Gamma-ray Irradiation Induces Useful Morphological Variation in Bermudagrass

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Abstract

Bermudagrass, Cynodon dactylon (L.) Pers. is a widely used warm-season turfgrass species in warmer regions of the world. Gamma (γ) irradiation has been used to generate useful variations in turfgrass breeding for various morphological traits. The objective of the present study was to measure and determine variations in morphology and turfgrass characteristics of a native drought resistant bermudagrass germplasm irradiated with 70, 90 or 110 Gy using a 60Co source. The stolons containing a single node were irradiated and immediately planted for regeneration in a greenhouse at the Akdeniz University, Antalya, Turkey. Selected mutants regenerated from the irradiated stolons were clonally propagated and transplanted into plastic pots for further observations of turfgrass characteristics. Survival rates of stolons exposed to 70, 90 and 110 Gy were 76%, 43% and 17% respectively, 6 weeks after treatment. Dosages of 85 and 57 Gy were determined as LD50 and LD20 for the cuttings, respectively. The linear reduction of survival rate with increasing gamma-rays was highly correlated (r²=0.99). A total of four mutant lines (0.3 % of the irradiated plants) showed a distinct dwarfed growth habit. Three of these lines were originated from 70 Gy and one from 110 Gy. These mutant lines exhibited more dwarf growth habit, higher shoot density, finer leaf texture than parental genotype. Mutant lines developed in this study can be used for the development of improved bermudagrass cultivars for landscaping and sports turf.

Keywords: breeding, dwarf mutants, 60Co, mutation, turfgrass

Introduction

Bermudagrass [Cynodon dactylon (L.) Pers.] is a warm-season grass widely used for pasture, forage, turfgrass, soil stabilization, and remediation (Burton, 1947; Taliaferro, 2003). Cynodon genotypes with all known ploidy levels exist in the eastern Mediterranean of Turkey (Gülsen et al., 2009). The tetraploid C. dactylon genotypes have been the sole genetic pool for development of seeded-type bermudagrass cultivars. Some of the native tetraploid C. dactylon genotypes collected from coastal Mediterranean region of Turkey possessed superior drought tolerance and darker green color than commercial bermudagrass cultivars (ex. ‘Princess 77’, ‘Riviera’) under Mediterranean conditions (Sever Mutlu et al., 2014). These wild germplasm of bermudagrass is well adapted to the region’s biotic and abiotic factors. However, their rapid vertical growth, coarse-leaf texture and long internodes are undesirable and limit their use as turfgrass. Rapid, upright shoot growth requires frequent mowing and irrigation, increasing energy cost during active growing season (Chen et al., 2009). Dwarf bermudagrass mutants induced by gamma radiation exhibited enhanced drought resistance compared to their wild-type parents (Lu et al., 2008). Therefore, there is an increasing demand for slow-growing dwarf type turf cultivars with high drought resistance. Irradiation mutagenesis has been used to create genetic diversity in major crops. Ahloowalia and Maluszynski (2001) reported that more than 1800 released cultivars developed either as direct mutants or derived from their crosses worldwide. Mutation breeding has successfully been used in turfgrasses (Busey, 1980; Dickens et al., 1981; Burton, 1985; van Harten, 1998; Hanna and Elsner, 1999). Gamma-rays irradiation has also been successfully used to generate new breed lines of bermudagrass from the wild germplasm (Lu et al., 2009). Each of these mutant turf cultivars possessed finer texture and superior turfgrass quality than their parental genotypes (Li et al., 2010). The most frequent mutant character from the irradiated plants is dwarfism (Powell et al., 1974). Many dwarf-type bermudagrass cultivars (ex. ‘Tifway II’,...
Materials and Methods

Plant material and mutation treatment

A local tetraploid bermudagrass genotype ‘B-165’ collected from Erzincan city, Turkey, was used as the plant material (Gulsen et al., 2009). The ‘B-165’ exhibits dark green color and was determined to be highly drought resistant after a multi-year/environment evaluation under Mediterranean conditions (Sever Mutlu, unpublished data). However, it exhibited an undesirable rapid upright growth habit with coarser leaf texture compared to commercial bermudagrass cultivars. Stolons of ‘B-165’ were collected from the mature field plot at Akdeniz University, Antalya, Turkey and attached soil was washed off with tap water. The stolons were cut into single node cuttings, placed in plastic petri dishes and irradiated with 0, 70, 90 or 110 Gy doses of γ-radiation from 60Co with a dose rate of approximately 35 Gy/h at 100 cm source-to-sample distance. Each treatment consisted of 450 cuttings.

The control cuttings were not irradiated, but placed in petri dishes and kept in 5°C to maintain moisture until planting. After irradiation, the cuttings were immediately planted in the mixture of peat, vermiculite and perlite (3:1:1, v/v/v) for regeneration in a greenhouse under natural light and at temperature 25-35°C. During the establishment period, plants were fertilized with 15N-6.6P-12.5K, a complex fertilizer, biweekly and irrigated daily to prevent visual wilt symptoms and mowed at 5 cm weekly. The plants were maintained outside the greenhouse under full sun conditions from Oct 2011 to Feb 2012 to detect variations in color retention, dormancy and relative chlorophyll content in comparison to their parental germplasm in the fall.

Data collection and statistical analyses

Irradiation dosage effect on single node cuttings

Survival of the cuttings was scored 6 weeks after irradiation treatment. Survival rate was calculated as a percentage of cuttings with re-growth divided by the total number. The data-points obtained in this way were fitted to the probit function: 

$$\text{probit}(p) = a + b \sqrt{2erf^{-1}(2p - 1)}$$

where $a$ is the y-intercept, $b$ is slope and $p$ is the normalized survival probability. The fit was performed by using the ROOT package (Brun, 1997). In addition an inverse fit was performed as a consistency check and for easy display of data. In all cases the probit-fit and the inverse fit agreed perfectly. The probit function can be easily used to determine any lethality dose (LD).

Morphological characterization of mutant plant lines

Canopy heights with and without seedhead measurements were made on five random plants for each pot. Longest leaf at third node below apical meristem on main upright tiller was used for the measurement of leaf length and leaf width. Seed head density was determined quantitatively by counting seed heads per pot. Five mature inflorescences were collected from each pot to determine the number of racemes per inflorescence, raceme length, seed head exertion from the base of the inflorescence to the flag leaf, and peduncle length. The measurements in each pot were made on five randomly selected stolons for internode length and diameter between 3rd and 4th fully extended nodes from apical meristem, stolon length from apical meristem to the 5th node; and for total stolon number by counting stolons per pot. Visual assessment of leaf texture was an estimate of leaf width.

The visual ratings of texture, shoot density, fall color retention and fall/winter dormancy were collected as described by the National Turfgrass Evaluation Program (NTEP) (Morris, 2004). Five measurements were taken within each pot for relative chlorophyll content using a chlorophyll meter (Field Scout CM 1000; Spectrum Technologies, Inc., Plainfield, IL).

Treatment differences were tested using analysis of variance procedures with PROC GLM (SAS release 8.0; SAS Institute, Cary, NC). Means were separated using Fisher’s protected least significant difference (P<0.05) procedure.

Results

Probit analysis indicated that LD$_{50}$ and LD$_{90}$ dosages were 85±7 and 57±14 Gy, respectively (Fig. 1). Survival rates of cuttings irradiated at 70, 90 and 110 Gy were 76%, 43% and 17%, respectively. The linear reduction of survival rate with increasing γ-rays was highly negatively correlated ($r^2=0.99$). Irradiation of 1350 single-node cuttings resulted in four morphological mutants (0.3 % of the total irradiated cuttings) with dwarf/semi dwarf growth habit. Three of these, 165-70-1, 165-70-5, and 165-70-6, were obtained from cuttings treated at 70 Gy, and one mutant line, 165-110-1, at 110 Gy. The mutant lines exhibited finer plant texture, a more dwarf habit and slower vertical growth than the wild type ‘B-165’ (Table 1). The mutant lines with and without seed heads, respectively, were 26% to 38%, and 30% to 51% shorter than wild type control.

The mutant lines exhibited significant morphological differences compared to wild type (Table 1). The leaf blade length reduced by 34%-46%, and 28% in the lines generated by 70 Gy and 110 Gy, respectively. The leaf blade width also narrowed by 25% to 31%. The mutant lines had significantly finer leaf texture (6.0 to 7.5 rating scale) compared to coarse textured parental genotype (4.0). The stolon number was both increased by 42% (165-70-6) and decreased by 30% (165-110-1). Stolon length was reduced by 27% to 42%, and by 52% in mutant lines originated from 70 Gy and 110 Gy, respectively. Stolon internodes were thinner and up to 31% and 54% shorter in mutant lines originated from 70 Gy and 110 Gy.
Table 1. Morphological characteristics of the dwarf mutant bermudagrass (C. dactylon) lines generated via γ-radiation

<table>
<thead>
<tr>
<th>Trait</th>
<th>Control</th>
<th>Mutant bermudagrass</th>
<th>% Difference from control</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>'B-105'</td>
<td>'165-70-1'</td>
<td>'165-70-5'</td>
</tr>
<tr>
<td></td>
<td>'165-70-14'</td>
<td>'165-110-1'</td>
<td>'165-70-5'</td>
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<td></td>
<td>'165-110-1'</td>
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<tr>
<td>CH</td>
<td>17.8</td>
<td>13.5</td>
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<tr>
<td>CHWS (cm)</td>
<td>13.0</td>
<td>10.7</td>
<td>-17%</td>
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<tr>
<td>LW (mm)</td>
<td>15.0</td>
<td>12.5</td>
<td>-17%</td>
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<tr>
<td>LL (mm)</td>
<td>13.0</td>
<td>10.7</td>
<td>-17%</td>
</tr>
<tr>
<td>SN</td>
<td>10.0</td>
<td>7.5</td>
<td>-25%</td>
</tr>
<tr>
<td>LL (cm)</td>
<td>13.3</td>
<td>10.7</td>
<td>-20%</td>
</tr>
<tr>
<td>SHE</td>
<td>50.0</td>
<td>40.0</td>
<td>-20%</td>
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<tr>
<td>SHE (mm)</td>
<td>1.0</td>
<td>0.8</td>
<td>-20%</td>
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<td>FCR (%)</td>
<td>6.0</td>
<td>4.0</td>
<td>-33%</td>
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</table>

*CH-canopy height without seed head; CHWS-canopy height with seed head; LW-leaf blade width at third node below apical meristem; LL-leaf blade length at third node below apical meristem; SN-stolon number per pot; SD-shoot density rated on a 1-9 scale with 9 equaling maximum density; T-leaf texture rated on a coarse to fine scale with 1 equaling coarse and 9 equaling fine texture; FD-fall/winter dormancy rated on 0-100% scale with 100 equaling complete dormancy, with straw brown color 50% less; FD (%): FD (%).

**Table 2. Correlation coefficients among morphological traits of mutant bermudagrass (C. dactylon) lines generated via γ-radiation**

<table>
<thead>
<tr>
<th>Trait</th>
<th>ID</th>
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<th>RN</th>
<th>SN</th>
<th>RL</th>
<th>SHD</th>
<th>SHE</th>
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<th>LW</th>
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<th>CHWS</th>
<th>CHL1</th>
<th>CHL2</th>
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<th>T</th>
<th>FD</th>
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<td>-0.56**</td>
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<tr>
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<td>SHD</td>
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<td>FDR</td>
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*IL and ID-internode length and internode diameter measured from between 3rd and 4th fully extended nodes from apical meristem. SL-leaf blade length; SN-stolon number per pot; RN-number of racemes per inflorescence; RL-raceme length; SHE-seed head exertion from the base of the inflorescence to the flag leaf; PL-peduncle length as the measurement of internode from base of whorl to first node; CHL1 and CHL2-relative chlorophyll content on 19 Oct 2011 and 4 Dec 2011, respectively; SD-shoot density rated on a 1 to 9 scale with 9 equaling maximum density. T-leaf texture rated on a 1 to 9 rating scale with 1 equaling coarse and 9 equaling fine texture. FDR-fall/winter dormancy rated on 0-100% scale with 100 equaling complete dormancy, with straw brown colour of entire leaves on 4 Dec 2011. FCR-Fall colour retention, rated on a 1 to 9 visual rating scale with 1 equating straw brown or no colour retention, and 9 equating dark green on 20 Nov 2011.

Significant changes in inflorescence characteristics were also observed in mutant lines (Table 1). Compared to wild-type, seed head density both reduced by 57% and increased threefold in mutant lines. The number of racemes per inflorescence increased up to 13%, and raceme length shortened up to 19%. In addition, the peduncle length and seed head exertion from the base of the inflorescence to the flag leaf were reduced in all mutant lines. Highest reduction in peduncle length (% 30) and seed head exertion (% 47) was identified in mutant line '165-70-1'. The mutant lines showed up to 58% higher shoot density. There was significant variation between mutant lines and control plants in terms of dormancy, relative chlorophyll content and color retention in late fall/winter period. Mutant lines retained their green color 28% to 56% better and developed up to 55% less dormancy in fall. Relative chlorophyll contents of mutant lines were up to 19% higher. Improvement in fall/winter performances as demonstrated by lower dormancy, higher chlorophyll content and darker green color in late fall was especially evident in mutant
bermudagrass cuttings (‘Bt165’)

Discussion

Increasing γ-ray dosage significantly reduced survival rates ($r^2=0.99$) in bermudagrass. Similar results were reported with other species of Poaceae family (Busey, 1980; Krishna et al., 1984; Hase et al., 1999; Zaka et al., 2002). Results showed that gamma irradiation doses of 70 to 113 Gy were successful in creating bermudagrass mutants, concurring with earlier reports (Powel et al., 1974; Burton, 1985; Hanna et al., 1997b; Caetano-Anolles, 1999; Hanna and Elsner, 1999; Lu et al., 2009). Four dwarf mutant bermudagrass lines (0.3%) were obtained, comparable to 0.4% dwarf bermudagrass and St. Augustinegrass [Stenotaphrum secundatum (Walt.) Kunze] mutants reported by Lu et al., (2009) and (Li et al., 2010), respectively. Dwarfisms have been associated with defects in biosynthesis or reception of gibberellins (Ross et al., 1997), brassinosteroids (Noguchi et al., 1999), and regulation of cell elongation (Takahashi et al., 1995) or with abnormal cell walls (Reiter et al., 1993).

The LD50 dose was determined to be 85 Gy. Bermudagrass exerted a higher tolerance to gamma irradiation compared to St. Augustinegrass (30–48.5 Gy) (Busey, 1979; Li et al., 2010). The most dwarf mutant line was 51% shorter, similar to 55% reduction in bermudagrass (Lu et al., 2009), and to 31-84% reduction in other species of grass family (Cheema and Atta, 2003; Al-Sahi et al., 2004; Borzouei et al., 2010; Nasab et al., 2010; Kim et al., 2015). Reduction in canopy height and slow-growing trait in dwarf lines may further improve drought resistance over parental genotype. Reports by Lu et al. (2008) and Chen et al. (2009) demonstrated enhanced drought resistance of dwarf mutants compared to their wild type parents, probably due to reduced leaf area. Studies with bentgrasses (Agrostis sp) also indicated that slow-growing turfgrass may be able to survive drought stress for prolonged periods of time (DaCosta and Huang, 2006, 2007).

Useful morphological variations were created by γ-radiation. The bermudagrass mutants had higher shoot density and finer plant texture with significantly smaller leaves and shortened internodes and stolons. As a result of gamma radiation, shorter internodes and smaller leaves were reported in bermudagrass (Hanna, 1990; Chen et al., 2009; Tiwari et al., 2014), centipede grass [Eremochloa ophiuroides (Munro) Hack.] (Pedersen and Dickens, 1985; van Haren, 1998) and St. Augustinegrass (Powell and Toler, 1980; Reynolds et al., 2009; Li et al., 2010) and enhanced shoot/tiller density in wheat (Triticum aestivum L.) and barley (Hordeum vulgare L.) (Breslavets, 1946); and purple false brome (Brachypodium distachyon) (Kim et al., 2015). The turfgrass quality is associated with compact growth pattern and finer leaves (Reynolds et al., 2009). Superior turfgrass quality of dwarf lines was also reported with other studies using gamma radiation in the development of dwarf cultivars of bermudagrass (Burton, 1985; Hanna et al., 1997b; Hanna and Elsner, 1999; Tiwari et al., 2014), St. Augustinegrass (Busey, 1980; Reynolds et al., 2009) and centipede grass (Dickens et al., 1981).

High variations in inflorescence characteristics were found between mutant lines and wild type parent. Among the dwarf mutants, 165-110-1, generated from 110 Gy is particularly interesting since it produced threefold more inflorescences with 13% increase in raceme number per inflorescences. Enhanced panicle length and number in rice (Oryza sativa L.) (Maity et al., 2005), and nearly eightfold more inflorescences in St. Augustinegrass were reported (Li et al., 2010) among the mutants. Wu et al. (2006) reported that inflorescence prolificacy had 51% positive correlation on seed yield, indicating that selection for increased inflorescence prolificacy would be one of the best indirect selection traits for improvement of seed yield in bermudagrass. Mutant line ‘165-110-1’ would enlarge the germplasm pool of bermudagrass and serve as a good breeding material to enhance seed yield.

The mutant lines exhibited better fall performance in comparison to the wild-type control, as manifested by better green color retention in conjunction with higher relative chlorophyll content and lower dormancy. Chlorophyll content is one of the important parameter reflecting the ability of plants to withstand stress. Dwarf mutant lines developed through gamma radiation with enhanced chlorophyll content, chilling and drought tolerance have been reported in Stylosanthes guianensis (Tan et al., 2009).
New genetic variations generated by mutation breeding are particularly advantageous in improving an already outstanding genotype (Busey, 1977; Ahloowalia and Maluszynski, 2001) such as 'TifSport', a fine-textured mutant bermudagrass, developed from a coarse-textured cold hardy cultivar 'Midiron' (Hanna et al., 1997b). Moreover, 'TifSport' has been reported to have significantly higher turfgrass quality than 'Midiron' with sufficient cold tolerance (Taliaferro, 2003). The genotype 'B-165' used as parent in the present study has excellent drought resistance but its coarse texture and rapid upright growth habit were undesirable. Similarly, the dwarf mutant lines generated from 'B-165' exhibited finer plant texture with slower vertical growth. Otherwise, transferring the high drought resistance of 'B-165' into commercially acceptable dwarf type background would have required lengthy phenotypic recurrent selections. Because the mutant lines derived from the drought resistant line 'B-165', they potentially bear a comparable drought resistance. Thus, evaluation of the mutants for drought resistance would be a necessary step to see whether or not they retained this important trait.

Conclusions

The results showed that dwarf mutants of bermudagrass generated through gamma irradiation induced mutation had higher shoot density, finer plant texture and exhibited better fall performance compared to the wild-type control. These lines can be used for the development of improved bermudagrass cultivars for landscaping and sports turf.

References

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