RESEARCH ARTICLE

Effect of Gamma Irradiation on Floral Morphology and Pollen Viability in Two Varieties of Pea (*Pisum sativum* L.)

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ABSTRACT

Mutation breeding by using radiations has been proved to be an important tool in introducing different desirable characters of agronomic values. Irradiation of plant materials by gamma rays is widely used to induce mutations at the genetic level which encounter with number of biochemical processes leading to the desirable changes in the genotype. Seeds of two varieties of Pea i.e. *P. sativum* var. *hortense* (garden pea) and *P. sativum* var. *arvense* (field pea) were exposed to different doses of gamma irradiation to evaluate the effect on morphological, floral characters and pollen viability. Gamma irradiation mostly has adverse effect on floral characters and pollen viability. It has been apparent from the data that different floral parts in both the varieties are showing dose dependent reducing trends. Lower doses of gamma irradiation are found to be enhancing the number of pollen grains produced in the anther. Pollen-ovule ratio has shown significant increase in var. *hortense* except in 200 Gy dose. Data on present studies has reveled that genotype of var. *arvense* is more sensitive to gamma irradiation than var. *hortense*.

Keywords: Floral characters, pollen viability, irradiation, sensitivity, mutagens, genotype.

INTRODUCTION

Pisum, an important genus of family Papilionaceae comprising seven species with their different cultivars cultivated all over the world. It is one of the important pulses, and many cultivars are mostly grown as a vegetable crop for its richness in protein content. Methionine, cysteine and tryptophan content of dry peas are reported to be 0.30, 0.124 & 0.039 %, respectively (Gupta and Das, 1955). It has more carbohydrate content (56.6%) than protein (19.7%) (Cherian *et al.*, 1955). The extracted pea oil has non-toxic and medicinally important bioactive compound, m-xylohydroquinone that is reported to be used to diminish spermatogenesis in males, and has not been reported to have an abortifacient action. According to the medicinal studies carried out by Sanyal (1960), the compound can also be used as an oral contraceptive. Similarly, the extract obtained from testa of pea seeds is a potent hyperglycemic and reported to be effective in lowering blood-sugar level (Mukerji, 1957).

Pisum is the pioneer material used for studying pattern of inheritance of characters by Mendel. Later, the genetics of this plant has been worked out by several workers. The crop is cultivated annual herb. Many cultivars are cultivated in western Maharashtra and Vidarbha region since its suitability in agroclimatic conditions of these geographical areas. Argonomically two main kinds of peas are known to be cultivated in different parts of the country. These are *P. sativum* var. *hortense* (garden pea) and *P. sativum* var. *arvense* (field pea). *P. sativum* var. *hortense* has no leaves but with large foliaceous and green stipules and all leaflets are modified into tendrils. Calyx consists of two types of unequal sized sepals i.e. upper two small and lower three large. Corolla is papilionaceous consisting five unequal petals i.e. standard, two wings and two keels. Flower size is bigger in var. *hortense* than in var. *arvense.* Androecium consists of diadelphous stamens (9) + 1, while gynoecium is monocarpellary with flat ovary. Length of stamens and carpel is greater in var. *arvense* than in var*. hortense.*

The physical mutagens, particularly, gamma irradiation mostly reported to cause gross structural changes in the morphology of chromosomes and leads to induce alteration in various morphological, floral and yield attributing characters. Earlier reports on induced mutation by gamma irradiation showed adverse effect on pollen viability and biochemical characters of various plants. The changes induced by mutagens, particularly, the primary injuries caused due to physiological damage are restricted to M_1 generation only and can be observed in more frequency. Some of them may get corrected through the auto repairing mechanism of the genetic material, but very few of them can be transferred to next generations, and can show stability over successive generations to yield a mutant plant with desirable characters. Selective mutagenic treatments with low physiological effects and strong genetical effects are desirable in producing the mutant with altered characters of agronomic importance. Earlier reports on mutation breeding suggest that the mutation spectrum with chemical mutagens is greater than physical mutagens in *Pisum*. *Pisum* is one of the most highly exploited crop plants frequently subjected to the breeding programmes for one or the other argonomically important characters. Fierlinger *et al*. (1966) recorded that eight different varieties of pea are reported to be more sensitive to gamma irradiation. The present investigation has been undertaken to assess the sensitivity of gamma irradiation and to induce the alteration in the genotypes to enhance the variability of agronomic traits in both the varieties.

MATERIALS AND METHODS

The present investigation on mutation breeding in pea was carried out during 1998 to 2000 at research field, department of Botany, Vidharbha Mahavidyalaya, Amravati (MS). The seeds of both the varieties were borrowed from Pulses Research Unit, Post Graduate Institute, Dr. Panjabrao Krishi Vidyapeeth, Akola (M.S.). The seeds of both varieties were screened and selected for their uniform size and healthy status, and were exposed to different doses of gamma irradiation from 50 Gy to 250 Gy, at an interval of 50 Gy, from Co^{60} source of 'gamma chamber', USIC, then Nagpur University, Nagpur. 100 seeds from each sample of different doses were kept for germination to decide LD_{50}. LD_{50} was determined based on % germination count, root length, shoot injury and root/shoot ratio. 150 Gy dose was found to be LD_{50} for both the varieties. Two lower doses (50 & 100 Gy) and two higher (200 & 250 Gy) were selected for final experimentation. 100 seeds from each dose of gamma irradiation and control of both the varieties were sown in experimental plot in triplicate sets, to raise the M_1 population. The data on following parameters was recorded from twenty five randomly selected healthy and mature plants from control and irradiated population, at regular intervals. All obtained data was subjected to the statistical analysis for mean and standard deviation by using Graphpad Prism 4 software.

Floral Characters

Quantitative data on number of flowers/plant, length of pedicel from three different flowers/ plant was measured. The floral characters such as dimension (length and breadth) of small and large sepals, three different types of petals, etc., as well as the characters associated with reproductive whorls viz., length of stamens and carpels, number of ovules/ ovary was observed and recorded from three different flowers/ plant, from both control and irradiated population.

Number of pollen grains/anther and pollen viability

Fully matured anthers of randomly selected 25 plants from each dose were collected. To determine pollen production per anther, three anthers from each flower before dehiscence were dissected on slide. Smears of those anthers were prepared in 1:1 mixture of glycerin and 45 % acetocarmine stain (Alexander, 1969). Pollen stainability as an index of viability/fertility was considered to record pollen viability. Shrunken, unstained and partially stained pollen grains were considered as sterile, while well stained, well-filled pollen grains were considered as fertile or viable. % frequency of pollen viability was calculated by using following formula.

$$\text{\% Pollen viability} = \frac{\text{No. of viable pollen grains}}{\text{Total no. of pollen grains scored}} \times 100$$

RESULTS AND DISCUSSION

Primary injury to plant material due to gamma irradiation is a physiological damage which is mainly restricted to M_1 generation. It can be identified and measured cytologically as well as from the response of the whole organism, especially growth retardation and at higher doses, the death (Sparrow, 1961). In the present investigation, 150 Gy dose was found to be LD_{50}. From the results of the present study, it is obvious that normal structure of floral parts of the plants in both the varieties was significantly disturbed due to gamma irradiation.

Floral Characters

The quantitative changes in characters of reproductive parts were observed in details, and the comparative data is depicted in table 1 & 2. The number of flowers and pedicel length was less in var. *hortense* than in var. *arvense*. A gradual and dose dependent decrease was observed with respect to these parameters, but it was found more significantly affected at higher doses of 200 and 250 Gy. Number of flowers in control plants of var. *arvense* and var. *hortense* was 37.00 and 28.33, respectively. But it was found reduced to 14.00 and 9.33 at 200 and 250 Gy doses respectively, in both the varieties. Similar reduction in number of flowers has been observed by Verma *et al*. (1999) in gamma irradiated population of *Lens culinaris.* Dose dependent reduction in length of pedicel of the flower also indicates the adverse effect of gamma irradiation on the morphological characters.

A floral morphology is unique and typical in family Papilionaceae. Calyx consists of two unequal sized sepals i.e. the upper two small and the lower three comparatively large, in both the varieties. A sizable difference in the sepals of two varieties under investigation is clearly observed. The length of both the small and the large sepals in both the varieties is approximately similar, but are showing differences in their breadth only. The lower doses i.e. 50 and 100 Gy had an enhancing effect on the size of both the types of sepals in var. *hortense*, while it was increased at 50 Gy dose only in var. *arvense*. A slight negative deviation has been recorded at the intermediate doses (100, 150 & 200 Gy) of gamma irradiation than control, but it was found significantly reduced at higher dose, 250 Gy, in both the varieties.

A papilionaceous corolla consists of three morphologically distinct and unequal sized petals viz., anterior, large-sized standard, two lateral medium-sized wings, and posterior two, small-sized keels. The dimension of all types of petals in var. *arvense* is greater than var. *hortense.* The dimension of all types of petals (standard, wings and keels) observed to be decreased gradually from lower to higher doses, in var. *hortense*, but was increased slightly in 50 Gy dose, in var. *arvense.* Negative response observed in petal size was found to be dose dependent. Tarar & Dnyansagar (1980) reported the significant reduction in regards to the size of floral parts in *Turnera ulmifolia* L. They have also noticed the formation of overall small-sized flowers in the mutagen treated population. The modifications in floral morphology caused by radiations have been reviewed by Gunckel & Sparrow (1953). They observed the modifications in floral parts due to irradiation and opined that the decrease or enlargements in floral parts are of common occurrence in irradiated plants. These workers attributed many of these positive and negative changes to the physiological or biochemical disturbances. Gupta and Samata (1967) noticed highly dissected flowers in *Cosmos* when irradiated with gamma rays. Giant flower mutants have been reported by early workers in mutagenized population of pea, tomato and barley. Sjodin (1971) reported significantly large-sized flowers in *Vicia feba*, showing all other morphological characters normal. The enhancement in the dimension of petals in both the varieties at lower doses, in the present investigation can be correlated with the flower mutant reported by Sjodin (1971).

Androecium consists of diadelphous stamens (9)+1, while gynoecium was monocarpellary with flat ovary. The var. *arvense* showed comparatively larger length

Table No. 1. Effect of different doses of gamma irradiation on some floral characters and pollen viability in *Pisum sativum var. hortense*

Dose (Gy)	Number of flowers	Length of Pedicel.	Sepals				Petals						Length of stamens	Length of carpel	Number of ovules/ ovary	Number of pollens/ anther	Pollen viability	P/O ratio
			Small		Large		Standard		Wing		Keel							
			Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth						
Control	**28.33** ± 0.577	**2.46** ± 0.042	**0.90** ± 0.015	**0.29** ± 0.015	**0.92** ± 0.005	**0.42** ± 0.005	**1.42** ± 0.010	**1.61** ± 0.020	**1.41** ± 0.015	**0.51** ± 0.015	**0.86** ±0.020	**0.63** ±0.020	**0.63** ±0.015	**0.44** ±0.005	**9.00** ±0.000	**391.33** ±5.033	**84.59** ±1.522	**43.48** ±0.556
50	**24.67** ± 1.528	**2.24** ± 0.042	**0.92** ± 0.010	**0.31** ± 0.005	**0.93** ± 0.005	**0.44** ± 0.005	**1.41** ± 0.011	**1.59** ± 0.017	**1.38** ± 0.015	**0.51** ± 0.032	**0.84** ±0.020	**0.62** ±0.026	**0.66** ±0.010	**0.42** ±0.000	**8.67** ±0.577	**405.33** ±12.220	**82.99** ±1.339	**46.85** ±1.914
100	**22.67** ± 0.577	**1.91** ± 0.056	**0.91** ± 0.010	**0.31** ± 0.015	**0.92** ± 0.015	**0.43** ± 0.015	**1.35** ± 0.011	**1.41** ± 0.081	**1.35** ± 0.030	**0.48** ± 0.036	**0.82** ±0.036	**0.61** ±0.010	**0.65** ±0.011	**0.41** ±0.017	**7.67** ±0.577	**414.67** ±10.260	**74.74** ±2.056	**54.37** ±5.633
150	**19.33** ± 1.155	**1.76** ± 0.056	**0.86** ± 0.020	**0.28** ± 0.015	**0.89** ± 0.015	**0.37** ± 0.015	**1.29** ± 0.011	**1.33** ± 0.096	**1.27** ± 0.011	**0.46** ± 0.020	**0.73** ±0.017	**0.55** ±0.010	**0.56** ±0.020	**0.36** ±0.015	**7.00** ±1.000	**374.33** ±9.504	**69.57** ±1.422	**54.15** ±7.067
200	**14.00** ± 1.000	**1.23** ± 0.042	**0.78** ± 0.025	**0.21** ± 0.010	**0.80** ± 0.011	**0.30** ± 0.011	**1.22** ± 0.017	**1.26** ± 0.102	**1.20** ± 0.015	**0.42** ± 0.017	**0.66** ±0.020	**0.52** ±0.005	**0.53** ±0.026	**0.34** ±0.037	**6.00** ±1.000	**328.00** ±13.110	**64.80** ±3.509	**55.59** ±8.404
250	**9.33** ± 1.528	**0.83** ± 0.014	**0.61** ± 0.023	**0.19** ± 0.005	**0.61** ± 0.011	**0.27** ± 0.011	**1.18** ± 0.028	**1.18** ± 0.040	**1.13** ± 0.015	**0.37** ± 0.015	**0.54** ±0.020	**0.49** ±0.020	**0.47** ±0.010	**0.30** ±0.032	**5.33** ±0.577	**298.67** ±3.055	**52.03** ±2.713	**56.38** ±5.240

Table No. 2. Effect of different doses of gamma irradiation on some floral characters and pollen viability in *Pisum sativum var. arvense*

Dose (Gy)	Number of flowers	Length of Pedicel.	Sepals				Petals						Length of stamens	Length of carpel	Number of ovules/ ovary	Number of pollens/ anther	Pollen viability	P/O ratio
			Small		Large		Standard		Wing		Keel							
			Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth						
Control	**37.00** ±2.000	**4.72** ±0.040	**0.99** ±0.010	**0.33** ±0.015	**1.01** ±0.010	**0.46** ±0.010	**1.73** ±0.015	**1.92** ±0.030	**1.56** ±0.041	**0.76** ±0.047	**1.35** ±0.015	**0.81** ±0.015	**0.77** ±0.005	**0.71** ±0.035	**11.33** ±0.577	**446.33** ±9.609	**87.73** ±1.055	**39.47** ±2.740
50	**31.33** ±1.528	**4.46** ±0.265	**1.01** ±0.020	**0.36** ±0.015	**1.03** ±0.030	**0.50** ±0.050	**1.81** ±0.030	**1.98** ±0.040	**1.60** ±0.051	**0.80** ±0.070	**1.41** ±0.041	**0.91** ±0.050	**0.75** ±0.030	**0.74** ±0.015	**10.33** ±0.577	**452.67** ±8.327	**82.11** ±0.544	**43.92** ±2.977
100	**29.67** ±2.517	**3.57** ±0.094	**0.96** ±0.020	**0.32** ±0.005	**0.98** ±0.005	**0.43** ±0.005	**1.68** ±0.092	**1.86** ±0.085	**1.54** ±0.036	**0.73** ±0.023	**1.32** ±0.015	**0.79** ±0.010	**0.72** ±0.005	**0.73** ±0.011	**9.67** ±0.577	**462.67** ±6.110	**75.99** ±1.589	**48.00** ±3.487
150	**22.33** ±1.528	**2.72** ±0.065	**0.93** ±0.041	**0.30** ±0.011	**0.90** ±0.017	**0.37** ±0.032	**1.55** ±0.035	**1.70** ±0.020	**1.47** ±0.020	**0.69** ±0.020	**1.29** ±0.030	**0.73** ±0.045	**0.67** ±0.011	**0.65** ±0.010	**8.00** ±0.000	**412.67** ±4.163	**72.79** ±3.942	**51.58** ±0.520
200	**14.00** ±1.000	**1.74** ±0.055	**0.85** ±0.035	**0.27** ±0.015	**0.83** ±0.035	**0.32** ±0.030	**1.49** ±0.023	**1.64** ±0.028	**1.41** ±0.017	**0.63** ±0.015	**1.21** ±0.011	**0.66** ±0.015	**0.64** ±0.025	**0.60** ±0.020	**8.00** ±0.000	**334.67** ±14.050	**61.77** ±1.174	**41.83** ±1.756
250	**9.33** ±1.528	**1.41** ±0.041	**0.75** ±0.030	**0.23** ±0.025	**0.77** ±0.017	**0.29** ±0.020	**1.42** ±0.015	**1.56** ±0.015	**1.36** ±0.015	**0.58** ±0.023	**1.19** ±0.020	**0.62** ±0.030	**0.58** ±0.015	**0.58** ±0.015	**6.00** ±0.000	**306.00** ±7.211	**48.67** ±2.281	**51.00** ±1.200

Abbreviations: P/O ratio=Pollen-ovule ratio, ± = Standard deviatio of stamens (0.77cm) and carpels (0.71cm) than in var. *hortense* (0.63 & 0.44 cm, respectively). A dose dependent but slight reduction in length of stamens and carpels was noticed in both varieties, but these sexual elements have been significantly affected at higher doses from 150 Gy onwards. The slight but negative changes in the length of stamens and carpels can also be due to the physiological or biochemical disturbances caused due to irradiation as mentioned by Gunckel & Sparrow (1953) and may affect the pollination and fertilization processes and consequently the reproductive potential of the plants.

So far as the reproductive potential of these two varieties under investigation is concerned, it has been observed that var. *arvense* is more potent than var. *hortense* since it has been noticed producing more number of ovules (11.33) per ovary and larger number of pollens (446.33) per anther than var. *hortense* (9.00 ovules/ovary & 391.33 pollens/anthers). Both the varieties showed gradual decrease in number of ovules/ovary, with the increase in dose rate, but it was remarkably reduced at higher doses of 200 & 250 Gy, and was almost 50% as compared to that in control plants (Table 1 & 2). The negative effect of gamma irradiation on the production of number of ovules in the ovary can affect the total number of seeds per pod and can be correlated with the decrease in the overall yield of the plant. The mean values of pollen grains produced per anther noticed in control plants in var. *arvense* and *hortense* were 446.33 and 391.33, respectively. So far as the amount of pollen grains produced per anther, a stimulatory effect has been observed at lower doses, 50 and 100 Gy. Maximum enhancement (462.67) and (414.67) was recorded in var. *arvense* and in var. *hortense*, respectively at 100 Gy dose (Fig.2). This parameter is gradually decreased at higher doses and has shown adverse effect, in both the varieties. The data on amount of pollen grains produced per anther and the number of ovules formed per ovary clearly indicates that female gametes are adversely affected by gamma irradiation. On the contrary, the effect on male gametes is stimulatory at the lower doses. The ratio of pollen grains produced per anther to the number of ovules produced per ovary has been reported to be more in control plants of var. *hortense* than that in var. *arvense*. All the doses of gamma irradiation have shown does dependent enhancing effect, in both the varieties. It was almost maximum (56.38) at higher dose 250 Gy as compared to that in control (43.48), in var. *hortense,* and was comparatively greater (51.00) at 250 Gy dose than in control (39.47) in var. *arvense*.

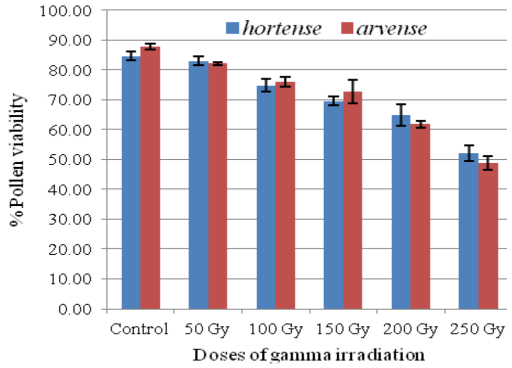

Fig. 1: Effect of different doses of gamma irradiation on pollen viability in two varieties of *P. sativum* var. *hortense* and var. *arvense*.

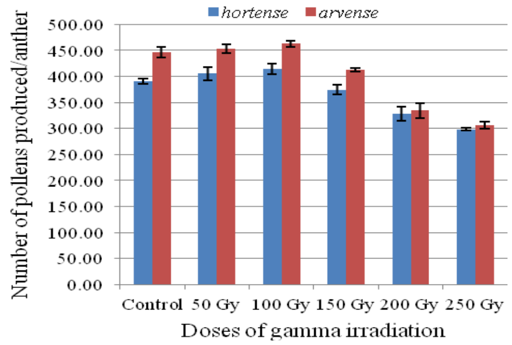

Fig. 2: Effect of different doses of gamma irradiation on number of pollens produced/anther in two varieties of *Pisum sativum* var. *hortense* and var. *arvense*

Pollen viability

It was apparent from the data depicted in table 1 & 2 on pollen viability that gamma irradiation had stronger effect on the viability of pollen grains. In the present investigation, the pollen viability was reported to be gradually decreased with the increase in dose rate, in both the varieties (Fig. 1). The lower doses had no significant effect on pollen viability but it was drastically affected at higher doses. The pollen viability in control plants (87.73 %) of var. *arvense* is comparatively greater than in control plants (84.59 %) of var. *hortense*. The treated plants of var. *arvense* (leafy variety) were found to be affected more than that of var. *hortense* (leafless variety), at higher doses. The adverse effects on pollen viability may be due to the effects of gamma irradiation at chromosomal level. Ehrenberg *et al.* (1961) and Tomkins and Grants (1972) reported upto 50 % reduction in pollen viability induced in *Hordeum vulgare* due to ionizing radiations. Radiation induced sterility in M_1 generation is mainly caused by small or minute deficiencies. According to Gaul and Mittelstensceid (1960) such radiation induced M_1 sterility is transferred into later generations. Ramesh and Reddy (2002) reported the increase in pollen sterility at lower doses in gamma irradiated rice. Kumar and Mani (1997) suggested that failure of homologous pairing during meiosis could be the main cause of high pollen sterility. Kumar and Dubey (1996) reported higher degree of pollen sterility in gamma irradiated *Lathyrus sativus* than application of chemical mutagens. Similar kind of results was also recorded by Farha Jabee *et al*. (2008) in *Trigonella foenum-graecum* L. The chromosomal abnormalities in PMCs resulted in increasing the pollen viability. Chary and Bhalla (1988) and Kumar *et al*. (2003) also reported an increase in pollen sterility with increase in mutagenic treatment. Rana & Swaminathan (1964), Ramana (1974) concluded that the deviation in karyokinesis and cytokinesis could produce non-viable microspores that results in pollen sterility. Sinha & Godward (1972) described that chromosomal aberrations, particularly, translocations to be responsible to decrease pollen fertility.

CONCLUSION

Pisum sativum is a valuable cultivated crop plant cultivated for its richness in protein content. It also possesses certain medicinal properties. Mutation breeding is a potent and successful method employed for improvement of one or the other argonomically important characters in the plant. The results obtained in the present investigation revealed the effectiveness of different doses of gamma irradiation on number of flowers, floral parts and pollen viability. Both the varieties showed dose dependent decrease in most of the floral characters, but the genotype of var. *arvense* (leafy) is observed to be more sensitive to the different doses of gamma irradiation than var. *hortense* (leafless). In conclusion, it can be said that the significant deviations in the floral parts and pollen viability induced through gamma irradiation consequently resulting in the alteration of some of the characters associated with the reproductive potential of the plant can provide sufficient scope for further improvement of this economic crop for different agronomic traits through mutagenesis.

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