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THE EFFECT OF SEED MOISTURE CONTENT AND POST-IRRADIATION STORAGE ON THE RECOVERY FROM RADIATION DAMAGE IN BARLEY *(HORDEUM VULGARE L.)*

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Barley seeds treated with 3 doses of gamma rays were employed to investigate the effects of two different levels of moisture contents at the time of irradiation (4 and 10%) and during post-irradiation storage (23 and 33%) on radiation induced seedling injury and chromosome aberrations on shoot meristem mitosis. It was observed that with increase in radiation dose, there was an almost linear increase in radiation damage. But with increase in seed moisture content from 4 to 10% during irradiation, there was decrease in radiation damage at all the doses. Post-irradiation hydration (20 hours), redrying and storage (1 week) of seeds at 23% moisture content potentiated the radiation damage (storage enhancement of damage) while storage at 33% moisture content resulted in recovery from the radiation induced damage.

Keywords :Barley; Post-irradiation storage, Radiation damage, recovery, Seed moisture content.

Introduction

The studies on the repair of mutagen damage in higher plants is of special value in understanding the mutagenic mechanism and in the experimental mutagenesis. Another incentive for the study of recovery from mutagen damage is that crop plants are being increasingly exposed to the effects of mutagenic pesticides and fungicides[1]. A model system has been developed in barley to study the recovery from mutagen by modifying the moisture contents of the mutagen treated seeds[2] during post-treatment storage.

In this study, barley seeds treated with three doses of gamma rays were employed to investigate the effect of seed moisture content at the time of irradiation and during post-irradiation storage on radiation-induced seedling injury and chromosome aberrations.

Materials and Methods

Pure line hull-less seeds (caryopses) of barley *(Hordeum vulgare L.)* strain IB-65 were used as the test material in all the experiments. Healthy seeds of uniform size and colour were selected. The moisture content of the seeds were equilibrated to 4 and 10% by keeping the seeds tied in a muslin cloth for 2-5 days in a desiccator containing fused calcium chloride. The moisture content was determined in small aliquots of seed samples (5 seeds × 3 replications) as the percentage of difference between the initial weight and the final weight after drying in an electric oven at a constant temperature of 60^0 C for 48 hrs.

After equilibration of seeds with the desired moisture content, 50 seeds each were put in three glass ampoules of 10 ml capacity for each treatment and were subject to evacuation using a glass manifold vacuum system as dune by Sah and Kesavan[3]. After continuous evacuation for 4 hours, the ampoules were sealed off. Evacuated and sealed ampoules with seeds were exposed to 3 doses (200, 400 and 600 Gy) of gamma rays using a Gammatron source (Siemens, Germany).

After irradiation, the ampoules were carefully broken and the irradiated seeds were placed in the hydration linear

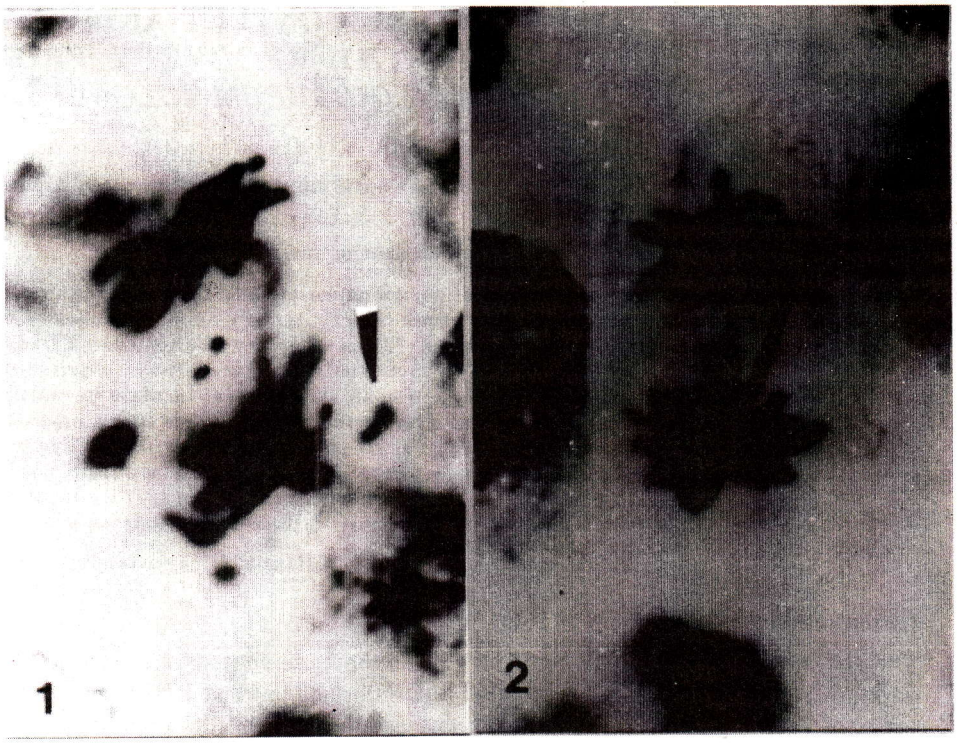

Fig. 1-2 : Chromosomal aberrations in barley, induced by Y-radiations
(1) Anaphase showing bridges and fragments (arrow) (x 1500)
(2) Shoot meristem anaphase showing bridges (x 1500)

medium (distilled water) for 20 hours at room temperature. The hydrated seeds were thoroughly washed with distilled water and then redried at 40^0 C to 23 or 33% water content and the redrying took 2-10 hrs. The storage of the seeds with 33% water content for 1 week was done in a desiccator over distilled water while storage of seeds with 23% moisture content in a desiccator over 7.5% H_2SO_4.

After the post-irradiation storage schedule, the seeds were washed in distilled water, and planted equidistantly on moist filter paper in sterile petri dishes and cultured at room temperature in day-light illumination.

The seven day seedling height was measured as the lenght of the first leaf (from the base of its apex) using a metal scale. Seedling height data were statistically analysed.

Radiation damage on seedling on seedling growth is expressed as follows.

$$\text{Seedling damage}(\%) = \frac{\text{Seedling height (unirreadiated)-seedling height (irradiated)} \times 100}{\text{Seeding height (unirradiated)}}$$

About 48 hrs after sowing, 15 germinating seeds from each treatment were fixed in acetic acid-alcohol (3 parts ethyl alcohol: 1 part acetic acid). About 9-12 slides out of 3 replicates of each treatment were made of the coleoptiles of the fixed shoots by the standard Feulgen squash technique. The slides were analysed randomly for bridges and fragments. Around 50 anaphase cells per treatment were scored.

Results and Discussion

At both the moisture contents (4 and 10%) at the time of irradiation, with increase in radiation dose, there was a marked

Table 1. Effect of seed moisture content at the time of irradiation on radiation damage in barley seeds.

Treat-ment	Radiation dose (Gy)	Seed moisture content at the time of irradiation (approximate %)	Post Irradiation hydration media	7-day seedling height (cm) Mean± S.E.	Seedling damage (%)	Chromosomal aberrations (bridges and fragments / cell
1	0	4	Water	14.89±0.37	0.00	0.0
2.	0	10	Water	15.01±0.42	0.00	0.00
3.	200	4	Water	5.82±0.25**	33.59	–
4.	200	10	Water	13.20±0.29	15.09	–
5.	400	4	Water	3.18±0.16	78.63	1.9
6.	400	10	Water	11.80±0.23**	45.03	1.7
7.	600	4	Water	2.45±0.05**	83.58	2.9
8.	600	10	Water	3.90±0.06	80.85	2.4

**Significant at 1% level

Table 2. Effect of seed moisture content during post-irradiation storage on radiation damage in barley seeds.

Treat-ment	Radiation dose (Gy)	Seed moisture content at the time of irradiation (approximate %)	Post Irradiation hydration media	7-day seedling height (cm) Mean± S.E.	Seedling damage (%)	Chromosomal aberrations (bridges and fragments / cell
1	0	23	Water	13.71±0.23	0.00	0.0
2.	0	33	Water	14.40±0.28	0.00	0.00
3.	200	23	Water	11.51±0.19	19.79	–
4.	200	33	Water	12.32±25*	14.40	–
5.	400	23	Water	3.70±0.06	73.06	0.9
6.	400	33	Water	5.52±0.16**	61.60	0.8
7.	600	23	Water	1.43±0.03**	89.60	1.3
8.	600	33	Water	3.42±0.09**	85.01	1.0

*Significant at 5% level

** Significant at 1% level

increase in seedling damage (Table 1). At 4% moisture content, the three radiation doses produced 34, 79 and 83% of seedling damage, respectively. When the seed moisture content was increased to 10% seedling damage was found to be reduced. The decrease in seedling injury was found to be maximum (30%) for the radiation doses of 400 Gy and minimum (3%) for 600 Gy.

Post-irradiation hydration, redrying and storage of the seeds for one week at 23% moisture content produced much more damage when compared to storage for one week at 33% moisture content. The percentage of seedling damage reversed as a result of increase in moisture content from 22 to 33% during storage, was significant for the radiation doses of 200 and 400 Gy (Table 2).

Shoot meristem mitotic aberrations (bridges and fragments per cell) (Fig. 1 & 2) produced by irradiation and the various post-treatments largely follow the same trend as the seedling injury (Table 1, 2). At 4% moisture content, increase in radiation dose produced an increased incidence of chromosomal aberrations The values of bridges and fragments per

anaphase cell ranged from 1.9 at 400 Gy to about 2.9 for 600 Gy. Increasing the seed moisture contennt at the time of irradiation to 10% caused a decrease in chromosomal aberrations.

The most distinct reaction of a multicellular plant to irradiation is manifested in the inhibition of growth processes[4] and hence seedling height is found to be the most consistent end point parameter to asses radiation damage and recovery[5]. Chromosome aberrations in shoot meristem mitosis and seedling injury largely followed a similar trend in barley[5,6]. Hence these two reliable morphological and cytological parameters were employed in the present study to assess the influence of seed moisture content on radiation effects in barley under different treatment conditions.

It is known that radiation induced very reactive oxygen sensitive free radicals, which in presence of oxygen magnify radiation damage[5]. DNA forms the prime target of radiation damage and membrane, the alternate target[7].

The results presented show that with increase in seed moisture content at the time of irradiation (4 to 10%) and during post-irradiation storage (23 to 33%) there was decrease in radiation damage. In dry seeds with 4% moisture content at the time of irradiation, the radiation induced free radicals are very stable and react with the oxygen contained in the hydration medium (distilled water, containing about 5mg of oxygen per litre) resulting in the full component of post-irradiation damage. The reduction in radiation damage by increasing the moisture content from 4 to 10% may be due to the decay of such radiation induced oxygen sensitive sites or free radicals.

The storage enhancement of damage observed in this study could be attributed to the action of radiation induced free radicals which can remain active for a longer period in non-metabolising barley seeds with 23% moisture content. The higher level of moisture content (33%) in the seeds enables increased metabolic activity, favouring the repair process and ATP synthesis[5]. Recovery from the induced mutagenic injury at 30% water content was made with chemical mutagen-treated barley seeds[8]. In similar studies, Sah and Kesavan[3] and Devasagatam and Kesavan[7] reported that the alkaloid caffeine which is now known to react with the radiolytically produced electrons and hydroxyl radicals is a radioprotector, against the oxic but a radiosensitiser of the anoxic components of damage.

The studies on repair processes in higher plants has been meagre until recently. It needs to be studied in depth, since it is crucial for protection of plants against the unfavourable effects of extrinsic mutgens.

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