

Gamma Ray Dosage and Mutation Breeding in St. Augustinegrass¹

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ABSTRACT

Stolon pieces of St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] were irradiated with gamma rays in an attempt to cause mutations. A practical dosage for most genotypes was 4,500 rads. This dosage caused considerable (50%) growth retardation and a mean survival of about 40% of single-node cuttings. However, 'Bitterblue' and another accession were entirely killed at 4,000 rads. At 4,500 rads, up to 7% recognizable mutants of accession FA-243 were obtained. This proportion resulted when irradiated cuttings were propagated clonally and observed for 1.5 years in replicated microplots. In addition to morphological variants, a chimeral anthocyanin change was noticed. From this chimera arose a stable genotype with green stolons and white stigmas, whereas the source genotype (FA-243) had red stolons and purple stigmas. Associated reduction in fertility from 56 to 0.6% suggested that the mutation arose as a small chromosome deletion. Mutation breeding is effective in improving St. Augustinegrass when easily recognizable variants are needed.

Additional index words: Anthocyanin, Chimera, Turf, *Stenotaphrum secundatum* (Walt.) Kuntze.

ST. AUGUSTINEGRASS [*Stenotaphrum secundatum* (Walt.) Kuntze] cultivars are normally propagated vegetatively. Unique quality characteristics and a need for uniformity in turf stands may contribute to the emphasis on vegetative propagation (3). St. Augustinegrass also reproduces sexually, but variations in chromosome number exist and are associated with high sterility (9). 'Floratum', a well-adapted cultivar, is aneuploid and is a poor seed producer (4). Numerous attempts to hybridize 'Floratum' and certain other accessions have failed (P. Busey and B. J. Myers, unpublished data). For these reasons, improvement of St. Augustinegrass cultivars has several restrictions.

Mutagenesis by gamma irradiation applied to stem pieces has shown promises for improving triploid turf-type bermudagrasses (10). Somatic mutations in such vegetatively propagated plants are immediately usable variations, particularly when a good genetic background is chosen for mutation breeding. Other ornamental plants have been studied from this perspective, e.g., *Dahlia* (1), along with other vegetatively propagated grasses (2). Although triploids appear to be better candidates for artificial mutagenesis, a large number of first generation (M_1) variants were recognized in a diploid turfgrass-centipede grass [*Eremochloa ophiuroides* (Munro) Hack.] (8).

The investigations reported here were conducted to determine the feasibility of using gamma irradiation to improve St. Augustinegrass. It was also desired to determine optimum dosage of gamma rays for inducing artificial mutations. Because of the problems

referred to in using sexual progeny in St. Augustinegrass improvement, the present investigations were limited to the study of M_1 plants.

MATERIALS AND METHODS

St. Augustinegrass stolon pieces were irradiated with varying dosages of gamma rays from a ¹³⁷Ce source that provided approximately 3,000 rads/min. Freshly uprooted stolons were sealed in plastic bags, stored 2 to 4 days at room temperature, and irradiated. Plants were maintained for several months to determine recovery rate and to identify somatic mutations.

Preliminary dosage experiments used FA-243 St. Augustinegrass. This is a rapid growing dwarf that is diploid ($2n = 18$). It was chosen because of several good performance characteristics, including moderate chinch bug resistance and high competitive ability. Stolon pieces and single-node cuttings were treated at various dosages from 1,500 to 16,500 rads. Irradiated stolons were cut into single-node segments and planted promptly in either: (i) aluminum trays filled to a depth of 15 cm with fumigated (methyl bromide) Hallandale fine sand, a siliceous, hyperthermic, typic psammaquent; or (ii) on a greenhouse bench covered with 10 cm of Zimpro® process heat treated and composted sewage sludge. Survival was rated after 81 to 114 days, after which stolon pieces were propagated for further observation in pots or miniature (0.6 × 0.6-m) field plots. For this accession (FA-243) the LD_{50} for single nodes was between 4,500 and 5,350 rads, based on observed survivals of approximately 100 and 2%, respectively.

Following preliminary dosage experiments, an irradiation series was applied to seven vegetatively propagated St. Augustinegrass genotypes. The seven genotypes included: 1806, Floratum, 'Floratine', 'Bitterblue', and FA-243. Also treated were two mutants (designated 2002-a and 2002-b, and herein referred to as the green mutant and the red mutant, respectively). These two mutants had arisen from a chimera from one of the preliminary irradiation experiments of FA-243. Six dosages were used as follows: 0, 3000, 4000, 4500, 5000, and 6000 rads. Each genotype-treatment combination involved 15 stolon pieces, about 15 cm long, each with about four healthy nodes. Stolons were removed from actively growing field plantings at the same location that (with the exception of 1806) had been planted at the same time and were maintained under the same regime. Stolon fresh weights were determined immediately after irradiation, and each genotype-treatment combination was planted in a galvanized flat (32 × 46.5 × 7 cm deep) filled with Peelings® commercial cypress bark mix. Stolons were uprooted, washed and weighed 120 days after irradiation, and surviving nodes were divided and planted in plastic pots. Average daily growth rate was computed, based on the original fresh weights before irradiation, and final fresh weights 120 days after irradiation. The daily growth rate was the compounded daily change in fresh weight, and was the same as the Relative Growth Rate (RGR) of Fisher (6), except for the use of fresh weight instead of dry weight (5). Number of single-node transplants that survived was counted 208 days after irradiation.

Chromosome numbers of St. Augustinegrass accessions were determined by means of squashes of pollen mother cells. Inflorescences in the boot stage were fixed in 3:1 absolute alcohol: glacial acetic acid for 24 hours, and were thereafter transferred to 70% ethanol. Chromosome squashes were made in 1% acetocarmine. The chromosomes in six to 20 well-spaced microspores of each accession were counted. In some cases mitotic chromosomes in associated tapetal tissue were also counted.

RESULTS AND DISCUSSION

Irradiated nodes reddened within 1 week after treatment and generally remained dormant for 1 to 2 months until either resuming growth or dying. Both

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survival and daily growth rate were significantly reduced at higher gamma ray dosages (Fig. 1). Coefficient of determination for linear regression was higher for survival ($r^2 = 0.933$) than for growth rate ($r^2 = 0.807$). Both responses (survival and growth rate) appeared related. Examination of individual genotype survivals and growth rates (Table 1) suggests that these responses to irradiation were shouldered type survival curves. Simple transformations—such as probit analysis—to linearize gamma ray response were tried but were ineffective. Logistic curves, including one based on a mechanistic model for unicellular systems (7) were cumbersome and would have required more data points. Visual inspection of survival and growth rate responses (Table 1) clearly indicates that genotypes differed in tolerance to gamma irradiation. Three genotypes, FA-243, Floratam, and the green mutant, sustained less than 50% mortality at 4,500 rads. Three others, 1806, Bitterblue, and the red mutant, underwent more than 50% mortality at only 3,000 rads. Since a sample of only seven genotypes was used, it would not be possible to conclude a relationship of gamma ray tolerance with chromosome number or other plant characteristics. Mean survivals for plants with different chromosome numbers are presented, however (Fig. 1). Genotypes used in this experiment with $2n = 18$ (FA-243, green mutant, and probably the red mutant) tended to be more tolerant of gamma rays than genotypes with $2n = 30$ (Bitterblue, Floratine, and 1806). Floratam, which has $2n = 32$ —a tentative count (4)—was fairly tolerant of gamma rays.

An induced mutation was observed in experimental FA-243 treated at 4,500 rads. The mutation was originally noticed as a stolon that was longitudinally striped red (with anthocyanin) and green (no apparent anthocyanin). This chimera was propagated and observed, and frequently produced offshoots that were all green or all red. The red offshoot form (the red mutant) had less pigmentation than the original strain FA-243 and appeared to be somewhat unstable, continuing to produce all-green sports at about 20% of the lateral branches. The red mutant was less competitive and was eventually lost. The green offshoot forms (the green mutant) were virtually devoid of reddening (Fig. 2), in both stolons and leaves. The green mutant was apparently stable—it did not produce obvious sports—and was expanded into plots 6 to 60 m² at several locations over a period of 2.5 years. Unlike the red mutant and FA-243, both of which had purple stigmas, the green mutant had white stigmas. All forms had the $2n = 18$ chromosome number, and normal pairing in meiosis. Whereas FA-243 produced an average of 56% filled seeds, fertility of the green mutant was only 0.6%. Another pleiotropic, or otherwise associated character was internode length, which was slightly longer in the green mutant than in FA-243 (Fig. 2). The source genotype FA-243 reddened considerably in the winter, and this gave stands an unattractive purplish off-color appearance. The green mutant did not show appreciable discoloration in the winters of 1977-78 through 1979-80, but remained green, while adjacent plots of FA-243 appeared purple.

Another mutation observed from FA-243, hereafter designated 2096, had extremely tall inflorescences

Table 1. Survival rate and daily growth rate of seven St. Augustine-grass strains following various dosages of gamma irradiation.

Genotype	γ radiation (rads)					
	0	3,000	4,000	4,500	5,000	6,000
1806	100%† (0.70)‡	6% (0.37)	0% (0.46)	0% (0.25)	0% (0.18)	0% (-0.04)
Bitterblue	100% (1.63)	47% (1.36)	0% (0.02)	0% (-0.07)	0% (0.10)	0% (-0.60)
Red mutant	100% (2.16)	30%§ (1.22)	3% (0.38)	27% (0.72)	0% (0.20)	0% (0.29)
Floratine	100% (1.20)	42% (1.14)	80% (1.64)	20% (0.37)	17% (0.34)	0% (0.16)
FA-243	100% (1.91)	100% (2.22)	55%§ (1.61)	77% (1.46)	6% (0.54)	0% (0.24)
Floratam	100% (1.49)	100% (1.37)	98% (1.45)	52% (1.36)	0% (0.16)	2% (0.54)
Green mutant	100% (2.13)	100% (2.04)	88% (1.94)	94% (1.66)	21% (0.66)	18% (0.75)
Avg.	100% (1.60)	60.7% (1.39)	46.3% (1.07)	38.6% (0.82)	6.3% (0.31)	2.9% (0.19)

† Survival of single-node cuttings 208 days after irradiation.

‡ Daily growth rate during 120 days after treatment.

§ Two survival percentages were estimated visually without reliance on direct counts. For the lower dosages (all survivals reported as 100%) extensive regrowth likewise resulted in a tangle of stolons that required estimation rather than direct counts.

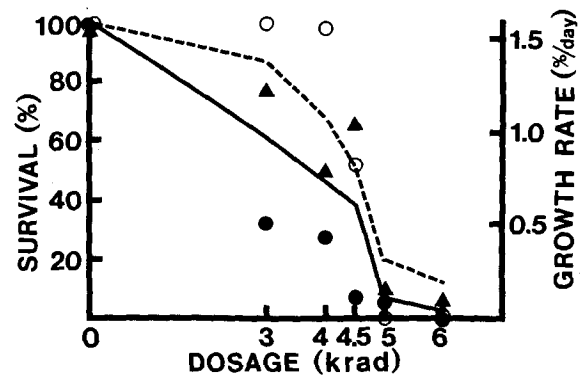


Fig. 1. Gamma ray dosage effect on St. Augustinegrass mean node survival (solid line) and growth rate during 120 days after irradiation (dashed line). The symbols (O, ●, and ▲) are used to locate mean survivals of plants with chromosome numbers $2n = 32$, $2n = 30$, and $2n = 18$, respectively.

with long floral internodes, but other plant parts were not similarly affected (Fig. 3). Although visual ratings in November 1977 showed that this clone flowered more intensively than its neighbors in microplots, it was not until April 1979, and 1.5 years of field culture, that the long inflorescences were noticed. Earlier visual evaluations had shown no significant differences among irradiated clones for inflorescence abundance, largely because of the scarcity of such variants and the skewed nature of the probability distribution. Although of no apparent economic value, mutants such as 2096 may be useful in genetic studies.

Other mutations included two ultra-dwarf chlorophyll defectives of FA-243, two mutants of Floratam (a narrower-leaved type and an early flowering type), and a wrinkled-leaf type of 1806. A total of 22 mutants were isolated from all irradiation treatments.



Fig. 2. Comparison between St. Augustinegrasses FA-243 (left three stolons) and the green mutant (right three stolons). Both sets of stolons were grown under identical conditions in full sunlight. Photograph taken with a green filter.

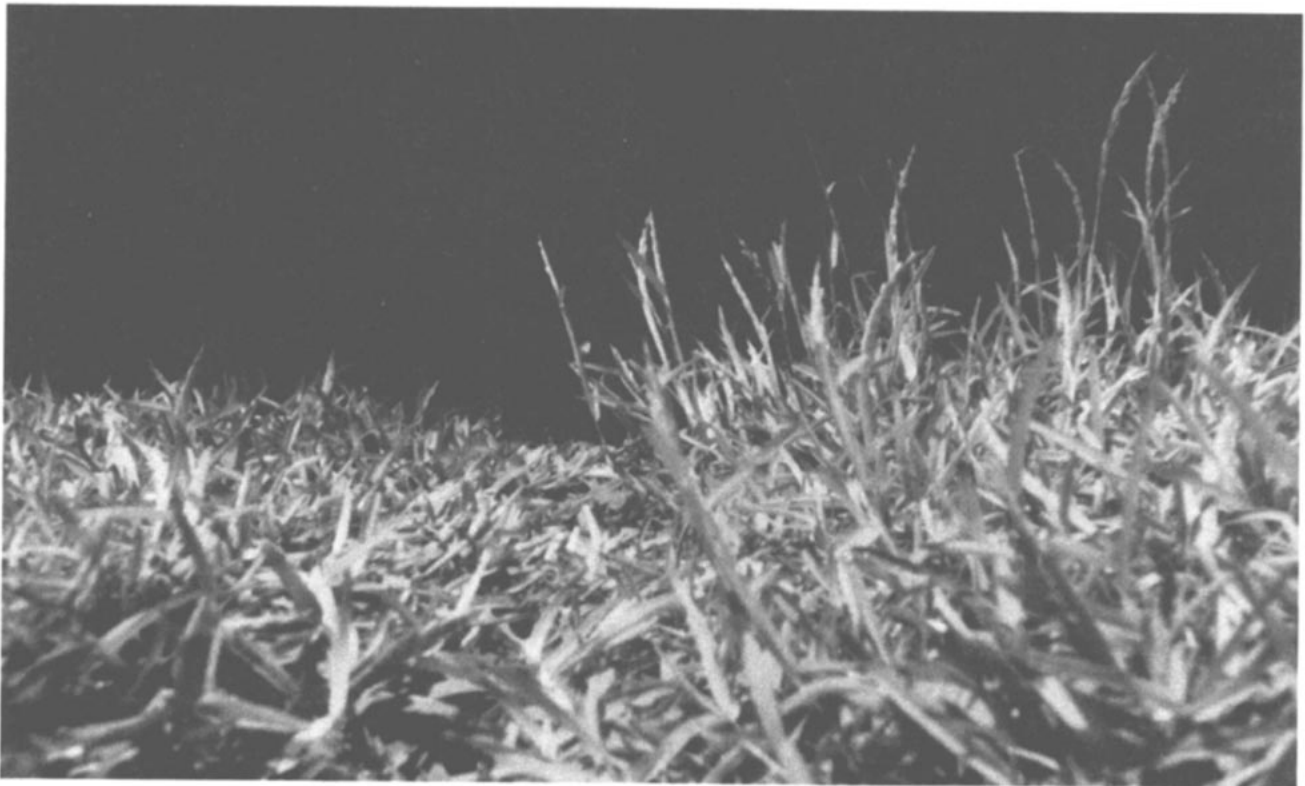


Fig. 3. Comparison between St. Augustinegrasses FA-243 (left microplot, an irradiated clone that showed no visual changes) and 2096, a tall-flowered mutant (right microplot). Photograph taken with a green filter.

The highest yield of mutants (7%) was 13 variants out of 190 clones of FA-243 treated at 4,500 rads, recognized in replicated microplots. Considerably smaller mutant yields were produced from other irradiation treatments in which clones were not compared for long periods under replicated field conditions. Additional field and laboratory studies are being performed in a search for genetic variants in terms of response to environmental stress.

CONCLUSIONS

Usable irradiation dosages for St. Augustinegrass can be indicated by survival and growth rate measurements. A dosage to provide considerable (greater than 50%) growth retardation, but substantial (about 40%) survival of most genotypes was 4500 rads. This dosage is recommended for inducing mutations in most St. Augustinegrasses, although 3,000 rads would be the maximum permissible dosage for Bitterblue and some other strains. The high tolerance of the green mutant (94% survival at 4,500 rads) is interesting and may have fundamental significance regarding the nature of the mutation. Gamma ray tolerance differences among genotypes did not seem closely associated with plant dimensions, color, or overall growth rate (Table 1), but a relationship with chromosome number is suggested. Since growth following irradiation did not occur continuously, growth rate as measured here (and survival) may be partly a measure of the duration of post-irradiation dormancy.

Gamma irradiation is a rapid method for inducing genetic changes in St. Augustinegrass. Mutants were noticed in the diploid strain FA-243, showing that expression of genetic changes is not limited to the presence of imbalanced sets of chromosomes (as in triploids and some aneuploids). Because the original FA-243 is heterozygous for color (P. Busey and B. J. Myers, unpublished data) and changes in fertility were observed in the green mutant, it is likely that the mutation was a small chromosome deletion.

Only morphological or color changes were directly picked out among irradiated clones, and differences in adaptive response would require much more extensive experimentation. Morphological changes may assist in the recognition of related, pleiotropic changes in adaptive response. But with the emphasis on appearance of turfgrasses, recognition of visual differences can be considered an important end in itself. The green mutant is similar morphologically and in growth rates to the parental form FA-243. Both maintain

very similar stand characteristics under turf maintenance. Yet, the greener winter color of the green mutant would be an important point of preference to observers. Associated changes in seed set are an unexpected benefit.

For characters that are easily discerned, mutation breeding offers several advantages as a plant improvement technique. But quantitative characteristics, or those with a high variability, are not easily detected, in part because familiar statistical techniques do not detect low frequency variants. Long-term evaluation of clones that survive irradiation can greatly increase the number of recognizable mutations. Repeat cycle mutation breeding can also be used, as it was in this study, to produce additional genetic changes. Although mutation breeding offers the promise of making useful genetic changes in ornamental plants, without altering coherent sets of quality characteristics, associated genetic changes are not necessarily avoided, and could be a problem.

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