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GROWTH AND YIELD RESPONSE OF FIELD PEA (*PISUM SATIVUM* L.) TO GAMMA IRRADIATION STRESS

ABSTRACT

Ionizing radiation has extensive applications in agriculture for inducing mutagenic changes in major field crops, potential breeding purposes, varietal development and crop improvement. This study was conducted to test the efficacy of 0.8, 1.6, 2.4 and 3.6 kGy gamma irradiation doses (Cobalt-60) upon growth and yield performance of edible pea (*Pisum sativum* L.) in pot culture experiment during 2009. Results demonstrated that higher radiation doses (1.6 and 3.2 kGy) significantly influenced the studied attributes of *P. sativum*. It was observed that 3.2 kGy had detrimental effects on shoot and pod lengths of pea which were reduced by 14.60 and 17.71 % respectively when compared to control. Differential response of the number of seeds pod^{-1}, 1000 grain weight and dry biomass of pea were recorded at the applied doses. Significant reduction in number of seeds (-14.21 %) but increase in 1000 grain weight (+13.93 %) and dry biomass (+11.32 %) of pea were recorded at 1.6 kGy which revealed stimulatory effects on grain weight and dry biomass. Conversely, radiation dose 3.2 kGy was found detrimental to all the studied parameters except number of pods plant^{-1} and number of seeds pod^{-1} which were not affected.

Key words: Biotechnology, Genetic variation, Ionizing radiation, Legumes, Mutation

INTRODUCTION

Pisum sativum L. in the family *Leguminosae*, holds an important position in human diet and agriculture. It possesses significant concentrations of proteins and other nutrients and is widely used as a food, vegetable and animal fodder (Schroeder *et al.*, 1993; Murtaza *et al.*, 2007). It is the most extensively grown crop in the world, ranked the 2nd in terms of cultivation after soybean (Smykal *et al.*, 2012). Nevertheless, having significant impact on human food and agricultural activities, global pea

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production is not encouraging relevant to other crops and there exists a production and consumption gap for this valuable crop. One of the several factors influencing decline in global pea production are the lack of high yielding varieties, different pathogenic diseases and soil salinity which potentially lower per acreage pea production (McPhee, 2003; Achakzai, 2012).

For improving production and quality of pea and other crops, different methods such as plant breeding, qualitative and quantitative genetic introgression manipulation and mutagenic improvement via chemical and physical mutagens have been extensively used in agriculture for decades (McPhee, 2003; Pratap *et al.,* 2010; Materne *et al.,* 2011; Smykal *et al.,* 2012). Induction of mutations in plants, particularly field crops, is an expanding area of crop research. The use of gamma irradiation for raising traits of interest through induced mutagenesis in crops is advantageous to other crop improving methods because gamma rays can create mutagenic changes in crops within short period of time and labor compared to extensive labor and time required in breeding methods for development of a cultivar of desired traits (Eroglu *et al.,* 2007; Alikamanoglu *et al.,* 2011; Moghaddam *et al.,* 2011). The use of gamma irradiation and other sources of ionizing radiation for improving crop productivity are well established (Ahloowalia and Maluszynski, 2001; Majeed *et al.*, 2014). Stimulatory effects of 3 Kr radiation dose on shoot length, leaves plant^{-1} and dry biomass of *Pisum sativum* L. have been reported under field conditions (El-Sadooni *et al.*, 2011). Soybean (*Glycine max*) subjected to radiation doses 0.2 – 0.8 kGy produced high yielding mutants in M2 generation (Mudibu *et al.,* 2012). Radiation dose 600 Gy has been documented to have positive effects on number of fruits and dry biomass of *Hibiscus sabdariffa* (El Sherif *et al.,* 2011). In a study, two cultivars of okra (*Abelmoschus esculentus*) performed well with increase in growth and yield and improved chemical profile in response to 300 Gy irradiation dose (Hegazi and Hamideldin, 2010). Rahimi and Bahrani (2011) stated that gamma irradiation dose of 100 Gy had stimulatory and healthy effects on shoot length, 1000-grain weight and general of Canola (*Brassica napus* L.).

Gamma rays are potentially vibrant ionizing radiations which are capable of changing genomic sequences of the subjected crops at higher frequencies; thus mutants with desired characteristics can be aroused once an optimum dose is determined. Although search for optimum doses of gamma irradiation to create high quality mutants of several crops have been carried out previously; however, such studies are limited on *P. sativum*. This work was conducted to assess different doses of Co-60 gamma irradiation for their potential effect on plant growth and yield of pea in pot culture.

MATERIALS AND METHODS

Plant materials and radiation treatment

The present work was carried out in the net house of Botany Department, Hazara University, Mansehra during 2009. Pea seeds (cultivar Climax) were collected Agriculture University, Peshawar. Viability of seeds was tested by growing on double layered moistened filter paper in petridishes at room temperature. Seeds were randomly selected from the lot and placed in paper bags at the rate of 100 seeds per bag. Seeds samples were exposed to gamma irradiation doses (0.8, 1.6, 2.4 and 3.2 kGy) at Nuclear Institute of Food and Agriculture (NIFA), Peshawar (Pakistan) in Novemeber, 2009 from a Co-60 installation.

Growth conditions and experimental design

Twenty plastic pots of uniform size (each 3 × 1 feet i.e. 90 cm × 30 cm) were filled with equal volume of loamy soil and were placed in net house at Botany Department under natural environmental conditions. Soil analysis was carried out at NIFA, Peshawar which revealed electrical conductivity (EC) as 1.79 dS × m^{-1}, pH 7.8, organic matte 1.70%, N: P: K 13:70:41 (kg × ha^{-1}) and total soluble salts 0.81%. Texture of soil was 25.7, 39.60 and 39.54 clay, silt and sand respectively.

Five seeds of each radiation treatment were sown in each pot with four replications. Non radiated seeds were kept as control for comparison with treated seeds. The experiment was established in randomize complete block (RCB) design. Pots were irrigated with tape water soon after seeds were sown. Further irrigation was done at 20 day intervals till the harvest of crop. At maturity, plant height [cm], pod length [cm], number of pods per plant, number of seeds per pod, 1000 seed weight [g] and dry biomass per plant [g] were determined.

Statistical analysis

SPSS software (SPSS V. 21.0, IBM Corp.) was used to analyze collected data. Uni-factor Analysis of variance (ANOVA) was applied to data on growth and yield parameters of pea under the influence of different doses of gamma irradiation. Significant variations among the treatments were determined by Least Significant Difference test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Values yielded by the analysis of variance (ANOVA) regarding plant height (shoot length), pod length, number of pods per plant, number of seeds per pod, 1000 seed weight and dry biomass per pea plant as effected by different doses of gamma irradiation are presented in Table 1. It is clear from Table 1 that radiation treatment had significant effects on all the studied parameters of test plant except number of pods × $plant^{-1}$ which were not affected by any of the radiation dose. Moreover, replicated data revealed insignificant differences.

Table 1

Analysis of variance (ANOVA) mean squares table for shoot and pod length, number of pods and seeds per plant, 1000 grain weight and dry biomass per plant of *Pisum sativum* L.

Source	DF	Shoot length [cm]	Pod length [cm]	No. of pods per plant	No. of seeds per pod	1000 seed weight [g]	Dry biomass per plant [g]
Replications	3	13.97^{ns}	1.149^{ns}	1.09^{ns}	0.837^{ns}	1.749^{ns}	1.427^{ns}
Doses	4	123.12^{*}	19.421^{*}	0.981^{ns}	22.312^{*}	19.762^{*}	167.873^{*}
Error	12	17.50	1.02	3.07	1.52	3.78	3.98
Total	19						
Coefficient of variation [%]		5.271	2.871	1.390	1.871	2.214	3.009

* - significant at α = 5%, n.s.—non significant

Shoot and pod length per plant

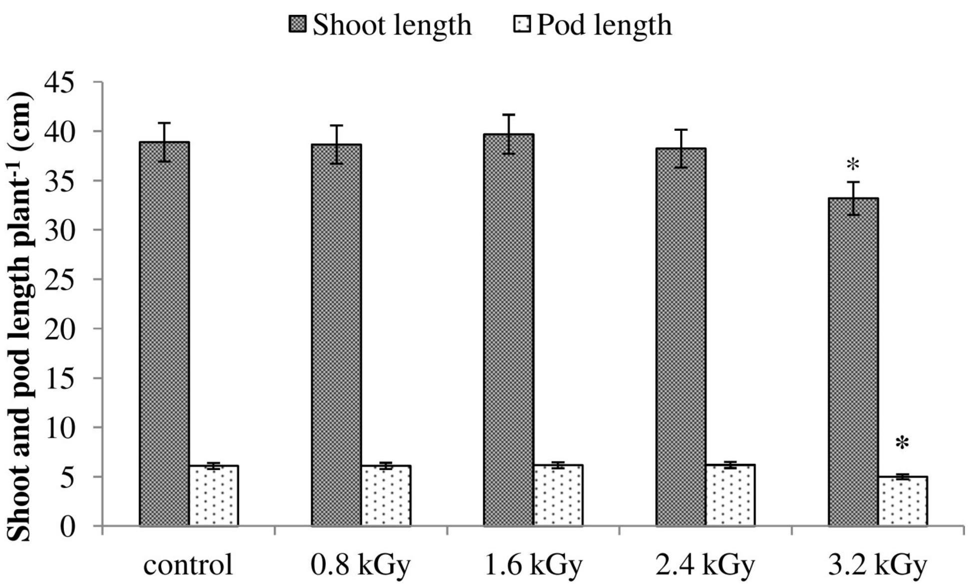

Fig. 1 Effect of different doses of gamma irradiation (0.8 – 3.2 kGy) on shoot and pod length [cm] of *Pisum sativum* L.; * statistically different from control ($p \leq 0.05$); error bars represent percentage

Shoot and pod length were not affected by radiation doses up to 2.4 kGy which revealed almost consistent values with those recorded in control, although slight variations occurred in the values but they were statically non-significant. However, both parameters were significantly reduced by 3.2 kGy which revealed shoot and pod length as 33.21 and 5.01 cm respectively when compared to control where shoot length was 38.89 cm and pod length 6.09 cm; the decrease corresponded 14.60 and 17.71% respectively over control (Fig. 1). In peas and other legumes, shoot and pod lengths are controlled by genetic factors and hormones. Any variation in genes and endogenous hormones in response to external stress may cause abnormalities in length of shoot and pods. Kurepin *et al.* (2013) suggested that gibberellins, an important class of plant hormones, are responsible for elongation of shoot, root and other organs of plants. Decrease in shoot and pod lengths in our study may be associated with activation of anti-Gibberellin chemicals as a result of higher dose of gamma irradiation which might have inhibited the gibberellin biosynthesis and consequently reduced lengths.

Effect on number of pods per plant and number of seeds per pod

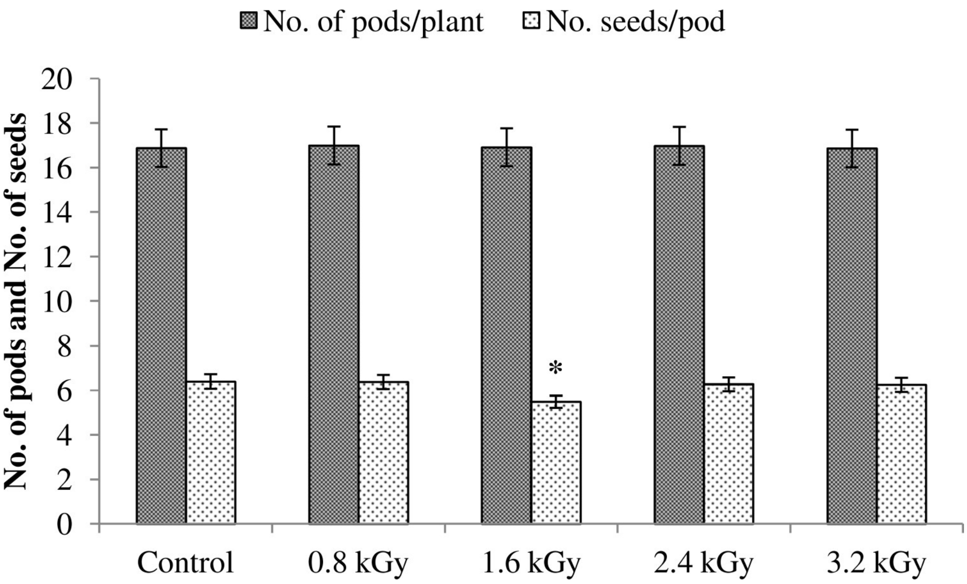

Fig. 2 Effect of different doses of gamma irradiation (0.8 – 3.2 kGy) on number of pods per plant and number of seeds per pod of *Pisum sativum* L.; * statistically different from control ($p \leq 0.05$); error bars represent percentage

Results on number of pods per plant and number of seeds per pod are shown in Fig. 2. It was observed that gamma irradiation doses did not alter number of pods × plant^{-1}. When compared to control, number of pods varied slightly but insignificantly in all treatments of radiation where it ranged 16.87-17.00. Results revealed that maximum number of seeds × pod^{-1} (6.40) were present in control following a declining trend with increase in radiation dose. However, the most drastic and significant effect was observed at 1.6 kGy which significantly reduced this parameter resulting in 5.49 number of seeds per pod. Percent reduction at this dose was 14.21. Number of pods and seeds are important traits which reflect the yield capacity of pea. These traits are dependent upon several factors, both endogenic and exogenic. Previously, multiple quantitative traits loci (QTL) for these traits have been identified in various pea populations (Symkal *et al.*, 2012). Depression in number of seeds in response to 1.6 kGy radiation stress may be attributed to alteration in QTL controlling this trait or possibly changing hormonal ratios required for normal growth and development of plants and its organs.

1000-grain weight and dry biomass per plant

Figs. 3 and 4 summarizes the effect of different doses of gamma irradiation on 1000-seed weight and dry biomass per plant. Variable responses at different doses were observed. These two attributes were first stimulated by 1.6 kGy increasing them by 13.93 and 11.32% over control respectively and when the dosage was increased to 3.2 kGy, significant suppression in these traits were recorded. At 3.2 kGy radiation, these parameters were reduced by 4.28 and 7.21% respectively when compared to control. The results demonstrated that 1.6 kGy had healthy and stimulatory effect on 1000 seed weight and dry biomass of pea. Other doses 0.8 and 2.4 kGy caused slight increase in test attributes; however, the increase was statistically non-significant.

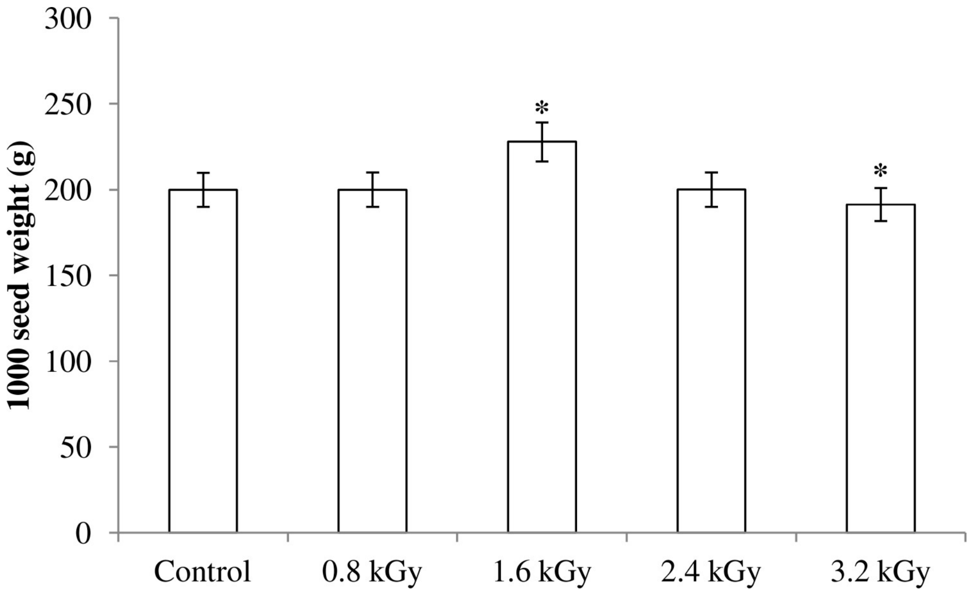

Fig. 3 Effect of different doses of gamma irradiation (0.8 – 3.2 kGy) on 1000-seed weight [g] of *Pisum sativum* L.; * statistically different from control (p≤0.05); error bars represent percentage

Seed weight and dry biomass are related to efficient physiological functions of plants, adequate uptake of minerals and water and photosynthetic rate. Disturbance in physiological activity of plant may lead to decrease photosynthesis and subsequently decreased seed weight and dry biomass. It may be inferred from the results of our study that at 1.6 kGy, test plant efficiently utilized the available resources with greater photosynthetic rate which resulted in enhancement of seed and dry biomass weight, while at the highest dose (3.2 kGy), physiological disturbance lead to reduce these traits.

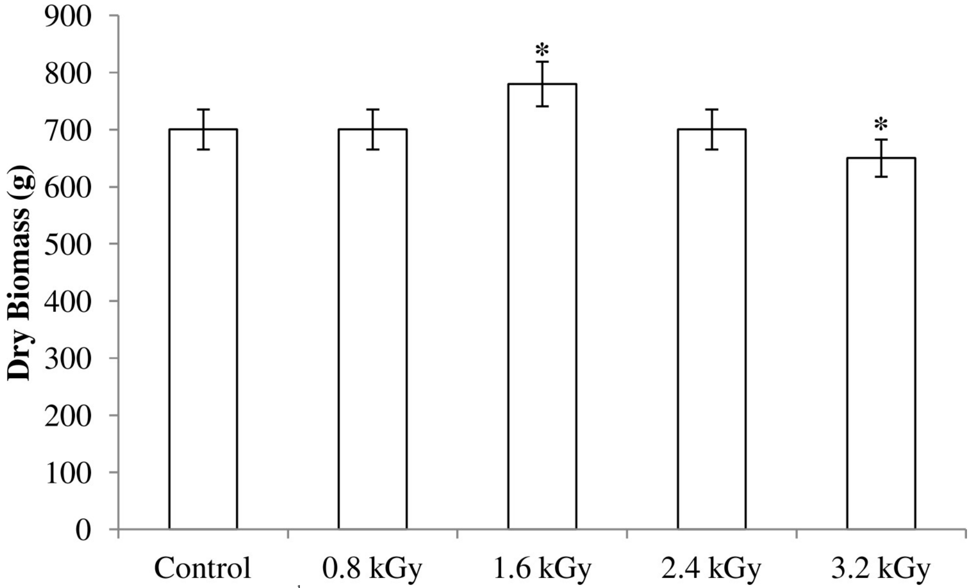

Fig. 4. Dry biomass × plant^{-1} [g] of *P. sativum* L. as affected by different doses of gamma irradiation (0.8 – 3.2 kGy); * statistically different from control ($p \leq 0.05$); error bars represent percentage

In general, ionizing radiation triggers changes in the genetic architecture and hormonal ratios of exposed plants which may determine stimulatory or inhibitory responses in the structural, morphological and developmental functions of plants. Responses are dependent on magnitude and duration of radiation dose and test plant species (Jan *et al.*, 2012). Detrimental effects of high radiation doses on plant growth and development has not been well understood, however it is assumed that ionizing radiation interact with atoms and molecules of the subjected plant tissues; producing highly reactive free species of oxygen, H_2O_2, hydrogen and hydroxyl radicals which can react with nucleic acids, proteins and lipids and may trigger functional or structural alteration in them with possible mutagenic effects (Zaka *et al.,* 2002; Alikamanoglu *et al.,* 2011; Moghaddam *et al.,* 2011). Inhibitory effects may also be attributed to cell cycle arrest or genomic alterations of the exposed plants (Preuss and Britt, 2003; Talebi and Talebi, 2012) directly by radiation doses or indirectly by creating free reactive radicals. Similarly, stimulatory effect of radiation doses on the studied parameters could be due to a number of reasons such as modifications in growth hormones patterns because of radiation exposure. It has been assumed that low radiation doses stimulate growth patterns of plants possibly by altering the hormonal signaling system or enhancing their anti-oxidative potentials to cope with stress conditions which delimit their growth (Wi *et al.*, 2007; Kim *et al.*, 2009; Jan *et al.,* 2012). The role of auxin synthesis may also be a possible explanation for plant growth depression or increment at variable doses of gamma irradiation as it is assumed that low doses partly inhibit its synthesis while higher doses completely destroy the activity of auxin (Jan *et al.,* 2012). Differential responses in growth and yield (both stimulatory and inhibitory) of *Pisum sativum* to different doses of gamma irradiation in this study are in agreement with previous research on the influence of gamma irradiation on Mungbean (Yaqoob and Ahmad, 2003; Tah, 2006), Long beans (Kon *et al.,* 2007) and Soybeans (Alikamanoglu *et al.,* 2011; Mudibu *et al.,* 2012.

CONCLUSION

In conclusion, our study revealed that lower doses (0.8 and 2.4 kGy) had no effect on growth and yield of pea. 1.6 Gy increased number of seeds pod^{-1}, 1000 seed weight and dry biomass to significant extent and thus, may be used an optimum dose for further studies on legumes. Conversely, 3.2 kGy had drastic effect on all the studied parameters except number of pods plant^{-1}.

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