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EFFECT OF GAMMA RADIATION ON ONION SEED VIABILITY, GERMINATION POTENTIAL, SEEDLING GROWTH AND MORPHOLOGY

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Seed of onion *(Allium cepa* L.) cv. Ailsa Craig were exposed to various doses of gamma radiation, i.e. 10,20.40.80. and 100 krad. Irradiation doses have no significant effect on seed viability, except the highest dose (100 krad) which resulted in reduced viabi lity. However, electrical conductivities of the leachates of seeds exposed to gamma radiation were higher than that of un irradiated seeds (control). Seed germination percentage was improved at lower doses of irradiation (10 and 20 krad) and was unaffected at higher doses. Number of abnormal seedlings increased with increasing irradiation dose. However. types of abnormalities could not be linked with a particular irradiation dose. Seedling growth was reduced severely with an increase in irradiation dose up to 40 krad. Root growth was more sensitive to gamma radiation than shoot growth.
Key Words: *Allium cepa*, gamma radiation, onion. seed viability, seedling growth and vigour.

INTRODUCTION

Ionizing radiations, i.e. Xvrays, gamma rays and ultraviolet rays have been widely used for producing mutations in crop plants. Among these. gamma radiation has been frequently used to create variation in gene pools of crop plants. Higher doses produce very drastic effects, usually causing plant death, and relatively lower doses often result in altered growth characteristics. The effects of gamma radiation on cytological characteristics vary from species to species and among different genotypes within the same species. Gamma radiation interferes with the process of cell division, resulting in cytological abnormalities and in a reduced frequency of dividing cells, which is ultimately reflected in reduced seedling growth and other morphological aberrations. Bajaj *et al.* (1970) found that *Phaseolus vulgaris* seeds gamma irradiated with 10-12 krad produced stunted seedlings which later exhibited delayed flowering and poor seed set. At 15 krad, although the seeds germinated. no normal seedlings were formed. Bhamburkar and Bhalla (1980) found that germination percentage, and seedling height and survival were affected, when seeds of three onion varieties were irradiated with gamma rays. They concluded that different varieties of *Allium* showed varying sensitivity to irradiation. Mandal and Basu (1986) found that the germination potential of rice was less affected than seedling growth. especially root growth, by various doses of gamma radiation. However, stimulation of growth by low doses of ionizing radiation has been noticed in wheat (Sheppard and Evenden, 1986) and barley (Sheppard and Regiting, 1987). In another experiment, doses from 1-4 krad also stimulated wheat and *Triticale* germination, whereas this was reduced by higher doses. and seedling height also showed a similar trend (Khanna, 1986). When seeds of *Phaseolus vulgaris* were irradiated with 0, 4, 8, 12, 16 and 20 krad gamma rays, seed germination. plant height, survival and yield all decreased as the dose of irradiation increased, but the effects were relatively small (Carneiro *et al.* 1987). Bhargava and Khalatkar (1987) treated genetically pure seeds of *Tecotna grandis* with 10, 20, 30, 40 and 50 krad doses of gamma radiation at the rate of 1.2 krad/rninute using a (;(lCo source. Irradiation at lower doses, improved the seed germination. Seedling growth was also affected to a significant extent by the irradiation doses used. However. Mahto *et al.* (1989) found that percentage germination of two chickpea cultivars in field trials was not affected by gamma irradiation at a dose of 15 krad, but this decreased progressively with increases in irradiation dose (30 krad to 75 krad with 15 had interval). Kawamura *et al.* (1992a) observed that rice seeds irradiated with gamma rays at 50 krad or more. resulted in reduced root. length. They (1992b) also found that in wheat seedlings, root and shoot lengths were more sensitive to gamma irradiation than the germination process itself AI-Safadi and Simon (1996) exposed dry seeds of carrot to various doses of gamma radiation. Lower doses (05 and I krad) enhanced seed germination, whereas higher doses delayed germination and also reduced plant size. They observed a highly negative correlation between irradiation dose and survival of plants. it is clear from the literature that the majority of seed irradiation experiments have used. germination or seedling height, or some measure of mutation numbers as methods of assessment of the effects of irradiation. Very few indeed have investigated any relation between irradiation and aspects of seed vigour, as presented in the present work.

MATERIALS AND METHODS

Onion *(Allium cepa* L.) cv. Ailsa Craig seeds were obtained from booker Seeds Ltd., Sleaford, England as a single large aluminum foil pack, containing one kilogram of seeds. Seeds were divided into small batches, which were stored in closed glass bottles in a cold room at 5°C.

Irradiation of seeds: Seed samples were removed from the cold room and the seeds, still in their airtight glass bottles, were kept closed overnight to equilibrate with laboratory temperatures before irradiation. Then these were irradiated by gamma rays using a ^{60}Co source at a dose rate of 2 krad/minute. The exposure doses used were 10. 20, 40, 80 and 100 krad. Moisture content of seeds at the time of irradiation was 11.1%.

Tetrazolium viability test: 1% solution of 2, 3, 5-triphenyl tetrazolium chloride (TTC) was prepared by dissolving 1 g in 100 ml of distilled water. Two replicates of 100 seeds were soaked in distilled water for 18-20 h before conducting the test. Each seed was cut longitudinally without completely separating the two halves. The seeds were then submerged in 1 % TTC solution for at least 8 h at 35°C in darkness, after which the staining patterns were recorded.

Electrical conductivity test: Two replicates of onion seeds, 100 seeds in each replicate, were placed in 100 ml beakers separately, each containing 75 ml distilled water. The seeds were gently stirred to ensure that all seeds were completely immersed and evenly distributed. The beakers were placed in an incubator at 20±2°C. After 24 h the seeds were gently stirred and the conductivity of the soaking water of each sample was measured without filtration using a digital conductivity meter (JENWAY, Model 4070).

Seed germination tests: Germination potential of the onion seeds was estimated in accordance with the International Rules for Seed Testing (ISTA, 1985). Germination percentages, using 3 replicates of 50 seeds, were determined by placing the seed samples in 90 mm Petri dishes on filter papers (Whatman No.1) moistened with 4 ml of distilled water. Seeds were distributed evenly within each dish. Petri dishes were covered with their lids and then placed in an incubator at 20 ~ 2° C with 12 h photoperiod by fluorescent light illumination. Germination in terms of radicle emergence (at least 2 mm) was assessed each day from the second day until no further radicle emergence was noted on two successive days. Germination capacity was expressed as a percentate of all seeds with fully emerged radicle in a given batch. The germination speed of the seed lots was derived from the formula of Kotowski (1926):

$$\text{Germination speed (G.S.)} = \frac{\Sigma n}{\Sigma (n \times Dn)} \times 100$$

where, n is the number of seedlings germinated on day Dn, Dn is the number of days from sowing, corresponding to n, and highest (G.S.) is the greatest speed.

Time to reach 50% of final germination (T_{50}) was calculated from the formula of Coolbear *et al.* (1984):

$$T_{50} = t_i + \left[\frac{(N+1)/2 - n_i}{n_j - n_i}\right] \times (t_j - t_i)$$

Where, $n_i < (N+1)/2 < n_j$, N is the number of seeds germinated. and n_i and n_j are total number of seeds germinated by adjacent counts at times t_i and t_j respectively.

Morphology of seedlings: After 10 days of beginning of germination test, the seedlings were classified as normal, weak normal or abnormal, following the guidelines given in the International Rules for Seed Testing (ISTA, 1985). Seedlings are classed as abnormal when one or more of the essential structures fails to develop normally because of previous damage to the embryo. or when development as a whole is weak or out of proportion compared with that of a normal seedling germinated at the same time and in the same conditions. Percentages and types of abnormalities were also recorded.

Measurements of seedling lengths as a vigour test: After 10 days from the initiation of imbibition, average shoot lengths, root lengths, and overall seedling lengths were measured using a ruler. Growth reduction in terms of the effects of irradiation doses on seedlings, e.g. the percentage change in mean seedling height as compared to the non-irradiated control were recorded as calculated from the following formula:

$$\text{Percentage change} = \frac{H_c - H_r}{H_c} \times 100$$

Where, Hc is the mean seedling height of the non-irradiated (control) and Hr is the mean seedling of the irradiated treatment.

Statistical analysis: The data collected were analysed statistically using Fisher's analysis of variance technique in a completely randomized design. Means were compared using Duncan's multiple range test at 5% probability level (Petersen, 1994).

RESULTS AND DISCUSSION

Seed viability (tetrazolium test): The first experiment was conducted to determine the direct effects of the irradiation doses used on the various parameters of seed quality. This so-called "direct" effect is said to be due to the immediate absorption of energy by the various cellular macromolecules themselves and not to the more long-term effects of the free radicals produced from irradiated water molecules (Levitt, 1972). In past, no attention has been given to study the direct effects of irradiation on seeds. In the present study, we have used tetrazolium viability test and conductivity test to study the direct effect of gamma radiation on onion seeds, irradiated at doses from 10 to 100 krad. The tetrazolium test is a biochemical test, which differentiates the living and dead tissues of a seed by the presence or absence of a red stain known as formazan. The test was conducted immediately after irradiation treatment. Seed viability was not much affected by the gamma irradiation doses used. However, at the highest dose (100 krad), the seed viability was significantly reduced as compared to control and other doses. While the seeds irradiated at all other doses behaved statistically alike and stood at par with control (Table I). This indicates that the direct effect of irradiation on onion seed is not so severe, except at the highest pose of 100 krad.

Table I: Viability, electrical conductivity of leachates and germination potential of onion seeds tested immediately after gamma irradiation at various doses.

Exposure Dose (krad)	Seed viability % (TTC test)	EC of seed Leachate (μS/cm)	Final Germination (%)	Germination Speed	T50 (days)
0 (control)	93 a'	54.0 b	83 b	19.25 a	3.06 a
10	93 a	58.9 ab	93 a	18.99 a	3.26 a
20	91 a	62.2 a	88 ab	18.97 a	3.33 a
40	89 a	59.8 a	82 b	18.97 a	3.41 a
80	91 a	61.4 a	84 b	18.69 a	3.44 a
100	83 b	62.4 a	82 b	19.14 a	3.49 a

'Means sharing similar letters are statistically non-significant at 5% probability (DMR test).

Table 2. Percentage of abnormal seedlings, with types of seedling abnormalities from onion seeds exposed to various doses of gamma radiation.

Exposure Dose (krad)	Seedlings classification			Types of abnormalities (%)		
	Normal (%)	Weak normal (%)	Abnormal (%)	Ia	Ib/IVc	VIa
0 (control)	81.1 a'	3.0 c	15.9 c	2.3 c	6.1 b	7.5 b
10	71.6 a	5.7 c	22.7 b	9.1 b	5.7 b	7.9 b
20	57.3 b	17.1 b	25.6 b	11.0 ab	7.3 b	7.3 b
40	42.2 c	20.5 b	37.3 a	13.3 a	12.0 a	12.0 a
80	35.6 c	28.8 a	35.6 a	13.8 a	12.6 a	9.2 ab
100	32.5 c	31.2 a	36.3 a	10.0 ab	16.3 a	10.0 ab

'Means sharing similar letters are statistically non-significant at 5% probability (DMR test).

Table 3. Seedling lengths and decrease in growth (%), from onion seeds exposed to various doses of gamma radiation, recorded after a period of 10 days of seed sowing.

Exposure Dose(krad)	Seedling lengths (mm)		Growth reduction	
	Shoot	Root	Overall	%
0 (control)	72.3 a'	36.0 b	108.3 a	-
10	47.6 b	43.2 a	90.8 b	16.2 c
20	33.9 c	23.4c	57.3 c	47.1 b
40	28.1 c	10.5 d	38.6 d	64.4 a
80	31.6 c	8.1 d	39.7 d	63.3 a
100	27.5 c	6.7 d	34.2 d	68.4 a

'Means sharing similar letters are statistically non-significant at 5% probability (DMR test).

Conductivity of seed leachate: The lowest electrical conductivity was recorded in the leachate of the seeds, which were not exposed to gamma radiation (control). The conductivities of seed leachates irradiated with gamma radiation were significantly higher except of those irradiated at the lowest dose (10 krad), which stood at par with control (Table 1). It appeared that the seeds irradiated at higher doses suffered greater losses than those irradiated at the lowest dose and therefore the leachates from the irradiated samples had higher conductivities. Like tetrazolium test, conductivity test is also frequently used to determine the quality of a seed lot but have not previously been used to study the direct effects of ionizing radiation on seeds.

Germination potential following irradiation: It is known that following irradiation, cells and tissues may continue to be subject to change, due to the activities of free radicals produced during the actual exposure period. It was necessary in the first instance to attempt to stimulate the metabolic processes of germination as quickly as possible after exposure to each dose of irradiation. Therefore, the seeds were immediately sown after irradiation treatments. The data on germination percentages of the seeds presented an interesting picture (Table 1). The germination of seed was improved by the lower doses (10 and 20 krad) and then again returned to the normal. The maximum germination percentage was recorded in seeds irradiated at a dose of 10 krad, followed by 20 krad. Seeds irradiated at doses higher than 20 krad had germination percentage almost same as unirradiated seeds (control). The improvement in seed germination by low doses of gamma radiation has already been reported by several workers in various crop plants (Sheppard and Evenden, 1986; Sheppard and Regiting, 1987; Bhargava and Khalatkar, 1987). The result regarding germination percentage of seeds in general also supports the biochemical tests. although slightly higher viabilities were shown in that test. However, germination speed and time to complete 50% germination (Tso) were not affected by various doses of gamma irradiation administered (Table I).

Seedling morphology: The seedlings were evaluated ten days after beginning of the germination test, and the numbers of apparently normal, weak, and abnormal seedlings were recorded for each radiation dose administered to the samples of onion seeds (Table 2). Number of normal seedlings recorded was the highest in case of unirradiated seeds (control). followed by those irradiated at the lowest dose (I0 had) and both these treatments stood statistically at par. However, as the irradiation dose was increased, number of normal seedlings progressively decreased. The minimum number of normal seedlings was recorded in case of the highest dose (100 krad), followed by 80 and 40 krad dose. These three doses also behaved statistically alike with non-significant behaviour to each other. An opposite trend was found for weak normal and abnormal seedlings. As the irradiation dose increased. the number of weak normal and abnormal seedlings increased (Table 2). Increase in number of abnormal seedlings with the increase in irradiation dose has already been reported in *Phaseolus vulgaris* by Bajaj *et al.* (1970). As far as the types of abnormalities are concerned. the following recognizable categories of abnormal seedlings were found; a) without primary root (la), b) primary root short and stunted (lb), c) poorly developed leaf-like cotyledon without a definite bend or "knee" (IVc), and d) short and weak, or spindly, or watery seedling (Via). Although their percentages varied with the irradiation doses but the types of abnormalities and their magnitude could not be linked with a particular dose.

Seedling growth: The results show that irradiation doses adm inistered caused severe reductions in overall seedling length compared with normal growth (Table 3). Both roots and shoots were affected, and it appeared that the growth of roots was improved at the lowest dose (10 kard). When seed were irradiated higher than this dose, the root growth was more affected than shoot growth. The maximum overall seedling length was recorded in seedlings resulting from unirradiated seeds (control) and as the irradiation dose increased. the seedling length decreased. The minimum seedling length was recorded when seeds were irradiated at the highest dose (100 krad) but this dose also stood at par with 80 and 40 krad. In the present study, the root growth of onion seedlings was affected to a greater extent than shoot growth in length, which has commonly been reported for other species (e.g., Mandal and Basu, 1986; Savaskan and Toker, 1991; Kawamura *et al.*, 1992a & b) and the effect was severe and consistent. The consistency of irradiation effect on growth makes this an attractive test for experiments on irradiation. However, it is evident that the sensitivity of the test varies according to the irradiation dose. It seems that the reduction in seedling growths is inversely proportional to the their lengths. As the seedling length decreased, the reduction in growth became more (Table 3). At the highest dose about 68% growth reduction was recorded. When seedling growth is compared directly with the irradiation dose given, the severe effects of increasing irradiation dose become very obvious. These results are in accordance with the findings of previous workers (Bajaj *et al.*, 1970; Khanna, 1986; Mandal and Basu, 1986; AI-Safadi and Sirnon, 1996), who recorded reduction in seedling growth with the increasing irradiation dose.

REFERENCES

Al-Safadi, B. and P. W. Simon. 1996. Gamma irradiation-induced variation in carrots *(Daucus carota* L.). J. *Amer. Soc. Hort. Sci.,* 121(4): 599-603.

Bajaj, Y.P.S., A.W. Saettler and M.W. Adams. 1970. Gamma irradiation studies on seeds, seedlings and callus tissue cultures of *Phaseolus vulgaris* L. *Radiat. Bot,* 10: 119-124.

Bharnburkar, S. and J.K. Bhalla. 1980. Differential mutagenic sensitivity of three varieties of *Allium cepa* L. 1. *Cytol & Genet.,* 15: 178- I85.

Bhargava, Y.R. and A.S. Khalatkar. 1987. Improved performance of *Tecoma grandis* seeds with gamma irradiation. *Acta Hort.,* 215: 30-35.

Carneiro, J.E.de, H.M. Barbosa, A.A. Cardoso and C. Vieira. 1987. The sensitivity of seeds of *Phaseolus vulgaris* L, cv. Milionario 1732 to gamma radiation. *Rev. Ceres. 34:* 306-312.

Coolbear, P., A. Francis and D. Grierson. 1984. The effect of low temperature pre-sowing treatment on the germination performance and membrane integrity of artificially aged tomato seeds. 1. *Exp. Bot,* 35: 1609-1617.

International Seed Testing Association (1ST A). 1985. International rules for seed testing. *Seed Sci. & Technol.,* 13: 299-355.

Kawarnura, Y., N. Suzuki, S. Uchiyama and Y. Saito. 1992a. Germination test for identification of gamma-irradiated rice. *Radiat. Phys. Chem.* 39: 203-207.

Kawarnura, Y., N. Suzuki, S. Uchiyarna and Y. Saito. 1992b. Germination test for identification of gamma-irradiated wheat. *Radiat. Phys. Chem.* 40: 17-22.

Khanna, V.K. 1986. Effect of gamma radiation on seedling growth and cell division in wheat and *Triticale. Acta Bot. Indica,* 14 (suppl.): 43-49.

Kotowski, F. 1926. Temperature relations to germination of vegetable seeds. *Proc. Amer. Soc. Hart. Sci.,* 23: 176-184.

Levitt, J. 1972. Ionizing radiation. In: Responses of Plants to Environmental Stresses. Academic Press, New York, London. pp. 460-488.

Mahto, R.N" MD.F. Hague and P. Prasad. 1989. Biological effects of gamma rays in chickpea. *Indian J Pulses Res,* 2: 97-101.

Mandal, K. and R.N. Basu. 1986. Control of age- and irradiation-induced seed deterioration in rice *(Ory::a sativa L.)* by hydration-dehydration treatments. *Seed Res.* 14: 197-205.

Petersen, R.G. 1994. Agricultural Field Experiments-Design and Analysis. Marcel and Dekker, New York.

Savaskan, C. and M.C. Toker. 1991. The effects of various doses of gamma irradiation on the seed germination and root tip chromosomes of rye. *Doga Turk Bot. Dergisi,* 15: 349-359.

Sheppard, S.c. and W.G. Evenden. 1986. Factors controlling the response of field crops to very low doses of gamma radiation of the seed. *Canad. J Plant Sci.,* 66: 431-441

Sheppard, S.c. and PJ. Regiting. 1987. Plant effects: factors controlling the hormesis response in irradiated seed. *Health Phys.,* 52: 599-605.