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M1 CYTOGENETIC AND PHYSIOLOGICAL EFFECTS OF GAMMA-RAYS IN SUDAN GRASS (*SORGHUM SUDANENSE* (PIPER.) STAPF)

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Abstract

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This paper presents data from investigations concerning the chromosome damage and physiological effects in the first generation (M1) of gamma-irradiated dry seeds of Sudan grass (*Sorghum sudanense* (Piper.) Stapf.), which is part of a study devoted to the assessment of the potential of a wide range of doses (100, 200, 300 and 400 Gy) of this mutagenic agent in inducing genetic changes in this crop. Three varieties of different origin, namely, Kazitachi (originating from Japan), Vercors (USA) and Voronejkaya (Russia) were subjects of our investigation. The anaphase analysis of the chromosome aberrations did not reveal significant differences in the radiosensitivity of the varieties studied Voronejkaya being however most resistant at the highest dose. The influence of gamma rays on three physiological parameters (germination, survival and sterility) of M1 progeny of the varieties used were investigated The data showed clearly pronounced "dose effect"; with increasing dose the values obtained for each of these biological parameters decrease and the differences are statistically proven. Survival data followed similar trends as those for field germination. The radiosensitivity of the cultivars is well expressed with the values of LD_{50}, which were calculated on the basis of M1 plant survival. This value is lowest for Kazitachi (307.52 Gy) while LD_{50} for Vercors and Voronejkaya 9 are 342.63 Gy and 340.97 Gy, respectively. These data provide valuable information when the optimal doses should be chosen for the purpose of mutation breeding programs of Sudan grass varieties.

Key word: gamma-rays, chromosome aberrations, M1 physiological damage, dose effects, *Sorghum sudanense*

Introduction

Sudan grass (*Sorghum sudanense* (Piper.) Stapf) is a diploid (2n=20) annual forage plant belonging to cereal family *Poaceae*, subfamily *Panicoide* (DeWet, 1978; Wiersema and Dahlberg, 2007; Parvatham and Sree Rangasamy, 2004; Zhan et al., 2006, 2008). In recent years the interest in this crop is growing globally since sustainable yields can be produced in the condition of water deficit and high temperature stresses (Swith and Frederiksen, 2000; Iskender and Andrews, 2001; Djukic et al., 2002). Because of its feed quality and undisputed advantages as an alternative forage crop under

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drought conditions, Sudan grass may attract the attention of Bulgarian farmers, especially in lowland areas of the country. The limited distribution of this crop in the country during the fifties of the last century was based exclusively on introduced Russian cultivars.

Different approaches have been used in breeding programs aimed at genetic improvement of Sudan grass the experimental mutagenesis being one of those which has given promising results (Kirillenko and Golovin, 1987; Golovin and Kirillenko, 1989). Nevertheless, the studies dealing with mutation induction in this species are scarce and the data obtained so far are rather contradictive, especially when dose effects of ionizing radiations are concerned (Lazanyi, 1987; Kirillenko and Golovin, 1987; Preobrazheskaya, 1971; Tabosa et al., 2007).

This paper presents data concerning chromosome damage and physiological effects in the first generation (M1) of gamma-irradiated dry seeds of Sudan grass, which is part of a study devoted to the assessment of the potential of this mutagenic agent in inducing genetic changes in this crop.

Materials and Methods

Plant materials. Three varieties of different origin, namely, Kazitachi (originating from Japan), Vercors (USA) and Voronejkaya 9 (Russia) that are renowned for their agronomic characteristics, including resistance to drought were used as experimental material.

Treatment procedures. Air-dried seeds of the varieties mentioned above were irradiated with four doses of gamma-rays (Caesium-137) – 100, 200, 300 and 400 Gy (dose rate 146 Gy/h). Radiation treatments were carried out with gamma-irradiation facility GOU-3M in Institute of Plant Physiology and Genetics - Sofia. Immediately after irradiation the seeds were soaked in distilled water for 30 min. and redried until their planting in the field or germinating in laboratory for cytological analysis.

Cytological analysis. For cytological analysis the seeds were germinated in Petri dishes at 24^{o}C (dark chamber). For scoring chromosome damage primary rootlets were fixed in Clarke solution (ethanol-acetic acid 3:1, by vol.) at different time after irradiation to cover the first mitotic cycle after radiation treatment (recovery times of 37, 40, 43 and 46 hrs). The chromosome aberrations were scored at late anaphase or early telophase (anaphase method). For karyotype analysis primary rootlets from non-irradiated seeds were immersed prior to fixation in a solution of 0.025% colchicine saturated with α-bromonaphthalene for 1.5h. After hydrolysis in 1N HCl (7 min at 60^{o}C water bath), staining with Shiff's reagent and maceration of the roots in 4% Pectinase, Feulgen squashes were prepared.

The inspection of slides and documentation of chromosomal aberrations of interest were done with microscope "Olympus BX41". The experiments were carried out at least in two replications.

Field experiments. Field experiments were carried out in two replications over two seasons in the period 2008-2010. One thousand seeds were sowed for each treatment. The field trials were set according to the standard method with consecutive arrangement of the variants (Shanin, 1977). Three M1 criteria were scored as percent of controls (non-irradiated seeds) – germination, sterility and survival. The sterility was scored on the basis of the number of M1-plants with complete reduction of seed setting. "Surviving plants" were counted at the time of harvest of M1 generation and survivors were defined as those plants that produce at least one inflorescence regardless of whether seeds are produced. Since the results obtained from field trials set in 2008 and 2010 show similar trends the data were pooled.

Statistical treatments. The influence of gamma rays on three physiological parameters (germination, survival and sterility) of M1 progeny was analyzed. For statistical evaluation of the data concerning germination, sterility and survival of M1 progeny criterion Fφ of Fisher was used (Plohinskii, 1967). Program products TRIMED SPEARMAN KARBER METHOD.VERSION 1.5" (Hamilton et al., 1978) were used to define LD50 and Microcsoft Office Excel 2003 to define correlation relationships, respectively..

Results and Discussion

There is convincing evidence of clearly pronounced correlation between M1 plant injury and mutation frequency in M2 induced by ionizing radiations. That is why, a quantitative determination of M1 damage is inevitable step in mutation breeding, especially for plant species, such as Sudan grass, that have not been subject of extensive studies by this approach for crop improvement.

The analysis of the first mitotic cycle in shoot or root meristem cells offers a reliable test to determine the effect of the mutagens (Gaul, 1977). This analysis provides valuable information about the nature of the mutagen interaction with the genetic structures of the treated biological objects and should be at least applied in cases when new mutagenic treatment is introduced, the effects of which are not yet well understood. At present, two methods, i.e. anaphase and metaphase scoring, are in use for analyzing induce chromosome aberrations (Nicoloff and Gecheff, 1976). The examination of the chromosome configuration of the structural mutations offered by metaphase analysis provides direct vision of the nature of induced chromosomal rearrangements and very few of them scored by this method escape detection. However, the metaphase analysis is applicable only to plant species having low number of comparatively large chromosomes. The karyotype analysis of somatic root-tip meristem cells of the Sudan grass varieties used in our study revealed in prometaphase and metaphase twenty pairs of homologous chromosomes with very small size and rather similar morphology (Figure 1). For this reason, we had to analyze the frequency and spectrum of chromosomal aberrations at anaphase.

Although the anaphase analysis escapes detection of a significant portion of the induced structural mutations it is widely used in mutation cytogenetics and breeding when the dose or concentration of mutagenic agents have to be defined. The kinetic of the induction of chromosomal aberrations in the varieties used in this study is represented in Figure 2. As can be seen, in all three varieties the peak of aberrations appears at 40 hrs after irradiation of seeds and according to the studies of Evans and Scott (1964) it could be assumed that late pre-synthetic stage of interphase (G1) appears to be the most sensitive to the action of gamma-rays. Moreover, the comparative analysis of the data shows no significant difference in the radiosensitivity of the varieties used. However, the variety Voronejkaya 9 showed the greatest resistance at the highest radiation dose of 400 Gy. The data in this figure are indicative also of a lin-

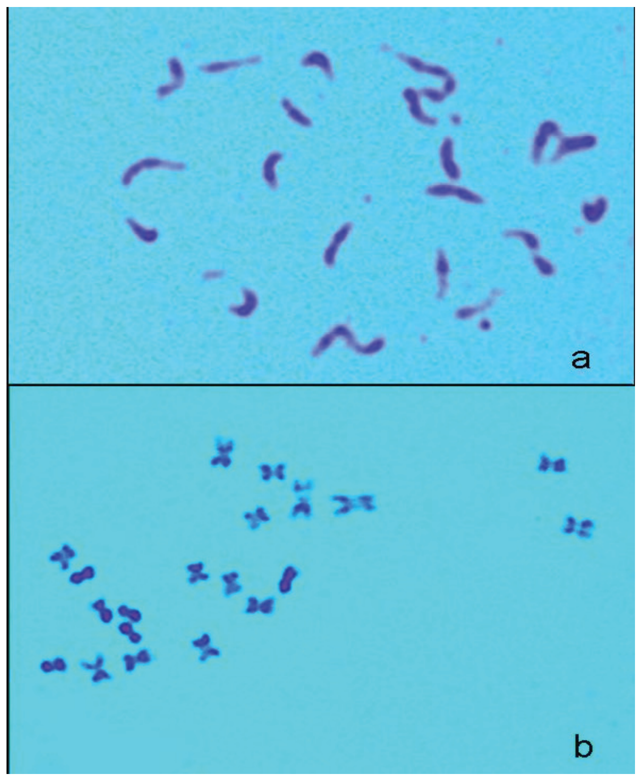

Fig. 1. Standard somatic prometaphase (a) and metaphase (b) of Sudan grass (variety Voronejkaya 9)

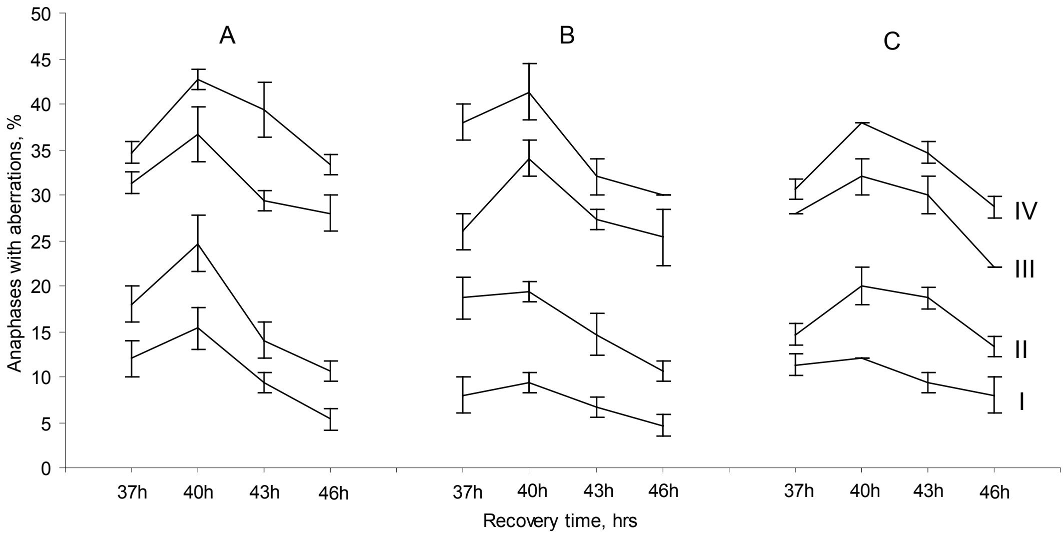

Fig. 2. The frequency of chromosome aberrations induced by different doses of gamma-radiation (I – 100 Gy; II – 200 Gy; III – 300 Gy; IV – 400 Gy) in three varieties of Sudan grass (A – Kazitachi; B – Vercors; C – Voronejskaya 9). The frequency of aberrations in non-irradiated seeds (controls) never surpasses 1%

ear function of dose in induction of chromosome damage which is widely established phenomenon (for review see Sasaki, 2009).

The anaphase analysis allows identification of only two main types of chromosome aberrations, which are based on the structural unit affected by mutagenic agents; chromosome types, scored as double bridges and fragments, and chromatid types, scored as single bridges and fragments. These two types of rearrangements were observed in Sudan grass and ana-telophase configurations that were most frequently occurred are given in Figure 3. Table 1 shows the relative frequency of different types of aberrations after irradiation with gamma rays of dry seeds of Sudan grass. According to our knowledge, the spectrum of chromosome aberrations induced by ionizing radiations in this species is not reported so far. However, the problem has been thoroughly studied in other higher plants and it has been established that these agents are able to produce rearrangements of chromosomes at any stage in development of interphase; chromosome type in G1 and chromatid type in S and G2 (Evans and Scott, 1964). What is remarkable in our study is that a sizable portion of gamma rays-induced aberrations in Sudan grass proved to be of chromatid type; 10.2% in Vercors and about 8.5% in Kazitachi and Voronejkaya 9. This means that some meristem cells of the seeds have

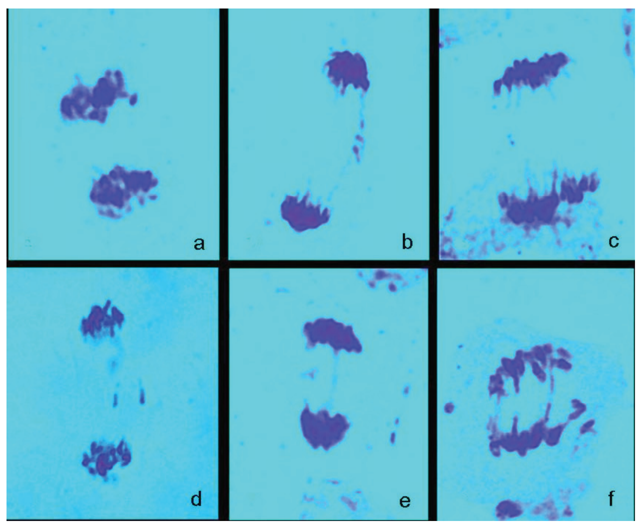

Fig. 3. The main types of chromosomal aberrations observed at late anaphase – early telophase of mitosis in Sudan grass: (a) undamaged cell; (b) chromadid (single) bridge; (c,d) chromosome (double) fragments; (e) chromosome (double) bridge with fragments; (f) anaphase with multiple aberrations

Table 1

The spectrum of chromosomal aberrations induced by different doses of gamma-rays in Sudan grass

Doses, Gy	Number of aberrations	Types of aberrations				
		Chromatid		Chromosome		
		Fragments	Bridges with fragments	Fragments	Bridges with fragments	Others
Kazitachi						
100	70	1	7	25	28	9
200	113	2	5	42	51	13
300	207	5	10	67	92	33
400	253	13	11	83	107	39
Vercors						
100	48	3	5	19	17	4
200	104	2	7	29	46	20
300	193	5	12	63	81	30
400	231	14	11	75	92	39
Voronejskaja 9						
100	67	4	4	23	30	6
200	110	5	6	35	46	18
300	185	7	7	72	80	19
400	215	6	11	71	91	36

undergone synthetic stage (S) of interphase during irradiation, which is unusual for cell population of dormant seeds, where a synchronized population of cells in pre-synthetic stage (G1) of interphase should be expected.

There is a few studies concerning the M1 physiological effects of gamma-rays in Sudan grass (Osborne et al., 1963; Preobrazhenskaya, 1971) and the 50% lethal dose (LD_{50}) has been found to vary in different studies within wide limits. In Table 2 are given the data concerning the influence of gamma rays on three physiological parameters (germination, survival and sterility) of M1 progeny of the varieties used in our study. To facilitate the comparative analysis of data the effects in the all three criteria are expressed as percentage of the control treatments. The data show clearly pronounced “dose effect”; with increasing dose the values obtained for each of these biological parameters decrease and the differences are statistically proven ($P<0.1\%$). The reduction of field germination is mostly pronounced in variety Vercors (50.5% after irradiation with 400 Gy).

Survival data followed similar trends as those for field germination; the number of surviving M1 plants decreases with increasing dose. Variety Kazitachi proved to be the most sensitive to gamma irradiation (40.8% surviving plants at dose 400 Gy). The radiosensitivity of the cultivars is well expressed with the values of LD_{50}, which were calculated on the basis of M1 plant survival. This value is lowest for Kazitachi (307.52 Gy), the most sensitive variety, while LD_{50} for Vercors and Voronejkaya 9 are 342.63 Gy and 340.97 Gy, respectively. These data provide valuable information when the optimal doses should be chosen for the purpose of mutation breeding program in Sudan grass and of the varieties studied, in particular.

The complex influence of the applied doses

Table 2
The influence of gamma-rays on the different physiologival parameters of M1-plants in Sudan grass under field conditions*

Varieties	Doses	Parameters						
		Germination, % of control	Fφ	Survival, % of control		Fφ	Sterility, % of control	Fφ
Kazitachi	100Gy	91	7.05	87.4	LD_{50}	9.39	4.7	3.41
	200Gy	82.4	13.85	77	307.52	17.24	9.4	6.69
	300Gy	59.7	31.8	49.1		37.83	11.9	7.44
	400Gy	55.6	34.96	40.8		43.43	16.6	9.9
Vercors	100Gy	89.3	8.85	85.5	LD_{50}	11.76	2.7	2.1
	200Gy	81.1	15.71	79	342.63	17.1	10.4	8.13
	300Gy	68.3	26.44	54.3		37.13	11.3	7.92
	400Gy	50.5	40.94	44.9		44.24	15.1	10.07
Voronejkaja 9	100Gy	96.5	2.87	92.7	LD_{50}	5.85	4.4	3.49
	200Gy	76.3	19.69	65.5	340.97	27.95	5.3	3.83
	300Gy	59.7	33.49	54.8		36.46	9.1	6.3
	400Gy	57.4	35.36	44.1		47.39	13.4	8.76

**Note:* The data concerning the field germination, survival and sterility in control treatments are as follows: for var. Kazitachi - 75.6%, 68.2% and 0.6%, respectively; for var. Vercors - 84.2%, 80.6% and 0.2%; for var. Voronejkaya 9 – 83.8%, 79.4% and 0.3%.

of gamma-rays in the all three varieties of Sudan grass has clearly proven non-proportional negative correlations with field germination (r values -0.967; -0.986 and -0.955 for Kazitachi, Vercors and Voronejkaya 9, respectively) and survival (r value -0.976, -0.974 and -0.968), while the parameter "sterility" is in a reverse correlation (r value 0.993, 0.946 and 0.968, respectively). It is interesting to note that the established trends in the influence of different doses of gamma rays on physiological parameters in M1 differ to some extent from the observed correlations in the effect of this mutagenic agent on the chromosome damage. These results are a further proof that the physiological damage of mutagenic agents in M1 has most probably both chromosomal and extra-chromosomal origin.

Conclusions

New information concerning cytogenetic and physiological effects of gamma-irradiation in M1 generation is obtained for three varieties of Sudan grass (*Sorghum sudanense* (Piper.) Stapf.), a crop plant which has not been so far subject of extensive mutation studies.

The anaphase analysis of chromosome aberrations in the first mitotic cycle after irradiation of dry seeds does not reveal significant differences in the radiosensitivity of the varieties studied one of them (Voronejkaya 9) being however most resistant at the highest dose (400 Gy). The spectrum of the observed aberrations is indicative of some heterogeneity of the meristem cell population which is unusual phenomenon for cell population of dormant seeds of higher plants, where a synchronized population of cells in pre-synthetic stage (G1) of interphase was established.

The influence of gamma rays on three physiological parameters (germination, survival and sterility) of M1 progeny of the varieties used were investigated and data show clearly pronounced "dose effect"; with increasing dose the values obtained for each of these biological parameters decrease and the differences are statistically proven. Survival data followed similar trends as those for field germination. The radiosensitivity of the cultivars is well expressed with the values of LD_{50}, which were calculated on the basis of M1 plant survival. This value is lowest for Kazitachi (307.52 Gy), the most radiosensitive variety, while LD_{50} for Vercors and Voronejkaya 9 are 340.97 Gy and 342.63 Gy, respectively. These data provide valuable information when the optimal doses should be chosen for the purpose of mutation breeding program of Sudan grass varieties.

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