Sorghum genotypes tolerant to soil salinity – Progenies developed under gamma rays doses

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Introduction

Soil salinity is one of the main problems for world agriculture (Ahloowalia et al. 2004). In the arid and semi-arid regions the salinization process occurs because incomplete lixiviation and intensive soil evaporation (Richards 1974). This process is characterized as the main factor of world soil degradation (Heinze 2002) of that affects around 60 to 80 million ha (Munns et al. 2002, FAO 2004). The irrigated agricultural area of the northeast region of Brazil is around 500 thousand ha, where 25–30% is in the process of salinization (Heinze 2002). The agricultural potentiality in the salinized areas is directly associated with production of salt resistant cultivars. The intensive crop yield in these salinized areas helps to resolve a great livestock regional problem of forage crop shortage, mainly in dry period of the year. According to the association Grupo Pró-Sorgo/APPS (2005), in 2004 the Brazilian sorghum area was 1,269,000 ha. According to Ahloowalia et al. (2004), there were 2,250 new mutants registered by gamma rays from 163 botanical species, from 62 countries. A new genetic material is considered when a change in basic genotype occurs (IAEA 1977). Bretaudeau and Traore (1990) declared that sorghum mutation induction by gamma rays doses of 200 to 300 Gy presented environmental stress viability tolerance. It is important to define which gamma ray dose is to be utilized to promote favorable changes. Moreover, the confirmation of genetic changes by gamma ray use is possible with microsatellite techniques (Ferreira and Grattapaglia 1998). In this work sorghum plants were evaluated for high salinity tolerance through electrical conductivity (EC) levels and gamma ray doses.

Material and methods

An experiment was carried out at the Nuclear Energy Department of the Federal University of the State of Pernambuco and with IPA support. The research actions were developed under greenhouse conditions. The soil used was neosoil eutophic, 0–30 cm, according to ZAPE (2001), in a 40-day period. The sorghum varieties (V) utilized were: V_1 – 467-4-2 (sweet stalk); V_2 – IPA 02-03-01 (dry stalk); V_3 – Sudan 4202 (early maturation). V_1 and V_2 belong to *Sorghum bicolor* and V_3 to *Sorghum sudanense*.

The seeds were submitted to doses of different gamma rays from ^{60}Co source (Cobalt Irradiator, Radionics Laboratory, Scott Plains, New Jersey, USA, dose of 13 Gy h^{-1}). The radiation doses utilized were 0, 450, 500 and 600 Gy. Each experimental unit is a pot with volume capacity of three liters of soil. The requirement for fertilization was based on soil analysis recommendation. The humidity was maintained in available water strip during the experimental period.

The salinity levels were preliminarily adjusted and defined by incubation test, carried out before beginning this activity. The salinity levels (N) were: 0, 15 and 30 dS m^{-1}.

Eight seeds were sown in each pot. Ten days after emergence, thinning was done leaving the four more vigorous plants. The NaCl addition was conducted 15 days after sowing.

The experimental design utilized was randomized block in factorial arrangement: three sorghum varieties (V_1, V_2 and V_3) × three salinity levels (0, 15 and 30 dS m^{-1}) × four gamma radiation doses (0, 450, 500 and 600 Gy), with three blocks.

The variables utilized in the sorghum variety evaluations were:

Final stand count – Surviving plant number at end of experiment at 40 days after emergence.

Scores of damage caused in the plants by salinity and radiosensitivity – The grading scale used was 1 to 5, according to Tabosa (1982) where 1 = absence of damage; 2 = plant with 25% of the foliage affected; 3 = plant burned on top with 26–50% of the foliage affected (chlorosis and/or necrosis of the leaf tissue); 4 = plant with 51–75% of the leaf tissue affected; 5 = plant with 76% or more of foliage affected (burn, chlorosis and/or necrosis of burned leaf tissue, stalk lodging with wilt presence or absence, progressive damage until death of plant).

Electrical conductivity of the soil saturated extract – This was obtained from (levels preliminarily adjusted to 0, 15 and 30 dS m^{-1}) collected soil samples in each pot (experimental unit) after harvesting. The methodology used was as cited by Richards (1974).

Genetic change identification in the sorghum varieties evaluated (control variety versus irradiated varieties) – Molecular marker type microsatellite SSR (simple sequence repeat) were used. These results were obtained by comparing genomic DNA from sorghum cultivars according to the following points: plants obtained from seeds not irradiated seeds; and plants obtained from seeds irradiated by 450 Gy dose. The methodology utilized in these determinations is according to Ferreira and Grattapaglia (1998). In the DNA extraction, the CTAB (cetyltrimethylammonium bromide) was used. After DNA quantification, PCR (polymerase chain reaction) was used with microsatellite primer.

Surviving plants selection and evaluation – The remaining plants that originated from EC of 30 dS m^{-1} and gamma radiation dose of 450 Gy were selected.

Results and discussion

Final stand count. The number of living plants 40 days after emergence for V_1, V_2 and V_3 varieties in presence of gamma radiation, independently of salinity levels, is presented in Table 1.

In the significant radiosensitivity interference on sorghum varieties plants, the following points were considered:

(a) In absence of radiation, plants of V_1 (467-4-2) and V_2 (02-03-08) varieties presented complete stand, above four plants per pot. These values (5.2 and 4.4) correspond to new tiller appearing during plant development. With dose of 450 Gy the V_1 (467-4-2) variety showed a greater number of plants per pot (3.8 plants/pot) when compared with V_2 (02-03-01) and V_3 (Sudan 4202) varieties. In addition, considering a 0–450 Gy dose interval, a stand reduction of V_1 of around 27% was observed. Results of plant stand reduction were also obtained by Rodrigues and Ando (2002), when three rice (*Oryza sativa*) variety plants were submitted to zero and 300 Gy interval with reductions of 14, 12 and 15%. It is important to point out that plant radiosensitivity varies between botanic species and variety levels too. In addition, V_1, V_2 and V_3 plant varieties, when submitted to 500 and 600 Gy radiation dose, although presenting survival, the development between them was subsequently interrupted and they died.

(b) Considering 0–600 Gy interval, it was observed that V_2 and V_3 varieties presented more reduction than V_1 variety.

(c) The interaction between final stand (plant number/pot) and salinity levels was not observed, independently of gamma radiation doses. The 450 Gy gamma radiation dose was characterized as a dose limit for radiosensitivity, considering V_1, V_2 and V_3 varieties. Irfaq and Nawab (2003) reported that on wheat (*Triticum aestivum*) varieties, at dose limit of up to 400 Gy, 20% survival was obtained.

Scores of damage caused in the plants by salinity and radiosensitivity. The analysis of variance indicated a significant interaction between salinity levels and gamma radiation doses (P <0.001) for V_2 and V_3 varieties. This

Table 1. Average results of final plant stand of sorghum varieties submitted to gamma radiation doses.

Sorghum variety	Numbeer of surviving plants per plot[1]				Function
	0 Gy	450 Gy	500 Gy	600 Gy	
V_1 (467-4-2)	5.2a	3.8a	3.1a	1.1a	2nd degree[2]
V_2 (02-03-01)	4.4a	0.6b	0.2b	0.0b	Linear[3]
V_3 (Sudan 4202)	0.9b	0.2b	0.0b	0.0b	Linear[4]

1. Means followed by the same letters do not differ by Tukey test (P >0.05); data transformed in $(x+1)^{1/2}$ to statistic analysis.
2. Y = 2.491128 + 0.0028016X – 0.00000755x2; dy/dx = 185; Y = 2.75; r^2 = 0.99.
3. Y = 2.326919 – 0.0023336 X; r^2 = 0.99 (P <0.000001).
4. Y = 1.389065 – 0.0006912 X; r^2 = 0.97 (P <0.01).

interaction was not significant for V_1 variety. The only significant variation source for V_1 variety was to gamma rays doses. When the damage levels after exposition of plants to salinity and radiosensitivity reached values greater than 4.5, the plants presented severe and irreversible damage. There was a damage evolution tendency in plants until death. In this phase the plant damage reached more than 75% in the aerial part. The average scores of damage to V_1 (467-4-2) variety plants by salinity levels and gamma radiation doses are presented in Table 2.

The following points were observed:

(a) With gamma radiation doses of 450 Gy, independently of salinity levels, surviving plants were detected, but with 500 and 600 Gy all plants died. These results indicate that the dose limit could have been exceeded, causing plant death. It is important to note that the radiosensitivity aspects occurred in different forms between botanic species and varieties, cultivars, etc. Thus, the optimal gamma radiation dose is the one that promotes positive effects on yield components.

(b) The main point of all results obtained with V_1 (467-4-2) variety is the survival of plants that were transplanted to a definitive place until seed production. Thus, according to IAEA (1977), the maximum variation of quantitative characters can show the mutation stability in subsequent generations.

The average scores of damage to V_2 variety plants by salinity levels and gamma radiation doses are presented in Table 3. In absence of radiation, plants with normal survival (scores from 2.3 to 2.7) were observed. When the plants were submitted to gamma radiation of 500 and 600 Gy, they presented severe damage and death. The damage score to all plants was 5, except to plants at salinity level of 30 dS m^{-1} and gamma radiation of 500 Gy (score of 4.3). However, these plants survived only for a short period of time. Independently of salinity level, gamma radiation dose of 450 Gy was characterized as surviving plant limit. At this dose there was no plant death. The rates were 3.6 and 3.3 in presence of salinity levels of 15 and 30 dS m^{-1}, respectively.

The results obtained for V_3 (Sudan 4202) variety plants are shown in Table 4. In the absence of radiation, low damage (up to score 3) was detected. This damage did not present interference on plant survival, mainly at salinity levels. Regression study revealed that salinity absence occurred at an effective level of 600 Gy interval. With gamma radiation dose of 450 Gy, surviving plants were observed at 15 and 30 dS m^{-1} salinity levels. Plant selection was performed using gamma radiation (450 Gy) and salinity level (30 dS m^{-1}) interaction. No live plants were observed when they were submitted to gamma radiation of 500 and 600 Gy. These doses, ie, above 450 Gy, are beyond radiosensitivity ability to variety V_3 (Sudan 4202) plants.

Electrical conductivity of the soil saturated extract. The EC average values obtained were 1.58, 14.56 and 26.34 dS m^{-1}, when compared with defined levels of 0, 15 and 30 dS m^{-1}, respectively. The estimated values from

Table 2. Average scores of damage to V_1 (467-4-2) variety plants by gamma ray doses independently of salinity levels.

	Damage score[1]			
Salinity levels (dS m^{-1})	0 Gy	450 Gy	500 Gy	600 Gy
Average[2]	2.8	3.1	5.0	5.0

1. According to Tabosa (1982), scored on a 1–5 scale where 1 = no damage; and 5 = plant with more than 75% damage.
2. $Y (V_1) = 2.873401 – 0.0041748 x + 0.00001351x2$; dy/dx = 154; Y = 2.5; $r^2 = 0.71$ ($P < 0.001$).

Table 3. Average scores of damage to V_2 (02-03-01) variety plants by salinity levels and gamma radiation doses.

	Damage score[1]				
Salinity level (dS m^{-1})	0 Gy	450 Gy	500 Gy	600 Gy	Equation
0	2.3	2.3	5.0	5.0	2nd degree[2]
15	2.6	3.6	5.0	5.0	Linear[3]
30	3.3	3.3	4.3	5.0	2nd degree[4]

1. According to Tabosa (1982), scored on a 1–5 scale where 1 = no damage; and 5 = plant with more than 75% damage.
2. $Y (V_2N_0) = 2.311676 – 0.0070063 X + 0.00001995X^2$; dy/dx = 175; Y = 1.7; $r^2 = 0.68$ ($P < 0.0005$).
3. $Y (V_2N_1) = 2.509144 + 0.0039764 X + r^2 = 0.81$ ($P < 0.0001$).
4. $Y (V_2N_2) = 2.66086 – 0.0037558 X + 0.00001281 X^2$; dy/dx = 146; Y = 2.3; $r^2 = 0.94$ ($P < 0.01$).

regression equation (Y = 1.786111 + 0.8251852 X; r^2 = 0.99; P <0.00001) were 1.78, 14.1 and 26.54 dS m^{-1}, respectively.

Surviving plants selection and evaluation. In this evaluation only the surviving and remaining plants that were submitted to 15 and 30 dS m^{-1} salinity level and gamma radiation dose of 450 Gy were considered. With the above combination it was possible to perform the selection of remaining plants. It is important to point out that these plants selected after experimental period recovered normal development. The damage noted in these plant selections were 3.1 (Table 2) to V_1 independently of salinity level; 3.3 to V_2 (Table 3) and 4.3 to V_3 (Table 4), when submitted to salinity levels of 30 dS m^{-1} in presence of 450 Gy dose.

In this condition, the few surviving plants reached normal development. It is important to point out that these remaining and surviving plants after experimental period were transplanted to definitive bed plant places. The plants in these places were developed until seeds reached viable production stage. These seeds were obtained by self-pollination.

Genetic change identification in the sorghum varieties. The identification of possible genetic changes was performed in the remaining sorghum plants from three varieties. The evaluation was conducted by comparing the plants in presence and absence of gamma radiation dose of 450 Gy, after surviving salinity level of 30 dS m^{-1}. The SSR markers were utilized with success to identify a mutant in barley (*Hordeum vulgare*) (Mlcochová et al. 2004). Twelve "primers" for microsatellite (SSR) were tested, and three of them presented polymorphism. The most important were "primer" 5L and 6R (Fig. 1).

Considering that gamma radiation presents interference directly on DNA, the following points are important: there is difference between genetic material control (without radiation) and irradiated with 450 Gy of ^{60}C0. V_1 (467-4-2) and V_2 (02-03-01) varieties that were submitted to 450 Gy gamma radiation presented less variation (in the amplification profile) in relation to their respective controls than plants of variety V_3 (Sudan 4202), under same conditions; this kind of change detected in these varieties can promote the appearance of new forms of genes that can probably be responsible for genetic variability. In addition, the plant performance on saline conditions is also reported by Zhu (2001), who points out that the expression of different genes shows different tolerance to NaCl.

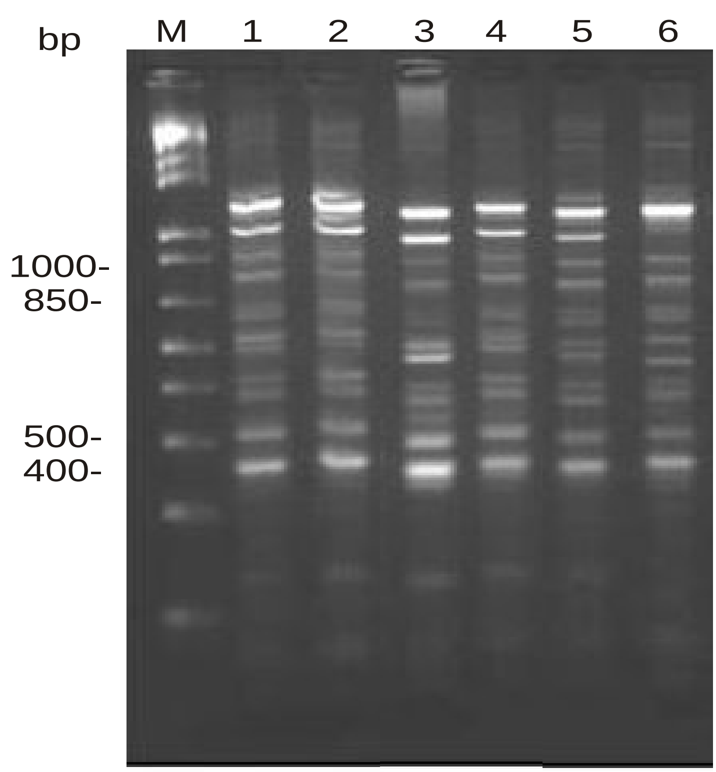

Figure 1. Standard band amplification products with microsatelite SSR markers in different sorghum varieties plants submitted to gamma radiation dose of 450 Gy. M – Marker of molecular weight (1 kb plus DNA Ladder); 1–6 amplification product with "primer" 5L and 6R in the different standard band: 1 – V_1 (467-4-2) (zero Gy); 2 – V_1 (467-4-2) (450 Gy); 3 – V_2 (02-03-01) (zero Gy); 4 – V_2 (02-03-01) (450 Gy); 5 – V_3 (Sudan 4202) (zero Gy); 6 – V_3 (Sudan 4202) (450 Gy).

Table 4. Average scores of damage to V_3 (Sudan 4202) variety plants by salinity levels and gamma radiation doses.

	Damage score[1]				
Salinity level (dS m^{-1})	0 Gy	450 Gy	500 Gy	600 Gy	Equation
0	1.6	4.3	5.0	5.0	Linear[2]
15	2.6	3.0	5.0	5.0	2^{nd} degree[3]
30	3.0	4.3	5.0	5.0	Linear[4]

1. According to Tabosa (1982), scored on a 1–5 scale where 1 = no damage; and 5 = plant with more than 75% damage.
2. $Y (V_3N_0) = 1.727925 + 0.0057129X$; $r^2 = 0.97$ ($P < 0.00001$).
3. $Y (V_3N_1) = 2.651385 - 0.0036902X + 0.00001294 X^2$; $dy/dx = 142$; $Y = 2.3$; $r^2 = 0.73$ ($P < 0.02$).
4. $Y (V_3N_2) = 3.001573 + 0.0033078X$; $r_2 = 0.96$ ($P < 0.0001$).

Conclusions

Electrical conductivity levels of the soil saturated extract of 14 to 26 dS m^{-1} can be used to select sorghum genotypes resistant to salinity. Plant count reduction and damage rate caused to them due to salinity allows to separate sorghum genotypes that are susceptible and resistant to soil salinity. Gamma radiation dose of 450 Gy promotes genetic change in plants of sorghum varieties V_1 (467-4-2), V_2 (02-03-01) and V_3 (Sudan 4202). These genetic changes between irradiated plants with 450 Gy of gamma radiation and non-irradiated plants are characterized by SSR molecular markers.

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