VOL. 6, NO. 8, AUGUST 2011 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science


www.arpnjournals.com

ELECTRON BEAM SENSITIVITY STUDIES ON *Knema attenuata* (HOOK. F. AND THOMSON) WARB. SEEDS - GERMINATION AND BIOCHEMICAL CHANGES

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ABSTRACT

The present study deals with the effect of electron beam irradiation (EBI) on germination and biochemical parameters of *Knema attenuata* (Hook. F. and Thomson) Warb. seeds. Seeds were exposed to 0.5kGy, 1kG, 2kGy, and 3kGy of EBI (8.0 MeV beam energy) at Microtron Center, Mangalore University, India. The study revealed that the germination and seedling growth were severely affected by EBI. There was no germination in the seeds treated with higher doses (1kG, 2kGy, and 3kGy). Although, seeds irradiated with 0.5kGy showed 10% germination, seedlings could not survive even for a month. The soluble protein decreased from first week to fourth week both in control and irradiated seeds. An initial rise followed by a decrease in soluble sugar was observed in the irradiated seeds compared to control. A steady increase in phenolic content in seed exudates was recorded during the course of storage. Decreased seed viability, germination potential and seedling growth of *Knema attenuata* was attributed to the damaging effect of electron beam irradiation.

Keywords: *Knema attenuata*, electron beam irradiation, storage, seed viability, germination potential.

INTRODUCTION

Physiological and biochemical processes in plants are significantly affected by radiation stress. High doses of radiations disturb the synthesis of DNA, RNA and protein (Roy *et al*., 1972), enzyme activity (Hameed *et al*., 2008). Relatively low doses usually alter growth characteristics where as, very low doses have been shown to stimulate plant growth (Munjeeb, 1974). However radio sensitivity varies from species to species and even among genotypes of the same species (Ahmad and Quireshi, 1992). The morphological, structural and functional changes depend on the strength and duration of radiation stress. Amjad and Anjum (2007) reported the effect of different doses of gamma radiation which affected the seed viability, conductivity, percentage of germination etc. in onion (*Allium cepa* L.) cv. seeds. Several workers have studied the effect of gamma rays on seed germination of angiosperms and gymnosperms (Thapa, 2004; Hameed *et al*., 2008).

Bhat and Sridhar (2007) investigated the effect of electron beam irradiation on the quality characteristics of a legume, *Mucuna pruriens* and opined that seeds loose germination capacity at about 2.5kGy of EBI. Cytogenic effects of electron beam radiation on dry seeds were studied by Baojiang *et al*., (1989). Soft electron treatment did not affect the germination capacity of adzuki bean seeds (Reddy *et al.*, 2006).

Knema attenuata (Hook. F. and Thomson) Warb. belongs to family Myristicaceae (nutmeg family, a family which has got the members of economically and medicinally important plant species) is a medium sized tree endemic to Western Ghats of penisular India. It is one of the ingredients of 'Ashwagandadhi nei' (medicated ghee) used in the treatment of spleen disorders, breathing disorders and tastelessness (Ravikumar and Ved, 2000).

As a component of marshy evergreen forests, the Myristica swamps are of great ecological as well as conservational significance (Verghese and Menon, 1999). Preliminary studies revealed that *Knema* seeds remain viable only for a week under normal laboratory conditions. However, it takes longer time to germinate when provided favorable environment (unpublished data). In this context, the present study was designed to evaluate the effects of electron beam irradiation (EBI) on the seed germination and biochemical changes.

MATERIALS AND METHODS

Mature, split opened and aril exposed fruits of *Knema attenuata* were hand harvested during the month of June 2009 from the selected trees of the Charmady forest of Dakshina Kannada district, Karnataka, India and brought to the laboratory in polythene bags. Fruit rind and arils were removed and cleaned. Seed Samples without any apparent physical damage or insect infestation were selected for the experiment. Samples were subdivided to analyze different parameters.

Seed samples were packed in specially designed bi-axially oriented polypropylene bags (BOPP, 25 µ, 6 x 6 cm) and exposed to electron beam irradiation (EBI) at the Microtron Center, Mangalore University (Microtron accelerator, designed by Raja Ramanna Centre for Advanced Technology, India) to doses of 0.5kGy, 1kGy, 2kGy, 3kGy at room temperature. Irradiation was carried out by exposing both the sides of the seeds for uniformity. Seed sample packed similarly without irradiation served as control.

Seeds (control and irradiated) were stored at 28±2ºC in sealed polythene bags (25 µ, 25x20 cm) until

use. Seed samples were removed at regular intervals of one week and used for the following studies: Moisture content, seed viability, germination potential and biochemical changes (Total soluble sugar, Proteins and phenolic content in seed exudates) in stored seeds. The experiment was continued for four weeks.

Moisture content (MC) of whole seed was determined following low constant oven drying method (ISTA 1991). Randomly selected five seeds were cut into quarters and dried at 103ºC for 17 hours in hot air oven. Seed MC was calculated on a fresh mass basis.

Seed viability was evaluated by treating dissected embryo (five numbers in each case) with 1% 2,3,5 - triphenyl tetrazolium chloride solution (TZ) for 14 hours at 32ºC in darkness and staining patterns were recorded (ISTA, 1991).

Thirty seeds were drawn from stored seeds and used for germination studies. Germination test was carried out in sand bed in accordance with the International Seed Testing Association procedures (ISTA, 1991). Germination was scored by emergence of radicle (2cm in length) and expressed as percentage of seeds germinated.

The seed sample for the estimation of total soluble sugar was extracted using 1g of dried powdered sample in 10 ml of boiling ethanol (80%) for 10 min and decanted. The extraction was repeated twice and extracted samples were evaporated and redissolved in distilled water and made up to 10 ml. The extract was then used for the estimation of total soluble sugars by anthrone method (Sadasivam and Manikam, 2008). The sample exacted in phosphate buffer (pH 7.0) was used for the estimation of soluble protein following the method of Lowry *et al*., (1951). For the estimation of phenolic content in seed exudates, two replicates of 15 whole seeds were weighed in each treatment and placed in 100 ml beaker separately, each containing 75 ml deionised water. The beakers were placed in the room temperature (28±2ºC) for 12 hours. Seed exudates were collected and used for the estimation of total phenolic content following Folin - Ciocalteu's method (Singleton *et al*., 1999).

The statistical significance of the results on the storage studies were tested using Analysis of variance (ANOVA), AGRES version.7.01. The *P* values (< 0.05) were considered as the level of significance.

RESULTS AND DISCUSSIONS

The initial moisture content of *Knema attenuata* was 31%. Initial increase in moisture content was observed in irradiated seeds against control in seven days of storage which decreased afterwards (Table-1). The accumulation of respiratory moisture over a period of storage might be the reason for observed increase in moisture content of stored seeds in sealed polythene bags. The mycelial growth was also observed. Similar observations were reported in *Aporusa lindleyana* seeds (Kumar *et al.,* 1996) and *Myristica malabarica* (Kumar *et al*., 2002).

Table-1. Percent moisture content of electron beam irradiated *Knema attenuata seeds* (n = 5; Mean ± SD).

Dose (kGy)	**Storage duration** (Days)				**CD** (0.05)	***P* value**
	7	**14**	**21**	**28**		
Control	31.10±0.13	33.87±1.28	32.37±1.02	30.17±0.93	1.70	0.007**
0.5	28.13±1.09	31.80±2.06	30.81±1.48	30.03±1.04	2.78	NS
1	32.81±3.10	31.15±0.92	30.70±1.80	30.44±0.67	3.51	NS
2	32.83±0.39	31.24±2.58	30.98±1.27	30.79±0.71	2.82	NS
3	34.51±0.75	33.58±1.06	33.01±1.16	32.62±0.70	1.70	NS

NS-Non significant, **- Significant

There was 70% germination in the case of un-irradiated seeds stored for seven days, which decreased to 48% after 28 days of storage. After 7 days of storage, seeds exposed to 0.5kGy of EBI showed 10% germination, which failed to germinate on further storage. Although, the seeds irradiated with 0.5kGy showed germination, the developed seedlings were abnormal and could not survive even for a month. There was no germination in the seeds treated with higher doses (1kG, 2kGy, and 3kGy). These results suggest that irradiation has some damaging effect on seeds resulted in the loss of the germination and viability. This was confirmed by the tetrazolium viability test. Seeds of *mucuna pruriens* showed 84% germination after EBI at 2.5kGy, but germination was delayed compared to control (Bhat and Sridhar, 2008). Seeds of *Knema attenuata* found to be more sensitive to EBI where, seedlings couldn't survive after exposure to 0.5 kGy. Dose depended decrease in germination was observed by several other previous workers (Thapa, 2004, Kon *et al*., 2007).

A significant (P< 0.05) initial increase in the protein content was observed in the seeds treated with 1kGy and 2 kGy of EBI compared to the control (Table-2). However, the protein content decreased there after. Increase in protein was also reported in seeds of desi and kabuli chick pea (*cicer arientinum*) genotypes irradiated with gamma radiation (hameed *et al*., 2008). A significant (P< 0.05) decrease in total soluble protein was found both in irradiated and control seeds during storage. The decreased level of soluble protein was attributed to the hydrolysis of proteins during ageing (Basavarajappa *et al*., 1991). Proteins break down and recycling, which depend

on the levels of proteolytic enzyme, are an essential part of the plant response to environmental stress (Hieng *et al.*, 2004). Similarly, in the present study, decrease of protein level in *Knema attenuata* seeds may be attributed to radiation stress.

Table-2. Soluble proteins (mg/100mg) in the seeds of *Knema attenuata* during storage after electron beam irradiation (n = 3; Mean ± SD).

Dose (kGy)	**Storage duration** (Days)				**CD** (0.05)	***P* value**
	7	**14**	**21**	**28**		
Control	0.45±0.03	0.43±0.03	0.41±0.03	0.37±0.02	0.06	NS
0.5	0.46±0.03	0.37±0.02	0.34±0.03	0.30±0.02	0.05	0.008**
1	0.48±0.02	0.41±0.03	0.37±0.02	0.34±0.03	0.04	0.000**
2	0.48±0.02	0.41±0.02	0.38±0.02	0.35±0.03	0.04	0.000**
3	0.35±0.02	0.32±0.03	0.28±0.02	0.24±0.02	0.04	0.001**

NS-Non significant, **- Significant

There was an initial rise in the soluble sugar content in the irradiated seeds compared to control (Table-3). A significantly ($P< 0.05$) higher concentration of soluble sugars was recorded in seeds exposed to 0.5 kGy of EBI compared to control seeds. Cloutier and Cox (1989) observed an initial rise followed by a decrease in the reducing sugar content and sucrose down to the control level after storage of four to five months in irradiated tubers. Similar observations were made in the present study also till the end of 4th week. There was a significant ($P< 0.05$) decrease in the soluble sugars during the storage both in control and irradiated seeds.

Table-3. Soluble sugars (mg/100mg) in the seeds of *Knema attenuata* during storage after electron beam irradiation (n = 3; Mean± SD).

Dose (kGy)	**Storage duration** (Days)				**CD** (0.05)	***P* value**
	7	**14**	**21**	**28**		
Control	3.18±0.12	2.30±0.02	2.29±0.04	1.55±0.05	0.48	0.000**
0.5	4.80±0.08	4.78±0.10	4.71±0.05	4.41±0.10	0.16	0.003**
1	3.70±0.06	3.91±0.02	3.2±0.11	3.15±0.13	0.17	0.000**
2	4.16±0.15	3.11±0.1	1.51±0.08	1.3±0.57	0.25	0.000**
3	4.21±0.16	3.21±0.1	1.5±0.08	1.35±0.45	0.48	0.000**

**- Significant

There was a steady increase in phenolic content in seed exudates during the course of storage both in control and irradiated seeds (Table-4).

Table-4. Changes in total phenolics (µg/ml) in seed exudates of *knema attenuata* during storage after electron beam irradiation. (n = 3; Mean± SD).

Dose (kGy)		**Storage duration** (Days)			**CD** (0.05)	***P* value**
	7	**14**	**21**	**28**		
Control	0.15±0.001	0.17±0.005	0.20±0.006	NA	0.02	0.001**
0.5	0.14±0.001	0.19±0.006	0.24±0.001	NA	0.01	0.000**
1	0.15±0.003	0.19±0.005	0.25±0.002	NA	0.01	0.000**
2	0.17±0.001	0.29±0.003	0.33±0.001	NA	0.02	0.000**
3	0.08±0.001	0.10±0.002	0.12±0.001	NA	0.01	0.000**

NA-Not analysed, **- Significant

A significant (P< 0.05) increase in phenolic content with increase in dose of EBI was observed upto 2kGy. There was a drastic reduction in the phenolic content of the seeds exposed to 3kGy of EBI. Ramamurthy *et al*., (1992) observed the increased formation of potato phenolics during storage of tubers following irradiation. It is believed that irradiation of biological systems can initiate senesce like lipid peroxidation (Ramarathanam *et al*., 1987). Since, irradiation causes rupture of lipid membranes, those authors assumed that cellular membrane damage led to the synthesis of phenolic compounds as part of the mechanism of curing. The increase in the phenolic content during storage and in response to radiation may be because of *de novo* synthesis of phenols in germinating seeds. However, it's difficult to interpret the reduction in the phenolic content in the exudates of the seeds treated with 3kGy at this stage, which warrants more research on these lines.

ACKNOWLEDGEMENTS

This work was financially supported by Board of Research in Nuclear Science (BRNS) in the form of a major project sanctioned to KRC.

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