With usual argument and redoing all steps as described in standard texts[3,5] for a conical horn, the radiation intensity is given by

$$\frac{\mathrm{d}w}{\mathrm{d}t} = \frac{p_{\max}^2}{2\sigma c} = \frac{A^2\sigma c}{2s\Omega}\frac{k^2}{(1+k^2z_0^2)}, \quad (37)$$

where z_0 is a point in the z-direction from the lower apex of the shell, where the opening of the mouth piece is located. The frequency–intensity curve as indicated by the theory is given in Figure 4.

It may be noticed that for the conch shell, $l - z_0$ is very small, may be of the order of less than 1 cm. In this case the response may be steep and the intensity may be uniform for all higher frequencies. This predicts that the ultrasonic frequencies and their overtones, if at all present, will appear with almost uniform intensity.

In this communication, we have presented the problem of propagation of sound waves in the coiled tunnel of a conch shell cavity; set up the Webster's horn equation in circular cylindrical coordinates and solved them. The solutions with application of proper boundary conditions, and physical situation, offer expressions for frequency, which computed with the conch parameters give the correct match with the observed frequency. Existence of the ultrasonic component in the conch spectrum is indicated by the theory. The theory further points out uniform intensity distribution for such high frequency components.

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Studies on Makhana (*Euryale ferox* Salisbury)

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Makhana (*Euryale ferox* Salisbury) grows as an exclusive aquatic cash crop in shallow water bodies in north Bihar and lower Assam regions of India. It has nutritional and medicinal properties and supports cottage industry. It is a monotypic genus and the available genetic variability is limited. An attempt was made to understand the cultural practices, genetic variability among the available germplasm and the biochemical changes during seed germination. It was included in an improvement programme using gamma ray induced mutagenesis. Different morphological parameters were selected to find out its sensitivity to different doses of gamma rays.

Keywords: Makhana, gamma rays, monotypic, mutation.

EURYALE FEROX Salisbury (Nymphaeaceae), known as Makhana, is distributed in tropical and subtropical regions of south-east and east Asia. It grows as an exclusive aquatic cash crop in shallow water bodies in north Bihar and lower Assam regions of India. It has nutritional and medicinal properties and supports cottage industry. It is cultivated in ponds, lakes, tanks and other aquatic bodies. Distribution, ecology, agronomy, biology, pests, production and processing of Makhana have been compiled earlier[1]. The major drawback with Makhana cultivation is that the interlacing ribs of leaves and petioles are prickly. The mature fruits are borne on long pedicels and are difficult to harvest due to the stout prickles on the outer surface. Makhana is a monotypic genus and the available genetic variability is limited. Although it is an important aquatic crop, work on its improvement was not initiated earlier using the conventional breeding and induced mutagenesis techniques. Because it is a monotypic genus, induced mutagenesis is the best available method for its improvement. An attempt was made to test the sensitivity of Makhana to physical mutagen and to induce desirable genetic variability (spineless strain, new better varieties, early flowering/early maturity strains, high yielding variety with increased seed number, increased seed weight, increased seed size, increased fruit number, increased floral stalk, increased berry size, etc.) through induced mutagenesis.

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To initiate improvement work on any crop, preliminary requirement is to understand the cultural practices, germplasm collection and genetic background of the available germplasm. Information on different aspects of Makhana-like strains, germination pattern, characterization on morphological, cytological, physiological, biochemical and molecular characters is scanty. Therefore, an attempt was made to collect information on all the above-mentioned aspects in addition to mutagen sensitivity.

A total of 17 accessions of seeds, maintained at the Makhana Research Centre (ICAR), Darbhanga (Bihar) were collected. Basic information regarding genetic variability of these 17 accessions was not available. Therefore, it was necessary to understand the genetic homogeneity of all accessions using a molecular technique – Random Amplification of Polymorphic DNA (RAPD).

Makhana is an aquatic plant and the seeds germinate when it is inside the water, hence an attempt was made to understand the different biochemical changes at different stages of seed germination. For biochemical analysis, four seedling samples were collected at 10, 16, 22 and 28 days after germination (Figure 1). The different parameters selected for biochemical analysis were protein content, MDA content, POD-, SOD-, GR-, APX- and CAT-activity.

For determination of antioxidant enzyme activities, 0.5 g of the material was homogenized in 1.5 ml of respective extraction buffer in a pre-chilled mortar and pestle using liquid nitrogen. The homogenate was filtered through four layers of cheesecloth and centrifuged at 22,000 *g* for 20 min at 4°C. The supernatant was recentrifuged again at 22,000 *g* for 20 min at 4°C for determination of antioxidant enzyme activities. Protein concentration of the enzyme extract was determined according to Bradford[2].

For superoxide dismutase (SOD) assay, fresh material (1 g) was homogenized in 25 ml polyvinylpyrrolidone (PVP) with a chilled pestle and mortar. The homogenate was centrifuged at 20,000 *g* for 20 min and the supernatant was collected and used for SOD assay following the method of Beyer and Fridovich[3]. Ascorbate peroxidase (APX) was assayed as described by Nakano and Asada[4]. Catalase (CAT) activity was determined spectrophotometrically following the method of Patterson *et al.*[5]. Guaiacol peroxidase (G-POD) activity was measured spectrophotometrically at 25°C by following the method of Tatiana *et al.*[6]. Lipid peroxidation was measured as the amount of malondialdehyde (MDA) produced by the thiobarbituric acid (TBA) reaction, as described by Dhindsa *et al.*[7].

No detailed information is available on the genetical aspects of the genus and about the different accessions that exist in nature. Realizing the necessity of identification of different accessions, 17 different accessions (randomly selected plants from different ponds of Makhana Research Institute, Dharbhanga) were collected and their RAPD patterns were studied.

Total genomic DNA was extracted from young leaves of Makhana by cetyltrimethyl ammonium bromide (CTAB) procedure with some modifications[8]. Extraction in chloroform : isoamyl alcohol (24 : 1) followed by centrifugation twice at 14,000 *g* helped to remove polysaccharides. RNA contaminants in all the samples were digested with 100 mg/ml RNase A for 30 min at 37°C, extracted once with phenol : chloroform : isoamyl alcohol (25 : 24 : 1). After ethanol precipitation, DNA was resuspended in 100 ml of TE (10 mM Tris–Cl + 1 mM EDTA) buffer (pH 8.0). Average yield was calculated using a spectrophotometer (Ultraspec 2000, Pharmacia Biotech) and DNA samples were stored at –20°C.

Thirty arbitrary decamer primers (Bangalore Genei, India) were used for polymerase chain reaction (PCR). PCR reaction was performed in 20 ml reaction mixture containing 5 ng template DNA, 1 unit of *Taq* DNA polymerase, 100 μM dNTPs, 1.0 μM primer, 2.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.01% gelatin. PCR amplification was performed using a PTC-100 Peltier Thermal Cycler (MJ Research, USA) using the following conditions: preheating of 4 min at 94°C; 45 cycles of 15 s at 94°C, 45 s at 36°C and 1.5 min at 72°C and elongation was completed by a final extension of 4 min at 72°C. The final reaction mixture was cooled down to 4°C. After amplification, the PCR product was resolved by electrophoresis in 1% agarose gel with 1× Tris-acetate EDTA (TAE) buffer. Bands were visualized by staining with ethidium bromide (0.5 μg/ml) under UV light and photographed. Only distinct bands were counted for data analysis, and faint bands were not considered. The size of the amplification products was estimated from a 100 bp DNA ladder (sigma). All the reactions were repeated at least twice and only those bands reproducible on all runs were considered for analysis.

Seeds were irradiated with 0, 100, 200 and 300 Gray of gamma rays (^{60}CO radiation source in Gamma chamber 900 model). Treated and control seeds were sown in a glass jar and the germinated juvenile seedlings were transplanted in a pond to determine the most suitable dose of gamma rays for large scale irradiation. Effects on morphological characters were recorded for various vegetative and floral parameters. Colours of foliage, petiole, bud, sepal and petal were compared with the Royal Horticultural Colour Chart[9]. Arnon's method[10] was followed to estimate chlorophyll (*a*, *b* and total) content by using Spectrophotometer Model-2000.

Seeds of Makhana are round in shape and their colour varies from brown to black. Seed coat is approximately 1 mm thick. Seed size ranges from 5 to 15 mm in diameter. Seed is endospermic and has starch in endosperm. Pericarp lies between the seed coat and endosperm. Operculum is clearly visible on the top of the seed and the hilum lies below it.

An attempt was made to study the seed germination in different months (July to March). Seed germination was

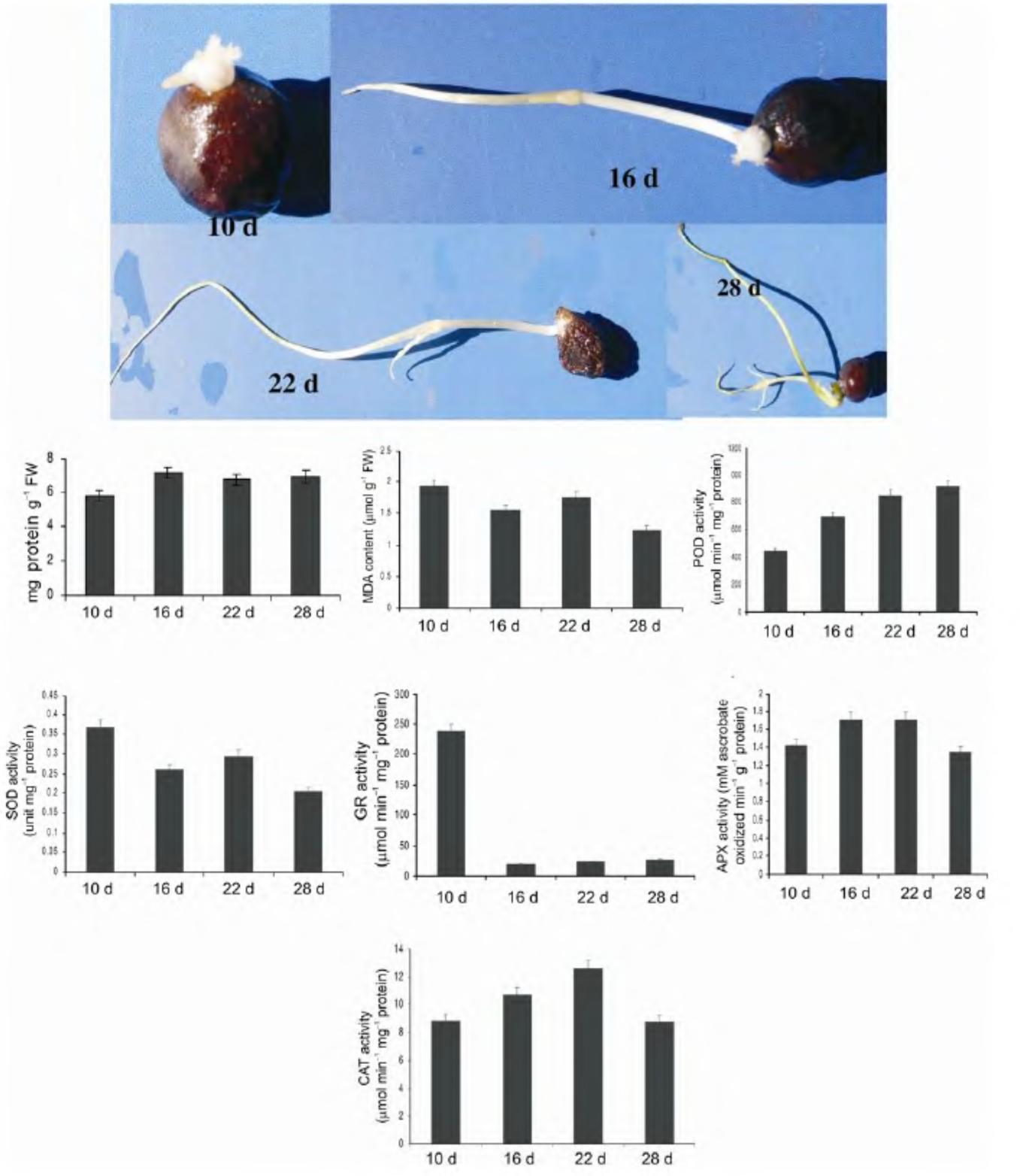

Figure 1. Stages of seed development and antioxidant enzyme activities at each stage.

earlier reported to be 60–70% under Lucknow conditions[11]. Maximum germination was observed during September and October (40–50%) and optimum germination (90%) was recorded during December–January. Germination of Makhana is very peculiar and does not resemble a normal dicot seed germination pattern. The nature of germination is hypogeal and takes two weeks time. Makhana produces five types of leaves during its life cycle.

Germination of seed begins with the formation of white callus type of projection. Development of the juvenile plant starts at the tip of this projection. Some filament-like structures start growing upwards inside the water. This is an indication of formation of the first type of leaf. The number of filamentous leaves varies from 5 to 8 and their colour is brown. At the same time, white roots emerge out from the base of white callus which grows downwards. The number of roots varies from 4 to 7. The 2nd category of leaves resembles the 1st category but it is sagitate, small and coppery. Both 1st and 2nd category of leaves are completely submerged in water. The 3rd category is found on the water surface which is light green and the shape varies between sagitate and orbicular. The 4th category is orbicular in shape having light yellow star at the centre whereas the rest of the lamina is dark green having small purple dark dots on the surface but the ventral side is spineless. The 5th category is permanent in nature; first develops in rolled form and then spreads out and remains throughout the life of the plant. The colour of the leaf is coppery bronze and the shape is orbicular and its surface is quite smooth with purple dots spread all over the surface. It is a good indication of a spine base; rather a marker indicating presence of well-grown spines on its reverse side. The margin is uninterrupted and its base is lobed. The mature leaves are green.

As Makhana is an aquatic plant and the seeds germinate when it is inside the water, an attempt was made to understand different biochemical changes at different stages of seed germination. Protein content was found to increase with age. MDA content and SOD activity decreased after 10 days. POD activity significantly increased after 10 days. GR activity drastically reduced after 10 days. APX activity increased noticeably at 16 and 22 days and then slightly reduced. CAT activity increased at 10–22 days after which it reduced (Figure 1). Lee *et al.*[12] reported that *E. ferox* has high levels of 1,1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging activity, inhibits lipid peroxidation, promotes cell viability, protects H_2O_2-induced apoptosis and enhances the effects of various antioxidant enzymes. Their findings strongly suggest that *E. ferox* has antioxidant activity.

PCR amplification of total genomic DNA from 17 accessions using 30 random decamer primers was carried out. Each primer yielded a wide array of strong and weak bands. However, only the data from 10 primers that gave reproducible product formation were included in the statistical analysis. Out of 30 primers, 10 produced 2–7 DNA bands per primer suitable for data analysis. However, no distinct variation was observed among the accessions tested in the present experiment, indicating homogeneity of all available accessions (Figure 2).

Seed germination was delayed with increase in gamma radiation and the delay was significant ($P < 0.001$) after treatment with 200 and 300 Gray. Percentage of seed germination decreased significantly ($P < 0.001$) after treatment with all the doses of gamma rays. Reduction in survival was recorded in all the treatment doses and the reduction was maximum in 300 Gray where only 20% plants survived. LD_{50} on survival basis was determined between 100 and 200 Gray of gamma rays. Significant ($P < 0.01$ and $P < 0.001$) reduction in plant height was recorded after treatment with 200 and 300 Gray. Control leaves were normal as mentioned here. Number of leaves decreased with increase in radiation doses. Significant ($P < 0.01$) reduction in leaf size was observed in highest exposure of gamma rays. In treated populations, there were different types of abnormalities in leaves. The leaf abnormalities included changes in shape and size, i.e. notching, asymmetrical development of leaf lamina, unequal development of leaf lobes, entire margin, red pigmentation on leaf surface, etc. Percentage of abnormal leaves and plants increased with increase in exposure to gamma rays. Number of spines on mature leaf surface increased at lower dose. However, it decreased after treatment with 200 and 300 Gray (Table 1). There was no change in leaf colour after gamma irradiation.

There was no flower formation after treatment with 300 Gray. Days to flower bud initiation, first colour shown and full bloom was significantly ($P < 0.001$) delayed after radiation. Size of sepals, petals and spines on sepals reduced, in some cases significantly ($P < 0.01$ and $P < 0.001$), after gamma irradiation and with increase in exposure. Gamma radiation induced floral abnormalities like changes in shape and size of flowers, numerical alteration in petals and stamens, narrower and/or twisted petal, obtuse petal tips, intensity of petal colours, etc. There was no change in sepal and petal colour. However, one plant from 100 Gray treatment showed lighter petal colour (Violet Group 86 A, Fan-2) in comparison to control (Violet Group 86 B, Fan-2a). Pollen grain sterility increased significantly ($P < 0.001$) after irradiation.

Seed production in Makhana was affected after gamma irradiation. Weight of raw seed with and without cover reduced after irradiation and significantly ($P < 0.01$) reduced after 100 Gray. Slight decrease in seed diameter was observed after 100 Gray exposure to gamma rays (Table 1). It was interesting to note that the seed coat weight decreased after irradiation and with increase in

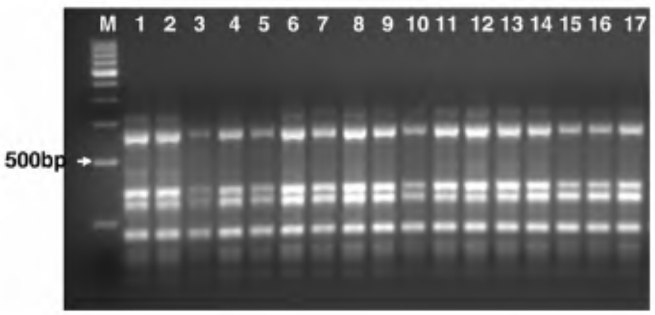

Figure 2. RAPD analysis of 17 accessions of Makhana.

Table 1. Effect of gamma irradiation on different characters of Makhana (*Euryale ferox* (Salisbury))

Characters		Control	Treatment gamma ray (Gray) 100	200	300
Days to seed germination		23.25 ± 4.67	27.33 ± 5.17	38.3 ± 2.02***	42.50 ± 4.02***
Germination percentage		57.3 ± 2.85	37.2 ± 3.22***	21.7 ± 4.60***	18.7 ± 4.30***
Mode of germination		Hypogeal	Hypogeal	Hypogeal	Hypogeal
Percentage of survival		100.00	60.00	40.00	20.00
Plant height (cm) ± SE		55.5 ± 2.10	54.6 ± 0.88	33.2 ± 3.24	23.2 ± 2.24***
Percentage of abnormal plant		0.00	10.00	24.00	50.00
Leaf number ± SE		34 ± 3.20	30 ± 2.15	20 ± 2.15	18 ± 2.15***
Leaf size (cm) ± SE	Length	24.6 ± 0.95	24.2 ± 1.28	20.2 ± 1.28**	14.2 ± 1.28***
	Width	23.3 ± 1.28	25.3 ± 1.76	22.3 ± 1.76	15.3 ± 1.76***
	Petiole	53.25 ± 1.25	51.8 ± 1.10	44.8 ± 1.10***	31.8 ± 1.10***
Number of spine on mature leaf surface		139.0 ± 18.6	169.6 ± 18.8	128.8 ± 19.0	113.8 ± 19.0
Percentage of leaf abnormalities		0.00	30.00	38.00	46.00
Seed weight with seed coat (g) ± SE		0.93 ± 0.08	0.77 ± 0.19	0.86 ± 0.06	0.00
Seed weight without seed coat		0.67 ± 0.09	0.57 ± 0.02***	0.85 ± 0.06	0.00
Seed colour		Brown group 200B, Fan-4	Black group 202A, Fan-4	Brown group 200B, Fan-4	Absent
Diameter (cm) ± SE		2.91 ± 0.46	2.93 ± 0.22	2.85 ± 0.03	0.00
Weight of 10 popped seeds (g) ± SE		0.29 ± 0.05	0.28 ± 0.04**	0.29 ± 0.05	0.00
Number of days for flower bud initiation		145 ± 1.45	154 ± 1.47***	164 ± 1.68***	–
Number of days for first appearance of colour		151 ± 1.38	164 ± 1.23***	171 ± 1.56***	–
Number of days for full bloom		156 ± 1.65	171.1.45***	178 ± 1.72**	–
Sepal length (cm) ± SE		3.44 ± 0.22	2.39 ± 0.11	2.37 ± 0.20	–
Sepal width (cm) ± SE		1.30 ± 0.14	1.09 ± 0.14	0.98 ± 0.08	–
Length of sepal spine (cm) ± SE		0.83 ± 0.07	0.53 ± 0.22	0.65 ± 0.08	–
Spine number on sepal surface		15.8 ± 1.8	18.4 ± 1.2	15.6 ± 0.85	–
Petal length (cm) ± SE		3.15 ± 0.11	1.84 ± 0.04***	1.62 ± 0.04***	–
Petal width (cm) ± SE		1.21 ± 0.03	1.14 ± 0.03	0.55 ± 0.01	–
Pollen grain sterility (%)		20.50	22.90***	32.00***	–
Sepal number		4.0 ± 0.01	3.69 ± 0.13	4.0 ± 0.01	–
Petal number		31.4 ± 2.16	31.04 ± 0.73	33.4 ± 2.97	–
Androecium number		79.0 ± 12.2	91.9 ± 3.84	89.2 ± 15.0	–

**$P < 0.01$; ***$P < 0.001$.

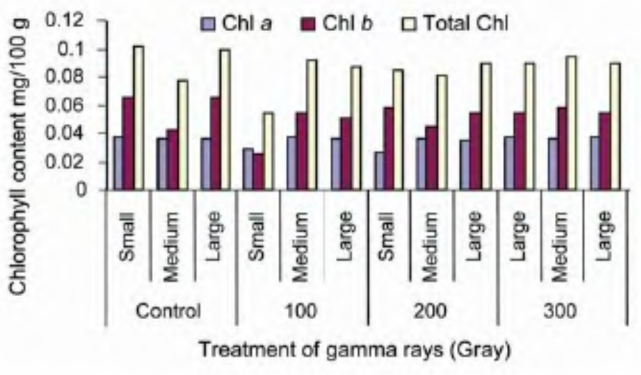

Figure 3. Chlorophyll content of control and irradiated population of Makhana.

doses. Ten popped seed weight was almost the same in control and treated populations. Nutritional value of control and 100 Gray treated seeds was estimated[13]. Protein (%), fat (%), carbohydrate (%), total ash (%), calcium (mg/100 g) and iron (mg/100 g) in control and 100 Gray (in parenthesis) treated seeds were 9.47 (9.49), 0.41 (0.59), 83.46 (83.73), 0.31 (0.29), 157.6 (177.8) and 1.31 (1.31) respectively. Present total ash was decreased after irradiation.

Chlorophyll content (Chl *a*, Chl *b* and total) was estimated from different types of Makhana leaves (young, medium and mature) from control and irradiated population. Gamma irradiation was found to be effective and both increase and decrease in Chl *a*, Chl *b* and total chlorophyll content were recorded in the treated population (Figure 3).

Radiosensitivity test is a prerequisite step before the large scale mutagenic treatment is started for crop improvement. The main purpose of this test was to study the most effective dosage of irradiation to be used and also to determine the induced genetic variability of different characters using gamma irradiation. Different characters of Makhana were found to be sensitive to gamma rays. Variants from the present experiment are being maintained to study their performance in subsequent generations. Thus, the present results clearly indicate that

gamma irradiation can develop new varieties of this crop with improved commercial traits.

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Effects of plot size and shape on the encounter rate of ungulate faecal pellet groups and abundance estimate precision

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Several pellet-plot (sampling unit) designs have been used by different workers to mitigate the underlying problems in the form of missed pellet groups on the larger plots or the interpretational error of the observer. We conducted a comparative study on four sympatric ungulate species in the Keoladeo National Park, Bharatpur, India. We explored different shapes and sizes of the sampling units, including groups lying on the plot boundary. We found a 5 m radius circular plot ideal (least percentage CV = 194) in comparison to circular plots of other sizes as well as their corresponding quadrat plots of the same area. Density estimates were systematically biased by plot size and shape, where smaller plots and higher edge-to-area ratios were found to have higher density estimates. We emphasize upon the inclusion of the pellet groups lying on the plot boundary to minimize the error caused by excluding them from the counts.

Keywords: Density, encounter rate, pellet-plot, strewn pellets, ungulates.

PELLET-GROUP counting is one of the several methods being widely used by field biologists to estimate the actual or relative abundance of animals[1–5] and by deer managers to take decisions relating to annual culls[4,6–9]. Two commonly used categories, faecal standing crop (FSC) and faecal accumulation rate (FAR)[4,9–11], have been studied well for their relative accuracy, precision and cost-effectiveness[12]. However, choice of the basic unit and the strewn condition of different pellet groups have not been discussed in detail. In this study, we have considered size and shape of the pellet plots and the strewn conditions associated with the pellet groups. Smith[13] identified two problems associated with the choice of plot size – missed groups and sampling efficiency. The error associated with the missing groups is the most important problem that affects pellet-count estimates. Several workers have documented evidence of bias resulting due to the missed groups, which is influenced by plot size and shape. Factors such as concerning peripheral groups, scattered groups and the minimum number of pellets to be counted as a group are the major sources of interpretational differences[1,14]. It is well known that larger plot sizes exhibit

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