Check for updates

Using Relaxation Time to characterize biological effects of different mutagens

OPEN

Xinglin Li[1✉], Shuguang Sun[2], Jingxia Yao[1] & Zhengfeng Sun[1]

All kinds of mutagenic factors may cause physiological, biochemical and genetic changes of all organisms. To characterize their characteristic biology effects, the concept of Relaxation Time (RT) was introduced for the first time, and the specific process was as follows. After mutation of organisms, the offsprings will be continuingly cultured (or cultivated) to the next generation (Rx). Once a biological effect began to show no significant difference compared to the untreated controls, the Rx was defined as the RT of the effect. In this paper, three kinds of mutagenic factors were selected to treat the seeds or seedlings of *Astragalus sinicus* L., subsequently, the corresponding RT was calibrated. The results showed that the RT was diverse not only among different biological effects but also among different mutagenic factors. For the RT of chemical mutagens and gamma rays, most of which are concentrated on R_1, whereas the heavy ion beams have significant differences among different tracks. Among biological effects, the SOD activity and superoxide anion free radical content in the Peak region are more prominent, and their RT reaches R_3 and R_4, respectively. Thus, the RT may characterize the characteristic biological effects from differently mutagenic factors.

Since X-ray increasing mutation rate was discovered in the last century[1–3], a series of mutations caused by mutagens have received extensive attention, and it has made many achievements in basic research and applied research[4–6]. And the application of mutagenic means are also constantly innovating in three fields: physical factors from x-rays, ultraviolet rays, gamma rays to various kinds of charged particles[1–3,7–9], chemical factors from DNA alkylating agents to alkali analogues[10–12], and biological factors from the introduction of exogenous genes to the knockdown of internal genes[13–15].

Although all kinds of mutagenic factors can cause biological physiological, biochemical and genetic changes, so far, only the change rate of these biological effect indicators is used to distinguish the differences among these factors or these indicators. However, the change rate of these indexes is influenced by different doses, there is a cross between these ratios. Thus, it is hardly possible to distinguish the characteristic biology effects of different mutagenic factors at present. There are direct and indirect effects in mutagenic biological effects, and direct effects will directly affect indirect effects. As a result, we try to grasp the characteristics of indirect effects to characterize the biological effects of different factors by the Relaxation Time (RT, a concept from thermodynamics) as a new characterization index. The RT indicates the time required for a system to move from an unstable state to a stable state. Since the life system is also a complex and open thermodynamic system[16–18], when undergoing mutagenesis, the change of the life system is a normal thermodynamic reaction, and when the action of mutagenesis weakens or disappears, the restoration of the initial stable state is also a normal thermodynamic reaction. In the biological system treated by mutagenesis, whether from the molecular expression or from the presentation of the complex traits of the organism, it is more statistically processed parental changes (i.e., the unstable stereotype of the system) from the population than from the offspring of the treatment (i.e., the stable stereotype of the system). Thus, when a mutant was acquired as a mutagenic purpose, only focusing on the individual selection of each breeding link in the offspring, it will completely conceals the process of the effect of the mutagenesis on the life system.

In this study, we selected three kinds of common mutagens, such as gamma-rays, med-high energy heavy ion beams and chemical mutagens, to treat the seeds or seedlings of *Astragalus sinicus* L., respectively. *Astragalus sinicus* L. is a self pollinated legume plant (before flowering, the pollen of every papilionaceous flower has been pollinated). Thus, to save treated time, we selected it as a kind of test materials in this paper. We continuously determined 5 physiological and biochemical indexes of five generations (in 2015–2019) at three developmental

[1]Tianjin University of Science and Technology, Tianjin 300457, China. [2]College of Forestry, Nanjing Forestry University, Nanjing 210037, Jiangsu, China. ✉email: lxlszf@tust.edu.cn
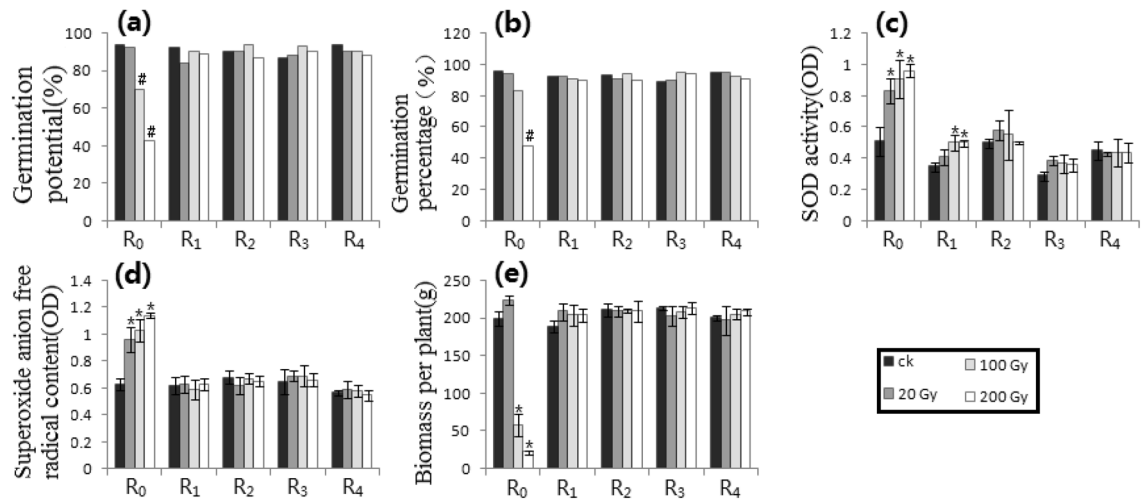
Figure 1. Gamma-ray processing. (**a**) GP_1. (**b**) GP_2. (**c**) SODA. (**d**) SAFRC. (**e**) BPP. # shows $\geq 15\%$ difference of mean, compared with respective control. * shows the difference of mean by $p \leq 0.05$ (Unpaired Sample T-Test), compared with respective control.
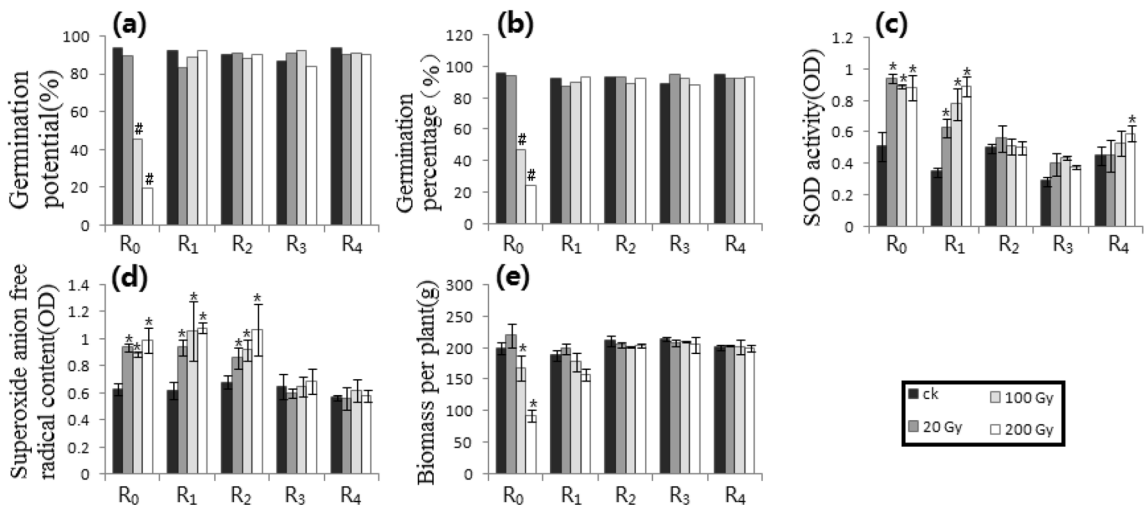

Figure 2. Irradiation processing of Petri dish 1 along the track of heavy ion beams. (**a**) GP_1. (**b**) GP_2. (**c**) SODA. (**d**) SAFRC. (**e**) BPP. # shows $\geq 15\%$ difference of mean, compared with respective control. * shows the difference of mean by $p \leq 0.05$ (Unpaired Sample T-Test), compared with respective control.

stages, and used stable generations (R_x) as the RT to characterize the characteristic biological effects of mutagens. Those seeds were irradiated using doses of 20, 100, and 200 Gy by gamma rays (i.e., ^{60}Co) and 80 meV/u carbon ion beams, respectively; whereas chemical mutagens treated seedlings using three concentrations of alkylating agent diethyl sulfate (DES) and base analogue maleyl hydrazine (MH), respectively.

Results

Overview. All raw data were lined in the Supplementary Materials (Additional information) "S-RD 1", "S-RD 2", and "S-RD 3". Starting from the raw data, compared with the respective control, the difference of germination potential (GP_1) or germination percentage (GP_2) was more than 15% and the difference of SOD activity, superoxide anion free radical content (SAFAC) or biomass per plant (BPP) reached a level of $p \leq 0.05$, which was listed as a significant difference, and the statistical results were shown in Figs. 1, 2, 3, 4, 5, 6, 7, 8 and 9 as follows.

Gamma-ray trials (Fig. 1). Compared with the control, only higher doses of the germination potential (GP_1) and the germination percentage (GP_2) had more than 15% differences in R_0 (i.e., RT = R_1), while other generations (i.e., RT = R_0) had no significant difference. For the SOD activity, R_0 (i.e., RT = R_1) at all doses and R_1 (i.e., RT = R_2) at high doses showed significant differences compared with controls ($p = 0.05$). For the superoxide anion free radical content (SAFRC) at all doses and the biomass per plant (BPP) at high doses, only there was a
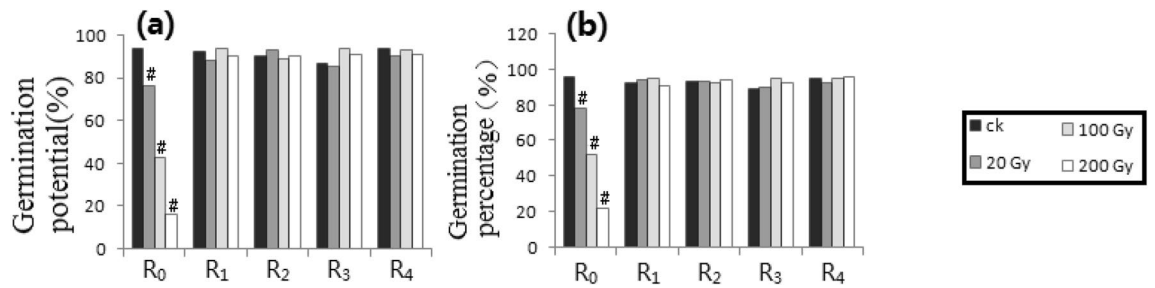
Figure 3. Irradiation processing of Petri dish 2 along the track of heavy ion beams. (**a**) GP_1. (**b**) GP_2. # shows $\geq 15\%$ difference of mean, compared with respective control.
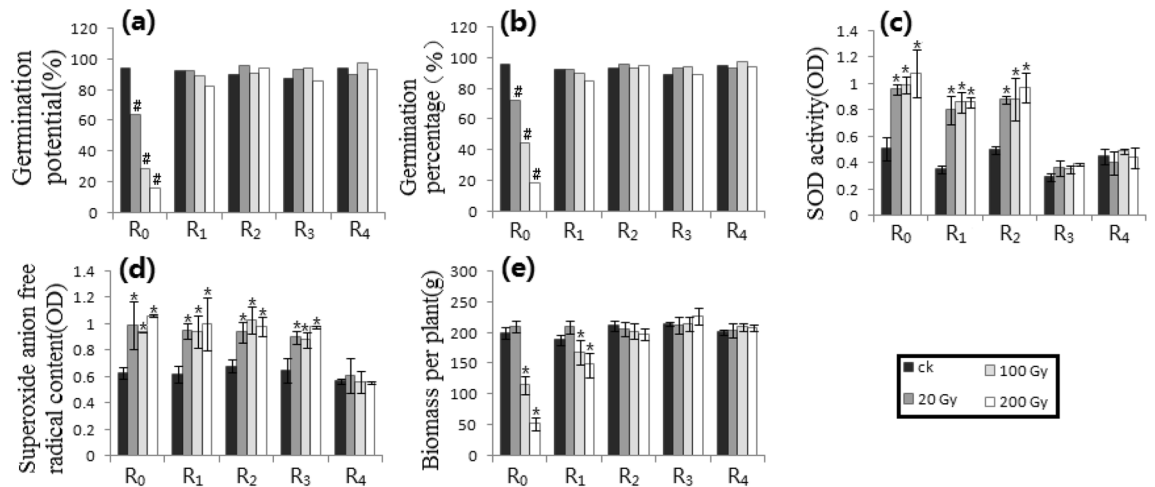

Figure 4. Irradiation processing of Petri dish 3 along the track of heavy ion beams. (**a**) GP_1. (**b**) GP_2. (**c**) SODA. (**d**) SAFRC. (**e**) BPP. # shows $\geq 15\%$ difference of mean, compared with respective control. * shows the difference of mean by $p \leq 0.05$ (Unpaired Sample T-Test), compared with respective control.
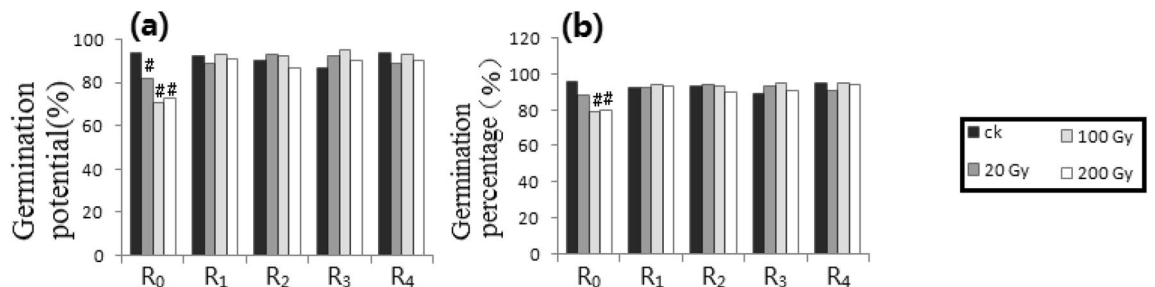

Figure 5. Irradiation processing of Petri dish 4 along the track of heavy ion beams. (**a**) GP_1. (**b**) GP_2. # shows $\geq 15\%$ difference of mean, compared with respective control.

significant difference in R_0 (i.e., $RT = R_0$) compared with the control, while there was no significant difference in the BPP at low dose (i.e., $RT = R_0$). The analysis results were listed in Table 1.

Heavy ion beam trials (Figs. 2, 3, 4, 5, 6, 7). Heavy ion beam irradiation is a complex process: six Petri dishes with seeds were stacked along 80 meV/u carbon ion beam tracks and separated into six regions to test after irradiation (see "Methods" for details), respectively. Petri dish 1 and 2, were equivalent to the Plateau regions of the Bragg curve; Petri dish 3 was close to the Peak of the Bragg curve; Petri dish 4 was the Peak-Tail junction region; and Petri dish 5 and 6 were the Tail regions of irradiation.

Figure 2 showed the results of Petri dish 1. Compared with the control, only R_0 (i.e., $RT = R_1$) of the GP_1 and the GP_2 had more than 15% differences at higher doses, while other generations (i.e., $RT = R_0$) had no significant differences. There were significant differences in R_0 and R_1 (i.e., $RT = R_2$) in the SOD activity compared to control ($p \leq 0.05$). There were significant differences in R_0, R_1 and R_2 (i.e., $RT = R_3$) of the SAFRC ($p \leq 0.05$). For the BPP, there was significant difference in R_0 (i.e., $RT = R_1$) at high doses compared with the respective control.

Figure 3 showed the results of Petri dish 2. Only the GP_1 and the GP_2 were measured. Compared with the control, only R_0 generations (i.e., $RT = R_1$) of the GP_1 and the GP_2 had more than 15% differences.
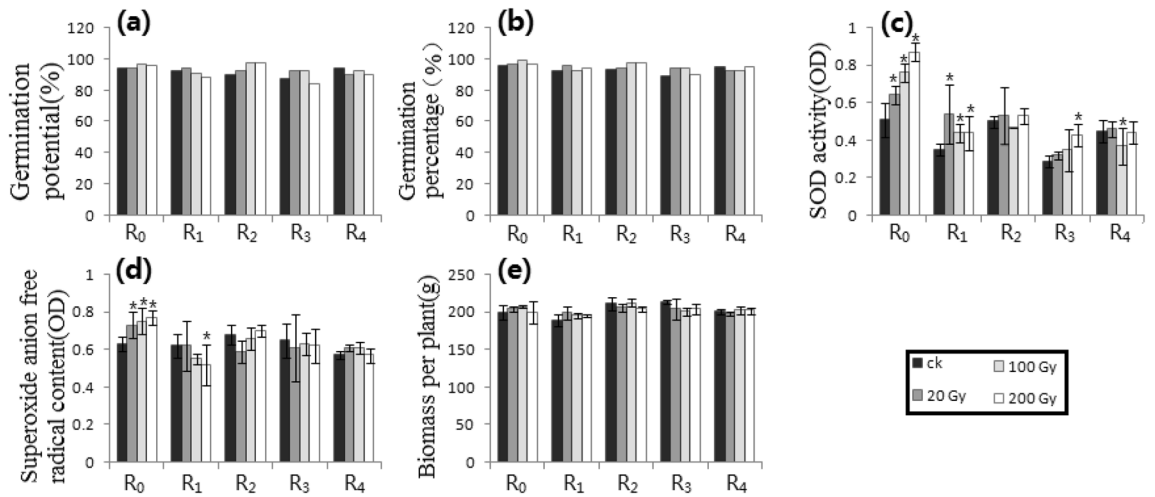
Figure 6. Irradiation processing of Petri dish 5 along the track of heavy ion beams. (**a**) GP_1. (**b**) GP_2. (**c**) SODA. (**d**) SAFRC. (**e**) BPP. # shows $\geq 15\%$ difference of mean, compared with respective control. * shows the difference of mean by $p \leq 0.05$ (Unpaired Sample T-Test), compared with respective control.

Figure 7. Irradiation processing of Petri dish 6 along the track of heavy ion beams. (**a**) GP_1. (**b**) GP_2. # shows $\geq 15\%$ difference of mean, compared with respective control.

Figure 8. DES processing trials. (**a**) GP_1. (**b**) GP_2. (**c**) SODA. (**d**) SAFRC. (**e**) BPP. # shows $\geq 15\%$ difference of mean, compared with respective control. * shows the difference of mean by $p \leq 0.05$ (Unpaired Sample T-Test), compared with respective control.
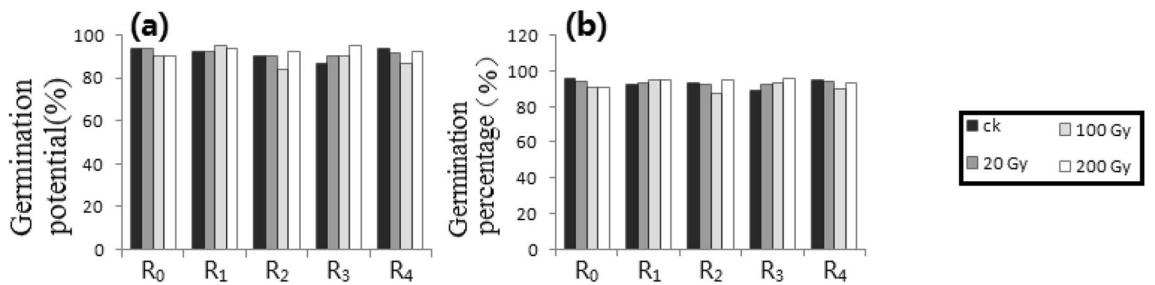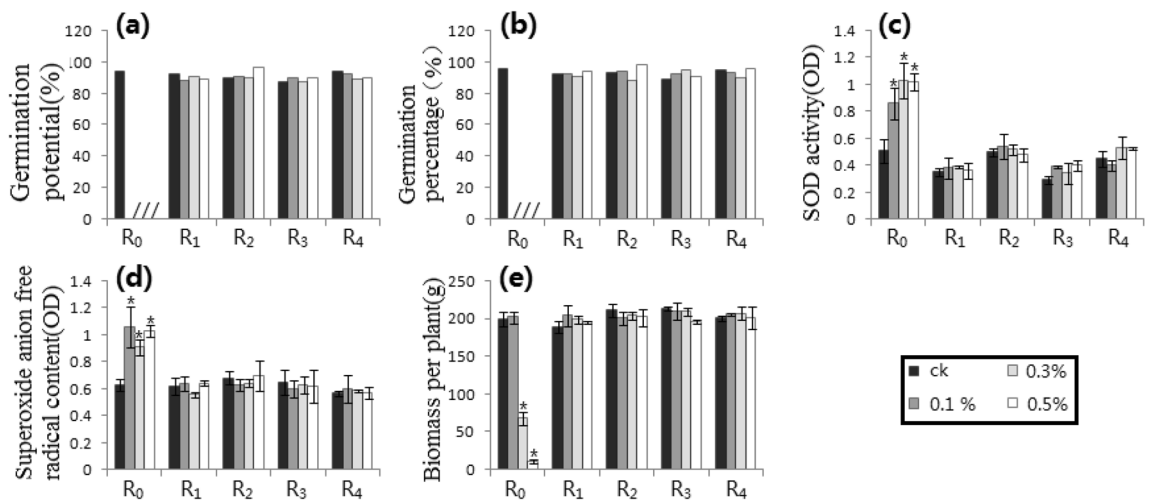
Figure 4 showed the results of Petri dish 3. For the GP_1 and the GP_2, only R_0 (i.e., $RT = R_1$) had more than 15% differences compared with the control. R_0, R_1, and R_2 (i.e., $RT = R_3$) of the SOD activity at all doses, R_0, R_1, R_2 and R_3 (i.e., $RT = R_4$) of the SAFRC at all doses, and R_0, R_1 (i.e., $RT = R_2$) of the BPP at high doses showed significant differences compared to controls ($p \leq 0.05$).
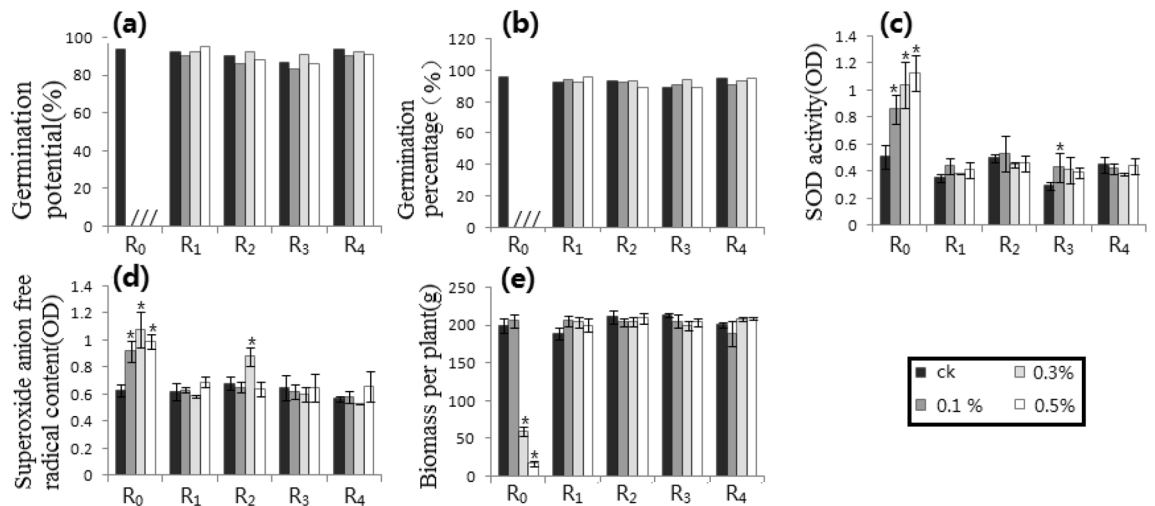
Figure 9. MH processing trials. (**a**) GP_1. (**b**) GP_2. (**c**) SODA. (**d**) SAFRC. (**e**) BPP. # shows ≥ 15% difference of mean, compared with respective control. * shows the difference of mean by $p \leq 0.05$ (Unpaired Sample T-Test), compared with respective control.

Figure 5 showed the results of Petri dish 4. Only the GP_1 and the GP_2 were also measured. Compared with the control, R_0 (i.e., $RT = R_1$) of the GP_1 at all doses and R_0 (i.e., $RT = R_1$) of the GP_2 at high doses had more than 15% differences.

Figure 6 showed the results of Petri dish 5. Compared with the control, there was no generations with more than 15% differences in the GP_1 (i.e., $RT = R_0$) and the GP_2 (i.e., $RT = R_0$). The results of the SOD activity was complicated: there were significant differences in R_0 and R_1, while the R_3 or the R_4 at a dose also showed significant differences ($p \leq 0.05$). After induction, we finalized the RT of the SOD activity as R_2. For the SAFRC, the significant differences were observed in R_0, R_1 (i.e., $RT = R_2$) at the highest dose and R_0 (i.e., $RT = R_1$) at other doses compared with the control ($p \leq 0.05$). For the BPP, there is no significant differences in all generations (i.e., $RT = R_0$) compared with the control ($p \leq 0.05$).

Figure 7 showed the results of Petri dish 6. Only the GP_1 and the GP_2 were also measured. There were no more than 15% different generations compared with the respective control, thus, the RT of both the GP_1 and the GP_2 were R_0. The results of the analysis in the Region 1–6 from heavy ion beam irradiation were also listed in Table 1.

DES processing trials (Fig. 8). Because the plantlets with normal germination were selected for chemical mutagens treatment, there was no R_0 determination of the GP_1 and the GP_2 (see "Methods" for details). Compared with the control, there were no generations (i.e., $RT = R_1$) with more than 15% difference in the GP_1 and the GP_2. For the SOD activity, the SAFRC and the BPP, compared with the respective controls, there were only significant differences in R_0 (i.e., $RT = R_1$) at high doses, whereas other treated generations (i.e., $RT = R_0$) had no significant differences ($p \leq 0.05$) at low doses. The analysis results were also listed in Table 1.

MH processing trials (Fig. 9). MH was completely consistent with DES processing results. The analysis results were also listed in Table 1.

RT analysis at seed germination stage. Using the RT of GP_1 or GP_2 may characterize this performance (Table 1 as shown). The GP_1 may reflect the uniformity of seed germination, whereas the GP_2 may reflect the survival ability of the seeds and the intensity of the action of the various mutagenic factors to the seeds. Table 1 indicated that the variation trend of the RTs of both the GP_1 and the GP_2 was consistent, compared with the untreated control group. For the irradiation in the gamma-rays and the Plateau regions and the Tail regions along carbon ion beam pathway at low doses, the RTs of the GP_1 and the GP_2 were R_0, whereas the RTs of the GP_1 and the GP_2 of the treatments from two chemical mutagens and the Peak regions along carbon ion beam pathway were R_1.

RT analysis at seedling stage. Using the RT of SOD activity or SAFRC may characterize their performances (Table 1 as shown). The SOD activity may reflect the cell stress facing to the mutagenic factors, while the SAFRC cannot only reflect the stress of the cells, but also reveals the indirect effects of various mutagenic factors, the results are shown in Table 1. It showed that the RT of SODA was R_1 after the treatments of low dose gamma rays, the Tail region along carbon ion beam pathway and two chemical mutagens, compared with untreated control group; the RT of the Peak region along carbon ion beam pathway was R_3; and the RT of the other treatment group was R_2. The treatment of gamma-ray, low-dose Tail region along carbon ion beam pathways and two chemical mutagens were performed, the RT of the SAFRC was R_1; the RT of the SAFRC the Plateau region and the Peak region along carbon ion beam pathway were R_3 and R_4, respectively.

Mutagens	Dosages	GP_1	GP_2	SODA	SAFRC	BPP
γ rays	20 Gy	R_0	R_0	R_1	R_1	R_0
	100 Gy	R_1	R_0	R_2	R_1	R_1
	200 Gy	R_1	R_1	R_2	R_1	R_1
HIB						
Petri dish 1: Plateau region 1	20 Gy	R_0	R_0	R_2	R_3	R_0
	100 Gy	R_1	R_1	R_2	R_3	R_1
	200 Gy	R_1	R_1	R_2	R_3	R_1
Petri dish 2: Plateau region 2	20 Gy	R_1	R_1	–	–	–
	100 Gy	R_1	R_1	–	–	–
	200 Gy	R_1	R_1	–	–	–
Petri dish 3: Peak region	20 Gy	R_1	R_1	R_3	R_4	R_0
	100 Gy	R_1	R_1	R_3	R_4	R_2
	200 Gy	R_1	R_1	R_3	R_4	R_2
Petri dish 4: Peak-Tail connect region	20 Gy	R_1	R_0	–	–	–
	100 Gy	R_1	R_1	–	–	–
	200 Gy	R_1	R_1	–	–	–
Petri dish 5: Tail region 1	20 Gy	R_0	R_0	R_2	R_1	R_0
	100 Gy	R_0	R_0	R_2	R_1	R_0
	200 Gy	R_0	R_0	R_2	R_2	R_0
Petri dish 6: Tail region 2	20 Gy	R_0	R_0	–	–	–
	100 Gy	R_0	R_0	–	–	–
	200 Gy	R_0	R_0	–	–	–
CM						
Diethyl sulfate (DES)	0.1%	R_1	R_1	R_1	R_1	R_0
	0.3%	R_1	R_1	R_1	R_1	R_1
	0.5%	R_1	R_1	R_1	R_1	R_1
Maleyl hydrazine (MH)	0.1%	R_1	R_1	R_1	R_1	R_0
	0.3%	R_1	R_1	R_1	R_1	R_1
	0.50%	R_1	R_1	R_1	R_1	R_1

Relaxation time	R_0	R_1	R_2	R_3	R_4
Color scales					

Table 1. Distribution of the Relaxation Time on physiological and biochemical indexes. HIB, Heavy ion beams. CM, Chemical mutagens. GP_1, Germination potential. GP_2, Germination percentage. SODA, SOD activity. SAFRC, Superoxide anion free radical content. BPP, Biomass per plant. "–" shows no determination.

RT analysis at plant maturation stage.   The performance of biomass per plant was characterized by the RT (Table 1 as shown). The BPP cannot only reflect the growth of *Astragalus sinicus* L. plants, but also reflect the synthesis and accumulation of plant cell metabolites. Table 1 revealed that the RT of the BPP of gamma-ray, the Plateau region and the Tail region along carbon ion beam pathway as well as chemical mutagens was mainly R_0 or R_1, compared with the untreated control group, whereas the RT of the BPP of the Peak region at high-doses along carbon ion beam pathway was R_2.

Discussion

In general, the RT is significantly different not only among different mutagenic factors and but also among different biological effects. The RT of gamma rays and chemical mutagens is relatively short. After planting a generation, except for mutants, the population affected by them can basically restore homeostasis. Whereas the results of the action of the heavy ion beams are the most complex, and the RT distribution of its biological effects is from R_0 to R_4, which may be related to the existence of a variety of physicochemical mechanisms such as energy deposition, mass deposition, charge effects, and momentum transfer along different tracks of the heavy ion beams[9,19], especially in the treatment of the Peak region, some biological effects requires a longer time to restore the population homeostasis. The longer RT after irradiation by heavy ion beams may reflect the accumulation of DNA mutations and chromosome rearrangements. Generally, heavy ion beams are more mutagenic than gamma rays, diethyl sulfate, or maleyl hydrazine[23–25].

According to the RT of different biological effects, it has positive application value in at least three aspects. First, during the breeding of mutagenesis mutants, the different strategy can be adopted: generally speaking, because of the longer stable time on the population, the biological trait with long Relaxation Time needs more generations to choose and accumulate useful individual plants. Second, in the study of stress and adaptability of biological individuals, it can be distinguished based on the length of Relaxation Time: the biological effects of short RT are stress traits, while the biological effects of long RT are adaptive traits. The third is that, in the biological study of phylogeny, because the organisms have experienced the action of natural environment factors for a long time, seeking and using some primitive or starting population as the control, the concept of the RT can still be introduced. By comparing the RT difference of specific biological effects among populations, the way of some population evolution can be speculated. Especially in the era of bioomics popularity, from the gene expression and metabolic pathway changes to metabolic network connectivity, we can quickly obtain the comparison results of related indicators such as nucleotide sequences between different species or individuals, which can be easily characterized by their RTs.

Not only is the biology a complex system[16–18], but its mutation is also a complex processing. as a result, there will be a rebalancing procedure among differently biological macromolecules (such as DNA, RNA, proteins and chromosome), different organelles (such as mitochondrion) and different metabolic pathways (such as antioxidant systems regulated by ROS). Thus, fully revealing the RTs of those life processes and inherited characters will be our important research objectives.

Methods

Gamma irradiation.   In 2015, the seeds of *Astragalus sinicus* L. harvested in 2014 were arranged around the irradiation chamber near the ^{60}Co source (the sources active was 221,754 Ci) at the Tianjin Irradiation Center. The radius of dose rates of 20, 10 and 2 Gy/min from the center of the ^{60}Co source bar was 0.87 m, 1.46 m and 4.19 m at the sites of 30 cm high, respectively. the seeds were placed in these three sites and irradiated for 10 min to obtain the irradiated materials with absorption doses of 200,100 and 20 Gy, respectively.

Irradiation by carbon ion beams.   Because 827 heavy ions of 80 meV/u carbon ions is equivalent to the dose of 1 Gy, an average dose rate was 0.5 Gy/min when the beam intensity of 400 ~ 500 ions/s used at this test. The doses of 20, 100 and 200 Gy needed 40, 200 and 400 s of irradiation time, respectively. In 2015, the seeds of *Astragalus sinicus* L. harvested in 2014 were filled with a Nunc plastic Petri dishes (34.4 mm diameter, 9.2 mm thick), and the six Petri dishes were stacked to be used in irradiation test with 80 meV/u carbon ion beams on the TR_4 terminal of the Lanzhou Heavy Ion Accelerator (Fig. 10). According to the theoretical calculation (Lise^{++}9.9: https://lise.nscl.msu.edu/lise.html) and the measurement of CR-39 heavy ion track detectors[23,24], when 87.5 meV/u of carbon ion beams was used to test, the Bragg peak of the energy loss appears near 2.2 cm depth in water and between 2.8 and 3.4 cm depth in Arabidopsis seeds along the tracks (Wang et al.[25]). While 80 meV/u of carbon ion beams was used to this test, the Bragg peak of the energy loss appears between 2.2 and 3.2 cm depth (not spread-out Bragg peak) in *Astragalus sinicus* L. seeds along the tracks (about at the Petri dish 3). After irradiation, the whole installation was divided into six Petri dishes, which were classified as Petri dish 1–6 from close to far away according to the contact of carbon ion beams: Petri dish 1 of the Plateau region 1, Petri dish 2 of the Plateau region 2, Petri dish 3 of the Peak region, Petri dish 4 of the Peak-tail connect region, Petri dish 5 of the tail region 1 and Petri dish 6 of the tail region 2. The seeds of each Petri dish were applied to subsequent experiments.

Treatment of chemical mutagens.   The whole seedlings of *Astragalus sinicus* L. (at 1–2 leaf stage) were treated with different concentrations (0–1.3%) of diethyl sulfate (DES) and maleyl hydrazine (MH) solutions at room temperature for 24 h, respectively. After washing with the sterilization water, their survival curves were plotted by statistics on survival ratios (as shown in Fig. 11). According to Fig. 11, the working concentrations of DES and MH of 0, 0.1%, 0.3% and 0.5% were used to test the seedlings. In 2015, the seeds of *Astragalus sinicus* L. harvested in 2014 were germinated. After about 10 days (at 1–2 leaf stage), the above working concentrations of DES and MH solution were sprayed to the whole seedlings of R_0 generation for 24 h, respectively. After washing
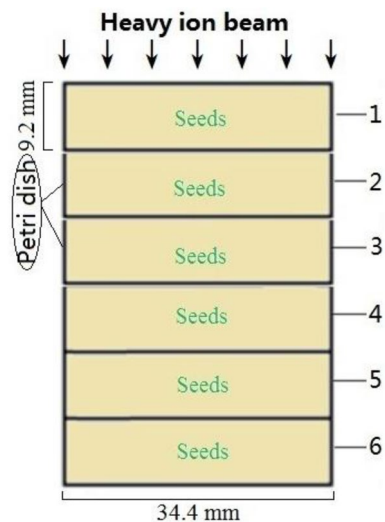
Figure 10. Schematic diagram of the seeds irradiated by heavy ion beams in Petri dishes.
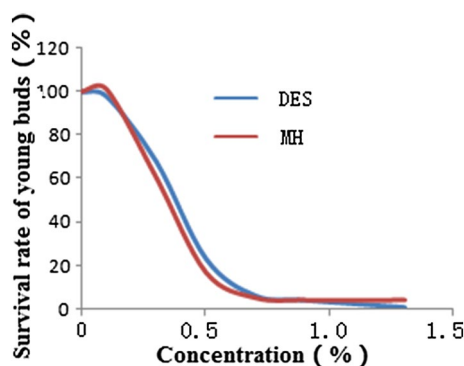

Figure 11. Survival curves of seedlings of *Astragalus sinicus* L.treated with chemical mutagens. DES, diethyl sulfate. MH, maleyl hydrazine.

with the sterilization water, we sampled and carried out cultivation successions according to different treatments for subsequent trials. Just because of this kind of treatment, the germination potential and germination percentage of R_0 generation were not counted in this paper.

Germination test. A Petri dish (a diameter of 150 mm and depth of 25 mm) with seeds was used for the germination test at room temperature and natural light. The germination potential (GP_1) was counted by the number of *Astragalus sinicus* L. germination within 7 days, and the germination percentage (GP_2) was counted by the number of all germination seeds about 20 days before transplanting. The chemical mutagen treatment group performed germination potential and germination percentage statistics from the R_1 generation seeds (i.e., the seeds harvested by the R_0-generation plants), while the other treatment groups performed germination potential and germination percentage statistics from the R_0 generation (the starting seeds harvested in 2014).

Determination of SOD activity and Superoxide anion free radical content. At the later stage of the germination test (about 20 days, including seedlings with chemical inducer treatment), the whole plant of 20 seedlings was randomly selected from each dish, and the crude extract of seedlings was extracted according to Xiao HS, et al.[26] and stored in a refrigerator at —20℃ for the determination of SOD activity (SODA) and Superoxide anion free radical content (SAFRC), which the determination process was carried out according to the method from Xiao HS, et al.[26] and Simova-Stoilova LK, et al.[27].

Field trials and determination of biomass per plant. All seedlings were transplanted into the field after about 20 days of indoor germination (including chemical mutagen-treated seedlings). three repeated, random block design with a plot of 12 m², and the plant spacing was 30 cm (North–South) × 20 cm (East–West) to ensure that each individual plant can grow normally and was rarely affected by other plants. Up to maturity,

the single plant was harvested, full dried indoor and performed test seeds, respectively. And finally the average biomass per plant (BPP) will be calculated according to the plot block.

Data statistics. A total of about 100 seeds of *Astragalus sinicus* L. were combined and added up after germination from two Petri dishes, and then the germination potential and germination percentage were counted. Compared with the control materials of the same generation without any treatment, when there was more than 15% mean of a treatment group, this treatment was acted as a significant difference (Indicated by #). SOD activity and Superoxide anion free radical content were took the OD value of survival seedling of 2 germination dishes to determine their effect, respectively, which every effect was calculated by its average value (Mean) and standard deviation (SD). The Mean and SD of Biomass per plant were calculated according to 3 plot blocks. Compared with control materials without any treatment at the same generation, when the statistics variance of SOD activity, Superoxide anion free radical content or Biomass per plant, was evaluated by $p \le 0.05$, the biological effect was regarded as a significant difference (Expressed by an asterisk *).

References

1. Muller, H. J. Artificial transmutation of the gene. *Science* **66**, 84–87 (1927).
2. Stadler, L. J. Mutation in barley induced by X-rays and radium. *Science* **67**, 186–187 (1928).
3. Swaminathan, M. S. & Natarajan, A. T. Effect of ultraviolet pretreatment on yield of mutations by X-rays in wheat. *Science* **130**, 1407–1409 (1959).
4. Tah, P. R. Induced macromutation in mungbean [*Vigna radiata* (L.) Wilczek]. *Int. J. Bot.* **2**, 219–228 (2006).
5. Kharkwal, M. C. & Shu, Q. Y. The role of induced mutations in world food security. In *Induced plant mutations in the genomics era* (ed. Shu, Q. Y.) 33–38 (Food and Agriculture Organization of the United Nations, Rome, 2009).
6. Roychowdhury, R. & Tah, J. Genetic variability study for yield and associated quantitative characters in mutant genotypes of *Dianthus caryophyllus* L. *Int. J. Biosci.* **1**(5), 38–44 (2011).
7. Kang, S. Y. *et al.* Gamma phytotron: a new chronic gamma irradiation facility. *Plant Mut. Rep.* **2**(2), 50–51 (2010).
8. Nakayama, M. *et al.* Comprehensive analyses of anthocyanin and related compounds to understand flower color change in ion-beam mutants of cyclamen (*Cyclamen* spp.) and carnation (*Dianthus caryophyllus*). *Plant Biotechnol.* **29**, 215–221 (2012).
9. Kazama, Y. *et al.* LET-dependent effects of heavy-ion beam irradiation in Arabidopsis thaliana. *Plant Biotechnol.* **25**, 113–117 (2008).
10. Roychowdhury, R. & Tah, J. Crop improvement: new approaches and modern techniques. In *Mutagenesis—A Potential Approach for Crop Improvement* (eds Hakeem, K. R. *et al.*) 149–188 (Springer, Berlin, 2013).
11. Sattler, S. E. *et al.* A nonsense mutation in a cinnamyl alcohol dehydrogenase gene is responsible for the sorghum brown midrib6 phenotype. *Plant Physiol.* **150**(2), 584–595 (2009).
12. Cozzi, R. *et al.* Desmutagenic activity of natural humic acids: Inhibition of mitomycin C and maleic hydrazide mutagenicity. *Mut. Res./Genet. Toxicol.* **299**(1), 37–44 (1993).
13. He, Y. *et al.* Large-scale production of functional human serum albumin from transgenic rice seeds. *Proc. Natl. Acad. Sci. USA* **108**(47), 19078–19083 (2011).
14. Horvath, H. *et al.* The production of recombinant proteins in transgenic barley grains. *Proc. Natl. Acad. Sci. USA* **97**, 1914–1919 (2000).
15. Galli-Taliadoros, L. A. *et al.* Gene knock-out technology: a methodological overview for the interested novice. *J. Immunol. Methods* **181**(1), 1–15 (1995).
16. Gladyshev, G. P. & Ershov, Y. A. Principles of the thermodynamics of biological systems. *J. Theor. Biol.* **94**(2), 301–343 (1982).
17. Ponizovskiy, M. R. The mechanisms operation of thermodynamic system of a human organism. *Eur. J. Biophys.* **2**(4), 29–37 (2014).
18. Josef, S. Life—a complex polymer system. *J. Polym. Sci. Part A Polym. Chem.* **42**(3), 471–478 (2004).
19. Sato, T., Watanabe, R. & Niita, K. Development of a calculation method for estimating specific energy distribution in complex radiation fields. *Radiat. Prot. Dosimetry* **122**, 41–45 (2006).
20. Xia, C. *et al.* Genetic polymorphisms in mutagenesis progeny of *Arabidopsis thaliana* irradiated by carbon-ion beams and γ-rays irradiations. *Int. J. Radiat. Biol.* **96**(2), 267–275 (2020).
21. Liangwen, C. *et al.* Induction of reproductive cell death in *Caenorhabditis elegans* across entire linear-energy-transfer range of carbon-ion irradiation. *DNA Repair* **63**, 39–46 (2018).
22. Yusuke, K. *et al.* Characterization of highly efficient heavy-ion mutagenesis in *Arabidopsis thaliana*. *BMC Plant Biol.* **11**, 161 (2011).
23. Waligorski, M. P. R., Hamm, R. N. & Katz, R. The radial distribution of dose around the path of a heavy ion in liquid water. *Nucl. Tracks Radiat. Meas.* **11**, 309–319 (1986).
24. Tse, K. C. C., Nikezic, D. & Yu, K. N. Comparative studies of etching mechanisms of CR-39 in NaOH/H$_2$O and NaOH/ethanol. *Nucl. Instrum. Methods B* **263**, 300–305 (2007).
25. Wang, T. *et al.* Homologous recombination in Arabidopsis seeds along the track of energetic carbon ions. *Mutat. Res.* **737**(1–2), 51–57 (2012).
26. Xiao, H. S. *et al.* A spectrophotometer method testing oxygen radicals. *Prog. Biochem. Biophys.* **26**, 180–183 (1999).
27. Simova-Stoilova, L. *et al.* Antioxidative protection in wheat varieties under severe recoverable drought at seedling stag. *Plant Soil Environ.* **54**(12), 529–536 (2008).

Author contributions

X. L. wrote the manuscript text and prepared all figures and all tables in this paper. S. S., J. Y. and Z. S. were involved in measuring all indicators in this study.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41598-020-70600-2.

Correspondence and requests for materials should be addressed to X.L.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.