Genetic resources and genetic transformation in bermudagrass – a review

Shilian Huang, Chen Wang and Junsong Liang

ABSTRACT
Bermudagrass is widely distributed as a warm-season turf and forage grass in the temperate and tropical zones around the world. Its strong vegetative reproduction and ability to withstand drought make it an ideal species as a most used warm-season forage grass and turfgrass. Genetic transformation is an important tool for the study of gene function and for germplasm improvement in bermudagrass. This paper attempts to present a recent review on genetic resources, plant regeneration and genetic transformation in bermudagrass. We first review the various genetic resources and collection of bermudagrass. Then the explants, basal medium and the effect of different cultivars and plant growth regulators on plant regeneration in bermudagrass are also summarized. Last, we outline the main areas of progress in genetic transformation with either the biolistic or Agrobacterium-mediated method in bermudagrass, and discuss various factors that influence Agrobacterium-mediated transformation. However, the question that still remains is why there have been no genetic modification reports on bermudagrass for 10 years.

Introduction
An increasing amount of researches in genetic analysis of bermudagrass (Cynodon dactylon (L.) Pers) have been produced in recent years due to its widespread distribution in warmer places and its use as forage grass and turfgrass. Bermudagrass is a typical warm-season turfgrass, widely distributed in warm, humid and semi-arid areas; it occurs across lands between 45° N and 45° S, penetrates to approximately 53° N in Europe and is found at 4000 m elevation in the Himalayas [1]. Bermudagrass is a perennial native to the Mediterranean area, north and east Africa, Asia, Australia and southern Europe. The genetic diversity centre of bermudagrass is considered to be Turkey, Iran, Afghanistan and the western part of Pakistan [1,2]. Bermudagrass has strong vegetative reproduction, tenacious vitality and ability to withstand drought. As its advanced stolon has a great extension capacity, it is widely used in sports fields, lawns, parks, golf courses, for environment restoration and as a general utility turf. It is also rich in nutritional value: the content of crude protein, crude fat, crude fibre, crude ash and ether extract are 83.2, 54.1, 206.2, 88.4 and 574.1 g kg⁻¹, respectively. With soft texture and good palatability, it is preferred by horses, cows, sheep, rabbits and so on. As a result, bermudagrass has become a most used warm-season forage grass and turfgrass [3]. In addition, Xie et al. [4] demonstrated that bermudagrass has the potential to be applied in revegetation of contaminated soil. Apart from that, it is also a traditional Chinese medicine.

Bermudagrass belongs to the genus Cynodon L. C. Rich., family Gramineae. The genus Cynodon consists of 9 species and 10 varieties, including C. aethiopicus Clayton et Harlan, C. arcuratus J. S. Presl. ex C. B. Presl., C. barberi Rang, et Tad., C. dactylon (L.) Pers (contains 6 varieties C. dactylon var. dactylon, C. dactylon var. afghanicus Harlan et de Wet, C. dactylon var. aridus Harlanet de Wet, C. dactylon var. coursii (A. Camus) Harlan et de Wet, C. dactylon var. elegans Rendle and C. dactylon var. polevansil (Stent)), C. incompletus Nees (contains C. incompletus var. incompletus and C. incompletus var. hirsutus (Stent) de Wet et Harlan), C. nlemfuensis Vanderyst (contains C. nlemfuensis var. nlemfuensis and C. nlemfuenensis var. robustus Clayton et Harlan), C. plectostachyus (K. Schum.) Pilger, C. transvaalensis Burtt-Davy, C. x magennensis Hurcombe. Among them, C. dactylon, C. incompletus, C. transvaalensis and C. dactylon × C. transvaalensis are commonly used as turfgrass [5]. The first cultivar ‘St Lucie’ which was used as a lawn grass in Florida, was identified by Tracy in 1917 [6]. Since then, more and
more cultivars have been produced. The search for superior Cynodon turfgrass cultivars can be traced back to the early twentieth century. Juska and Hanson [7] note that P.I. 213391, C. transvaalensis, commonly called ‘Florida’ in South Africa, was collected near Johannesburg, South Africa, about 1907. Presumably, it was introduced into the United States and later exported back to South Africa [5].

Due to the importance of bermudagrass, molecular techniques involving amplified fragment length polymorphism (AFLP), inter-simple sequence repeat (ISSR), random amplified polymorphic DNA (RAPD), sequence related amplified polymorphism (SRAP) and simple sequence repeats (SSRs) have long been applied in improving bermudagrass, with a focus on enhancing its stress resistance and analysing its germplasm and genetic diversity for constructing linkage maps. Recently, RNA-Seq have been used for analysing the molecular basis of the physiological processes [8,9]. These studies have provided insights into the differences between species or varieties and the reasons of their stress tolerance, which may help accelerate the genetic improvement of bermudagrass.

Transgenic technology can transfer exogenous DNA into plants, which could influence the expression of endogenous genes. This way, resources possessing a phenotype of interest can be obtained. The technology has been used to develop many varieties of forage, turf and bioenergy crops. Several varieties with improved productivity, quality and stress tolerance have been produced with transgenic technology [10]. Since the production of the first transgenic forage-type tall fescue plants [11], tremendous progress has been made in genetic engineering of forage, turf and bioenergy crops in the last two decades (reviewed in [10]). Callus induction, plant regeneration and transformation have been realized in plenty of bermudagrass cultivars between 2004 and 2006. From then on, it seems this technology disappeared from bermudagrass investigation. This paper reviews the genetic resources and the progress of genetic transformation studies in bermudagrass. The starting point is to provide a solid reference for investigating genetic modified bermudagrass and discuss the reasons that prevent the progress of transgene technology in the study of bermudagrass.

### Bermudagrass genetic resources

To enable efficient use of genetic resources and to increase access for breeders, the U. S. National Plant Germplasm System (NPGS) have collected 1116 accessions (a great progress compared to 435 accessions in June 2009) of Cynodon as indicated by GRIN database records current to March 2017. Among these, 988 are classified in the Cynodon genus, or are listed as C. spp. or C. hybrids (Table 1). Of the 988 accessions, C. aethiopicus, C. barberi, C. incompletes, C. magennisii, C. nlemfuensis, C. plectostachyus, C. radiatus and C. transvaalensis are represented by 2 (0.20%), 13 (1.32%), 30 (3.04%), 12 (1.21%), 13 (1.32%), 83 (8.40%), 19 (1.92%) and 38 accessions (3.85%), respectively. C. dactylon is the best represented taxon having 778 (78.74%) accessions and 6 varieties. C. radiatus Roth ex Roem. and Schult was described as an annual species and as a synonym of C. arcuatus [12].

There are two types of commercial bermudagrasses: seeded bermudagrasses and vegetative bermudagrasses. Seeded bermudagrass varieties generally are coarser in texture and have a lower shoot density than vegetative bermudagrasses. Vegetative bermudagrasses must be propagated by vegetative planting as they cannot produce fertile seeds. Although vegetative bermudagrasses require more maintenance, such as frequent mowings, fertilization, edging and dethatching for the best appearance and quality, their great advantages lie in disease resistance, plant density, weed resistance and fewer seedheads, giving them great potential for use as lawn and forage grass [13]. Some varieties of the two types are listed in Table 2 [7,14,15].

### Evaluation and use of genetic resources

DNA markers have been widely utilized for germplasm classification and evaluation [16,17], biodiversity analysis [18,19], high-density linkage map construction [20], genome mapping and marker-assisted selection to investigate structural genomics [21], crop varieties and

| Cynodon species | Number of chromosomes | Number of accessions |
|-----------------|-----------------------|---------------------|
| C. aethiopicus  | 18, 36                | 2                   |
| C. barberi      | 18                    | 13                  |
| C. dactylon     | 36                    | 261                 |
| C. dactylon var. dactylon | 36  | 494                |
| C. dactylon var. africanus | 18, 36  | 1       |
| C. dactylon var. afidus | 18  | 1       |
| C. dactylon var. coursii | 36  | 13      |
| C. dactylon var. elegans | 36  | 2       |
| C. dactylon var. polevansii | 36  | 6       |
| C. hybrids     | 10                    |                     |
| C. incompletus  | 4                     |                     |
| C. incompletus var. hirsutus | 18, 36 | 18     |
| C. incompletus var. incompletus | 18 | 8      |
| C. incompletus var. incompletus | 18 | 8      |
| C. incompletus var. incompletus | 18 | 8      |
| C. nlemfuensis var. nlemfuensis | 18, 36 | 12   |
| C. nlemfuensis var. nlemfuensis | 18, 36 | 12   |
| C. nlemfuensis var. robustus | 18, 36 | 1     |
| C. plectostachyus | 18  | 83      |
| C. radiatus     | 36                    | 19                  |
| C. transvaalensis | 18  | 38      |
| C. spp.        | 118                   |                     |
purification identification [22] in plants (reviewed in [23]). In earlier bermudagrass studies, Caetano-Anollés et al. [24] first applied DAF (DNA amplification fingerprinting) in studying the relatedness of 13 genotypes of C. transvaalensis, C. dactylon and hybrid derivatives of the two species, and they separated C. transvaalensis and C. dactylon into distinct clusters, one containing the African-type bermudagrasses and another containing the common-type bermudagrasses (including an interspecies C. transvaalensis × C. dactylon cross). The result demonstrated the efficiency of DAF as a technique for genetic variation study of bermudagrass. In 1997, Caetano-Anollés et al. [25] found that off-types were genetically distant to cultivar ‘Tifway’ and represent a heterogeneous group of bermudagrass probably of inter-specific hybrid origin with analysis of 13 bermudagrass cultivars using DAF. DAF has also been employed to investigate the relationships among 18 Cynodon cultivars in Australia and among 62 accessions representing 8 species and 7 varieties maintained at the Oklahoma State University [16,17]. In the former research, a primer that is able to discriminate between all the cultivars except ‘Tifdwarf’ and its off-types was identified and, finally, 18 cultivars were separated into two distinct groups based on DNA amplification data [26]. The later one demonstrated that C. arcuratus separated from another seven species, and the species had different chromosome numbers clustered in all instances, indicating a close relationship between 2x and 4x [27]. Since then, more and more molecular markers have been used in genetic diversity analysis of Cynodon accessions, such as AFLP, ISSR, RAPD, SRAP and SSR (details in Table 3) [28–43]. Khanal et al. [44] successfully applied sugarcane EST-SSR analysing genetic diversity among triploid hybrids, tetraploid C. dactylon and diploid C. transvaalensis. These researches provide valuable information for future genomic and breeding studies.

Table 2. Characteristics of some seeded and vegetative bermudagrass varieties.

| Bermudagrass varieties | Seeded or vegetative | Leaf texture | Major characteristics |
|------------------------|----------------------|--------------|-----------------------|
| Common                 | Seeded               | Coarse       | Moderate cold hardiness; best adapted south of about 34° N lat. and in sub-humid climates; used as a turf and forage grass |
| Cheyenne               | Seeded               | Medium       | Initially released for turf and described as having good cold tolerance |
| Guymon                 | Seeded               | Medium       | Very cold tolerant, released primarily for erosion control and soil stabilization |
| Giant                  | Seeded               | Medium       | Productive, disease resistant, but lower cold tolerance |
| Riviera                | Seeded               | Fine         | Fairways, lawns, parks, roughs, sports turfs |
| Sonesta                | Seeded               | Medium       | Roughs, lawns |
| Wrangler               | Seeded               | Medium       | High yield |
| Yukon                  | Seeded               | Fine         | Fairways, lawns, parks, roughs, sports turfs |
| GN-1                   | Vegetative           | Medium       | The dark-green variety has medium-fine texture, good low-temperature tolerance, excellent wearability and improved nematode resistance; the variety is intended for use in commercial and home lawns, athletic fields, fairways and tees |
| Midlawn                | Vegetative           | Medium       | This slow-growing, dark-green hybrid ranks high for overall turf quality, texture, density, spring green-up and spring dead spot resistance |
| Patriot                | Vegetative           | Fine         | Low-temperature-hardy |
| Quickstand             | Vegetative           | Medium       | Establishes quickly, and is low growing (5–6 inches) making it better suited for grazing than for hay |
| Tifgreen               | Vegetative           | Fine         | Forest green and has soft, narrow leaves, short mowing heights |
| Tifsport               | Vegetative           | Fine         | Tolerance to close mowing, resistance to southern mole cricket, spring green-up |
| Tifway                 | Vegetative           | Fine         | Dark-green, disease-resistant, cold hardiness superior to ‘Tifgreen’ |
| TifwayII               | Vegetative           | Fine         | More resistant to root knot, ring and sting nematodes and more tolerant of frost than ‘Tifway’ |

The tolerance of several cultivars to abiotic stresses has been investigated by different authors. Among nine bermudagrass varieties, ‘Tifgreen’ and ‘Tifway’ showed tolerance to drought, whereas ‘LaPaloma’, ‘Riviera’, ‘SR9554’, ‘Laprima’ and ‘Veracruz’ showed moderate tolerance and ‘Wrangler’ and ‘Yukon’ proved susceptible based on measurement of the leaf water content (LWC), electrolyte leakage (EL) and accumulation of hydrogen peroxide (H2O2) and malondialdehyde (MDA) after drought stress treatment [45]. Compared to the parent control, TifEagle, 10-17, 89-02 and 117-08 among eight somaclonal variants with enhanced drought tolerance reportedly show higher relative water contents and relative growth and lower ion leakage [46]. ‘Tifway’ showed higher drought tolerance than a common bermudagrass variety, ‘C299’ [47]. The greatest reported level of freeze tolerance is −11 ºC for ‘Midron’ and −7 ºC for ‘Tifgreen’ in December and January [48]. Brookings’ showed greatest cold tolerance among 8 bermudagrass cultivars (Tifway, ‘La Junta’, ‘Santa Ana’, ‘NEJC’, ‘Brookings’, ‘Tifgreen’, ‘Tifdwarf’ and ‘PeeDee 102’), and it could endure the low temperature of −17 ºC [49]. Native Cynodon dactylon varieties all over Xinjiang, China have been able to survive by cleaning snow in field under −32 ºC air temperature [50]. Besides drought and cold tolerance, bermudagrass also demonstrates strong salt tolerance. In 1967, Youngner and Lunt [51] found ‘Sunturf’ and ‘Tifway’ had superior salt tolerance among 9 cultivars, after comparing the aerial part and root growth under different salinity conditions. By adding NaCl to basic nutrient solution gradually, Dudeck et al. [52] found that ‘Tifdwarf’ and ‘Tifgreen’ were most tolerant, whereas ‘Common’ and ‘Ormond’ were most sensitive. By
measurement of the shoot dry weight reduction relative to control plants and per cent green leaf canopy area of 35 bermudagrass cultivars exposed to different salinity levels, Marcum and Pessarakli [53] found a wide range in salinity tolerance within them. FloraDwarf, ‘Champion Dwarf’, Novotek and ‘TifEagle’ showed the highest salt tolerance and ‘Patriot’, ‘Santa Ana’, ‘Tifgreen’ and TifSport the lowest tolerance within the 12 bermudagrass hybrids, based on determination of dry matter production and the dry weight of roots and crowns below clipping height. Francois [54] found that Tifton 86, which originated near the Dead Sea, Israel, showed greatest salt tolerance. Also, Ramakrishnan and Nagpal [55], Hameed and Asbral [56], Akram et al. [57] and Zhou et al. [58] found that cultivars form saline-alkali soil possessed stronger salt tolerance. By placing calli (TifEagle) on solid medium containing 0.3 mol L$^{-1}$ NaCl for 10 days followed by a recovery for 2 weeks, Lu et al. [59] selected three somaclonal variant lines that showed higher relative growth and less injury than TifEagle under salt stress. This might provide us a useful method for resistance breeding, especially for salt stress. By impacting the antioxidant system, photosystem II and metabolic homeostasis, exogenous melatonin improves the cold stress tolerance in $C$. dactylon, whereas exogenous AM1 (ABA mimic 1) improves the cold tolerance by keeping the homeostasis of reactive oxygen metabolism, accumulating proline and modulating stress-inducible genes [60,61].

**Tissue culture and plant regeneration**

In order to obtain a transgenic plant for improving bermudagrass, a regeneration system needs to be established. Regeneration systems have seen great development over the last 30 years, from in vitro cultured organs, tissues and cells for various forage and turf grasses. Researches focusing on bermudagrass

---

### Table 3. Different DNA markers used in analysing genetic diversity among Cynodon accessions.

| DNA marker | Material | Conclusion | References |
|------------|----------|------------|------------|
| AFLP       | Twenty-seven bermudagrass (Cynodon spp.) cultivars | The 27 genotypes were grouped into three major clusters, many of which were in agreement with known pedigrees. | [28] |
|            | Twenty-eight *C. dactylon* var. *dactylon* accessions | Accesions originating from different continents were clustered into different branches based on 443 polymorphic AFLP fragments from 8 primer combinations. | [29] |
|            | One hundred and nineteen Chinese bermudagrass accessions | The accessions were grouped into five clusters based on 466 polymorphic AFLP bands. | [30] |
|            | Forty-three bermudagrass ecotypes from Korea | Nuclear DNA contents were in the ranges of 1.42–1.56, 1.94–2.19, 2.54 and 2.77–2.85 pg/2C for the triploid, tetraploid, pentaploid and hexaploid accessions, respectively. The genetic similarity ranged from 0.42 to 0.94 with an average of 0.64. | [31] |
| ISSR       | Fifty-five accessions of *Cynodon dactylon* from 17 countries | A total of 236 ISSR fragments were generated with 14 primers. GSC (genetic similarity coefficients) ranged from 0.52 to 0.95. | [32] |
|            | Ninety-five wild bermudagrass accessions collected from 11 provinces in China and ‘Tift 3’ | The results indicated that 97.6% bands generated from 29 ISSR primers were polymorphic. The GSC ranged from 0.51 to 0.97. All accessions clustered into 11 groups with the UPGMA (unweighted pair-group arithmetic averages) method. Fourteen ISSR primers amplified 389 fragments of which 313 (80.5%) were polymorphic. | [33] |
|            | Twenty-seven bermudagrass accessions from Iran | | |
|            | Thirty-three *Cynodon dactylon* accessions and 22 cultivars from four different countries | The results showed that 97.7% of the 9 SSR primers and 86.9% of the 23 ISSR primers were polymorphic. The GSC were 0.58–0.97 for ISSR and 0.52–0.97 for SSR. | [34] |
| RAPD       | Twelve well-known cultivars in South Africa and 10 potential new cultivars | The cultivars ‘Silvertone Blue’ and ‘Bayview’ exhibited the greatest genetic variation and two potential new cultivars were identified. | [35] |
|            | Six merit selections of *C. dactylon* in China | Four hundred thirty-eight bands were amplified by 15 primer sequences, 415 polymorphic bands were produced. The GSC were 0.408–0.672. | [36] |
| SRAP       | Thirty-two wild *C. dactylon* accessions from four provinces of China | GSC ranged from 0.591 to 0.957. Four clusters implied a correlation among wild resources, geographical and ecological environment. | [37] |
|            | Fifty-seven *C. dactylon* accessions from 17 countries, 5 continents | The Chinese accessions formed 5 groups (a total of 7) and displayed greater genetic variation than others. | [38] |
|            | Thirty-three Chinese accessions of *C. dactylon* and 22 cultivars developed in China | The percentage of polymorphic loci (PPL) for the domestic and introduced accessions was 95 and 83%, respectively. The GSC ranged from 0.57 to 0.97. | [39] |
|            | Four hundred and thirty *C. dactylon* accessions collected from 22 Chinese provinces | Three distinct branches clustered with UPGMA. The GSC ranged from 0.53 to 0.96. The accessions were separated into 8 distinct groups with arithmetic averages (UPGMA) and principle coordinate analysis (PCoA) methods. | [40] |
| SSR        | One hundred and fifty-seven genotypes from 20 provinces in China | Twenty-six SRAP primer pairs produced 340 bands, of which 328 (96.58%) were polymorphic bands were produced. The GSC were 0.408–0.672. | [41] |
|            | One hundred and twenty *C. dactylon* accessions in China | The 104 SSR primers amplified 1474 alleles. Cluster analysis showed that the distance of genetic relatedness was affected by the natural habitats and the edaphic conditions. | [42] |
regeneration system establishment have been published mostly between the year 2000 and 2010.

The first step for regeneration system establishment is callus induction. Different explants have been used for bermudagrass callus induction, for example, young inflorescence [47–49], mature hulled seed [50] and the nodes of stolons [53,54]. From the summary provided in Table 4 [62–71] we can see that, for bermudagrass, young inflorescences and nodes of stolons are most used in callus induction, rarely seeds. Murashige and Skoog (MS)-based media are commonly used in tissue culture. Lu et al. [46] also used N6 macroelements [73] and B5 microelements [74], Fe–ethylene-diaminetetraacetic acid (EDTA), supplemented with Gamborg’s vitamins to induce callus from ‘TifEagle’ (Cynodon dactylon × C. transvaalensis cv. TifEagle) stolon node segments. 2,4-Dichlorophenoxyacetic acid (2,4-D) and 6-benzyladenine (BA) are the most frequently supplemented exogenous hormones for callus induction and maintenance, although other auxins, for example, 3,6-dichloro-o-anisic acid (dicamba), have also been used [46]. However, there are large differences in the concentrations used even for the same cultivar. This may be related to the environment of plant growth and the laboratory. During the subculture process, the suspension culture method is also widely used.

BA is mostly used in the regeneration medium, whereas the rooting medium is typically MS medium with or without auxin.

**Factors influencing plant regeneration of bermudagrass**

Firstly, an efficient and stable plant regeneration procedure of bermudagrass should be provided for reliable genetic transformation. Several problems have been solved with attempts to regenerate bermudagrass plants, but the regeneration systems developed so far are still immature due to the genotypes of bermudagrass, the ploidy levels and the growth regulators used. Zhang et al. [71] compared callus induction and plant regeneration with three triploid (‘TifSport’, ‘TifEagle’, and ‘Tift97-4’) and four tetraploid (‘Tift93-132’, ‘Tift93-135’, ‘Tift93-156’ and ‘Tift93-157’) bermudagrass cultivars. MS media supplemented with 1, 1.5 or 2 mg L⁻¹ 2,4-D, 0, 0.01 or 0.02 mg L⁻¹ BA, 11.6 g L⁻¹ proline, 30 g L⁻¹ sucrose and 3 g L⁻¹ Gelrite were tested for callus induction. For triploid cultivars, the highest percentage of callus induction from cultured inflorescences, 100% for ‘TifEagle’, 72.5% for ‘TifSport’ and 50.0% for ‘Tift97-4’, occurred on MS medium with 1.5 mg L⁻¹ 2,4-D and 0.01 mg L⁻¹ BA; and in node culture, high callus induction rates were obtained on medium with 1 mg L⁻¹ 2,4-

### Table 4. Media used for induction and regeneration of bermudagrass.

| Cultivar | Induction material and medium (mg L⁻¹) | Subculture medium (mg L⁻¹) | Regeneration medium (mg L⁻¹) | Root induction medium (mg L⁻¹) | References |
|----------|---------------------------------------|---------------------------|-----------------------------|-------------------------------|------------|
| Zebra    | Young inflorescences; MS + 2,4-D (3) + CH (200) + 0.7% agar | Same as induction medium | Same as induction medium | MS + 2,4-D (0.5) + zeatin (1.2) + 0.7% agar | [62] |
| J1224    | Young inflorescences; MS + 2,4-D (1) + BA (0.009–0.203) + ABA (0.5) + sucrose (30,000) + phytagel (3000) | Induction medium + an elevated BA level (0.5) Solid or liquid (for suspension culture) | MS + BA (1) + NAA (0.2) + GA3 (0.45) + sucrose (30,000) + phytagel (3000) | MS + NAA (0.2), sucrose (30,000), hygromycin B (50) and phytagel (3000) | [63] |
| J1224    | Young inflorescences; MS + 2,4-D (1) + AA liquid medium + 2,4-D (1) and sucrose (30,000) + phytagel (3000) | Arizona Mature hulled seeds; MS + 2,4-D (1) + BA (0.1) + maltose (30,000) + gelrite (2500) | Same as induction medium | MS + BA (1) + NAA (0.2) + GA3 (0.45) + sucrose (30,000) + phytagel (3000) | [64] |
| Tifgreen | Young inflorescences; MS + 2,4-D (1) + BA (0.01) + sucrose (30,000) + phytagel (3000) | Same as induction medium | Same as induction medium | MS + BA (2.5) + sucrose (30,000) + phytagel (3000) | [66] |
| Tifway   | Young inflorescences; MS + 2,4-D (1) + BA (0.01) + sucrose (30,000) + phytagel (3000) | Same as induction medium | Same as induction medium | MS + BA (2.5) sucrose (30,000) + phytagel (3000) | [67] |
| TifEagle | Nodes of stolons; MS + dicamba (6.63) + BA (4.5) + myoinositol (100) + l-xylitol (1000) + sucrose (30,000) + phytagel (2500) | Equal to MS + BA (2.0) + hygromycin (200) + sucrose (30,000) + phytagel (2500) | Same as induction medium | MS + BA (2,4-D (0.13) + BA (0.5) or 0.01 mg L⁻¹ 2,4-D and 0.02 mg L⁻¹ BA + 0.5 mg L⁻¹ GA3 (0.45) + sucrose (30,000) + phytagel (3000) | [68] |
| Tifway   | Nodes of stolons; MS + 2,4-D (1) + BA (0.01) + sucrose (40,000) + agar (7500) + PPM (1 ml L⁻¹) | Same as induction medium | Same as induction medium | MS + 2,4-D (0.13) + BA (0.5) or 0.01 mg L⁻¹ 2,4-D and 0.02 mg L⁻¹ BA + 0.5 mg L⁻¹ GA3 (0.45) + sucrose (40,000) + agar (7500) | [69] |
| TifEagle | Nodes of stolons; MS + 2,4-D (1) + BA (0.1) + L-proline (2000) + inositol (100) + CH (200) + phytagel (3000) + sucrose (30,000) | Same as induction medium | Same as induction medium | MS + 2,4-D (0.13) + BA (0.5) or 0.01 mg L⁻¹ 2,4-D and 0.02 mg L⁻¹ BA + 0.5 mg L⁻¹ GA3 (0.45) + sucrose (40,000) + agar (7500) | [70] |
| Seven cultivars | MS + 2,4-D (1, 1.5, 2) + BA (0, 0.01, 0.02) + proline (1.13) + sucrose (30,000) + Gelrite (3000) | MS + 2,4-D (1–1.5) + BA (0.02–0.5) + Gelrite (2000) or agar (5000) | MS + 2,4-D (0.1) + BA (0.5–4.0) | MS + 0.52 MS or 1/2 MS | [71] |
D and 0.01 mg L⁻¹ BA, i.e. 68.3% for ‘TifSport’, 78.3% for ‘TifEagle’ and no vigorous callus was observed for ‘Tift97-4’. Four tetraploid genotypes showed high callus induction frequencies from foliage on MS medium with 1.5 mg L⁻¹ 2,4-D and 0.01 mg L⁻¹ BA, ranging from a high of 93% for ‘Tift93-132’, 87% for ‘Tift93-157’, 64% for ‘Tift93-135’, to 54% for ‘Tift93-135’. Different BA concentrations in MS supplemented with 0.1 mg L⁻¹ 2,4-D were also tested for shoot regeneration. The concentration of BA had a significant effect on green spot formation in ‘TifEagle’, ‘TifSport’ but not in ‘Tift93-132’, whose calluses formed green spots on all media. The concentration of BA had low significance for ‘TifSport’ but was highly significant for ‘TifEagle’ and ‘Tift93-132’. The combination of 0.1 mg L⁻¹ 2,4-D and 2–4 mg L⁻¹ BA yielded the highest percentage of shoot regeneration for ‘TifEagle’, whereas for ‘Tift93-132’, 0.1 mg L⁻¹ 2,4-D and 0.5 mg L⁻¹ BA gave the best shoot regeneration [71].

Platform of bermudagrass genetic transformation

Biolistics, or microprojectile bombardment, transfers exogenous DNA attached to metal particles into explants for stable inheritance [75–77]. Cells and tissues that have the ability to divide and differentiate could be used as explants for biolistics, such as embryogenic cultures and meristematic cells and so on [78]. Without considering protoplast culture and Agrobacterium host specificity, biolistics could produce transgenic plants from recalcitrant species [79]. The first transgenic triploid bermudagrass plant gained by biotic transformation and using phosphotransferase (hpt) as the selected marker gene was reported in 2003 [68]. The next year, Goldman et al. [69] transformed the bar gene into the hybrid bermudagrass cultivar ‘TifEagle’ by biotic transformation to produce a herbicide-resistance plant. The same year, the method was applied to common bermudagrass [63].

Agrobacterium-mediated transformation is often preferred because the approach often yields plants with low number of transgene copies, fewer rearrangements and an improved stability of transgene expression over generations than the free DNA delivery methods [80,81]; also it does not require expensive equipment. This method is based on the fact that Agrobacterium tumefaciens and A. rhizogenes contain Ti and Ri plasmids, respectively, which contain transfer DNA (T-DNA). The T-DNA is inserted into the plant genome via Agrobacterium infection.

To find factors influencing Agrobacterium-mediated transformation of bermudagrass, Zhang [82] tested several aspects, time of co-culture, acetosyringone, pre-culture, Agrobacterium concentration and light conditions of co-culture. He found that the optimal conditions to obtain higher transformation efficiency were co-cultivation with OD₆₀₀ 0.5–0.8 A. tumefaciens liquid culture for 2 d in darkness after pre-culture for 10 d. The pre-culture medium was induction medium supplemented with 30 g L⁻¹ mannitol. The transformation frequency was significantly improved by adding 100 mmol L⁻¹ acetosyringone into the co-cultivation medium [82]. Benefiting from this method, Hu et al. [70] transformed the bar gene into triploid bermudagrass and gained transgenic plants highly resistant to the glufosinate herbicide. Expression of the cry1Ac gene in the common bermudagrass cultivar ‘Arizona common’ via Agrobacterium-mediated transformation gave the transgenic plant the ability to resist to Black Cutworm (Agrotis ipsilon Hufnagel) [65].

Prospects

Bermudagrass has already been widely used as a turf and forage grass, and like many grasses shows great potential with phytoremediation. Climate change puts great press on us to create bermudagrass lines which fulfill our needs as soon as possible; however the difficulty with breeding bermudagrass is its low transformation efficiency which makes it difficult to establish transgenic lines. The Agrobacterium-mediated strategy is preferred, as it is convenient and economical; however, the transformation efficiency is seriously affected by the types and the genotypes of plant materials. Young inflorescences, nodes of stolons and seeds are the most widely used explants for callus induction for bermudagrass. To overcome the problem with the low transformation efficiency, maybe different Agrobacterium strains, various transformation methods such as ultrasonic oscillation and vacuum infiltration should be attempted. Protoplast transformation may be another choice under the precondition of the implementation of the protoplast regeneration. With the great progress in next-generation sequencing, large amounts of transcriptomics and proteomics statistical data related to bermudagrass resistance to low temperature [8,9,52], drought [83,84] and salt stress [85–87], have been published. We can take full advantage of these data for studying the molecular mechanism of bermudagrass resistance and resistance breeding. True, we still do not know what the main factors influencing the transformation efficiency are. Recently, Zhang et al. [88] found a method of efficient virus-induced gene silencing in Cynodon dactylon. They achieved significant reduction in the expression of the phytoene desaturase (PDS) gene in leaves by testing a modified rice tungro bacilliform virus (RTBV) vector through agroinfiltration. It may give us help in Agrobacterium-mediated transformation [88]. Overcoming the obstacle will be a major breakthrough in bermudagrass investigations.
Conclusions

Until March 2017, more than one thousand Cynodon accessions have been collected in the National Plant Germplasm System, belonging to nine species. As it has vegetative reproduction, tenacious vitality and strong drought tolerance, it is widely used in sports fields, lawns, parks, golf courses and for environment restoration; as it is rich in nutritional value, it becomes a most used warm-season forage grass. Due to the importance of bermudagrass, several DNA markers (such as AFLP, ISSR, RAPD, SRAP and SSR) have been used in germplasm evaluation, genetic diversity and phylogenetic analysis, high-density linkage map construction and genome mapping. In addition, resistance research has also provided valuable information for future genomic and breeding studies.

Transgenic technology offers the opportunity to introduce novel genetic variation into plant breeding; it can help us improve the productivity, quality and stress tolerance of plants. For bermudagrass, tissue culture and plant regeneration systems have been established. Also, there are currently two reported platforms for bermudagrass genetic transformation, biolistics and Agrobacterium-mediated transformation. The latter one is preferred, as it yields plants with low a number of transgene copies, fewer rearrangements, an improved stability of transgene expression and no need for expensive equipment. However, the Agrobacterium-mediated strategy has a very low transformation efficiency in bermudagrass, which is a great limitation for gene function research. Perhaps different Agrobacterium strains and various transformation methods should be attempted to overcome this setback. If we implement protoplast regeneration, protoplast transformation should be a great choice.

Acknowledgement

The research was supported by Yulin Normal University and South China Agricultural University and funded by the Natural Science Foundation of Guangxi Province [grant number 2013GXNSFAA019090] and Research Project of Higher Education of Guangxi [grant number YB2014315].

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Natural Science Foundation of Guangxi Province [grant number 2013GXNSFAA019090]; Research Project of Higher Education of Guangxi [grant number YB2014315].

References

[1] Harlan JR, de Wet JMJ, Rawal KM. Origin and distribution of the seleucidus race of Cynodon dactylon (L.) Pers. var. dactylon (Gramineae). Euphytica. 1970;19(4):465–469.
[2] Harlan JR, de Wet JMJ. Sources of variation in Cynodon dactylon (L.) Pers. Crop Sci. 1969;9(6):774–778.
[3] Huang CQ, Zhang YF, Liu GD. Nutrition value evaluation of Cynodon dactylon germplasm resource. Chin J Tropical Crops. 2011;32(8):1418–1425. Chinese.
[4] Xie Y, Luo HJ, Hu LX, et al. Classification of genetic variation for cadmium tolerance in Bermudagrass [Cynodon dactylon (L.) Pers.] using physiological traits and molecular markers. Ecotoxicology. 2014;23(6):1030–1043.
[5] Taliaferro CM. Diversity and vulnerability of bermuda turfgrass species. Crop Sci. 1995;35(2):327–332.
[6] Tracy SM. Bermuda grass. Farmers bulletin 814. Washington (DC): Usda Farmers Bulletins; 1917.
[7] Juska FV, Hanson AA. Evaluation of bermudagrass varieties for general-purpose turf. Washington (DC): U.S. Government Publishing Office; 1964.
[8] Chen L, Fan JB, Hu LX, et al. A transcriptomic analysis of bermudagrass (Cynodon dactylon) provides novel insights into the basis of low temperature tolerance. BMC Plant Biol. 2015 [cited 2017 Mar 23];15:216. DOI:10.1186/s12870-015-0598-y
[9] Zhu H, Yu X, Xu T, et al. Transcriptome profiling of cold acclimation in bermudagrass (Cynodon dactylon). Sci Hort. 2015;194:230–236.
[10] Wang ZY, Brummer EC. Is genetic engineering ever going to take off in forage, turf and bioenergy crop breeding? Ann Bot. 2012;110(6):1317–1325.
[11] Wang Z, Takamizo T, Igleisias VA, et al. Transgenic plants of tall fescue (Festuca arundinacea Schreb.) obtained by direct gene transfer to protoplasts. Nat Biotechnol. 1992;10(6):691–696.
[12] Kole C. Wild crop relatives: genomic and breeding resources for general-purpose turf. Washington (DC): U.S. Government Publishing Ofce; 1964.
[13] Han YD, Huckabay E. Bermudagrass lawns. The Alabama cooperative extension system. 2008. Available from:http://www.aces.edu/pubs/docs/A/ANR-0029/ANR-0029.pdf
[14] Huang CQ, Zhang YF, Liu GD. Research and improvement in germplasm resources of Cynodon dactylon. Acta Agric Sin. 2011;19(3):531–538. Chinese.
[15] Liu W, Zhang XQ, Wu YQ, et al. Botanical diversity and breeding of Cynodon. Acta Hort. Sci. 2003;30(5):623–628. Chinese.
[16] Gogorcena Y, Arulesek S, Dandekar AM, et al. Molecular markers for grape characterization. Vitis. 1993;32:183–185.
[17] Deng ZN, Gentile A, Nicolosi E, et al. Identification of in vivo and in vitro lemon mutants by RAPD markers. J Hort Sci. 1995;70:117–125.
[18] Luro F, Laigret F, Bove JM, et al. DNA fingerprinting a useful tool for genetic origin and diversity analysis in citrus. HortScience. 1995;30:1063–1067.
[19] Novy RG, Vorsa N, Patten K. Identifying genotypic heterogeneity in ‘McFarlin’ cranberry: a random amplified polymorphic DNA and phenotypic analysis. J Am Soc Hort Sci. 1996;121:210–215.
[20] Patrick JC, Susan KB, Norman FW. Random amplified polymorphic DNA-based genetic linkage maps of three cultivars. J Am Hort Sci. 1997;122(3):350–359.
[21] Lin ZX, Zhang YX, Zhang XL, et al. A high-density integrative linkage map for Gossypium hirsutum. Euphytica. 2009;166:35–45.

[22] Koller B, Lehmann A, Mcpermott JM, et al. Identification of apple cultivars using RAPD markers. Theor Appl Genet. 1993;85:901–904.

[23] Kim SK, Nair RM, Lee J, et al. Genomic resources in mungbean for future breeding programs. Front Plant Sci. 2015 [cited 2017 Mar 23];6:626. DOI:10.3389/fpls.2015.00626.

[24] Caetano-Anolles G, Callahan LM, Gresshoff PM. The origin of Bermudagrass (Cynodon) off-types inferred by DNA amplification fingerprinting. Crop Sci. 1997;37(1):81–87.

[25] Ho CY, McMaugh SJ, Wilton AN, et al. DNA amplification fingerprinting within cultivars of turf-type couch grasses (Cynodon spp.). Plant Cell Rep. 1997;16(11):797–801.

[26] Huang CQ, Huang DY, Zhang YF. Genetic analysis for 57 accessions of Cynodon dactylon from 17 countries in 5 continents by SRAP markers. Trop Grasslands. 2010;44:274–281.

[27] Zheng YQ, Yu SJ, Liu J, et al. Genetic diversity and population structure of Chinese natural bermudagrass [Cynodon dactylon (L.) Pers.] germplasm based on SRAP markers. PLoS ONE. 2017 [cited 2017 Mar 23];12(5):e0177508. DOI:10.1371/journal.pone.0177508.

[28] Caetano-Anolles G, Callahan LM, Gresshoff PM. The origin of Bermudagrass (Cynodon) off-types inferred by DNA amplification fingerprinting. Crop Sci. 1997;37(1):81–87.

[29] Ho CY, McMaugh SJ, Wilton AN, et al. DNA amplification fingerprinting within cultivars of turf-type couch grasses (Cynodon spp.). Plant Cell Rep. 1997;16(11):797–801.

[30] Huang CQ, Huang DY, Zhang YF. Genetic analysis for 57 accessions of Cynodon dactylon from 17 countries in 5 continents by SRAP markers. Trop Grasslands. 2010;44:274–281.

[31] Koller B, Lehmann A, Mcpermott JM, et al. Identification of apple cultivars using RAPD markers. Theor Appl Genet. 1993;85:901–904.

[32] Huang C, Liu G, Bai C, et al. Genetic analysis of 430 Chinese Cynodon dactylon accessions using sequence-related amplified polymorphism (SRAP) analysis. Afr J Biotechnol. 2011;10(75):17106–17115.

[33] Huang C, Liu G, Bai C, et al. Genetic analysis of 430 Chinese Cynodon dactylon accessions using sequence-related amplified polymorphism markers. Int J Mol Sci. 2014;15(10):19134–19146.

[34] Zhang LH, Ozias-Akins P, Kochert G, et al. Differentiation of bermudagrass (Cynodon) genotypes by RAPD markers. Theor Appl Genet. 1999;99(2):465–474.

[35] Zhang LH, Ozias-Akins P, Kochert G, et al. Differentiation of bermudagrass (Cynodon) genotypes by RAPD markers. Theor Appl Genet. 1999;99(2):465–474.

[36] Huang C, Liu G, Bai C, et al. Genetic analysis of 430 Chinese Cynodon dactylon accessions using sequence-related amplified polymorphism (SRAP) analysis. Afr J Biotechnol. 2011;10(75):17106–17115.

[37] Zheng YQ, Yu SJ, Liu J, et al. Genetic diversity and population structure of Chinese natural bermudagrass [Cynodon dactylon (L.) Pers.] germplasm based on SRAP markers. PLoS ONE. 2017 [cited 2017 Mar 23];12(5):e0177508. DOI:10.1371/journal.pone.0177508.

[38] Yi YH, Zhang XQ, Huang LK. Genetic diversity of wild Cynodon dactylon germplasm detected by SRAP markers. Yichuan. 2008;30(1):94–100. Chinese.

[39] Huang CQ, Huang DY, Zhang YF. Genetic analysis for 57 accessions of Cynodon dactylon from 17 countries in 5 continents by SRAP markers. Trop Grasslands. 2010;44:274–281.

[40] Wang Z, Liao L, Yuan X, et al. Genetic relationships of bermudagrass (Cynodon dactylon var. dactylon) from different countries revealed by sequence-related amplified polymorphism (SRAP) analysis. Afr J Biotechnol. 2011;10(75):17106–17115.

[41] Huang C, Liu G, Bai C, et al. Genetic analysis of 430 Chinese Cynodon dactylon accessions using sequence-related amplified polymorphism markers. Int J Mol Sci. 2014;15(10):19134–19146.

[42] Zheng YQ, Yu SJ, Liu J, et al. Genetic diversity and population structure of Chinese natural bermudagrass [Cynodon dactylon (L.) Pers.] germplasm based on SRAP markers. PLoS ONE. 2017 [cited 2017 Mar 23];12(5):e0177508. DOI:10.1371/journal.pone.0177508.

[43] Xie Y, Sun X, Ren J, et al. Genetic diversity and association mapping of cadmium tolerance in bermudagrass [Cynodon dactylon (L.) Pers.] Plant Soil. 2015;390(1–2):307–321.

[44] Khanal S, Schwartz BM, Kim C, et al. Cross-taxon application of sugarcane EST-SSR to genetic diversity analysis of bermudagrass (Cynodon spp.). Genet Resour Crop Evol. 2017;1:1–12. DOI:10.1007/s10722-017-0496-2.

[45] Shi H, Wang Y, Cheng Z, et al. Analysis of natural variation in bermudagrass (Cynodon dactylon) reveals physiological responses underlying drought tolerance. PLoS One. 2012 [cited 2017 Mar 23];7(12):e53422. DOI:10.1371/journal.pone.0053422.

[46] Lu S, Wang Z, Peng X, et al. An efficient callus suspension culture system for triploid bermudagrass (Cynodon transvaalen sis C. dactylon) and somaclonal variations. Plant Cell Tiss Organ Cult. 2006;87(1):77–84.

[47] Lu S, Chen C, Wang Z, et al. Physiological responses of somaclonal variants of triploid bermudagrass (Cynodon transvaalen sis C. dactylon) to drought stress. Plant Cell Rep. 2009;28(3):517–526.

[48] Anderson JA, Kenna MP, Taliaferro, CM. Cold hardiness of ‘Midiron’ and ‘Tifgreen’ bermudagrass. Hortscience. 1988;23:748–750.

[49] Ibitayo OO, Butler JD, Burke MJ. Cold hardiness of bermuda grass Cynodon spp and Paspalum vaginatum Sw. Hortscience. 1981;16:683–684.

[50] Abulaiti, Shi DS, Yang G, et al. Preliminary research report on the native Cynodon dactylon in Xinjiang. J Xinjiang Agric Univ. 1998;21(2):124–127. Chinese.

[51] Youngner VB, Lunt OR. Salinity effects on roots and tops of Bermudagrass. Grass Forage Sci. 1967;22(4):257–259.

[52] Dudeck AE, Singh S, Giordano CE, et al. Effects of sodium chloride on Cynodon turfgrass. Agrono J. 1983;75(6):927–930.

[53] Marcum KB, Pessarakli M. Salt tolerance and salt gland excetration efficiency of bermudagrass turf cultivars. Crop Sci. 2006;46(6):2571–2574.

[54] Francois LE. Salinity effects on three turf bermudagrass. HortScience. 1988;23:706–708.

[55] Ramakrishnan PS, Nagpal R. Adaptation to excess salts in an alkaline soil population of Cynodon dactylon (L.) Pers. J Ecol. 1973;61:369–381.

[56] Hameed M, Ashraf M. Physiological and biochemical adaptations of Cynodon dactylon (L.) Pers. from the Salt Range (Pakistan) to salinity stress. Flora. 2008;203:683–694.
Zhang G, Lu S, Chen TA, et al. Transformation of triploid bermudagrass (Cynodon dactylon) for salt-tolerance. Chin J Trop Agric. 2010;30(4):20–24.

Chu CC, Wang CC, Sun C, et al. Establishment of an efficient experiments on the nitrogen sources. Sci Sin. 1975;18:659–668.

Gamborg OL, Miller RA, Ojima K. Nutrient requirement suspension cultures of soybean root cells. Exp Cell Res. 1968;50(1):151–158.

Sanford, JC. The bifloric process. Tibtech. 1988;6(12):299–302.

Christou P. Genetic transformation of crop plants using microprojectile bombardment. Plant J. 1992;2(3):275–281.

Klein TM, Fitzpatrick-McElligott S. Particle bombardment – a universal approach for gene transfer to cells and tissues. Curr Opin Biotech. 1993;4(5):583–590.

Spangenberg G, Wang ZY. Biologic transformation of embryogenic cell suspensions. In: Celis JE, editor. Cell biology: a laboratory handbook. 2nd ed. Vol. 2. San Diego (CA): Academic Press; 1998. p. 162–168.

Vasil IK. Cellular and molecular genetic improvement of cereals. In: TerziM, Cella R, Falavigna A, editors. Current issues in plant molecular and cellular biology. Vol. 22. Current plant science and biotechnology in agriculture. Dordrecht: Springer; 1995. p. 5–18.

Dai S, Zheng P, Marmey P, et al. Comparative analysis of transgenic rice plants obtained by Agrobacterium-mediated transformation and particle bombardment. Mol Breeding. 2001;7(1):25–33.

Hu T, Metz S, Chay C, et al. Agrobacterium-mediated large-scale transformation of wheat (Triticum aestivum) using glyphosate selection. Plant Cell Rep. 2003;21(10):1010–1019.

Zhang ZX. Factors influencing Agrobacterium-mediated transformation of Cynodon dactylon (L.) Pers. J Plant Physiol. 2005;41(4):449–452. Chinese.

Shi H, Ye T, Chan Z. Comparative proteomic responses of two bermudagrass (Cynodon dactylon (L.) Pers.) varieties contrasting in drought stress resistance. Plant Physiol Biochem. 2014;82:218–228.

Zhao Y, Du H, Wang Z, et al. Identification of proteins associated with water-deficit tolerance in C4 perennial grass species, Cynodon dactylon × Cynodon transvaalensis and Cynodon dactylon. Physiol Plant. 2011;141(1):40–55.

Hu L, Li H, Chen L, et al. RNA-seq for gene identification and transcript profiling in relation to root growth of bermudagrass (Cynodon dactylon) under salinity stress. BMC Genomics. 2015 [cited 2017 Mar 23];16(1):575. DOI:10.1186/s12864-015-1799-3

Liu A, Hu ZR, Bi AY, et al. Photosynthesis, antioxidant system and gene expression of bermudagrass in response to low temperature and salt stress. Ecotoxicology. 2016;25:1445–1457.

Ye TT, Shi HT, Wang YP, et al. Contrasting proteomic and metabolomic responses of bermudagrass to drought and salt stresses. Front Plant Sci. 2016 [cited 2017 Mar 23];7:1694. DOI:10.3389/fpls.2016.01694

Zhang B, Shi JA, Chen JB, et al. Efficient virus-induced gene silencing in Cynodon dactylon and Zoysia japonica using rice tungro bacilliform virus vectors. Sci Hort. 2016;207:97–103.