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Detection of DNA and Ploidy Variation within Vegetatively Propagated Zoysiagrass Cultivars

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Abstract. **Zoysiagrass (*Zoysia* sp.) is used as a warm-season turfgrass for lawns, parks, and golf courses in the warm, humid and transitional climatic regions of the United States. Zoysiagrass is an allotetraploid species (*2n = 4x = 40*) and some cultivars are known to easily self- and cross-pollinate. Previous studies showed that genetic variability in the clonal cultivars Emerald and Diamond was likely the result of contamination (seed production or mechanical transfer) or mislabeling. To determine the extent of genetic variability of vegetatively propagated zoysiagrass cultivars, samples were collected from six commercially available zoysiagrass cultivars (Diamond, Emerald, Empire, JaMur, Meyer, Zeon) from five states (Arkansas, Florida, Georgia, North Carolina, Texas). Two of the newest cultivar releases (Geo and Atlantic) were to serve as outgroups. Where available, one sample from university research plots and two samples from sod farms were collected for each cultivar per state. Forty zoysiagrass simple sequence repeat (SSR) markers and flow cytometry were used to compare genetic and ploidy variation of each collected sample to a reference sample. Seventy-five samples were genotyped and an unweighted pair group method with arithmetic mean clustering revealed four groups. Group I (*Z. japonica*) included samples of 'Meyer' and Empire11 ('Empire' sample at location #11), Group II (*Z. japonica* × *Z. pacifica*) included samples of 'Emerald' and 'Geo', Group III (*Z. matrella*) included samples of 'Diamond' and 'Zeon', and Group IV (*Z. japonica*) consisted of samples from 'Empire', 'JaMur', 'Atlantic', and Meyer3 ('Meyer' at sample location #3). Samples of 'Empire', 'Atlantic', and 'JaMur' were indistinguishable with the markers used. Four samples were found to have alleles different from the respective reference cultivar, including two samples of 'Meyer', one sample of 'Empire', and one sample of 'Emerald'. Three of these samples were from Texas and one of these samples was from Florida. Three of the four samples that were different from the reference cultivar were university samples. In addition, one sample, Empire11, was found to be an octoploid (*2n = 8x = 80*). For those samples that had a fingerprint different from the reference cultivar, contamination, selfing, and/or hybridization with other zoysiagrasses may have occurred.**

In the United States, zoysiagrass refers to two perennial species from the genus *Zoysia* (*Z. japonica* and *Z. matrella*) that are used as a warm-season turfgrass for lawns, parks, and golfing surfaces (tees, fairways, roughs, bunker faces) in the warm, humid and transitional climatic regions. In Japan and other countries of East Asia, *Z. japonica* is also used as a forage grass (Cai et al., 2005; Tsuruta et al., 2005). Zoysiagrasses are slow to establish, require full sun to partial shade, and have high heat and drought tolerance. Differences between the species exist because *Z. matrella* has narrower leaves, grows more slowly, and is less cold-hardy than *Z. japonica*, yet *Z. matrella* is more tolerant to salinity and insect pests (Patton, 2009). Zoysiagrass has been slowly increasing in use across the United States since it was introduced ≈1900. The cultivars Meyer and Emerald were released in the 1950s and are considered to be the industry standards for zoysiagrass (Patton, 2009).

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Zoysiagrass is an allotetraploid species (*2n = 4x = 40*) (Arumuganathan et al., 1999; Forbes, 1952) with an estimated genome size of 421 Mb for cultivar Zenith (Arumuganathan et al., 1999). Zoysiagrass has the most genetic tools as compared with the other widely used warm-season turfgrasses such as st. augustine grass (*Stenotaphrum secundatum*), centipedegrass (*Eremochloa ophiuroides*), seashore paspalum (*Paspalum vaginatum*), and common bermudagrass (*Cynodon dactylon*). The genetic diversity of zoysiagrass species has been characterized with amplified fragment length polymorphisms (Chen et al., 2009), restriction fragment length polymorphisms, random amplified polymorphic DNA markers (Weng et al., 2007; Yaneshita et al., 1999), and even by flavonoids (Anderson et al., 2007). A high-density SSR genomic map has been completed (Li et al., 2009, 2010). Additionally, markers have been identified that are linked to a locus for fall armyworm (*Spodoptera frugiperda*) resistance (Jessup et al., 2011). Although 1044 SSRs have been developed for zoysiagrass (Cai et al., 2005), most are not publicly available (Jessup et al., 2011); however, a small number of zoysiagrass SSRs have been developed and released (Tsuruta et al., 2005).

From 2001–05, homeowners and landscapers in Georgia and Alabama noticed inconsistent spring green-up patterns in the cultivar Emerald and it was determined that many different genotypes are present, arising from either contamination or mislabeling (Waltz et al., 2005). A previous study examining marker transferability between bermudagrass and zoysiagrass found that sources of the vegetatively propagated zoysiagrass cultivar Diamond from North Carolina and Georgia breeding programs were genetically different (Harris-Shultz et al., 2012). Additionally, some cultivars of zoysiagrass are known to easily self- and cross-pollinate (Harris-Shultz et al., 2012; Kimball et al., 2012a). Because zoysiagrass is not apomictic, it is possible that sod fields could become contaminated by germinating seedlings if zoysiagrass inflorescences were allowed to mature. The objective of this study was to determine the genetic variability and ploidy variation within vegetatively propagated zoysiagrass cultivars.

Materials and Methods

Sample collection and DNA extraction. Six popular clonal zoysiagrass cultivars were collected from five states and were examined for within-cultivar variability. 'Diamond', 'Emerald', 'Empire', 'JaMur', 'Meyer', and 'Zeon' (Table 1) were collected from Arkansas, Florida, Georgia, North Carolina, and Texas. These will be referred to in the remainder of the article by the cultivar name and a unique identifier number, which describes the location/source of the specimen collection (Supplemental Table 1). The representative person from each state was responsible for providing a university sample of each cultivar and two samples of each cultivar from sod producers in their state. Because each of the six cultivars selected for this study was not grown for sale in every state, samples were collected based on their availability. Each sample consisted of one or two 10-cm-diameter plugs from a field location of the collector's or sod producer's choosing (Supplemental Table 1). Additionally, a reference sample for each cultivar was collected from the breeder or foundation fields or from K. Morris at the National Turfgrass Evaluation Program. All samples were sent to K. Harris-Shultz and maintained in a temperature-controlled greenhouse. DNA was extracted using a Pure Link Plant DNA Purification kit (Life Technologies Corp., Grand Island, NY), run on a 1% agarose gel to control for quality, and analyzed using a spectrophotometer (Nanodrop 2000c; Thermo Scientific, Wilmington, DE) to control for DNA quantity.

Forty primer pairs were selected that had been previously identified as being the most polymorphic in a collection of zoysia accessions (Kimball et al., 2012a) (Supplemental Table 2). These primers had been previously developed by Li et al. (2009) and Ma et al. (2007). Each primer pair was added in a 10-μL reaction volume that contained 2 μL of 5× reaction buffer (Clear GoTaq®; Promega Corp., Madison, WI), 1 μL of 25 mM $MgCl_2$, 0.8 μL of 2.5 mM dNTP mix, 0.5 μL of M13-tagged forward primer, 2.0 μL of reverse primer at 1 μM, 1.8 μL of 1 μM M13 primer (M13-TGTAAAACGACGGCCAGT) fluorescently labeled with the IRDye® 700 CW fluorophore (Eurofins MWG Operon, Huntsville, AL), 0.04 μL of GoTaq® DNA polymerase (Promega Corp.), 0.86 μL of sterile water, and 1 μL of 2.5 ng·μL^{-1} DNA. Thermocycler conditions were: an initial denaturation at 94 °C for 3 min, 39 cycles of 94 °C for 30 s, 45 to 60 °C (see Supplemental Table 2) for 1 min, 72 °C for 1 min 10 s, and a final elongation step at 72 °C for 10 min. The thermocycler used was a Gene Amp PCR System 9700 dual block (Applied Biosystems, Foster City, CA). Two microliters of polymerase chain reaction (PCR) product were combined with 5 μL of Blue Stop (LI-COR® Biosciences, Lincoln, NE) and 0.35 μL of this mixture was loaded on a 6.5% v/v

Table 1. Vegetatively propagated zoysiagrass cultivars examined with 40 simple sequence repeat markers.[z]

Cultivar	*Zoysia* species	Breeder or supplier	Yr
Diamond	*Z. matrella*	Texas AgriLife, Dallas, TX	1996
Emerald	*Z. japonica* × *Z. pacifica*[y]	USDA-ARS, Beltsville, MD	1955
JaMur	*Z. japonica*	Bladerunner Farms, Poteet, TX	2002
Meyer	*Z. japonica*	USDA-ARS, Beltsville, MD	1951
Zeon	*Z. matrella*	Bladerunner Farms	1996
Empire	*Z. japonica*	Sod Solutions, Mount Pleasant, SC	2000
Geo	*Z. japonica* × *Z. pacifica*	Sod Solutions	2011
Atlantic	*Z. japonica*	Sod Solutions	NR[x]

[z]Table information from Patton (2009) with the exception of the cultivars Empire and JaMur, which was obtained from information obtained from plant patents (Doguet, 2002; Gurgel and Ito, 2000).
[y]Previously referred to as a cross between *Z. japonica* and *Z. tenuifolia*. In the literature, *Z. pacifica* has been previously incorrectly referred to as *Z. tenuifolia* (Anderson, 2000).
[x]Not yet released.

acrylamide gel and run on a LI-COR® Biosciences 4300 DNA Analyzer. Gel images were scored visually and coded as a "1" for presence of a band or "0" for absence of a band for each accession for each marker. Sequences for the 10 chloroplast-specific simple sequence repeat length polymorphism (CpSSRLP) markers (Karaca et al., 2002) and 11 zoysiagrass SSR markers (Jessup et al., 2011; La Mantia et al., 2011; Tsuruta et al., 2005) were amplified on samples JaMur1 and Empire1 using the parameters as described previously with the exception of the La Mantia et al. (2011) primers. For the La Mantia et al. (2011) zoysiagrass primers, each reaction contained 1.0 µL of 5× Clear GoTaq® reaction buffer, 1 µL of 25 mM $MgCl_2$, 1.0 µL of 2.5 mM dNTP mix, 0.5 µL of M13-tagged forward primer, 2.0 µL of reverse primer at 1 µM, 1.8 µL of 1 µM M13 primer (M13-TGTAAAACGACGGCCAGT) fluorescently labeled with the IRDye® 700 CW fluorophore, 0.05 µL of GoTaq® DNA polymerase, 0.86 µL of sterile water, and 1 µL of 2.5 ng·µL^{-1} DNA. Thermocycler conditions were: an initial denaturation at 94 °C for 3 min, 35 cycles of 94 °C for 40 s, 53 °C for 40 s, 72 °C for 30 s, and a final elongation step at 72 °C for 4 min.

Data analysis. Marker data were imported into NTSYSpc (Rohlf, 2008). Genetic similarity between each pair of accessions was calculated using the SIMQUAL module using the DICE coefficient of similarity (Nei and Li, 1979). A dendrogram was generated from the similarity matrix by using the unweighted pair group method with arithmetic mean clustering procedure in the SAHN module of NTSYSpc. Bootstrap resampling was performed using the software program FreeTree (Hampl et al., 2001) with 500 bootstrap repetitions. Because FreeTree does not allow missing data, those markers that contained missing data were removed. Bootstrap values greater than 50 are noted in Figure 1.

Flow cytometry. Zoysiagrass samples were processed and analyzed on a flow cytometer (BD Accuri C6; Becton Dickinson and Co., Franklin Lakes, NJ) by first adding 1000 µL of Tris-$MgCl_2$ nuclei extraction buffer (Pfosser et al., 1995) to 0.02 to 0.03 g of fresh leaf tissue using a modified protocol (Galbraith, 2009). For each sample of a cultivar, the reference stock of that cultivar was cochopped with each sample. For comparison of the ploidy between the cultivars, the reference stock of each cultivar was cochopped with the reference stock of another cultivar. This procedure was repeated until the ploidies of all cultivars were examined. Tissue was chopped using a double-edged razor blade (Astra Superior Platinum; ZAO Petersburg Products, St. Petersburg, Russia) until fully macerated. The resulting slurry was then pipetted into a 50-µm disposable filter (CellTrics; Partec, Munster, Germany) sitting on top of a plastic

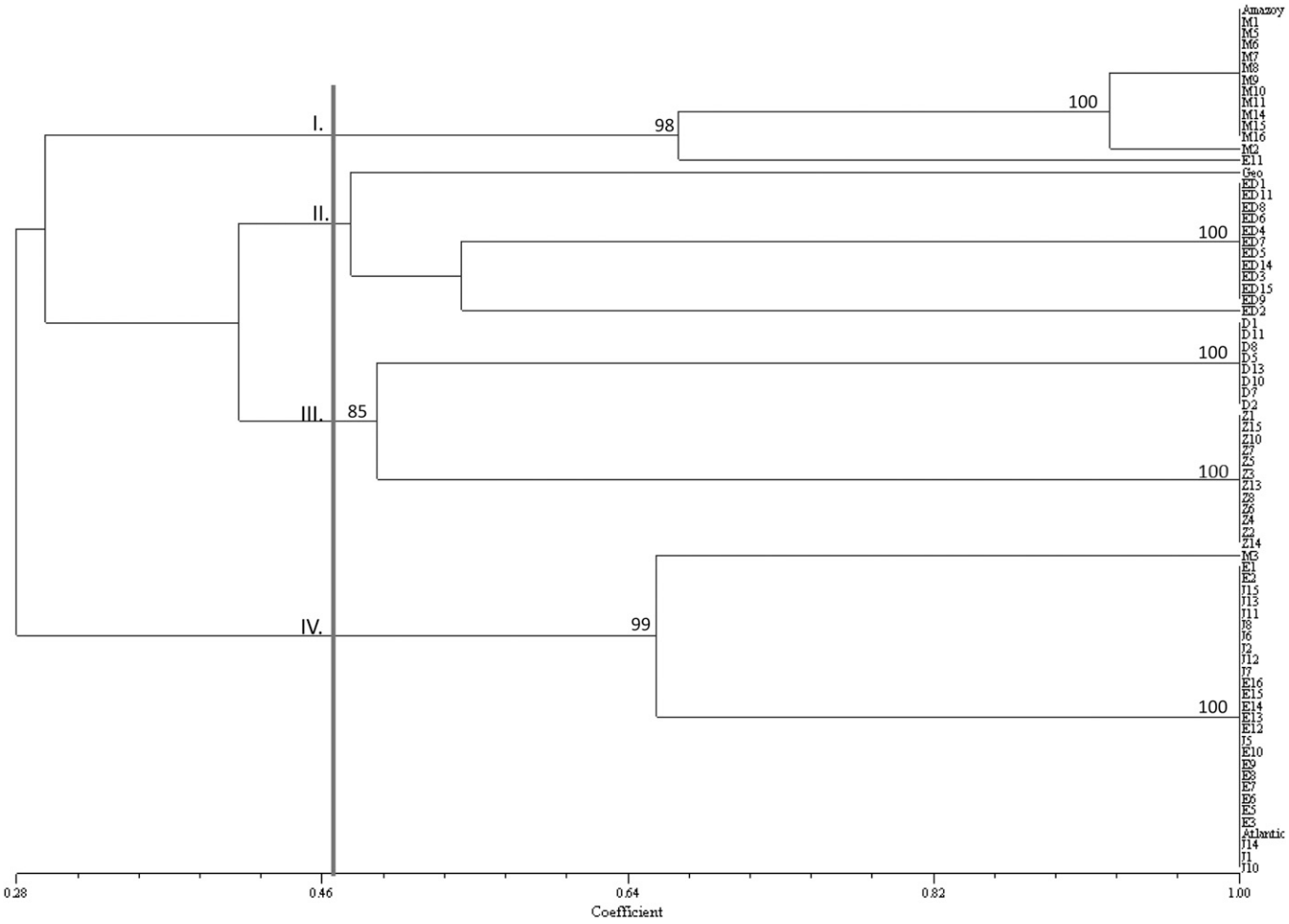

Fig. 1. Unweighted pair group method with arithmetic averages (UPGMA) dendrogram of 75 vegetatively propagated zoysiagrass samples representing six cultivars. Bootstrap values greater than 50% are shown. All samples were found to be tetraploids except sample E11, which is an octoploid; M = 'Meyer', ED = 'Emerald', D = 'Diamond', Z = 'Zeon', E = 'Empire', J = 'JaMur'.

12 × 75-mm culture tube. The filter was removed and 500 µL of Rnase/propidium iodide solution (BD Biosciences, San Jose, CA) was added to each filtered sample. Samples were then placed on ice for 15 min and then run on the device using gating that selected objects that exhibited a strong correlation between the FL-2, fluorescence signals screened with a 585/20-nm bandpass filter, and FL-3 signals, fluorescence signals screened with a 670-nm longpass filter. At least 50,000 events were collected for each sample, the flow rate was set to slow (14 $\mu L \cdot min^{-1}$), and the mean FL-2 and CV were recorded for the Gap 1 (G1) and G2 peaks. Ploidy level was determined based on the G1 and G2 sample peaks relative to the control G1 and G2 peaks.

Cytology. The zoysiagrass plants Empire11 and Meyer1 were shipped to Texas, subdivided, and initially grown in small pots (≈15 cm diameter) of commercial potting soil (Fafard 52; Sun Gro Horticulture, Agawam, MA) in a heated glasshouse, but growth was anemic and so they were moved into an indoor hallway where they were illuminated by light-emitting diode (LED) grow lights (LumiGrow ES330; LumiGrow, Novato, CA) using maximum levels of red and blue LED lighting. Once plants were rejuvenated, 1.5-cm root tips were harvested and pre-treated with alpha-bromonaphthalene (saturated) for 90 min at room temperature, then fixed in 95% ethanol-acetic acid (3:1) fixative for at least 24 h, and then rinsed in water for 60 min. Excised meristematic tips were placed in 0.2 N HCl for 30 min at 37 °C, rinsed three times in water 15 min each, and placed in an enzyme solution of 30% cellulase (Sigma-Aldrich, St. Louis, MO) and 15% pectolyase (Sigma-Aldrich) in 0.1 M citrate buffer at 37 °C for 40 to 50 min, rinsed with distilled water, placed on a clean glass slide with a drop of ethanol-acetic acid (3:1) fixative, macerated using fine-pointed forceps, and allowed to air-dry. Dried slides were then stained with azure B (Sigma-Aldrich), 25 to 200 $\mu g \cdot mL^{-1}$ of 0.1 M phosphate buffer at pH 6.4 to 7.0 Azure B (Halfmann et al., 2007). Stained chromosome spreads were viewed under brightfield illumination using a light microscope (Universal II; Zeiss, Oberkochen, Germany); good spreads were evaluated under oil immersion optics (numerical aperture = 1.40) and digital images of the best spreads were captured using a color camera (Microfire; Optronics, Goleta, CA).

Results and Discussion

Seventy-five samples were examined of the cultivars Diamond, Emerald, Empire, Meyer, JaMur, and Zeon. which also included two newly released zoysiagrass cultivars, Geo and Atlantic (Sod Solutions, Mount Pleasant, SC). which served as outgroups (Supplemental Table 1). A single university sample and two samples from sod farms were obtained, if available, for the six cultivars from each state. For all of the accessions, a maximum of only two alleles was seen for most primer pairs with the exception of Meyer2, which frequently displayed a third faint allele. More than two fragments were seen per accession for five primer pairs (b01012, b06n05, GBZJM029, GBZJM039, GBZJM110) but these are likely non-specific amplification or homologous loci given the wide range of size variation. Although zoysiagrass is a polyploid, many studies that have used SSR markers have identified only one or two alleles per cultivar (Cai et al., 2005; Tsuruta et al., 2005). Linkage analysis supports disomic inheritance (Cai et al., 2005), but observations of more than two alleles per accession have been reported (La Mantia et al., 2011; Li et al., 2009). Forty SSR markers were amplified on these 75 samples and a dendrogram was produced (Fig. 1). Using a genetic similarity cutoff of 0.48, the samples were differentiated into four groups. Group I contained the 'Meyer' samples (*Z. japonica*) and Empire11. Group II consisted of the 'Emerald' (*Z. japonica* × *Z. pacifica*) and 'Geo' (*Z. japonica* × *Z. pacifica*) samples. Group III consisted of the 'Diamond' and 'Zeon' samples (*Z. matrella*), and Group IV consisted of samples of 'Empire', 'JaMur' as well as the 'Atlantic' and Meyer3 sample (*Z. japonica*). Of interest, cultivars Empire, Atlantic, and JaMur were indistinguishable using these 40 primer pairs. Both 'Empire' and 'JaMur' are cultivars that were both filed for patent in 1998 (Doguet, 2002; Gurgel and Ito, 2000). 'JaMur' was discovered from a collection of plants in Japan made by Jack Murray, whereas 'Empire' was discovered on a Brazilian turf farm (Doguet, 2002; Gurgel and Ito, 2000). The pedigree of cultivar Atlantic is unknown because its patent is pending. Kimball et al. (2012a) reported a single sample of 'Empire' and a single sample of 'JaMur' had a genetic similarity of 0.75. A list of primers was obtained for SSRs that were polymorphic between the two samples in their study. Most of these primers have been run in this current study and no polymorphism was observed. DNA was re-extracted from JaMur1, JaMur5, Empire1, Empire5, and Emerald9 and DNA was amplified with a subset of these primers (seven primer pairs). No polymorphisms were seen between the 'JaMur' and 'Empire' samples. To identify polymorphic fragments between 'Empire' and 'JaMur', 10 CpSSRLP markers (Karaca et al., 2002) and 11 zoysiagrass SSR markers (Jessup et al., 2011; La Mantia et al., 2011; Tsuruta et al., 2005) were used to amplify DNA from the reference cultivar samples of 'Empire' and 'JaMur'. No differences were seen between the 'Empire' and 'JaMur' reference cultivar samples using these markers. A plausible explanation of why differences were seen between 'Empire' and 'JaMur' in the Kimball et al. (2012a) study, despite using the same primer pairs, is that the authors used vegetative clones of a different genotype than the samples of 'Empire' and 'JaMur' evaluated in this study.

Cultivars that are genetically similar, and often very difficult to distinguish genotypically or phenotypically, are commonly found in turfgrass. Cultivar genetic similarity is the result of the use of natural or induced off-types or well-defined areas with noticeable differences in plant morphology in cultivars that are then released as new cultivars or the accidental release of existing cultivars. For example, the bermudagrass cultivar Tifdwarf is a natural somatic mutant of 'Tifgreen' and was found in golf greens (Burton, 1966). Examination of 'Tifdwarf' with 70 SSR markers identified only two fragments that were unique to 'Tifdwarf' as compared with 'Tifgreen' (Harris-Shultz et al., 2011). Furthermore, many of these nearly genetically identical cultivars have reported pedigrees that are incorrect. For example, the bermudagrass 'TifEagle' was reported to be an off-type derived from 'Tifway 2' (Hanna and Elsner, 1999) but is actually derived from 'Tifgreen' (Kamps et al., 2011; Wang et al., 2010) and the seashore paspalum cultivars Seadwarf, Temple2, SeaIsle 1, and Taliaferra were indistinguishable using 80 SSR markers despite diverse reported collection locations (Harris-Shultz et al., 2013).

From the dendrogram, it was immediately apparent that samples Meyer2, Meyer3, Empire11, and Emerald2 did not match the fingerprint of the reference cultivar. For all the primer pairs examined, sample Meyer2 had all of the reference

'Meyer' alleles and often (nine of 40 SSR primer pairs) an additional faint allele indicating that this sample is likely 'Meyer' with a contaminating zoysiagrass. This contaminating zoysiagrass did not match any of the genotypes examined. Empire11 is genetically similar (0.679) to 'Meyer' and may be derived from a 'Meyer' cross with an unknown grass, but for two primer combinations, a03k14 and b02c05, a 'Meyer' allele was not seen. Emerald2 is likely a cross of 'Emerald' with another zoysiagrass, because Emerald2 had an 'Emerald' allele for the 40 primer pairs used. Meyer3 may be derived from a cross with 'Empire'/'JaMur'/'Atlantic', yet for two primer pairs (b06n05, Ta148), an 'Empire'/'JaMur'/'Atlantic' allele was not seen.

A previous study had noted that 'Empire' and 'JaMur' had statistically different 2C DNA contents (0.88 and 0.84 pg, respectively) (Schwartz et al., 2010). Examination of all 'Empire' and 'JaMur' samples from the greenhouse found no difference between the FL-2 means of 'Empire' (mean = 28,742.00; SD = 1328.11) and 'JaMur' samples (mean = 29,227.20; SD = 1951.424) on the flow cytometer. Furthermore, when the samples were cochopped, a single G1 peak was seen. All samples were cochopped with their reference cultivar to eliminate artifacts. No difference in ploidy was seen between any of the samples except sample Empire11, which consistently ran as twice the FL-2 mean of 'Empire' when the samples were cochopped ('Empire': FL-2 mean 29,000, 1.94% CV; Empire11: FL-2 mean 59,000, 1.97% CV) or run alone (Empire1 alone: FL-2 mean 32,000, 3.6% CV; Empire11 alone: FL-2 mean 63,000, 3.11% CV). Empire11 was obtained from the University of Florida and all reported cytological studies of *Zoysia* species have determined $2n = 4x = 40$ or $2n = 2x = 20$ (Gould and Soderstrom, 1974; Schwartz et al., 2010). The chromosome number of Empire11 was determined using root tip spreads and was found to be $2n = 8x = 80$ (Fig. 2). The mechanism (i.e., somatic chromosome doubling or the fertilization of unreduced gametes) of polyploidization of Empire11 is unknown but dinitroaniline herbicides are commonly used as a pre-emergent herbicide in turfgrass (McCullough et al., 2013). Dinitroaniline herbicides are mitosis-inhibiting herbicides that are used for in vitro chromosome doubling (Dhooghe et al., 2011). Indeed, the field from which Empire11 was harvested had been treated with prodiamine (Barricade; Syngenta Crop Protection, Greensboro, NC) at least once per year since it was installed in 2005 (K. Kenworthy, personal communication). The discovery of a natural octoploid zoysiagrass is particularly exciting because all zoysiagrass cultivars are fertile and *4x* and thus capable of exchanging pollen to create seedlings with a different genotype than the parent. Zoysiagrass seedlings with different genotypes than the parental genotype could look different and thus detract from visual uniformity, which is demanded in turfgrass. To eliminate or reduce possible outcrossing events, breeders could develop sterile hybrids by crossing different plants of different ploidy levels, which has been done extensively in bermudagrass (Burton, 1974). Crosses between different ploidy levels in turfgrass have been quite common and the progeny are screened for desirable turfgrass traits (Taliaferro, 1995).

As for the purity of the six cultivars in five states, only four were identified as having genotypes different from the reference cultivar. Excluding the reference sample, two of 12 samples of 'Meyer' were different from the reference sample, one of 14 samples of 'Empire' was different from the reference sample, and one of 11 samples of 'Emerald' was different from the reference sample. Of the four samples, three were from Texas and one was from Florida. Of these four samples that possessed genotypes different from the reference, three of these were university samples and only one sample currently under sod production in Texas varied from the reference sample. No differences were detected between samples of 'Diamond', 'Zeon', or 'JaMur' when compared with the respective reference genotypes. These data indicate, in general, most of the samples received were genotypically similar to the reference cultivar despite these cultivars being fully fertile indicating that management practices are sufficient for genetic purity. Past problems with the production of 'U-3' common bermudagrass (Anderson et al., 2001), 'Raleigh' st. augustinegrass (Kimball et al., 2012b), and 'Emerald' zoysiagrass (Waltz et al., 2005) indicate that outcrossing, selfing, or contamination in production fields can occur leading to highly variable turf performance. Furthermore, these data indicate that the genetic purity of 'Emerald' has improved since the Waltz et al. (2005) study. This study also illustrates the usefulness of molecular markers

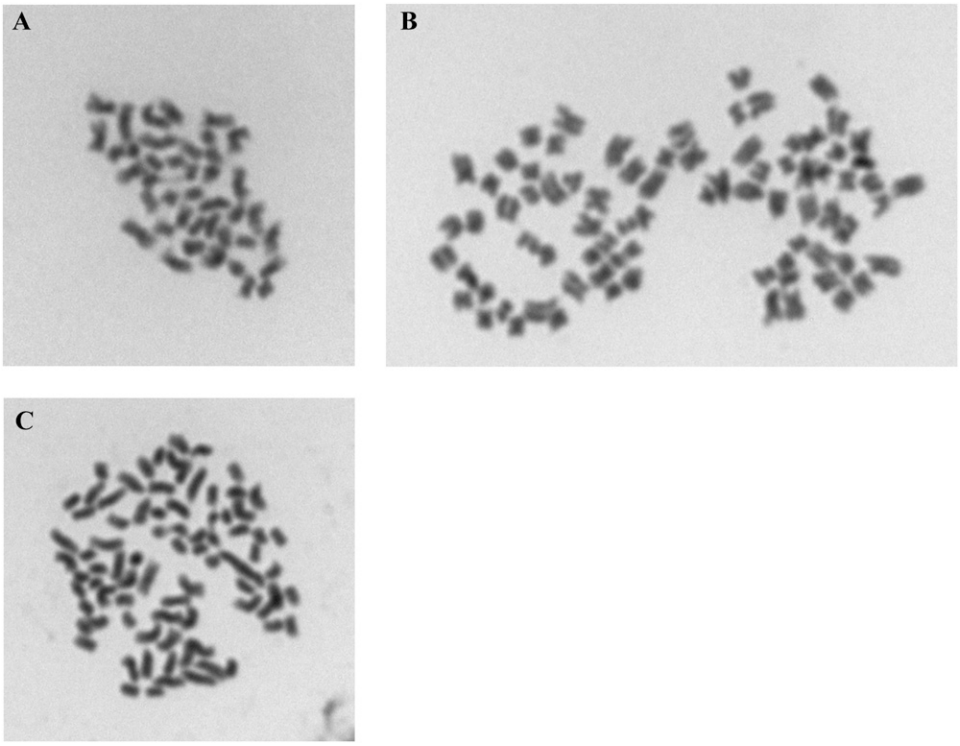

Fig. 2. Images of root-tip chromosome spreads from zoysiagrass Meyer1 (**A**) and Empire11 (**B–C**) at metaphase taken at 100× magnification (numerical aperture = 1.40).

for identifying and preserving vegetatively propagated cultivars and for the detection of genetic variants in turfgrass breeding programs and sod production fields. Owners of the four samples different from the reference sample were notified so that this material can be relabeled or perhaps used for other purposes.

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Supplemental Table 1. Source information for samples collected from six zoysiagrass cultivars from five different states.[z]

Sample name	Sample type[y]	Source[x]
Amazoy	Commercial Meyer	Zoysia Farm Nurseries, Taneytown, MD
Atlantic	Outgroup	Sod Solutions, Mount Pleasant, SC
Diamond1	Original stock	Trinity Turf, Pilot Point, TX
Diamond2	University	Texas AgriLife, Dallas
Diamond5	University	NCSU
Diamond7	University	University of Arkansas, Fayetteville
Diamond8	Sod farm	Double Springs Grass Farm, Judsonia, AR
Diamond10	University	University of Florida, Gainesville
Diamond11	Sod farm	SuperTurf, Indiantown, FL
Diamond13	University	UGA
Emerald1	Original stock	NTEP
Emerald2	University	Texas AgriLife
Emerald3	Sod farm	Rod Grass Farm, El Campo, TX
Emerald4	Sod farm	Wittig Grass Sales, Boling, TX
Emerald5	University	NCSU
Emerald6	Sod farm	Lawn Pro, Hubert, NC
Emerald7	Sod farm	Quality Turf, Burgaw, NC
Emerald8	University	University of Arkansas
Emerald9	Sod farm	Winrock Grass Farm, Little Rock, AR
Emerald11	University	University of Florida
Emerald14	University	UGA
Emerald15	Sod farm	Patten Seed, Lakeland, GA
Empire1	Original stock	Sod Solutions
Empire2	University	Texas AgriLife
Empire3	Sod farm	Rod Grass Farm
Empire5	University	NCSU
Empire6	Sod farm	Oakland Plantation, Council, NC
Empire7	Sod farm	Quality Turf
Empire8	University	University of Arkansas
Empire9	Sod farm	Searck Sod Farm, Dermott, AR
Empire10	Sod farm	Geisler Bros., Wheatley, AR
Empire11	University	University of Florida
Empire12	Sod farm	Bethel Farms, Arcadia, FL
Empire13	Sod farm	Bayside Sod, Sarasota, FL
Empire14	University	UGA
Empire15	Sod farm	Legacy Turf, Cartersville, GA
Empire16	Sod farm	McIntyre Turf, Abbeville, GA
Geo	Outgroup	Sod Solutions
JaMur1	Original stock	Bladerunner Farms, Poteet, TX
JaMur2	University	Texas AgriLife
JaMur5	University	NCSU
JaMur6	Sod farm	Cape Fear Turf Farm, Council, NC
JaMur7	Sod farm	Tri-State Turf, Dunn, NC
JaMur8	University	University of Arkansas
JaMur10	University	University of Florida
JaMur11	Sod farm	Cura Sod, Tampa, FL
JaMur12	Sod farm	S&K Sod, St. Cloud, FL
JaMur13	University	UGA
JaMur14	Sod farm	NG Turf, Whitesburg, GA
JaMur15	Sod farm	Sumter Sod, Leslie, GA
Meyer1	Original stock	NTEP
Meyer2	University	Texas AgriLife
Meyer3	Sod farm	Unlisted[w]
Meyer5	University	NCSU
Meyer6	Sod farm	Carolina Turf Farms, Raeford, NC
Meyer7	Sod farm	Quality Turf
Meyer8	University	University of Arkansas
Meyer9	Sod farm	Double Springs Sod Farm

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Supplemental Table 1. Continued.

Sample name	Sample type[y]	Source[x]
Meyer10	Sod farm	Winrock Grass Farm
Meyer11	University	University of Florida
Meyer14	University	UGA
Meyer15	Sod farm	NG Turf
Meyer16	Sod farm	Sod Atlanta, Cartersville, GA
Zeon1	Original stock	Bladerunner Farms
Zeon2	University	Texas AgriLife
Zeon3	Sod farm	Tri-Tex Grass, Tioga, TX
Zeon4	Sod farm	Rod Grass Farm
Zeon5	University	NCSU
Zeon6	Sod farm	Sandhills Turf, Eagle Springs, NC
Zeon7	Sod farm	Tri-State Turf
Zeon8	University	University of Arkansas
Zeon10	University	University of Florida
Zeon13	University	UGA
Zeon14	Sod farm	Sod Atlanta
Zeon15	Sod farm	NG Turf

[z]K. Harris-Shultz and C. Waltz were responsible for samples from Georgia, A. Patton was responsible for the Arkansas samples, S. Milla-Lewis was responsible for samples from North Carolina, A. Chandra was responsible from samples obtained from Texas, and K. Kenworthy and K. Harris-Shultz were responsible for samples from Florida.

[y]Commercial Meyer = 'Meyer' grown under another name and sold; Outgroup = a cultivar that was not one of the six cultivars examined that was presumed to be genetically different from the six cultivars; Original stock = reference sample used to compare the genetic purity of samples collected from universities and sod farms.

[x]NCSU = North Carolina State University, Raleigh; UGA = University of Georgia, Tifton; NTEP = National Turfgrass Evaluation Program, Beltsville, MD.

[w]Sod farm owner asked to be unlisted.

Supplemental Table 2. Zoysiagrass simple sequence repeat (SSR) or chloroplast specific SSR length polymorphism markers used to distinguish zoysiagrass cultivars.

Primer name	Repeat	Forward sequence (5′-3′)	Reverse sequence (5′-3′)	Source	LG in zoysia SSR map	Estimated allele size of reference sample (bp)[z]							
						Diamond	Emerald	Empire	Jamur	Meyer	Zeon	Geo	Atlantic
a01a07	$(TG)_{14}$	CATTCCTGATCATCTGAGACTTC	AATTACTGCCATTTCTGGC	Li et al., 2009	14	140, 149	173, 185	147	147	143	153, 185	150, 185	147
a01n09	$(CA)_{13}$	CGACTCCCTGGTGTTCTACAA	TTGAGATACGTCCAAGGAAC	Li et al., 2009	11	111	111, 141	111, 126	111, 126	111, 122	111, 122	111, 126	111, 126
a02f14	$(TG)_{12}$	CATGTATTACACATGATGGTTGC	AAACATTGTTAGCACATTACCA	Li et al., 2009	19	265, 306	265, 283	255, 281	255, 281	255, 295	255, 265	275	255, 281
a02m03	$(TC)_6(TG)_{20}(TC)_{16}$	CTCCATATTTTACCCGCTAGA	CTTCTCAATCGATGCCTG	Li et al., 2009	9	122, 157	98, 147	98, 141	98, 141	98	98, 157	100, 147	98, 141
a03f03	$(TG)_{14}$	CATCAAGGTAACAAGATCACGA	GAGAAGGACGTAACGTAACAA	Li et al., 2009	21	130, 147	147, 149	145, 147	145, 147	147	NS	149	145, 147
a03k14	$(TG)_{32}$	CAGGACCAGTGAACATAGTGG	TGATTGAACAGAAAATTCACC	Li et al., 2009	2	96, 169	126, 173	107, 130	107, 130	120, 149	124, 169	136, 173	107, 130
b01c16	$(GA)_{20}$	CAGGACAAGTGAGGCAGTAGA	AAAGGTCAGTCTCCGTCC	Li et al., 2009	11	126, 134	130, 147	126, 132	126, 132	132, 151	126, 147	126, 147	126, 132
b01o12	$(GA)_{15}$	CTGCTGTCCTTTAGTCCAAGT	TCTGCTTCAGCTCTGTCAC	Li et al., 2009	14	180	145, 170	143, 151	143, 151	122, 145	147, 180	170	143, 151
b02c05	$(TC)_{23}$	CCATATCTGGTCGATCGATTT	AAAAGATCCAGAGGTGCC	Li et al., 2009	6	155, 181	181	155	155	161, 175	155, 161	171, 181	155
b02e03	$(TG)_8(GA)_{14}$	CTATTTTGGTTTCTCAACTGTCA	CAGGGATAGTTGTTGCATG	Li et al., 2009	7	240, 271	255, 271	240, 265	240, 265	238, 255	263, 271	267, 271	240, 265
b03b03	$(TC)_{24}$	CAGCCTCTGTCTTCCATGG	GAATAGCGGGTACACCTG	Li et al., 2009	7	161	159	145	145	141, 161	159	145, 163	145
b03i14	$(GA)_{12}$	CTGTGTCTCCCAGAGATGG	AGGAGAGCTGTCGTTCTGT	Li et al., 2009	13	255	253, 255	249	249	253, 263	255	253, 257	249
b03o03a	$(GA)_{10}$	CTGCAACAGGTAGCTGGTAG	GCATCAGCAGGTAGATTCTC	Li et al., 2009	1	257	255	259	259	257	257	234, 257	259
b06n05	$(GA)_{22}$	CAAGCAACAAGCAAGCAAG	GACGTCCACAGACACCAC	Li et al., 2009	7	NS	183	214	214	170	179	183	214
b08k21	$(TC)_{13}$	CGAGAACGACGTCCTGGAC	ACTTCGCACACAGAGAAGAC	Li et al., 2009	18	151	167	151, 187	151, 187	155, 167	151, 155	155	151, 187
b09e08	$(GA)_{15}$	CAGAACGTTAGATGTAGAGCCC	CTCTTATCCCAAAATCACTCC	Li et al., 2009	8	142	142, 144	141, 143	141, 143	147	150	142, 145	141, 143
b10k05	$(TC)_{13}$	CATGTCCTAATGGACAATTGG	GAGACTGCATTGGACTGTG	Li et al., 2009	2	140, 147	147	140	140	147	147, 155	147, 149	140
b10m09	$(GA)_{12}$	CCAGCATAGGAAAGGAAGCTA	AGTGTAAGCCGTTGCTTG	Li et al., 2009	1	164, 190	190	170	170	186, 196	164, 170	170	170
c01l07	$(TTC)_6$	CTTATGGTGCTGCTGTGTAAG	AGAAGAGCATTGAGCTGGT	Li et al., 2009	11	NS	NS	215	215	196, 200	202	197	215

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Supplemental Table 2. Continued.

Primer name	Repeat	Forward sequence (5′-3′)	Reverse sequence (5′-3′)	Source	LG in zoysia SSR map	Estimated allele size of reference sample (bp)[z]							
						Diamond	Emerald	Empire	Jamur	Meyer	Zeon	Geo	Atlantic
c02p14	$(TTC)_{11}$	CTGTTCTACACAGGCAGAGC	TACGTACTAGCCAGCTATTGG	Li et al., 2009	4	244	234, 244	226, 242	226, 242	234, 242	234, 244	236, 242	226, 242
GB-ZJM-007	$(GGC)_5$ AND $(GTGC)_3$	CGCCCTGCTTCGTCTTCTT	AAACAACCCCTACCACCG	Ma et al., 2007		230, 250	232	232	232	232	232	232	232
GB-ZJM-009	$(GTG)_4$	CCTGCGTATCCTCCAGCAG	ACTCCACCTCCATTGCCT	Ma et al., 2007		255	255	255	255	257	255	255	255
GB-ZJM-017	$(CTGC)_4$	CTCTGCTCTCTCTGGCTGC	ATCGACCACGACGATGAG	Ma et al., 2007		265	265, 267	235, 267	235, 267	267	265, 267	265, 267	235, 267
GB-ZJM-019	$(GAGT)_3$	CACAGCAGGAGAGGAAGGC	GCCATGACTTTGAGGCAG	Ma et al., 2007		308	306, 308	308	308	308	308	306, 308	308
GB-ZJM-020	$(TCC)_6$	CGTCATGGTAGCTGCGCTT	CGAGGTCAGGAGGCTTG	Ma et al., 2007		184	182, 188	188	188	188	184, 188	182, 188	188
GB-ZJM-025	$(CGG)_6$	CAGGTCGGAGATGGAGAGC	CCCGCCTCATGATAAATG	Ma et al., 2007		206, 208	196, 218	206, 218	206, 218	216, 218	196, 218	196, 216	206, 218
GB-ZJM-029	$(CTG)_4$ $(CTACTA)(CTG)_3$	CGGGGTCGATTCCTCTGTC	CTGCTGCGAAACCTGAAC	Ma et al., 2007		153, 173, 249	153, 173	153, 173, 249	153, 173, 249	173, 249	153, 173,	153, 173, 249	153, 173, 249
GB-ZJM-039	$(CTG)_6$	CATTGCGGAGCTGTTCAGA	AAAGACAGTTCGGTGGCA	Ma et al., 2007		300, 302	302	300, 302	300, 302	302	302	302, 306	300, 302
GB-ZJM-051	$(TG)5(GG)(GGA)_3$	CGCTTGCAGGATAAGGGGA	CATGCATAAATGTGGGGG	Ma et al., 2007		255, 259	261, 269	255, 277	255, 277	263, 269	259, 269	257, 261	255, 277
GB-ZJM-095	$(CTT)_5$	CCCAGAACAGCAGGCTACG	GCATCGACAACAGGATGG	Ma et al., 2007		171	169, 171	171	171	171	169, 171	171	171
GB-ZJM-099	$(TC)_6$	CCAACAGGTGTAGTGGGGC	CAATCTCGGGAACACCAA	Ma et al., 2007		300, 302	302, 302	295	295	302, 352	300	300, 352	295
GB-ZJM-110	(GAC)5	CATTTGCGGGAATACCCAT	TGTGATCTCCAAGCGTCC	Ma et al., 2007		NS	226, 253	251	251	253	253	NS	251
GB-ZJM-116	$(GCA)_2(GAG)(GCA)_3$	CCCGGTGTTGTTCCTTTCA	GCTGGAGCCGGATAGTTT	Ma et al., 2007		192	192, 200	192, 200	192, 200	200	192, 200	192, 200	192, 200
GB-ZJM-125	$(TG)_9$	CGGCTGGAAGCGGTAAAAT	CCTTTACAGTGCGCCAAC	Ma et al., 2007		NS[y]	277	275	275	277	275	279	275
GB-ZJM-136	$(GGC)_2(GCG)(GGC)_3$	CCGTTGATATCGAACCGGA	ACCGTCGAGCACTAGCAA	Ma et al., 2007		394	400	400	400	400	400	NS	400
T-a08	$(TC)_{28}(CA)_{19}$	CAGATCGACATTGCTGCAT	CCTTTCTCTCTCGACCTGC	Li et al., 2009	18	191	155	141, 181	141, 181	141, 157	141, 191	141, 191	141, 181
T-a111	$(CA)_{20}$	CAACTGGGTTAGTCCCGAG	TGGTAATAGATAGGAAGCGG	Li et al., 2009	8	126, 134	126, 167	145, 163	145, 163	171	126, 163	126, 165	145, 163
T-a124	$(TC)_{13}(CA)_{27}$	CGGACAGTCGCTCTCAATTAG	GTAGCTAGCCTTTTCGTGAA	Li et al., 2009	19	220, 224	210, 242	216, 220	216, 220	242	220, 224	224, 236	216, 220
T-a148	$(TC)_6(TG)_{13}$	CTTCAGCCTCTTCGACAAG	GCATCGTGCTCCTACAAC	Li et al., 2009	5	165, 189	165, 173	181, 185	181, 185	173, 179	165, 175	173, 195	181, 185

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Supplemental Table 2. Continued.

Primer name	Repeat	Forward sequence (5′-3′)	Reverse sequence (5′-3′)	Source	LG in zoysia SSR map	Estimated allele size of reference sample (bp)[z]							
						Diamond	Emerald	Empire	Jamur	Meyer	Zeon	Geo	Atlantic
T-a164	$(TG)_{13}$	CTGTAACGGAAATGTTAGAGACA	AAGTTACTGCGAACTGAAGC	Li et al., 2009	14	118	116, 157	118, 147	118, 147	116, 155	116, 122	116, 126	118, 147
CCMP1	(TA)10	CCGAAGTCAAAAGAGCGATT	CAGGTAAACTTCTCAACGGA	Karaca et al., 2002				204	204				
CCMP10	$(T)_{14}$	TTCGTCGCCGTAGTAAATAG	TTTTTTTTTAGTGAACGTGTCA	Karaca et al., 2002				NS	NS				
CCMP2	(A)11	ATCGTACCGAGGGTTCGAAT	GATCCCGGACGTAATCCTG	Karaca et al., 2002				204	204				
CCMP3	(T)11	GTTTCATTCGGCTCCTTTAT	CAGACCAAAAGCTGACATAG	Karaca et al., 2002				NS	NS				
CCMP4	(T)13	CCAAAATATTTCGGAGGACTCT	AATGCTGAATCGATGACCTA	Karaca et al., 2002				236	236				
CCMP5	(C)7 $(T)_{10}$	AGGTTCCATCGGAACAATTAT	TGTTCCAATATCTTCTTGTCATTT	Karaca et al., 2002				165	165				
CCMP6	$(T)5C(T)_{17}$	CATTACGTGCGACTATCTCC	CGATGCATATGTAGAAAGCC	Karaca et al., 2002				111	111				
CCMP7	$(A)_{13}$	ACATCATTATTGTATACTCTTTC	CAACATATACCACTGTCAAG	Karaca et al., 2002				NS	NS				
CCMP8	$(T)6C(T)_{14}$	TTCTTTCTTATTTCGCAGGAA	TTGGCTACTCTAACCTTCCC	Karaca et al., 2002				NS	NS				
CCMP9	$(T)_{11}$	CTCAACTCTAAGAAATACTTG	GGATTTGTACATATAGGACA	Karaca et al., 2002				NS	NS				
OsSai27	None	GCAGGTACTCCCAGCTCTCA	CTCGAGGACCACACCTCCTA	Jessup et al., 2011				327	327				
XM_00246502	(CG)4	GGTAAGGGAGGGCAGGAC	AGCTCGTTGGGCCCGTAT	Not previously published				264	264				
ZjAG136	$(AG)_{28}$	AAAAGAACTTACCCACCGATACC	ACTAGTAGGCCCACTCATTACCA	Tsuruta et al., 2005				236, 251	236, 251				
Zm12	$(AC)_{16}$	CGATATCGTTCAACATCTACACCG	ATATCTCGAGGGAGACTGCAACAG	LaMantia et al., 2011				171	171				
Zm13	$(CA)_{7}$	TTTTAAAACTTTGCACCCTTGCAC	AGAATAAAGTCCAGCTCCCTGACC	LaMantia et al., 2011				296	296				
Zm25	$(GT)_{56}$	GCACCTTAACCCATCCGAAATA	AGCTTTCACACCCCTAGCACTC	LaMantia et al., 2011				215	215				
Zm34	$(CT)_{20}$	TTCTTACGTTTTAGTTGCTCATGC	GGCGTTACAGATGTTAAGAGTTGA	LaMantia et al., 2011				298	298				
Zm53	$(AC)_{12}$	AGTGGGTTTGGCAGCTAGTAGAGT	GAATGTTTGTATTTGCAGGTTGCT	LaMantia et al., 2011				185	185				
Zm61	$(AG)_{6}$	AACACAGAATGAAGGAAAGCAGGT	AGAGTGATAAACAATGGCTGTGGA	LaMantia et al., 2011				NS	NS				

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Supplemental Table 2. Continued.

Primer name	Repeat	Forward sequence (5′-3′)	Reverse sequence (5′-3′)	Source	LG in zoysia SSR map	Estimated allele size of reference sample (bp)[z]							
						Diamond	Emerald	Empire	Jamur	Meyer	Zeon	Geo	Atlantic
Zm65	$(AC)_{16}$	GCGCAACACATGATCCAATAGTC	AGCAGGAAAGTGTGATGGTGATG	LaMantia et al., 2011				NS	NS				
Zm71	$(TG)_6$	TGCTGGTGTGTCTGTATGTGTGAG	GTGAGGGAGTGTGTTGGAGAGC	LaMantia et al., 2011				275	275				

[z]Estimated allele size of scored fragments. An 18-bp M13 tag was added to the forward primers.
[y]Allele not scored or no allele amplified.