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

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Project Milestones Since Field Day 2017:

- ✓ Planted ca. 750 bermudagrass hybrids generated by open pollination of selected collection accessions.
- ✓ Continued crossing of UCR bermudagrass accessions, with emphasis on genotypes possessing desirable winter color retention, early spring green-up, and drought tolerance.
- ✓ Evaluating ca. 1,000 bermudagrass and zoysiagrass accessions in replicate plots from University of Florida, Oklahoma State University, Texas A&M, and UCR for winter color retention.
- ✓ Second screening of UCR bermudagrass hybrids, this time for suitability for lawns.
- ✓ Continued evaluation of 12 of our most promising bermudagrass hybrids or accessions in comparison to Tifway, TifTuf, and Bandera cultivars for fairways/athletic fields (0.5 in mowing height) and lawns/rough (2.0 in mowing height).
- ✓ Evaluating UCR kikuyugrass collection accessions for drought tolerance and winter color retention.
- ✓ Started crossing of UCR kikuyugrass accessions selected for desirable quality traits, drought tolerance and winter color retention.
- ✓ Planted ca. 100 selected kikuyugrass seedlings obtained from wild-type seed stocks.

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 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.

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. 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) genetic markers to aid in breeding efforts and marker-assisted selection.

Bermudagrass:

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 greenup. After evaluating hybrids and collection accessions for winter color retention and visual quality they are being intercrossed on the assumption that the next generation hybrids may show reduced dormancy period. New sets of hybrids were also generated, 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. In the meantime, the best-looking hybrids were 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 were clearly evident and the best of these hybrids are being used in subsequent tests. Two years ago relative drought tolerance of selected hybrids and collection accessions was tested and two of our hybrids survived it in good shape. Because of new plantings in this area test couldn't be repeated. New dry-down testing area will be established early next year to repeat the test on previously evaluated hybrids and the best of new hybrids obtained this year. To establish the parentage of the existing hybrids, the collection and a sample of hybrids were genotyped using 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. Second genotyping performed last winter, including new samples from USDA, suggests that some accessions may be indeed designated incorrectly, since they grouped closely with USDA samples, but with species other than listed, as in the first genotyping results. Analysis showed also that our best hybrids grouped together with *C. transvaalensis* accessions.

Twelve of our most promising accessions or hybrids chosen in 2017 were further evaluated 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, 2017 in Riverside; June 14, 2017 in Coachella Valley; and June 22, 2017 in Fairfax. During the first year of the test dynamics of establishment were measured and after obtaining full cover, visual turf quality, winter color retention, Normalized Difference Vegetation Index (NDVI) and Dark Green Color Index (DGCI, using Digital Image Analysis) were evaluated. In spring 2018 plots at University of California Riverside and West Coast Turf were divided in half to test suitability both for golf courses and lawns. For this purpose half of the each plot was mowed in 0.5 in 3 times a week and the other half in 2.0 in once a week. The higher mowed part of the plot, beside visual quality, NDVI and DGCI was also evaluated for color, scalping injuries and flowering in the spring.

First year of the study showed that among evaluated hybrids TP 6-3, TP 3-2 and NRCC12 were the fastest growing accessions in Southern California, while 10-9 and 15-4 in Northern California (data not shown). During further evaluation only TP 6-3 showed good visual turf quality and winter color retention, supported by high NDVI and DGCI values in all three areas, placing this hybrid in the highest position of the ranking (Table 1). Over the year of testing also 17-8 and BF1 turned out to be among the best evaluated hybrids, both in Southern and Northern California. BF1 seems to be better adapted to cooler areas. The best of commercial cultivars, TifTuf, showed high ranks in visual quality and NDVI, especially in the desert and in Northern California, however taking into account all analyzed factors, it doesn't outmatch UCR entries. When tested for lawns and mowed at 2.0 in, our entries haven't exceeded the best of commercial cultivars, Tifway 419 (Table 2). However, two of them (17-8 and BF2) seem to be more suitable for lawns than other tested commercial cultivars Bandera and TifTuf.

Table 1. Ranking of twelve bermudagrass hybrids and three commercial cultivars – fairway height (0.5 in mowing height).

Code	Visual Quality (1-9)			Visual Color (1-9)			NDVI (0-1)			DGCI (0-1)			**General ranking**
	UCR	WCT	MC	UCR	WCT	MC	UCR	WCT	MC	UCR	WCT	MC	
10-9	11	8	1	7	7.5	5.5	4	7	10.5	3	7	4	**75.5**
15-4	7	13	6	8	10	7	7	9	13	9	9	8	**106.0**
16-6	8.5	7	6	11	7.5	11.5	11	12	9	11	10	10	**114.5**
17-8	2	3	2	3	6	3	2	4	5	2	6	5	**43.0**
TP1-1	14	11	11	14	15	13.5	14	14	12	14	14	15	**161.5**
TP1-2	12	9	13.5	13	12	11.5	13	13	15	15	12	13	**152.0**
TP3-2	8.5	10	13.5	12	9	8.5	12	10	10.5	12	11	11	**128.0**
TP5-4	15	14	12	15	13	13.5	15	15	14	13	13	12	**164.5**
TP6-3	1	2	6	1	3	4	1	3	3	1	3	3	**31.0**
BF1	3	6	3.5	4	2	2	8	2	4	7	1	1	**43.5**
BF2	4	4	10	2	4	5.5	6	6	6	6	2	6	**61.5**
NRCC12	13	15	15	10	14	10	10	11	7	10	15	14	**144.0**
Bandera	10	12	9	9	11	1	9	8	8	4	8	2	**91.0**
Tifway 419	5	5	8	5	1	15	5	5	2	5	4	7	**67.0**
Tif Tuf	6	1	3.5	6	5	8.5	3	1	1	8	5	9	**57.0**

UCR – University of California Riverside; WCT – West Coast Turf, Thermal; MC – Meadow Club, Fairfax
NDVI – Normalized Difference Vegetation Index; DGCI – Dark Green Color Index
– Hybrids with the highest ranks

Table 2. Ranking of twelve bermudagrass hybrids and three commercial cultivars – lawns (2.0 in mowing height).

Code	Quality (1-9)		Color (1-9)		NDVI (1-9)		DGCI (0-1)		Scalping (1-9)		Flowering (1-9)		**General Ranking**
	UCR	WCT	UCR	WCT	UCR	WCT	UCR	WCT	UCR	WCT	UCR	WCT	
10-9	7	8	14	10	3.5	8	6	5	1.5	9	3	6.5	**81.5**
15-4	11	12	3	12	8	9	8	7	10.5	8	4	1.5	**94.0**
16-6	13	9	10	11	13	11	11	11	8.5	13	12.5	6.5	**129.5**
17-8	3	3	3	3.5	1	2	1	9	4	7	1.5	12	**50.0**
TP1-1	12	12	15	14.5	12	13	14	13	7	11	11	4	**138.5**
TP1-2	9.5	7	8.5	6	14	12	15	10	14.5	14	7	10.5	**128.0**
TP3-2	8	12	11.5	13	11	14	12	14	6	12	12.5	8.5	**134.5**
TP5-4	14	14	8.5	9	15	15	9	12	13	15	7	10.5	**142.0**
TP6-3	2	6	3	6	6	5	4	8	12	10	1.5	8.5	**72.0**
BF1	4	5	3	6	7	6	7	3	8.5	5.5	9	3	**67.0**
BF2	1	4	3	8	2	4	3	2	1.5	5.5	7	5	**46.0**
NRCC12	15	15	13	14.5	9.5	10	13	15	14.5	1	14	15	**149.5**
Bandera	9.5	10	6.5	3.5	9.5	7	5	4	10.5	3.5	5	14	**88.0**
Tifway 419	5.5	2	6.5	1.5	5	3	2	1	4	3.5	10	1.5	**45.5**
TifTuf	5.5	1	11.5	1.5	3.5	1	10	6	4	2	15	13	**74.0**

UCR – University of California Riverside; WCT – West Coast Turf, Thermal; MC – Meadow Club, Fairfax
NDVI - Normalized Difference Vegetation Index; DGCI - Dark Green Color Index
– Hybrids with the highest ranks

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) markers 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. Last year we established a collection of available genotypes representing the greatest genetic diversity and conducted dry down events to select for improved drought tolerance. Accessions were also evaluated for turf quality and winter color retention. Stolons from genotypes showing the best quality, drought tolerance and color retention were planted in pots and are being used for crossing. Last year, we located seed stocks of the grass (from about 20-25 years back) and established ca. 400 individual seedlings. These were individually assessed, selected for best suitability for turf, and added to our collection. Selected accessions will be intercrossed and new hybrids screened and selected for further evaluation. 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. Two attempts to reduce ploidy via androgenesis have been made. There is no known technology adapted to this species and the species appears to be recalcitrant. We managed to determine the best methods to collect the material and apply external stresses to induce the switch from gametophytic to sporophytic pathway of microspore development, however none of these microspores managed to survive and form a new plant. 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 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 reenters 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 and zoysiagrass germplasm from Texas A&M University and the University of Florida is now under evaluation in Riverside, CA together with bermudagrass, zoysiagrass, and kikuyugrass germplasm from UCR. Replicate space plantings were established in fall 2016. Starting the winter 2017/2018 accessions are being evaluated for winter color retention and spring green-up, along with turf quality evaluation during the summer season. In addition, tolerance to deficit irrigation will be evaluated. Ratings include visual and NDVI analysis.

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