped using the DArT technology. The results were confusing suggesting that some 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. We have requested new samples from USDA and hope to straighten the matter during upcoming winter.

This year, we chose 12 of our most promising accessions or hybrids for further evaluation in larger, replicated plots (for more realistic cultural care and better evaluation of quality characteristics) across several climatic zones in California. UCR entries included: 10-9, 15-4, 16-6, 17-8, TP1-1, TP1-2, TP3-2, TP5-4, TP6-3, BF1, BF2 and NRCC12. These are being compared with four widely used or new cultivars: Bandera, Santa Ana, TifTuf and Tifway. Experiments were designed as randomized blocks with three replications. Three locations in California were chosen for establishing the trial: University of California, Riverside (Riverside, Inland Southern California); Coachella Valley (Thermal, Low Desert) and Fairfax (Northern California). Plots (5’ x 5’) were established from 2.5-inch plugs on May 22 in Riverside; June 14 in Coachella Valley; and June 22 in Fairfax. During the first year of the test dynamics of establishment are being measured using Digital Image Analysis (DIA) and turf quality is being evaluated after obtaining full cover.

Preliminary results thus far indicate that the bermudagrasses grow fastest in the warmest climates (Table 1), but Riverside was the only location where the accessions reached 100% establishment by the end of August 2017. Tested accessions and hybrids showed different growth dynamics (Table 2). TP 6-3, a UCR hybrid, turned out to be the fastest growing accession in Riverside, reaching 75% cover 51 days after planting (DAP). DIA measurements taken in Coachella Valley also revealed that TP 6-3 is the most rapidly growing entry so far (data not shown). Other faster growing entries were TP 3-2 (75% cover reached at 56 DAP) and NRCC12 (75% cover reached at 57 DAP). TP 6-3 and TP 3-2 were also characterized by high turf quality evaluated in Riverside (UCR), higher than Tifway (Table 3). All tested hybrids and accessions have demonstrated turf quality ratings of 6 (minimally acceptable) or higher and are comparable to commercial cultivars in this study.

Table 1. Differences in average bermudagrass cover (0-100%) among the three test locations in CA. 2017. Based on DIA averaged across 4, 6, and 8 weeks after planting.

Location	Cover (%)
Riverside	37.42 b
Coachella Valley	51.96 c
Fairfax	19.39 a

Means followed by the same letter in a column are not statistically Different (P =0.05).

Table 2. Days after planting (DAP) to reach 75% plot cover based on regression analysis in Riverside, CA. Bermudagrass plugs were planted on 22 May 2017.

Accession code	DAP to reach 75% cover
10-9	63
15-4	63
16-6	63
17-8	61
TP1-1	63
TP1-2	59
TP3-2	56
TP5-4	61
TP6-3	51
BF1	64
BF2	63
NRCC12	57
Bandera	61
Santa Ana	52
TifTuf	55
Tifway	58

Table 3. Visual quality (1-9, 9 = best) of bermudagrass Accessions averaged over three rating dates in Riverside, CA. 2017.

Accession code	Visual quality (1-9)
10-9	6.1 e
15-4	6.4 cde
16-6	7.2 abc
17-8	7.0 abcd
TP1-1	6.8 abcde
TP1-2	6.7 abcde
TP3-2	7.4 a
TP5-4	6.8 abcde
TP6-3	7.5 a
BF1	6.8 abcde
BF2	6.6 bcde
NRCC12	6.3 de
Bandera	7.3 ab
Santa Ana	6.7 abcde
TifTuf	6.8 abcde
Tifway	6.4 cde

Means followed by the same letter in a column are not statistically Different (P =0.05).

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).

In previous years we have sampled kikuyugrass from throughout California, from our collection at UCR (ca. 20-25 yrs. old), as well as 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 showed that the 336 accessions 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. This means that there is relatively little variation among known sources of the grass. Accessions from Australia and Hawaii showed a much broader degree of genetic diversity than our California samples 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. This year we established a collection of available genotypes representing the greatest genetic diversity and are conducting dry down events to select for improved drought tolerance. In addition, recently we have located all seed stocks of the grass (from about 20-25 years back) and established ca. 280 individual seedlings. These will be individually assessed, selected for best suitability for turf, and added to our collection. 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 have repeated last year's attempt to generate haploids (which here would carry two genomes, as the starting material is tetraploid) via androgenesis. There is no known technology adapted to this species and the species appears to be recalcitrant. This year again we brought in a specialist in androgenesis, some 13,000 anthers were plated but we have no haploids as yet. The consolation is that we now have tested protocols for material collection, application of external stresses to induce the switch from the gametophytic to sporophytic pathway of microspore development, and selected best culture media. We must try this approach in different seasons; perhaps the microspores will be more amenable to manipulation than in summer.

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 but a handful of germplasm from those breeding efforts has disappeared and if the breeding is to be initiated again, a new germplasm collection has to be established. As described below, we have acquired 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. If UCR re-enters zoysiagrass breeding, early on progress will be slow, given the long establishment time for zoysiagrass. However, once interesting accessions are identified and hybrids are made (by us or other breeding programs), 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 is now under evaluation in Riverside, CA together with bermudagrass, zoysiagrass, and kikuyugrass germplasm from UCR. Replicate space plantings were established in fall 2016 and starting in fall 2017 accessions will be evaluated for 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.

References

Garner, E.S. (1925) Kikuyu Grass. USGA Green Section Record. 5:252-253.

Gibeault V. 2003. Zoysiagrass for California. Calif. Turfgrass Cult. 53:2:1-3.

Gross, P. J. 2003. Looking kindly at kikuyugrass. USGA Greens Section Record. 41:2-8.

Kopecky, D., A.J. Lukaszewski and V. Gibeault. 2005. Reduction of ploidy level by androgenesis in intergeneric Lolium-Festuca hybrids for turf grass breeding. Crop. Sci. 45:274-281.

Mears, P.T. 1970. Kikuyu- (Pennisetum Clandestinum) as a Pasture Grass- A Review. Trop. Grassl. 4:139-152.

United States Department of Agriculture. 2012. Federal Noxious Weed List. https://plants.usda.gov/java/noxious?rptType=Federal (accessed12 March. 2014).

Wilen, C.A., and J.S. Holt. 1996. Physiological mechanisms for the rapid growth of Pennisetum clandestinum in Mediterranean climates. Weed Res. 36:213-225.

Youngner, V.B., W.W. Wright, and E. Zimmerman. 1971. Kikuyugrass-its management and control. Calif. Turfgrass Cult. 21:1-3

Acknowledgments:

Thanks to the CTLF, USGA, MWD, WMWD, West Coast Turf, Meadow Club, and Arizona Country Club for their support of this research.