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Effect of gamma radiation on mutagen sensitivity and mutability in field pea (*Pisum sativum* L.)

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Pea (*Pisum sativum* L.) is one of the earliest known human foods that thrives well in places with cool climate; in India, it is grown in an area of 0.77 million ha with an annual production of 0.71 million tonnes and average productivity of 915kg ha^{-1} (www.faostat.fao.org). It is rich in protein, carbohydrate and digestible nutrient content but low in fibre content, which make it an excellent livestock feed.

Most of the pea growing area in our country is occupied by traditional and high yielding varieties of field pea which suffers from some constraints like late maturity, lodging, susceptibility to rust *etc*. Induced mutations may play a vital role for the improvement of pea variety. Induced mutations offer possibility for the induction of desired changes in various attributes, which can be exploited as such or through recombination breeding (Akbar and Manzoor, 2003; Khin, 2006).

A large range of chemical and physical mutagens have been investigated for their use in crop improvement. Physical mutagens, specially the ionizing radiation, have been widely and routinely used to generate genetic variability in various crop species including pulses (Tomlekova, 2010).

MATERIALS AND METHODS

The study was carried out at Crop Research Centre, G. B. Pant University of Agriculture and Technology, Pantnagar (29^0 N latitutde, 79.30^0E longitude and at an altitude of 243.84m above the mean sea level) in three consecutive *rabi* seasons.

Plant materials and their basic characteristics

HFP4- A popular bold seeded (100 seed wt.-20g) variety was developed at CCS HAU, Hisar from a cross between EC109196 and T163. It is late maturing (145days), dwarf, semi-leafless, powdery mildew resistant but susceptible to rust disease; gives average yield of 26q ha^{-1}.

DDR13- A bold seeded (100 seed wt.-18g) variety was developed at IARI, New Delhi from a cross between P965 and PH429. It is medium duration (125-130days), dwarf, powdery mildew resistant, but susceptible to rust disease; gives average yield of 24q ha^{-1}.

There are different types of ionizing radiation (*viz*., X rays, gamma rays, protons, neutrons, alpha and beta particles) but gamma rays are widely employed for mutation studies as they have shorter wave length and therefore, posses more energy per photon than X rays and penetrate deep into the tissue (Khin, 2006; Zhou *et al*., 2006).

In order to induce variability for efficient plant breeding, systematic study of mutagenic sensitivity and/or mutability of various crop plants and different cultivars within a crop are essential (Brock, 1971). Although studies have been made on the biological effects of radiations and the relative mutagens sensitivity in lentil (Sharma and Sharma, 1981), mung bean (Ignacimuthu and Babu, 1989), urd bean (Singh *et al*., 1999) and chickpea (Kharkwal, 1998), such reports are limited in field pea. Therefore, in the present investigation, an attempt has been made to study the mutagen sensitivity in the two high yielding varieties of field pea. The similarities or differences between genotypes with regards to radio-sensitivity, radio-mutability and the relationship between mutagen sensitivity and mutability following gamma treatment to those bold seeded field pea varieties were worked out.

Dry, uniform and healthy seeds of these two cultivars of field pea (HFP4 and DDR13) were irradiated with different doses (5, 10, 15 and 20 kR) of gamma rays using ^{60}Co gamma-chamber 4000 (B.A.R.C., Trombay, India) at a dose rate of 2.368 kR per minute. Four hundred seeds were taken for each treatment as well as for control. Irradiated seeds (M_0) along with the controls (un-irradiated) were sown (15 days after the exposure) in the field (treatment and variety wise) in a Randomised Block Design with three replications in two rows plot of 5m length keeping plant to plant and row to row distance of 10 and 30 cm., respectively. Before flowering, 10 plants from each row of the each treatment in the M_1 generation were covered with muslin cloth bags to ensure selfing. Seeds of the selfed plants were harvested separately while seeds from the rest of the material under each treatment were bulked (M_2

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populations). Plant to progeny from selfed seeds were grown in separate rows of 3m length, and bulked seeds of each treatment were sown in 5 rows of 3m length along with the control in RBD (M_2 generation). Seeds from plant to progeny rows were harvested separately and seeds from bulk populations under each treatment were harvested as bulk (M_3 populations) and were grown in the next season following the same pattern followed in the M_2 generation to constitute the M_3 generation. Germination (%), pollen fertility (%), plant survival (at maturity), root length, shoot length were studied in M_1 generation. Bulk populations were used to record data on quantitative traits, whereas individual plant progenies were used to record qualitative characters (chlorophyll and other morphological mutations).

Data on germination on each treatment were recorded after 20 days of sowing and germination percentage was calculated. For recording pollen fertility, flower buds (collected from 10 plants in each replication under each dose) were kept in the Carnoy's fixative (3 part ethyl alcohol+ 1 part acetic acid) for 24 h. The material was then transferred to 70% ethyl alcohol and kept in refrigerator until subsequent study. Study on pollen fertility was done by using 2% iodine solution. Pollen grains stained were considered fertile while deformed or poorly stained or translucent ones were sterile. Pollen counts were taken under the stereoscopic microscope from five randomly selected plots and fertility percentage was calculated. Seeds from M_1 plants were grown in order to score the chlorophyll mutations in M_2 generation. The frequency of chlorophyll mutations was calculated as percentage of families segregating for any type of chlorophyll mutation (M_2 family basis) as well as the percentage of chlorophyll mutants in the population of a particular treatment (M_2 mutant basis).

Characters such as root and shoot length of 7-day old seedlings grown at 25 ± 1^0C temperature and 97% relative humidity in the laboratory, germination at 20^{th} day in the field, plant survival at maturity and pollen fertility were used to assess the dose response. Since the doses requirement for inhibition of shoot length and root length was very high (beyond 20kR), ID_{30} was calculated instead of ID50 value using Probit analysis (Finney, 1971) after Abbott's (1925) correction of each treatment with respect to control response. Besides these, correlation coefficients were computed to study the genetic damage caused by the mutagens. Level of sensitivity of both the cultivars was also compared with the help of paired-t test (Gomez and Gomez, 1984).

This dose-response may vary for different biological parameters considered for bioassay test. Sensitivity of the test materials may also vary even against a similar dosage. This indicates that some samples may show less response while others show more response than the average response against a particular dose. The average response can be found out from regression line, Y= a+ bX (Y= respone; X= dose), by using probit analysis. In the present investigation, the average response for germination, pollen fertility and plant stand at harvest were observed from median inhibition doses (ID_{50}), which fluctuated between a confidence interval. The upper and lower limits of that interval are known as upper fiducial and lower fiducial, respectively. These two limits can be expressed as m ± t x SE (m), where 'm' indicates log ID_{50}.

RESULTS AND DISCUSSION

Mutagen sensitivity

In order to determine irradiation doses, it is very useful to understand general radiation sensitivities of plants against radiation. Radiation sensitivities of plants differ greatly not only among plant species, but also within plant materials (seeds, plantlets, tissues, etc.). Irradiation dose should be carefully determined according to the kinds of ion species and energies, plant species, plant varieties, plant state of materials such as cell cycle, and water content (Magori *et al*., 2008).

In the present investigation, it has been found that germination, root and shoot length, pollen fertility and plant survival decreased progressively with increasing doses of gamma-rays in both the varieties of field pea under study. This is indicative from the significant correlation coefficients of biological parameters with the doses of gamma rays and genetic sensitivity of the germplasms to mutagen is clearly understood from the steepness of the probit lines (Fig.1). Similar results have been reported in other leguminous crops.

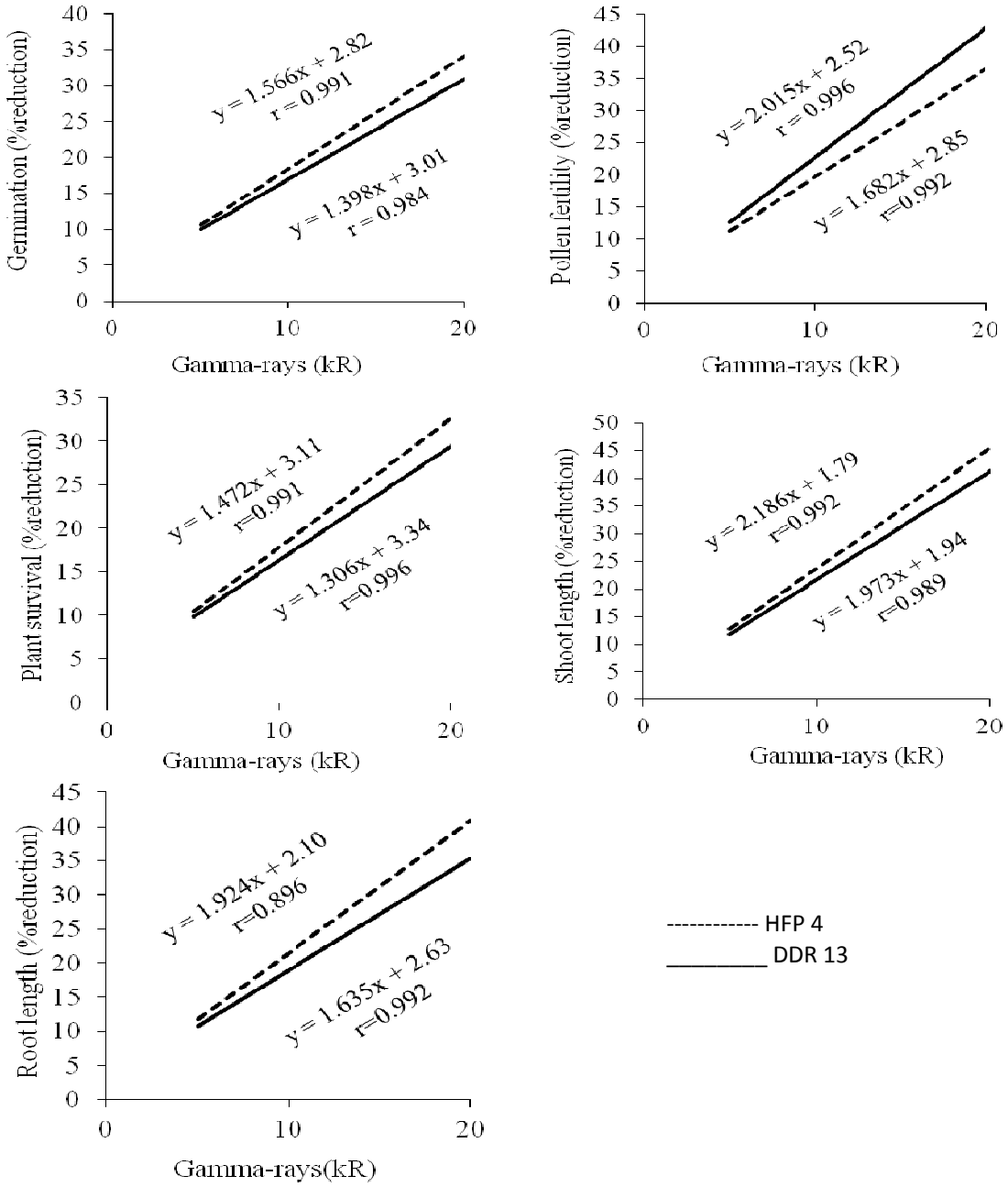

Fig.1. Effect of gamma-rays on different parameters in field pea in M_1 gene

Perusal of table-1 reveals the different ID_{50} along with their fiducial values for different biological parameters in M_1 generation. Reduction in the 50% germination (ID_{50}) occurred at 24.44 kR gamma rays in the cultivar HFP4, whereas slightly higher dose (26.47 kR) was needed for the cultivar DDR13. Another immediate effect of irradiation in the M_1 generation was also observed through reduction in pollen fertility for which ID_{50} being 18.78 kR for HFP4, and 16.84 for DDR13. Similar trends were also observed by Davies (1973), Blixt and Gottschalk (1975), Brock (1980), Ignacimuthu and Babu (1989), Akbar and Manzoor (2003). Chromosomal aberrations, changes involving DNA and/or RNA synthesis, meiotic abnormalities might be the causes of reduction in pollen fertility owing to radiation.

Positive and highly significant correlation between chromosomal abnormality and pollen sterility (r = 0.82- 0.98) has been reported in mung bean (Ignacimuthu and Babu, 1989).

The ID_{50} for plant survival was 18.36 kR in HFP4 and 18.99 kR in DDR13, which is almost similar to ID_{50} for reduction in pollen fertility. In the case of shoot and root length, doses higher than 20kR were required to obtain 50% reduction. The ID_{30} for shoot length in HFP4 and DDR13 being 16.79 kR and 18.99 kR, respectively, and for root length 16.55 kR and 17.21 kR, respectively. Paired-t east for comparison of different kinds of biological responses due to mutagens between the germplasms proved significant (Table1). This indicates that the germplasm HFP4 was more sensitive at different level of mutagen than DDR13. In the present investigation, it was observed that retardation in the root length was more pronounced than that found in the shoots. The root system appears to be relatively more sensitive to gamma rays. This can possibly be due to an inhibition of division in root cells by mutagens, which exert less effect on the elongation of shoot cells. The shoot growth is reported to be mainly due to the cell elongation while root growth is more dependent on cell division. A greater delay in rooting than that of shooting was observed in *Kalanchoe diagremntiana* leaves by Mishra *et al.* (1980) following irradiation with gamma rays.

In the present investigation, it was found that for germination, plant survival at maturity, shoot length and root length, the cultivar HFP4 was more radiosensitive than the cultivar DDR13, whereas for pollen fertility, the cultivar DDR13 was more radiosensitive than HFP4. It appears from the above results that mutagen sensitivity, at least for these cultivars, is independent of the genotypic background as well as of biological parameters under investigation.

Table 1: Median inhibition dose (ID_{50}) and radio-sensitivity of two field pea genotypes for different biological parameters in M_1 generation

Biological parameters	Genotype		Radio-sensitivity	% reduction over control
	HFP4	DDR13		Paired-t value
Germination	24.44 (18.78-41.36)	26.47 (19.49-52.37)	HFP4> DDR13	3.523*
Pollen fertility	18.78 (15.37-26.27)	16.84 (14.37-21.18)	DDR13 > HFP4	3.565*
Plant survival	18.36 (14.39-29.39)	18.99 (15.16-28.65)	HFP4>DDR13	3.445*
Shoot length#	16.79 (8.04-35.07)	18.99 (7.83-46.05)	HFP4> DDR13	3.647*
Root length#	16.55 (6.54-41.85)	17.21 (7.07-41.92)	HFP4>DDR13	3.333*

Note: #ID_{30} (kR) value calculated; Data in parentheses indicate fiducial low and up values (kR), Significant at 5% level*

Mutability

Mutability in terms of chlorophyll mutations, serves as an important index in the estimation of genetic damage caused by the mutagenic agents. In the present investigation, the mutability (calculated as percentage of mutated progenies) of the cultivar DDR13 (Table 2) induced with different doses of gamma rays was lower (6.3-12.5) than that of HFP4 (8-13.5). Similarly, radio-mutability expressed as percent mutants in DDR13 was less (0.58-2.75) than that of HFP4 (1.20-3.14). It is also evident from the data (Table 2) that differential response of genotypes *i.e.* marked varietal differences were present in terms of induction of chlorophyll mutations at different doses of gamma rays. The highest frequency of chlorophyll mutations (3.14%) was induced at 5 kR in HFP4, while 15 kR gamma rays induced highest frequency (2.75%) in the cultivar DDR13. The result also reveals that the mutagen reached its saturation point even at lower doses in the highly mutable genotype of HFP4, and further increase in dose did not add to the mutation frequency. However, no chlorophyll mutations was noticed at 5 kR in DDR13, and chlorophyll mutation rate increased with an increase in the dose of mutagen up to certain level, beyond which it decreased or remained constant (Table 2). Thus induction of chlorophyll mutations was independent of different doses of gamma rays as this occurred at random. Similar observations were also made by Sharma and Sharma (1981), Sarkar and Sharma (1989) in lentil and by Kharkwal (1998) in chickpea. In general, relative differences in mutability of genes for chlorophyll mutations with different doses of mutagen as well as different genotypes were clearly observed. The phenomenon can be attributed to the intra-somatic selection, genetic background, nuclear volume and reduction in the number of M_2 plants produced by high sterile M_1 plants and other processes of gamete as well as zygote elimination.

Table 2: Chlorophyll mutation frequency of two field pea genotypes in M_2 generation

Mutagen treatment	Mutant (%)		Mutagen progenies (%)	
	HFP4	DDR13	HFP4	DDR13
Control	0.00	0.00	0.0	0.0
5kR	3.14	0.00	13.5	0.0
10kR	2.05	0.58	10.3	6.3
15kR	1.20	2.75	8.0	12.5
20kR	1.57	1.77	9.5	12.5

Note: HFP4 and DDR13 are two field pea genotypes

The root system was more sensitive to the mutagen than the shoot. The cultivar HFP4 showed more sensitivity to any level of mutagen than DDR13 for several biological characters except pollen fertility. Chlorophyll mutations showed independent response to gamma rays as they occurred at random, and the mutability (expressed as % mutated progenies as well as % mutants) of the cultivar DDR13 induced with gamma rays was lower than that of HFP4. Due to saturation in the mutational events in the biological material, the mutation frequency either decreased or remained constant at higher doses of the mutagens.

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