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Short Communication

Phenotypic and genetic variability induced in Lehmann's love grass (*Eragrostis lehmanniana*) through gamma irradiation

Variabilidad fenotípica y genética inducida en pasto amorseco africano (Eragrostis lehmanniana) *mediante irradiación gamma*

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Abstract

This study assessed the morphological and nutritional diversity induced through gamma irradiation in Lehmann's love grass. Seed were irradiated at doses of 0, 100, 200, 300, 450, 600, 900, and 1400 Gy. Ten agronomic traits related with forage quality were evaluated and used to select the mutants, which were confirmed by cluster analysis and multivariate analysis of variance and then characterized by nutritional and molecular characterization. Mutants with 16–20% less ($p<0.05$) lignin and 36–68% more protein content than the control genotype were found. Genetic distances of 0.38 and 0.49 also revealed differences ($p<0.05$) between the mutants and control genotype. The phenotypic and genetic variability, induced through gamma irradiation, resulted in the identification of two first generation mutants with outstanding agronomic traits and nutritional quality.

Key words: AFLP, Cobalt 60, forage quality, grass species, mutation induction.

Resumen

Este estudio evaluó la variabilidad morfológica y nutricional inducida en *Eragrostis lehmanniana* mediante irradiación gamma. Para ello, semillas fueron irradiadas a 0, 100, 200, 300, 450, 600, 900 y 1400 Gy. Se evaluaron 10 características agronómicas relacionadas con calidad de forraje. Esto sirvió para seleccionar mutantes M1 sobresalientes, los cuales fueron confirmados con análisis cluster y análisis multivariado y posteriormente caracterizados nutricional y molecularmente. Estos mutantes presentaron entre 16 y 20% menos ($p<0.05$) lignina y entre 36 y 68% más proteína que el genotipo control. Además, se encontraron distancias genéticas de entre 0.38 y 0.49 (Coeficiente de Dice) y diferencias significativas ($p<0.05$) entre los mutantes y el genotipo control. La variabilidad fenotípica y genética, inducida a través de irradiación gamma, resultó en la identificación de dos mutantes de primera generación con características agronómicas y nutricionales sobresalientes.

Palabras clave: AFLP, calidad de forraje, cobalto 60, especies de pastos, inducción de mutaciones.

Introduction

Lehmann's love grass (*Eragrostis lehmanniana* Nees.), which is native to Africa, has been used to revegetate degraded grasslands due to its excellent establishment capacity in areas where native plants cannot be established (McGlone and Huenneke 2004). However, it is invasive and the use of this grass is ecologically risky because it can be

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dispersed to adjacent areas and displace native vegetation (Guevara et al. 2007). The main cause of Lehmann's love grass invasiveness may be due to low consumption of the mature grass by cattle (Chávez et al. 2000). Although young plants of this grass are moderately palatable, mature plants have high tiller density and fiber content, with low leaf-stem ratio and protein content (O'Reagain and Mentis 1989; González-García et al. 2017). In addition, genotypes of *E. lehmanniana* brought to America for erosion control were apomictic (Burson and Voigt, 1996). Individuals from apomictic seeds are genetically identical to the maternal plant, indicating that populations established in the Americas may have low genetic variability. Hence, if diversity could be induced, Lehmann's love grass could be included in a breeding programme to increase its forage quality and its acceptability by cattle.

A fundamental requisite for breeding is the existence of genetic variability, which can be increased by a collection of more genotypes from the wild or recombination of existing germplasm through plant breeding. When such variability is not present, an alternative is to induce it. Mutation induction has been an important tool in plant breeding because it provides a simple and low-cost mechanism to induce genetic variability (Xi et al. 2012) and has been used to modify the nutritive value of forage crops (Golubinova et al. 2017; Lee et al. 2017). Mutagenesis may be useful to obtain new Lehmann's love grass genotypes with better forage quality. The objective was to evaluate the agronomic, nutritional and molecular variability induced through gamma irradiation in Lehmann's love grass within the framework of a breeding programme focused on nutritional quality.

Materials and Methods

Seven samples of approximatively 100 g of seed of a commonly used variety of Lehmann's love grass were irradiated with doses of: 100, 200, 300, 450, 600, 900 and 1400 Gray (Gy) using a panoramic irradiator (Gamma Beam, model GB-127 MDS, Nordion). A sample of unirradiated seed (0 Gy) was used as control. The exposure times required to apply the doses were determined by using a Gafchromic dosimetry system and an ionization chamber (Model Acudose 4094118, RADCAL). Exposure times were calculated based on the activity of the radioactive source and its distance to the seed samples. The radioactive source was cobalt 60 (60Co) with an activity of 15,000 Curies. The irradiation stage was carried out at the MOSCAFRUT SAGARPA/IICA complex in Chiapas, Mexico.

The seed utilized for germination were randomly selected from the irradiated samples. Given that radiation does not affect all the individuals irradiated equally, ten plants were randomly selected from the germinated seedlings and from each irradiation dose to evaluate and then select those plants that can be considered as mutants. The evaluation was carried out using a completely randomized experimental design, where the treatments were the irradiation doses. The mean temperature (T) during the experiment was 23.7 ± 5.6 °C, with a minimum of 10.1 and a maximum of 44.7 °C. The mean relative humidity (RH) was 52.0 ± 16.8%. Measurements of T and RH were performed with a HMP60 probe (Vaisala, Woburn, MA, USA). Data were recorded in a CR200X datalogger (Campbell Scientific Inc., Logan, UT, USA). The plants were grown in pots of 26 cm height and 18 cm diameter in a greenhouse. Pots were filled with sandy-loam soil of alluvial origin to 23 cm height and watered until soil saturation every three days throughout the experiment. Sowing was done during June 2016 and the evaluations were carried out in October 2017. The following agronomic descriptors were measured: stem weight (g/plant), leaf weight (g/plant), forage yield (g/plant), leaf-stem ratio, leaf length (cm), leaf width (mm), plant height (cm), seed production (g/plant), foliage height (cm) and foliage-plant height ratio. To quantify the stem weight, leaf weight and forage yield for each plant, shoots were cut at 0.05 m above ground and leaf and stems separated. The harvested samples were dried in a forced air oven at 65 °C for 72 h and dried samples were weighed using an analytical balance. Leaf length was measured from the ligule to the apex of the leaf while leaf width was measured at the middle of the leaf sheath. These two variables were recorded from three randomly selected leaves and their values averaged. Plant height was measured from the ground to the tip of the tallest stem while foliage height was measured from the ground to the second leaf of the tallest stem. Individual plants with the greatest leaf weight, leaf-stem ratio, leaf length, leaf width, foliage height, and foliage-plant height ratio were selected for the subsequent nutritional and molecular characterization.

Nutritional characterization was performed by near-infrared spectroscopy (NIRs) (SpectraStar 2600 XT, Unity Scientific). Only the selected outstanding mutant individuals identified from the morphological characterization, as well as the plants from the control treatment, were nutritionally characterized. The dried leaf and stem samples from each plant were remixed separately for the nutritional analysis. The forage from each individual sample (mutant and control plants) was

divided into two sub-samples and then analyzed. The variables evaluated were neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose, hemicellulose and crude protein (CP).

Molecular characterization was also performed only for the selected outstanding individual mutants identified from the morphological characterization using Amplified Fragment Length Polymorphism (AFLP) molecular markers. Approximately 100 mg/pl of fresh leaf material (from three leaves harvested before the morphological and nutritional analysis) was used for the DNA extraction. The genomic DNA was extracted with a DNeasy® Plant Mini Kit (QIAGEN Inc.) following the manufacturer's instructions. The AFLP analysis was performed using an AFLP template kit (LI-COR Biosciences), according to the manufacturer's instructions. Mutants were individually analyzed while a bulk analysis was carried out with the DNA extracted from the ten control plants. The restriction enzymes EcoRI and MseI and four fluorescent labeled primers combinations (MseI + CAG-EcoRI + AGC, MseI + CAG-EcoRI + AGA, MseI + CAG-EcoRI + ACA, MseI + CAG-EcoRI + ACT) were used for the analysis. The AFLP fragments were analyzed on a DNA Analyzer (Model 3730xl, Applied Biosystems).

Agronomic and nutritional data were subjected to a cluster analysis following Ward's method. The number of groups was determined based on the pseudo F and T^2. A discriminant analysis was performed to verify the classification generated by the cluster analysis. The resulting clusters, corrected by the discriminant analysis, were compared by an analysis of variance (ANOVA) and a Tukey test (α=0.05), using the statistical software SAS, version 9.1.3 (SAS, 2004). To analyze the molecular data, the presence or absence of bands detected in the electropherograms was scored and a binary matrix was elaborated. The matrix was then statistically analyzed with NTSYSpc, version 2.1. Genetic similarity among populations was estimated based on the Dice similarity coefficient. The unweighted pair group method with arithmetic mean (UPGMA) was used as the clustering method. The population groups, clustered after the analysis, were compared through an analysis of molecular variance (AMOVA) (Excoffier et al. 1992). Finally, a Mantel test was performed to correlate the genetic matrix with the morphological and nutritional matrices. The genetic matrix was constructed with the values of the Dice's coefficient of genetic similarity. The morphological and nutritional matrices were elaborated with the Euclidean distances obtained from the cluster analysis of the morphological and nutritional data, respectively.

Results

The clustering pattern based on agronomic characterization separated the individuals into four groups (R^2= 0.51) (Figure 1). In all groups, at least one control plant was included, except in Group II, which included mutants exclusively. The mutants included in Group II were 100-3, 100-6, 200-2, 200-6, 300-7, 450-7, 1400-2, and 1400-10. However, the individuals 100-3 and 200-2 were misclassified, according to the discriminant analysis and they belonged to Group IV. Group II was represented by individuals with low stem weight and high leaf weight and leaf-stem ratio. In addition, individuals in this group showed the greatest ($p<0.05$) leaf length and the lowest seed production. Only the six mutants included in Group II, after the discriminant analysis, were selected to be included in the nutritional and molecular characterization. The irradiation treatments had no effect on the frequency of the mutations because the selected mutants were generated by doses from 100 to 1400 Gy.

Based on the nutritional characterization, the clustering pattern combined the individuals in only two groups (R^2= 0.82) (Figure 2). Group II was clustered by mutant plants while all the control plants and two mutants were clustered into Group I. Group II presented lower ($p<0.05$) NDF, ADF, ADL and higher crude protein ($p<0.05$) than Group I. Thus, only the 6 mutants included in Group II (100-6, 200-6, 300-7, 450-7, 1400-2, and 1400-10) were selected for molecular analysis.

The AFLP analysis detected a total of 256 polymorphic bands. The resulting values of the Dice similarity coefficient ranged from 0.43 to 0.73. Cluster analysis based on molecular data separated the mutants and the control genotype into two groups. Group II included only the mutants 200-6 and 300-7 while Group I included the rest of the mutants and the control genotype (Figure 3). The AMOVA revealed differences ($p<0.05$) among Groups I and II. The Mantel test revealed a significant correlation ($p=0.03$) between the genetic matrix and the matrix elaborated with the morphological distances. The correlation coefficient between the genetic matrix and the morphological distance matrix was 0.32. A significant correlation ($r= 0.58$; $p=0.0008$) was found between the genetic matrix and the matrix elaborated with the nutritional distances (Figure 4).

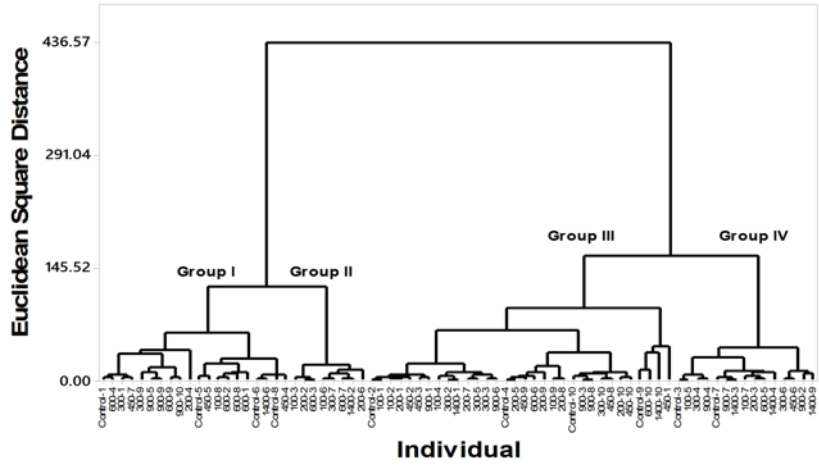

Figure 1. Dendrogram of 70 mutants and 10 individuals germinated from unirradiated seed (control) of Lehmann's love grass using 12 quantitative morphological variables. The dendrogram was constructed following Ward's method.

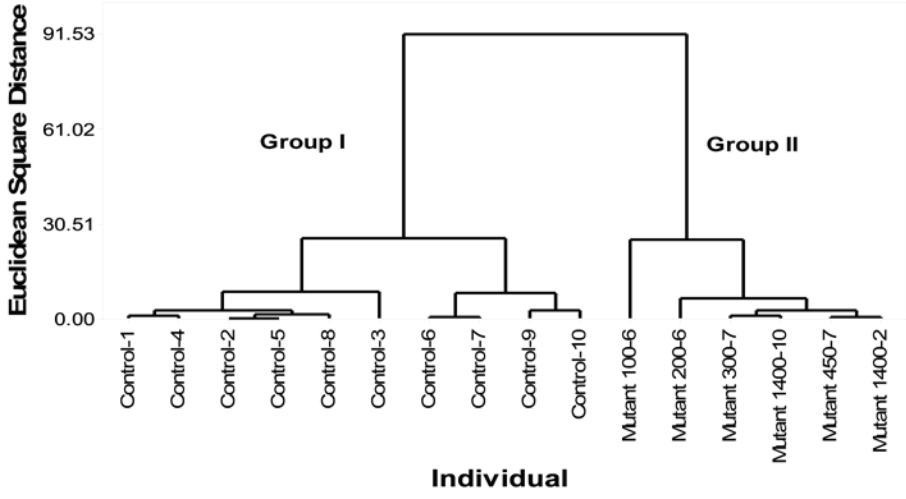

Figure 2. Dendrogram of eight mutants and 10 individuals germinated from unirradiated seed (control) of Lehmann's love grass evaluated for 6 nutritional variables at maturity. The analysis was constructed following Ward's method.

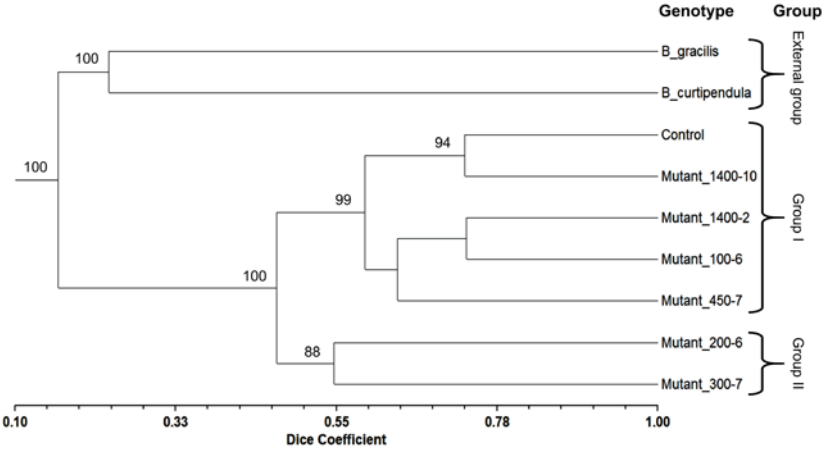

Figure 3. UPGMA dendrogram of six mutants and a genotype germinated from unirradiated seed (control) of Lehmann's love grass computed using 279 AFLP markers. Bootstrap values greater than 80% are shown. *Bouteloua gracilis* and *B. curtipendula* were included as external species to validate if the analysis was correct by verifying if these species were clustered together.

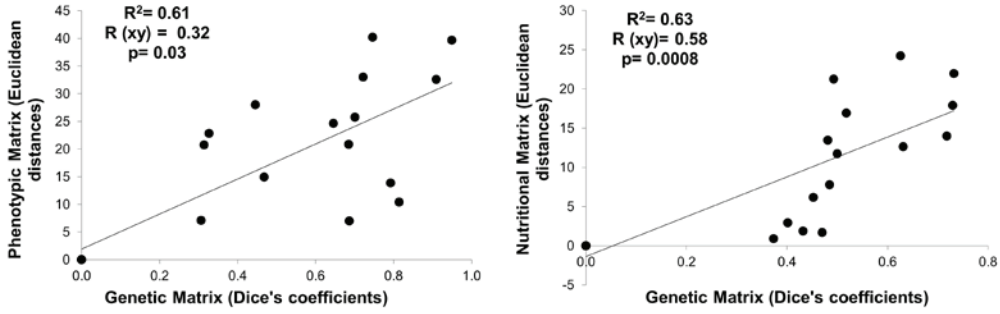

Figure 4. Relationship between **A)** morphological and genetic AFLP distances and **B)** nutritional and genetic AFLP distances among mutant and plants germinated from unirradiated seed (control) of Lehmann's love grass.

Discussion

A high morphological variability among mutants was found. Previous studies have reported a relationship between morphological traits and forage nutritive quality. Batistoti et al. (2012) found that leaf area is positively correlated with CP in guinea grass and the grass structure affects its acceptability by cattle. O'Reagain and Mentis (1989) evaluated nine African grass species, four from the genus *Eragrostis*, and reported a positive relationship among leaf-stem ratio, forage height and crude protein with grass acceptability by cattle. This relationship was used for the selection of traits in the morphological characterization for the identification of mutants.

According to the nutritional characterization, the selected mutants presented between 4 and 5% less fiber than the control genotype. This may represent an increase in nutritional value because forage digestibility is inversely related to fiber content (Ávila et al. 2013). Crude protein content was significantly increased in all of the selected mutants, with an increase from 36 to 68% compared to the control. Grass acceptability by cattle is negatively related with fiber content and positivity related with protein content (O'Reagain and Mentis 1989; Ávila et al. 2013) indicating the mutants may have a higher nutritional quality and could be more acceptable by cattle compared to the control. Lehmann's love grass is considered an invasive species (Guevara et al. 2007) and has been used to revegetate degraded grasslands due to its good establishment capacity (McGlone and Huenneke 2004). These new genotypes with a higher acceptance by cattle could be used to revegetate highly degraded areas, where the native vegetation cannot be established, with a lower risk of invasiveness.

The AFLP analysis revealed significant genetic variation between the mutants and the control genotype since genetic similarities from 0.43 to 0.73 were found. This result agrees with previous findings where genetic variation was induced through gamma irradiation in grass species. Zhang et al. (2012) increased the genetic diversity of 72 *Brachypodium* sp. accessions collected from different countries, by using gamma radiation. Pongtongkam et al. (2006) induced genetic variability in Napier grass (*Pennisetum purpureum*) and found genetic similarities from 0.56 to 0.78 (Dice´s coefficient) between mutants and unirradiated plants. The significant correlation found between the molecular and the morphological distances, together with the nutritional distances, suggests that some of the phenotypic differences between mutants and control plants could be produced by genetic variability induced through gamma irradiation. Nonetheless, the weak correlation found between the phenotypic and molecular distances is likely because only a few genes may control agronomic and biochemical traits, while the AFLP markers randomly sample areas along the genome (Harris et al. 2010).

Conclusions

Gamma irradiation induced phenotypic and genetic variability in Lehmann's love grass. The induced variation allowed the identification of the first generation of mutants with more desirable agronomic traits and nutritional quality. To further evaluate the following generations of these materials, it will be necessary to verify if the desired characters become fixed to provide new improved germplasm for future grassland revegetation programmes.

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(Note of the editors: All hyperlinks were verified 7 January 2022).

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