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Effects of Electron Beam Irradiation on Germination and Vigour of Greengram (*Vigna radiata*) Seeds

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ABSTRACT The full genetic potential of a crop can be realised by pre-treatment of seeds with plant growth regulators and chemicals enhancing the seed germination and seedling vigour. Therefore, the present study was conducted to evaluate the effects of electron beam (EB) irradiation on greengram (*Vigna radiata*) seeds. For this purpose, dry seeds of greengram were exposed in a single layer to EB doses, *viz.* 170, 340, 510, 680, 850, 1020, 1190 and 1360 Gy at an energy level of 500 keV produced from a 750 keV, 20 kW DC accelerator. Irradiated seeds along with the control (unirradiated) were subjected to standard seed germination test and shoot length, root length, seedling vigour and vigour index. The seeds were also subjected to electrical conductivity and tetrazolium test for viability. Results showed that irradiation with EB doses as above have no significant effects on seed germination but affected seedling vigour and vigour index. The lower doses, *viz.* 170, 340 and 510 Gy had stimulatory effect on seedling vigour which were 37.69, 35.69, and 33.70 as compared to 30.19 in the control. Corresponding to this the seedling vigour index showed a decline @ 680 Gy. The electrical conductivity of seed leachate of the control and irradiated samples exhibited nearly a similar trend.

Key words: Electron beam, irradiation, greengram, germination, vigour

Electron beam irradiation of agricultural commodities and food products is a novel approach for food preservation and is gaining global importance. The benefits of these soft electron radiations with low penetrating power can be harnessed for several agricultural applications. This technology has been found successful in facilitating safe storage of pulses through insect-disinfestations. However, the effect of electron beam irradiation on seed quality *per se* has not yet been explored. Hence, present study aims to evaluate the implications of electron beam irradiation for enhancing germination and vigour of greengram seeds.

The full genetic potential of the greengram [*Vigna radiata* (L.) Wilkzec] crop is not realized due to several factors including insect-pest infestation. Amongst the major pests, pulse beetle (*Callosobruchus maculates*) is the most destructive storage pest of greengram, since it can cause up to cent per cent loss. Whereas efforts were being made for developing the disinfestations protocols using electron beam technology, it was found that irradiation enhances seed quality considerably. Hence, it was imperative to analyze the dosage effect of the irradiation on basic physiological attributes of the seed.

MATERIALS AND METHODS

The seeds of greengram [*Vigna radiata* (L.) Wilczek] cv. Pusa Ratna were obtained from the Division of Genetics, Indian Agricultural Research Institute,

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New Delhi. The seeds were stored at a temperature of 4°C until further use. The seeds were exposed in a single layer to electron beam doses, *viz.* 170, 340, 510, 680, 850, 1020, 1190 and 1360 Gy at an energy level of 500 keV produced from a 750 keV, 20 kW DC accelerator fabricated indigenously at the Raja Ramanna Centre for Advanced Technology, Indore. Each dose was replicated six times. The surface dose was determined to be 170 Gy/pass by pre-calibrated FWT-60 radiochromic film dosimeters [1]. The irradiated and unirradiated seeds were stored at 4°C until use.

Both irradiated and unirradiated seeds were subjected to standard seed germination test in paper towels as per the International Seed Testing Association (ISTA) rules [2]. For seedling vigour, 25 seeds per replication per treatment, in 10 replications, were tested. The observations were recorded on 08 day based on the parameters of seed germination and vigour index. The vigour index was calculated following Abdul-Baki and Anderson [3]. The treated seeds were also subjected to the electrical conductivity test and tetrazolium test for viability.

Electrical conductivity of the irradiated seeds was tested by soaking 25 seeds of each treatment, in four replicates, in 25 ml-distilled water for overnight at 20°C. The electrical conductivity from seed leachates was measured with a conductivity meter calibrated with 0.01 M potassium chloride. The conductivity is expressed as µS/cm/g fresh weight of the sample. To determine the viability of seeds through tetrazolium staining, seeds were soaked in distilled water for 24 hr. The seed coat was removed and decoated seeds were dipped in 0.5 per cent solution of tetrazolium salt prepared in 0.067M phosphate buffer of pH 7.0, followed by incubation for 24 hr at 25°C in dark.

These were washed several times with distilled water and evaluated for staining. The seeds irradiated at different doses were grown in the National Containment Facility at NBPGR, New Delhi, in randomised block design to observe for chlorophyll mutations, if any.

The results were subjected to analysis of variance using the SPSS software and treatment means were compared using the least significance difference (LSD) values at a significance level of $P<0.01$.

RESULTS

The data on seed germination and vigour of the greengram seeds irradiated with different doses of EB are presented in Table 1 and Fig. 1. Irradiation with EB doses (as above) had no significant effects on per cent seed germination. However, these doses affected the seedling length and vigour index (Table 1 and Fig. 1). The lower doses, *viz.* 170, 340 and 510 Gy had stimulatory effects on seedling length and vigour index. The seedling vigour at low EB doses were 37.69, 35.69 and 33.70 as compared to 30.19 in the control. The mean shoot length was highest in 170 Gy treated seeds which was highly significant as compared to the control. The improvement of shoot length was also significant in 340 and 510 Gy treated seeds. The EB doses greater than 510 Gy significantly reduced the shoot length as compared to the control and other beneficial treatments (Table 1). The lowest vigour was observed for seeds treated at 1360 Gy.

Irradiation at different doses evoked similar response in root length. Maximum mean root length was observed for seedlings raised from 170 Gy treated seeds and this effect declined gradually up to 510 Gy where the root length was at par with the untreated seeds. Minimum root length was observed for seeds irradiated at 1360 Gy. Also the vigour index at these doses was significantly higher as compared to the control; being 3730, 3545, 3335 at 170, 340 and 510 Gy, respectively, in comparison to 2994 in the control (Table 1 and Fig. 1).

Although the higher EB doses, *viz.* 680, 850, 1020, 1190 and 1360 Gy at an energy level of 500 keV did not drastically affect the per cent seed germination, the vigour index was significantly reduced. The vigour index at these doses were 2667, 2493, 2078, 1933 and 1848, respectively, which were significantly lower as compared to the control (Table 1 and Fig. 1). Thus, the soft electrons at lower doses only, *viz.* 170, 340 and 510 Gy at

Table 1. Effects of different doses of EB on greengram seeds: physiological parameters

Dose (Gy)	Germination (%)	Average shoot length (cm)	Average root length (cm)	Seedling vigour
0	98.50	16.220	13.970	30.190
170	99.00	21.555	16.135	37.690
340	98.50	20.215	15.480	35.695
510	99.00	19.256	14.452	33.708
680	100.00	14.765	11.905	26.670
850	100.00	14.760	10.175	24.935
1020	95.00	12.240	8.830	21.070
1190	99.50	11.090	8.335	19.425
1360	98.50	10.595	8.121	18.716
CD at 1%	N.S.	1.660	1.294	2.98
CV (%)	7.8	8.73	8.92	9.38

500 keV had stimulatory effects on seed vigour.

The electrical conductivity from the seed leachate of the control and irradiated samples also exhibited a similar trend. The changes in the electrical conductivity values (from seed leachates) recorded from seeds treated at different doses are given (Table 2). It is evident from the data that there was a significant increase in the values particularly at doses higher than 1020 Gy and noticeable decrease at lower doses as compared to the untreated (control) seeds. The tetrazolium test results indicated that the seed viability at different doses did not differ significantly and was comparable to the actual germination percentage value (Table 2).

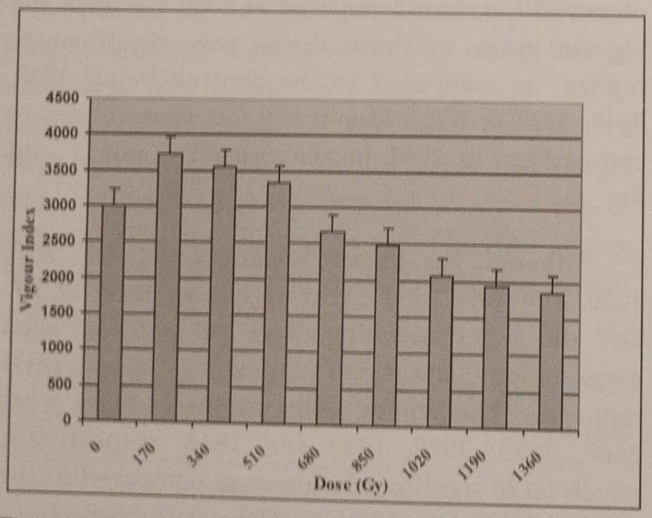

Fig. 1. Effects of electron beam irradiation on vigour index of greengram seeds

Table 2. Effects of different doses of EB on greengram seeds: biochemical parameters

Dose (Gy)	Electrical conductivity (μS/cm/g fwt)	Viability (%)
0	344.4 ± 5.8	100.0
170	315.2 ± 4.7	100.0
340	320.3 ± 5.1	100.0
510	325.5 ± 5.7	100.0
680	351.8 ± 5.8	96.6
850	355.4 ± 4.9	100.0
1020	357.3 ± 3.4	100.0
1190	353.9 ± 3.8	95.3
1360	366.5 ± 3.8	97.5

No chlorophyll mutations were observed in the plants raised from the seeds irradiated with different doses of EB.

DISCUSSION

Electron beam irradiation technology has wide range of applications in various fields. However, it is relatively less exploited for seed quality enhancement although sporadic reports are available in the literature. Bhat and Sridhar [4] reported 90 per cent seed germination in untreated control as against 84 per cent germination recorded for *Mucuna* seeds treated at a higher dose of 2.5 k Gy. The treatment resulted in reduced mean shoot

and root length as compared to the control. No seed germination was observed at doses higher than 2.5 k Gy. Kotapalli *et al.* [5], on the contrary observed a slight increase in the germination of dry barley samples treated at doses ranging from 6-8 k Gy.

Germination tests on the low dose treated soybean showed that doses from 1-10 k Gy clearly reduced the ability of soybean to germinate, increasing the abnormal seedlings and dead seeds [6]. Kikuchi *et al.* [7] observed no change in soybean seed quality during gamma radiation and electron beam irradiation with soft electrons at 60 keV and 26 k Gy; but later gamma radiation exhibited changes in grain properties. Takeshita *et al.* [8] observed growth promotion for irradiated seeds of maize and soybean at the doses ranging from 2 to 10 k Gy. The germination capacity of adzuki bean seeds remained unchanged when they were treated with soft electrons for disinfestation treatment [9].

A clear cut inhibitory effect of EB irradiation on wheat seed viability and vigour at doses ranging from 2 to 10.2 k Gy has been reported by Sitton *et al.* [10]. It is thus evident that the effects of electron bean irradiation are crop, dose and energy level dependent. In the present study, where greengram seeds were exposed to low energy electrons at doses ranging from 170 Gy to 1360 Gy, at an energy level of 500 keV, germination was unaffected, as evident by the data on standard germination as well as the viability test (Tables 1, 2), whereas vigour parameters were significantly affected at doses above 510 Gy. This is in agreement with most of the similar studies conducted by other groups as cited above.

Increase in electrical conductivity of seed leachates from seeds treated at higher doses indicate the onset of membrane deterioration, which could be due to the formation of free radicals, which play a major role in triggering the process of lipid peroxidation. This is corroborated by the observations of Bhat and Sridhar [4] in *Mucuna pruriens*, where a dose dependent increase in the free radical content in the seed coat as well as the cotyledons of treated seeds occurred.

Whether irradiation *per se* can stimulate crop yield is a matter of considerable interest. It has been found in some cases that the effects is stimulatory, whereas in others it is inhibitory. Further studies on the field evaluation of the seeds irradiated at different doses of electron beam are being pursued. The results of the present study clearly indicated the beneficial and safe effects of electron beam irradiation on seed quality enhancement on greengram.

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