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IRRADIATION DOSE AND EFFECT ON GERMINATION AND GROWTH OF DESERT SHRUB *NITRARIA TANGUTORUM* BOBR. WITH TWO GAMMA IRRADIATION MODES

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Abstract

The seeds of *Nitraria tangutorum* were exposed to different doses of gamma radiation from ^{60}Co gamma rays based on the two irradiation modes of without dose rate and with dose rate of 25Gy/hr. The effects of different irradiation modes and irradiation dose on the seed germination rate, emerged seedling rate and phenotypic characters of seedling have been studied. The semi-lethal dose and critical dose were determined for different irradiation mode. It has been found that there were the differences of irradiation effects between the two irradiation modes. Promotion effect on the seed germination rate, emerged seedling rate, seedling height and ground diameter in lower dose treatment were observed, while inhibition in higher dose. As compared to control, the irradiation effects on the seed germination rate and seedling height and ground diameter present the very remarkable differences at above 1500Gy dose without dose rate mode, while above 2400Gy with dose rate mode. The negative relativity exists between the relative emerged seedling rate and irradiation dose, the emerged seedling rate decreased with increasing dose. The semi-lethal dose based on the emerged seedling rate was 985Gy and 1363Gy for the irradiation without dose rate and with 25Gy/hr dose rate, respectively. The Appropriate ^{60}Co gamma irradiation dose for the *Nitraria tangutorum* seeds was ranging from 950 Gy to 1500Gy without dose rate irradiation mode, while 1300Gy to 2400Gy with 25Gy/hr dose rate mode.

Introduction

Spontaneous genetic mutation of plants can be induced and exploited through the changes of external environment and the instability of genetic structure of plants under natural conditions. But some reports showed that the frequency of such mutations is extremely low and different from the plant species and genes (Drake *et al.*, 1998). Generally speaking, the mutation rate is ranging from 10^{-3} to 10^{-4} events for a single plant, while 10^{-5} to 10^{-6} events for a single gene (Ma *et al.*, 1998; Kovalchuk *et al.*, 2000a). However, the rate can be enhanced by more than a thousand times by a variety of radiation rays. Gamma rays are the most energetic form of electromagnetic radiation, possesses the energy level from 10 kilo electron volts (KeV) to several hundred KeV. So they are considered as the most penetrating in comparison to other radiation such as alpha and beta rays (Kovács & Keresztes, 2002). As the commonest radiation source for induced-mutation, ^{60}Co gamma rays are widely used in forestry and agriculture, especially in ornamental and economic plants, as well as crops (Ghosh & Sen, 1978; Lata, 1980; Cuero *et al.*, 1986; Asghar & Khan, 1988; Thapa, 2004; Borzouei *et al.*, 2010), which possess many characters, such as creating genetic mutation, expanding variation and improving plant quality, stable variability traits, simple method and so on. Seeds, pollens, clonal reproductive organs, culture tissue and live plants can all be applied as irradiated materials (Mergen & Strøm Johansen, 1964; Koti *et al.*, 2004; Kazama *et al.*, 2008; Afrasiab & Iqbal, 2012). However, radiation-induced breeding greatly depends on the selection of the appropriate radiation dose. Simultaneously, some report indicated that the dose rate is also a key question in the induced radiation (Russell *et al.*, 1958; Bari, 1971; Amiro, 1986; Kovalchuk *et al.*, 2000b; Yamaguchi *et al.*, 2008).

Nitraria tangutorum Bobr., the shrub species of the Genus *Nitraria* L., Family Zygophyllaceae, mainly grows in sandy areas around lakes, oases, river terraces, sandy plains, aeolian basins, clay deserts, semi-arid desertificated areas with scarce rainfall (Liu, 1987; Ma, 1989). It is also the most common constructive species and a good sand-fixing woody plant in the expansive sandy areas in China. But the excellent drought-resistant cultivated germplasm is important for the sand-fixing afforestation. Simultaneously its fruits are rich in nutrients and have great potential for development and upgrade. Radiation breeding for the stress-tolerant genotype is also efficient method (Shereen *et al.*, 2009; Melki & Marouani, 2010). But there haven't been any reports on the artificial radiation tests of *Nitraria tangutorum*. The purpose of the external radiation experiment in 2009 was evaluating the appropriate radiation dose and the effect on seed germination in relation to subsequent seedling growth characters. The results of such experiments could be used to lay a foundation of the new genotype breeding and cultivation for follow-up work in the future.

Materials and Methods

Plant materials: The experiment was based on the seeds of *Nitraria tangutorum* collected from the Ulan Buh Desert areas, Inner Mongolia Autonomous Region, China. After dried to 13% water moisture content by vacuum drying in the dryer with water and glycerol (1:1), the mature seeds were removed to small parchment paper bags and subjected to external radiation of ^{60}Co gamma rays.

Experimental methods: The irradiation tests were carried out at the State Forestry Radiation Center in Hefei City, Anhui Province, China. The seeds have been treated with two different irradiation modes. One is the irradiation without dose rate, and the other is with the dose rate of 25Gy/hr. The seeds treated by each modes were subjected to 0, 400, 500, 600, 700, 800, 1000, 1200, 1500, 1800, 2100 or 2400 Gray (Gy) irradiation doses.

Among them, the seeds irradiated with 0 Gy radiation dose were served as control (CK).

After irradiation, 100 seeds from each treatment were selected for germination experiment with 3 replications. Most plants' seeds can be sown in Petri dishes with wet filter paper (Cuero *et al*., 1986), which is liable to mildew for seeds of *Nitraria tangutorum*. So the germination experiments of *Nitraria tangutorum* seeds were conducted by mixing seeds with sand in packets to prevent from getting mildewed. Then the packets with seeds were placed in incubators for germination at the temperature range of 24℃ to 26℃. Subsequently, the cultivation experiment for seedlings in culture cups were carried out for the observation in M1 generation. The irradiated seeds and seeds for control were sown in the plastic culture cups filled with sandy soil to strengthen the centralized management of seedlings. 250 seeds were sown in three replications per treatment. All the experiments were carried out in Experimental Center of Desert Forestry, Chinese Academy of Forestry located in Dengkou County, Inner Mongolia Autonomous Region, China.

The numbers of seeds germination were counted per 10 days in the germination tests. And the germination rates were statistically recorded 30 days later. The percentage ratio between germination rates under different irradiation doses and the germination rate for control was calculated as the relative germination rates.

Emerged seedling rates were recorded 40 days after sowing in the culture cups. The relative emerged seedling rates (the percentage ratio between emerged seedling rates under different irradiation doses and the emerged seedling rates for control) have been used as the analytic indicators. The correlation coefficients between relative emerged seedling rate and radiation dose were calculated by the following formula:

$$r=\frac{\sum xy-\frac{\sum x\cdot\sum y}{N}}{\sqrt{\left[\sum x^2-\frac{\left(\sum x\right)^2}{N}\right]\left[\sum y^2-\frac{\left(\sum y\right)^2}{N}\right]}}$$

Take the different doses as independent variables, the relative emerged seedling rates as the dependent variables. The semi-lethal doses were calculated by the linear regression model and the following formula:

$$D_{50}=\frac{\mathrm{LD}_{50}-a}{b}$$

Where: b- regression coefficient;
a - constant;
D_{50} - semi-lethal dose;

$$b=\frac{\sum xy-\frac{\sum x*\sum y}{n}}{\sum x^2-\frac{\left(\sum x\right)^2}{n}}$$

$$a=\frac{\sum y}{n}-b*\frac{\sum x}{n}$$

The phenotypic characters of seedling, such as seedling height and ground diameter were investigated at the end of growing season. The data were analyzed statistically by ANOVA and multiple comparisons with SAS software.

Results and Discussion

Radiation Effects on germination: The relative germination rate was served as the indicator for the impact of irradiation on germination. And the correlation between the relative germination rate and radiation dose exists (Fig. 1).

For two irradiation modes, the relative germination rate of seeds irradiated with small doses was higher than that of the control. Then the rate decreased rapidly with increasing irradiation dose until it was significantly lower than the control (Fig. 1). Those indicated that seed germination can be promoted by irradiation with low doses, whereas higher radiation doses significantly inhibited seed germination and negatively correlated with the germination rate. The results were in accordance with some previous reports, which showed that the higher exposures are usually inhibitor on seed germination (Iqbal & Aziz. 1981; Thapa, 2004; Khawar *et al*., 2010) whereas lower exposures are sometimes stimulatory (Chauhan & Singh, 1980). The stimulating causes of gamma ray on germination may be certified to the activation of RNA or protein synthesis, which occurred during the early stage of germination after seeds irradiated (Borzouei *et al*., 2010).

The relative germination rate of irradiated seeds revealed a general downward trend with increasing of the dose. The significant correlation was found between the dose and relative germination rate for two irradiation modes. The fitted curves of the two indices for irradiation without dose rate mode followed a power law, whereas that for irradiation with dose rate of 25 Gy/hr followed a logarithmic law (Fig.1). The correlation coefficients (*R*) were 0.949 and 0.983 for the irradiation without dose rate mode and with dose rate of 25 Gy/hr mode，respectively. As illustrated from the fitted curve in Fig.1, as the dose increased, the relative germination rate decreased more rapidly within the lower dose range for the irradiation without dose rate mode than that with dose rate mode.

The relative germination rate of seeds exposed to the irradiation without dose rate mode was significantly affected with all doses. Similar results have been found in the seeds exposed to irradiation with dose rate mode (Table 1). The multiple comparison results revealed that the differences was significant between dose treatment above 1500 Gy and control without dose rate mode, while between dose treatment above 2400 Gy and control with the dose rate of 25 Gy/hr. It illustrated that the critical dose of the irradiation effects on relative germination rate with dose rate mode was higher than that without dose rate mode.

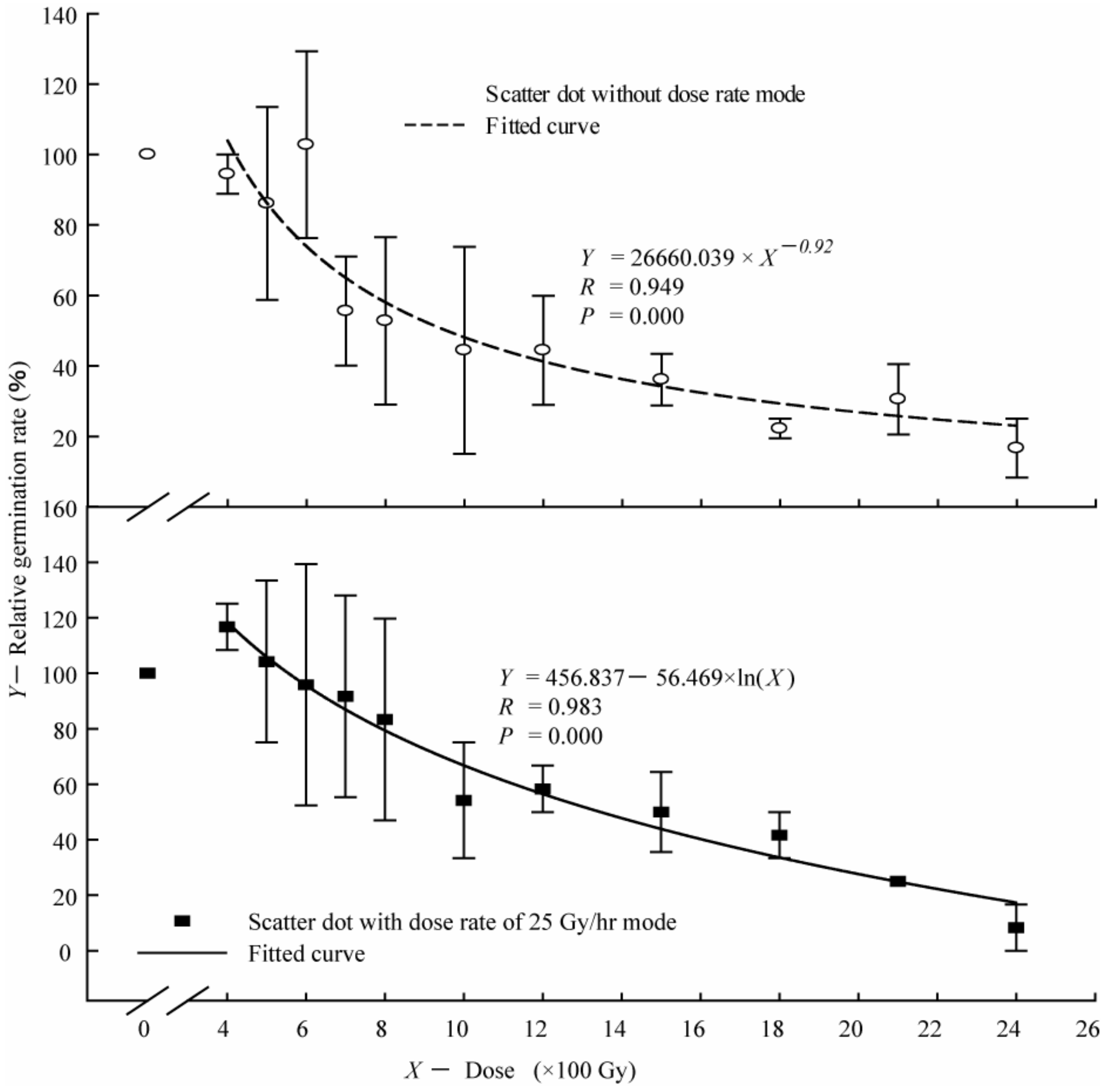

Fig. 1 The correlation between the relative seed germination rate and irradiation dose for two irradiation modes

Table 1 *F* values and Duncan multiple range test of One-way ANOVA of the effects of irradiation dose on relative germination rate of seeds and biological characters of seedling for *Nitraria tangutorum*

Dose	Irradiation without dose rate			Irradiation with dose rate of 25 Gy/hr		
	Seeds	Seedlings		Seeds	Seedlings	
	Relative germination rates (%)	Ground diameter (mm)	Seedling height (cm)	Relative germination rates (%)	Ground diameter (mm)	Seedling height (cm)
CK	100.00^{ab}	2.11^{abc}	13.75^{abc}	100.00^{ab}	1.53^{ab}	10.20^{ab}
400	94.44^{ab}	2.29^{ab}	14.75^{abc}	116.67^{a}	1.73^{a}	12.00^{a}
500	86.11^{abc}	2.37^{a}	17.00^{a}	104.17^{a}	1.58^{ab}	10.25^{ab}
600	102.78^{a}	2.02^{abc}	10.00^{abc}	95.83^{ab}	1.50^{abc}	8.00^{abcd}
700	55.56^{abcd}	2.03^{abc}	16.75^{ab}	91.67^{ab}	1.27^{abc}	8.33^{abcd}
800	52.78^{abcd}	2.04^{abc}	16.75^{ab}	83.33^{abc}	1.33^{abc}	10.00^{abc}
1000	44.44^{bcd}	0.42^{bcd}	2.33^{bc}	54.17^{abc}	1.07^{abc}	8.00^{abcd}
1200	44.44^{bcd}	0.32^{cd}	1.00^{c}	58.33^{abc}	0.75^{abc}	7.67^{abcd}
1500	36.11^{cd}	0.00^{d}	0.00^{c}	50.00^{abc}	0.20^{bc}	1.33^{bcd}
1800	22.22^{d}	0.00^{d}	0.00^{c}	41.67^{abc}	0.17^{bc}	1.33^{bcd}
2100	30.56^{cd}	0.00^{d}	0.00^{c}	25.00^{bc}	0.19^{bc}	1.00^{cd}
2400	16.67^{d}	0.00^{d}	0.00^{c}	8.33^{c}	0.00^{c}	0.00^{d}
F value	3.17	3.29	3.02	2.24	2.14	2.54
Pr>F	0.0088	0.0047	0.0079	0.0474	0.0482	0.021

The radiobiological responses of a large number of species of higher plants have been studied after acute or chronic irradiation. Although different views were found for the effect of irradiation of different dose rate on the seeds germination, it is a common view that seed germination was decreased with increasing total dose. Asghar and Khan (1988) reported that no differences were found in germination percentage between the chronic and acute gamma irradiation, which is mainly for the lower treatment. Mergen and Strøm Johansen (1964) observed that seed germination was reduced after a lower total exposure at higher dose rate for the chronic irradiation. The effects on the seed germination were related to dose rate rather than total dose for the irradiated forest (Sheppard *et al.*, 1992). In present study, the effect of different irradiation mode on the seed germination is different. Ghosh and Sen (1978) reported that different effects existed at different seeds germination, in the second phase of germination, a dose rate relationship was apparent. The low dose irradiation did not cause these changes in the ultra-structure of chloroplasts whereas the irradiation of seeds with high doses of gamma rays disturbs the synthesis of protein, hormone balance, leaf gas-exchange, water exchange and enzyme activity (Borzouei *et al.*, 2010).

Radiation Effects on emerged seedling rate: The emerged seedling rates were recorded according to the cultivation tests for seedlings in culture cups. Generally the relative emerged seedling rates decreased with increasing dose (Table 2), viz. seedling was significantly inhibited by irradiation. The coefficient of correlation between them was -0.847 for irradiation without dose rate mode, while -0.923 for irradiation with the dose rate mode. A significantly negative correlation was observed between the relative emerged seedling rate and the irradiation dose by *t*-test (p=0.01). The results were consistent with germinate rate.

Table 2 Relative emerged seedling rate（RESR）and semi-lethal dose and correlation coefficient between RESR and dose of *Nitraria tangutorum*

Dose	Relative emerged seedling rate（Taking CK as 100%）(%)	
	Irradiation without dose rate	Irradiation with dose rate of 25 Gy/hr
CK	100	100
400	125	100
500	93.54	111.11
600	41.67	133.33
700	35.09	100
800	50	77.78
1000	41.67	66.67
1200	25	44.44
1500	16.67	33.33
1800	8.33	11.11
2100	8.33	11.11
2400	0	0
D_{50}	985	1363
r	-0.847**	-0.923**

**Represents $|t|>t_{0.01}$, which showed very remarkable correlation

Determination of semi-lethal dose: Appropriate radiation dose is the premise for the production of more effective variations. Materials treated with appropriate dose of radiation can gain valuable mutants in a short time and enrich germplasm sources. It is an important indicator to determine the effects of radiation damage. The critical dose and semi-lethal dose is commonly used to determine appropriate dose of radiation breeding. Semi-lethal dose is a main indicator to determine the radiosensitivity, as well as the reference of suitable radiation dose for induced mutation breeding.

The semi-lethal doses of *Nitraria tangutorum* were calculated according to the linear regression equation, which were respectively 985 Gy and 1363 Gy for the irradiation without dose rate and with 25 Gy/hr dose rate (Table 2). It seemed that the semi-lethal dose of the irradiation mode with dose rate was higher than the mode without dose rate. The result can be used to determine the appropriate radiation doses for genus *Nitraria* L. and provide an important basis to understand their radio sensitivities.

Effects on biological characters of seedling: The seedling growth status was investigated in the late growing season based on the cultivation seedlings test in the culture cups. The irradiation effects of ^{60}Co gamma rays on seedling growth were explored according to the indicators of seedling height and ground diameter, which provide references for further screening of beneficial mutants.

Comparisons among different dose of the two irradiation modes have revealed that a lower dose range (400 Gy to 500 Gy) was found very effective for improving the growth of ground diameter and seedling height. But a gradual reduction of these traits was observed with the increase of the irradiation dose (Table 1). This indicated that such growth was significantly inhibited by higher dose irradiation. A negative correlation was observed between them. And the inhibition was stronger with the increase of the dose (Wi *et al.*, 2007). These results were in accordance with the results obtained by Borzouei *et al*., (2010). The dose above 1500 Gy proved to be lethal as none of the plants in these treatments survived for irradiation without dose rate, while 2400 Gy with dose rate of 25 Gy/hr.

Multiple comparison analysis revealed that the irradiation effects on the seedling height and ground diameter present the very remarkable between dose treatment above 1500 Gy and control without dose rate mode, while between treatment above 2400 Gy dose and control with dose rate mode (Table 1). This revealed that the critical dose with dose rate mode was 2400 Gy higher than that without dose rate as 1500 Gy. It also showed that the damage of the irradiation with dose rate was less than that without dose rate. The seedlings height and ground diameters of treatment below 1500 Gy dose without dose rate mode is higher than that of treatment with dose rate mode. It was on contrary for treatment above 1500 Gy dose with dose rate of 25 Gy/hr.

The biological responses of many species have been studied after acute or chronic irradiation of growing plants or seed irradiated plant. Bari (1971) showed that there was a considerable increase of variability in morphological characters of the M1 and M2 generation of seed subjected to gamma irradiation. There was a significant increase in plant height and the number of primary branches for chronically irradiated plant. Kovalchuk *et al*., (2000b) found that exposure of seeds and seedlings to 0.1 to 10.0 Gy ^{60}Co resulted in increased homologous recombination frequency. In the present study, the biological variability as measured by mean values of height and ground diameter of M1 generation seedling increased at lower dose, and subsequently decreased with increase in the radiation dose at higher dose for 2 irradiation modes. Tabasum *et al*., (2011) illustrated that the differences among the different irradiation treatment were highly significant for the seedling traits among the rice genotypes, which indicated that genotypes displayed variable response towards gamma radiations. Even As Amiro (1986) reported, seedling growth was inhibited at the higher doses, and was inversely correlated with total dose and dose rate.

In the present study, the lethal dose for M1 generation seedling of irradiation without dose rate was lower than that with dose rate of 25Gy/hr, same as the critical dose for significant difference between dose treatment and control in the germination experiment. The results indicated that the critical dose of the irradiation with dose rate is more effective than that without dose rate. Cordero (1982) reported that the chronic irradiation made a reduction in the number of leaves formed, and also affected the stem tissues. In another study by Kovalchuk *et al*., (2000b) observed that a much higher frequency of homologous recombination in plants exposed to chronic irradiation when compared to acutely irradiated plants.

Conclusions

The growth traits of seeds and seedlings are both affected by ^{60}Co gamma irradiation (Irfaq & Nawab, 2001; Mokobia & Anomohanran, 2005). And there are slight differences in the two irradiation modes. Firstly, the semi-lethal dose for the irradiation with dose rate of 25Gy/hr is higher than that without dose rate, which are 1363 Gy and 985 Gy respectively. Secondly, the germination rate and phenotypic characters of seedling were improved at a lower dose range (400 - 500Gy) and significantly inhibited by the increasing irradiation dose for the two irradiation modes. The critical dose of significant radiation effects on such traits was 1500 Gy dose without dose rate mode, while 2400 Gy with dose rate mode. The appropriate dose of *Nitraria tangutorum* seeds is ranging from 950 Gy to 1500 Gy without dose rate mode, while 1300Gy to 2400Gy with dose rate of 25Gy/hr.

Whether putative mutants with desirable properties have been produced is still unclear, because the young seedlings are too young at current stage. The irradiation-induced breeding of *Nitraria tangutorum* is a strong complement to the conventional breeding. Induced mutations are used to obtain high frequency gene mutant and chromosomal alteration and provide a number of mutant varieties, which offer various breeding materials. The directional cultivation of new germplasm of genus *Nitraria* L. through the combination mutation breeding and molecular biology technologies should be greatly focused on in the future, which can greatly enrich the plant germplasm resources.

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