USE OF GAMMA-RADIATION TO INCREASE GENETIC VARIABILITY IN JARAGUA GRASS (*Hyparrhenia rufa*)

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SUMMARY - Spontaneous or induced mutations provide important genetic variability, useful to many plant species breeding programs. With the objective to observe mutagenic effect of gamma radiation in Jaragua grass (*Hyparrhenia rufa*), seeds were irradiated with the dose of 400 Gy from ^{60}Co source. The M_1 generation was evaluated in the field. Different morphological characters were recorded, such as divergent racemes, awnless spikelets, low growth, narrow leaves, and chimerism. No variation in flowering time was observed. A preliminary progeny test with M_2 off-type and control plants showed variation within progeny mainly for the character divergent racemes, which presented Mendelian segregation. Embryo sac analysis, by the clearing technique with phase contrast microscopy, showed the presence of single and multiple aposporous embryo sacs as well as a lower frequency of meiotic embryo sacs indicating the facultative apomictic breeding system of this species. Further investigation is needed to verify whether irradiation has caused a break in apomixis.

Index terms: apomixis, gamma radiation, genetic variability, *Hyparrhenia rufa*.

USO DE RADIAÇÃO GAMA PARA AUMENTAR A VARIABILIDADE GENÉTICA EM CAPIM JARAGUÁ (Hyparrhenia rufa)

RESUMO - Mutações espontâneas ou induzidas podem gerar importante variabilidade genética, útil para muitos programas de melhoramento de plantas. Objetivando avaliar o efeito mutagênico da radiação gama em capim-jaraguá (*Hyparrhenia rufa*), sementes férteis foram irradiadas com 400 Gy pela exposição à fonte de ^{60}Co. A geração M_1 foi avaliada no campo. Foram registrados diferentes caracteres morfológicos, tais como racemos divergentes, espiguetas sem arista, crescimento lento, folhas estreitas e com manchas amareladas. Não foi observada variação da época de florescimento. O teste de progênie com plantas M_1 e plantas controle mostrou variações entre as progênies principalmente para o caráter racemo divergente, os quais apresentaram segregação Mendeliana em M_2. A análise de saco embrionário de flores M_1, pela técnica de clareamento com microscópio de contraste de fase, mostrou a presença de sacos embrionários apospóricos simples e múltiplos e uma menor freqüência de sacos embrionários meióticos, indicando um sistema de reprodução apomítico facultativo para essa espécie. Maiores investigações são necessárias para verificar se a irradiação causou uma interrupção na apomixia.

Termos para indexação: apomixia, radiação gama, variabilidade genética, *Hyparrhenia rufa*.

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INTRODUCTION

Hyparrhenia rufa is one of the most common and widespread species of forage grasses in tropical and sub-tropical Africa. It was introduced early in Brazil, and is frequently considered part of the native flora (PARSONS 1972). This species has been established in extensive poor soil areas of Brazil, due to its rusticity (BIANCHINI et al. 1980). It has high nutritive value, but shows limited vegetative growth period determined by short winter days (VELLOSO et al. 1982). According to AGREDA & CUANY (1962), Jaragua grass was found to be a short day plant, although individual desirable late-flowering plants have been selected.

Jaragua grass shows great uniformity in the different edapho-climatic conditions in Brazil but its potential should be further studied, considering that during long time it has been exposed to different ecological pressure (ARONOVICH & ROCHA, 1985). CUANY (1967) found considerable natural variability in *H. rufa*, despite the great uniformity observed in seedling populations. It is considered a facultative apomictic species (BROWN & EMERY 1957), presenting the "Panicum type" of apospory (NOGLER 1984).

Mutation induction has been applied to apomictic species that present low sexuality rates (BASHAW & HOFF, 1962; SINGH & MEHRA, 1971; HANNA & POWELL, 1973), and has provided substantial number of mutant progeny with variation in earliness, and morphological characters (BASHAW & HOFF, 1962; BURTON & HANNA, 1975; BUSEY, 1980), introducing genetic variability in species which depend almost exclusively on spontaneous mutations (BROERTYES & HARTEN 1988).

The purpose of the present work was to increase genetic variability and/or apomixis break in Jaragua grass by seed irradiation. The contribution of irradiation to breeding programs of this species is discussed.

METHODS

Fertile seeds of Jaragua, with 12% of moisture, were exposed to 40 Krad dose from ^{60}Co source (5.26 KGy.h-1, dose rate) at Nuclear Energy Center for Agriculture (CENA), Piracicaba, São Paulo, Brazil. This dose was choosen based in a first experiment with this species and different doses, where the occurrence of abnormal seedlings above 400 Gy was observed. After treatment, irradiated and non-irradiated seeds were planted in plastic pots in the greenhouse. A total of 1030 irradiated and 150 non-irradiated young plants were separately transplanted in the field. Morphological and phenological observations were taken. Spikelets were collected for embryo-sac analysis in order to determine the mode of reproduction. Mature M2 seeds were collected individually from each plant.

A preliminary progeny test was conducted with the M2 seeds from eight off-type (atypical) M1 plants and the progenies of two non-irradiated plants. Each progeny was represented by a maximum of 10 M2 plants. Germination and plant survival was low in some progenies. Morphological observations were obtained from each plant. Young spikelets were collected for embryo-sac analysis by the clearing technique (HERR, 1971) modified by SAVIDAN (1975), and the use of phase contrast microscopy for the study of apomixis.

RESULTS

From the morphological observations in the M1 plants, some off-type or atypical plants were observed, with single and combined characters such as low growth, narrow leaves, divergent racemes, awnless spikelets, and yellow spots. The frequency of these off-type plants is presented in TABLE 1. The chlorophyll deficiency in the M1 plants observed were represented by the 'albina', 'chlorina' and 'striata' types, according to BASU & BASU (1969). Whether this chimeric formation was a result of physiological effect or genetic mutation was not determined. All M1 plants flowered around April and May and no late flowering plants were observed.

TABLE 1 - Off-type plant characters recorded from irradiated M_1 Jaragua grass (*Hyparrhenia rufa*) plants

Type	Number of plants	Frequency (%)
Low growth and narrow leaves	23	2.2
Divergent racemes	21	2.0
Chimerism	3	0.3
Awnless spikelets	1	0.1

The description of the off-type M1 plants used in the progeny test is presented in TABLE 2. Results of this preliminary test are presented in TABLE 3. Variation within progeny for the character divergent racemes was observed for progenies 3, 5 and 6. Progenies 8 and 9 also showed variation. Embryo sac analysis was done in some of these progeny plants to detect possible presence of sexuality. The results are reported in TABLE 4. High percentage of multiple embryo sacs was observed in all plants analysed. The frequency of single aposporous embryo sacs was lower. Within plants of progeny number 3, higher frequency of meiotic embryo sacs was observed, around 10%. Meiotic embryo sacs were considered as those ovules with single meiotic embryo sacs or with multiple sacs where the meiotic embryo sac was near the micropylar end. Aposporous embryo sacs with two polar nuclei, as well as meiotic embryo sacs with one polar nucleus were observed. The presence of two or more meiotic embryo sacs within one ovule were also observed.

TABLE 2 - Off-type irradiated M_1 plants of Jaragua grass used in the progeny test

Plant number	Main characteristics
1	control - normal phenotype
2	control - normal phenotype
3	control - normal phenotype
4	divergent racemes
5	divergent racemes
6	divergent racemes
7	divergent racemes, low growth
8	low growth, narrow leaves
9	low growth, narrow leaves

TABLE 3 - Results of preliminary progeny test of off-type M_1 Jaragua grass: low growth and narrow leaves (L), divergent racemes (D) and normal type (N). Progenies 1 and 2 are controls

Progeny number	Plant number									
	1	2	3	4	5	6	7	8	9	10
1	N	N	-	N	N	N	N	N	N	N
2	N	N	N	N	N	N	N	N	N	N
3	D	D	D	N	D	N	D	N	N	N
4	N	N	N	N	N	N	N	N	L	N
5	N	D	D	D	D	D	N	N	N	D
6	D	D	D	N	D	D	N	D	D	D
7	L	L	-	-	-	-	-	-	-	-
8	L	N	L	-	N	N	N	N	-	-
9	-	L	N	N	-	N	N	-	-	-

- No survival in field

TABLE 4 - Embryo sac analysis of M_1 Jaragua grass plants

Progeny number (plant number)	Total number of ovules analysed	(%)*			
		sm	a	m	sm/A
controls	43	70	23	7	75
2 (10)	15	100	-	-	100
3 (2)	113	68	15	17	82
3 (3)	77	82	8	10	91
3 (4)	71	83	13	4	87
3 (10)	27	78	7	15	91
4 (6)	9	100	-	-	100
9 (1)	11	100	-	-	100

* - sm, percentage of ovules with multiple embryo sacs; a, % of ovules with a single aposporous embryo sac; m, % of ovules with meiotic embryo sac; sm/A, % of ovules with multiple embryo sacs in all "aposporous" ovules

Further studies are needed to conclude whether these results are due to radiation effects or represent the status of natural populations of *H. rufa*.

DISCUSSION

Relatively high frequency, 2.2 and 2.0%, was observed in the M1 generation for the characters low growth associated with narrow leaves, and divergent racemes, respectively suggesting that radiation could increase the frequency of these types. The characters chimerism and awnless spikelets appeared in very low frequencies probably due to induced mutations or physiological effects of radiation. The character awnless spikelets was maintained in the M2 generation and could be associated with low growth, narrow leaves, and also poor vigour. It might be interesting to maintain this character, from the seed technological point of view, although the awns may be important for burying the seed and for the retention of dew improving the efficiency of the hygroscopic mechanism in the early dry season when the grain is shed (CLAYTON, 1969). According to this author, there are only two species of *Hyparrhenia* (*H. exarmata* and *H. mutica*) where the awn is absent, suggesting that this plant was a result of possible spontaneous mutations.

CUANY (1967), studying natural variability in *H. rufa*, observed various combinations of the characters: short racemes, divergent racemes, late flowering, low growth, narrow and broad leaves, white and purple stigmas, suggesting that intrinsic genetic traits are involved, and that they could be used as marker types in progeny tests to detect the stability of apomixis. The variations presented in the progeny tests with M2 seeds, in fact, suggest the presence of sexual reproduction in the M1 plants. Such variations also suggest the Mendelian segregation pattern of 1:1 and 3:1. Further studies on the genetic basis of these characteristics should be carried out.

BASHAW & HOFF (1962) obtained extensive changes in growth habit and morphological characters of *Paspalum dilatatum* by irradiation with gamma-rays and neutrons, but no effect was observed on pattern of reproduction. Similarly, BURTON & JACKSON (1962) did not observe a break in apomixis in irradiated *P. dilatatum*. HANSON & JUSKA (1962) reported a slight increase in the number of sexually formed aberrant offspring in X-ray treatment of *Poa pratensis* cv. Merion, which, however, reverted again rather rapidly to apomixis. According to NOGLER (1984), further research on mutagenesis concerning genes involved in reproduction would be highly desirable.

These preliminary results show that *Hyparrhenia rufa* is a facultative apomictic species, confirming the reports of BROWN & EMERY (1957), with the presence of single and multiple aposporous embryo sacs, as well as meiotic embryo sacs. However, a higher frequency of meiotic embryo sacs was observed in irradiated plants. Further studies are still needed to conclude if gamma-ray treatment induces break in apomixis, as suggested by the present work.

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