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Studies on the Effects of Ultraviolet Irradiation on Pea (*Pisum sativum* L.)

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Abstract

Pea (*Pisum sativum* L.) is one of the earliest domesticated cool season annual legume crop produced worldwide, mainly in temperate regions. In common with other grain legumes, pea plays an important role in food and nutritional security of humans as well as livestock. Pea is an annual plant which exhibits mainly self-pollination, although cross pollination through insects also occurs in nature. The improvement of pea through plant breeding requires considerable genetic variations in the key quantitative traits and the expression of those polygenic traits also depends on the environmental interactions. Therefore, UV irradiations have been employed in the present study to assess the genotypic sensitivity of the pea cultivar for possible application in mutation breeding of pea. Seed germination and seedling growth at different duration of exposures were calculated for estimating the effect of UV irradiations stress on pea. The findings of the present study conclude that the UV irradiation can be useful as non-ionizing physical mutagen for induction of selectable macromutations in local pea cultivars and the exposure to the increasing UV stress in the nature will be detrimental for the crop productions.

Introduction

Pea (*Pisum sativum* L.) is generally considered to have originated in the Near East region and domesticated as early as 7000-6000 BC [1]. In India, it is grown in an area of 1.10 million ha with an annual production of 1.02 million tones and average productivity of 927.2 kg/ha. The major producers are Canada, China, India, Russian Federation, the USA, and France. Dry pea seeds are rich in protein (18-33%), starch (35-50%) and digestible nutrient content and low in fibre (4-7%), which make it an excellent livestock feed also. Most of the pea growing area in developing countries, including India, is occupied by traditional varieties, with narrow genetic base, which suffers from some abiotic and biotic constraints like late maturity, lodging, susceptibility to rust etc. Genetic bottleneck, mostly in Indian cultivars, accumulated due to traditional breeding methods over the years, is one of the major constraints commonly limiting the breeder's efforts. The availability as well as accessibility to the genetic variation within the genepool of a crop species is the prerequisite for initiating an improvement programmed. Induce mutagenesis has proved to be a powerful complimentary breeding tool for creating new genetic blend within a short period of time without disturbing overall genetic architecture of the crop. Thus, it allows the plant breeders to screen and select desirable combination of expressed economic traits for further introgression into the proper breeding stock.

UV radiation, first described by Johann Wilhelm Ritter in 1801, is light energy emitted between the wavelengths of 100 and 400 nm, i.e. between the electromagnetic spectra of X-ray and visible light. UV radiation, in comparison γ- and X-rays, is of relatively low energy and is not ionizing, i.e. does not dislodge electrons. Based on the wavelength, UV radiation is further divided into Ultraviolet A (UVA) 315-400 nm, Ultraviolet B (UVB) 280-315 nm and Ultraviolet C (UVC) 100-280 nm. UVC is the most energetic and biologically damaging, it is not found in sunlight as it is absorbed by the ozone layer; UVB is the major mutagenic fraction of sunlight; and UVA also has deleterious effects primarily because it creates oxygen radicals, which can indirectly damage DNA. However, due to continuous depletion of ozone layer, the strength of UV radiation reaching Earth's surface increasing everyday which have deleterious effects on both animals and plants. Enhanced UV radiation can alter plant growth and development as

well as reproduction [2]; this has serious implications for plant yields and agricultural economies.

[3] discovered the mutagenic effect of UV radiation on polar cap cells of fruit fly eggs. The mutagenic potential of these rays has since been confirmed in many organisms in which germ tissue could be easily exposed to the low-penetrating ultraviolet light. Since, UV wavelengths are absorbed by bases in DNA molecules and by aromatic amino acids of proteins, it reacts with DNA and other biological molecules to induce mutagenic effects in the organism. Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing improved cultivars of cereals, pulses, fruits and other crops [4]. These mutations provide beneficial variation for practical plant breeding purpose. The technology is simple, relatively cheap to perform and equally usable on a small and large scale [5]. Expression of traits is a complex process which involves many interdependent genes and the responses of each gene varies towards different mutagens [6]. Therefore, selection of mutagens and their optimal doses is very crucial for unleashing the huge possibilities in mutation breeding. The germination and seedling growth inhibition percentage of the treated seeds has been considered as one of the most dependable indexes to estimate the sensitivity of any crop genotype towards any mutagens. Keeping in view the economic value of pea and mutagenic potential of UV irradiation, the present study was designed to estimate the impact of UV irradiation stress on pea genotype for understanding the future threat of increasing UV incidence on agricultural production and to assess the mutagenic potency of UV irradiation at different treatment durations for possible genetic improvement of local pea cultivars through UV irradiation based mutation breeding.

Materials and Methods

Dry (moisture content 10-12%) and healthy seeds of the local pea cultivar, obtained from Seed Store, Hailakandi, Assam, were used for mutagenic treatments of ultraviolet radiation (UV). A germicidal ultraviolet lamp which emits high intensity ultraviolet radiation concentrated around the wavelength of 253.7 nm (i.e., UV-C radiation) was used in the present experiment. The seeds were divided into three sets of 15 seeds each and seeds from each set were distributed in three petriplates with 5 seeds each. Therefore, three replications of each seed set were prepared for the experiment. Two sets were used for UV radiation treatment of different durations and the remaining one set was considered as control. UV radiation treatment of two durations (30 mins and 60 min) per day for 7 days was employed and distance of 30 cm was maintained between lamp and petriplates. Therefore, C, T_1 and T_2 abbreviations for Control, UV (30mins/day/7days) and UV (60mins/day/7days), respectively, will be used in the following texts of the study. In between the treatments, the petriplates were kept in the B.O.D. incubator at 27±1°C temperature in Department of Botany, S. S. College, Hailakandi, for seed germination and seedling growth. The seeds were allowed to grow for 14 days with initial 7 days under UV stress for genotypic sensitivity assessments.

Statistical analysis, namely, Mean (X), Standard Error (SE), Standard Deviation (SD), Coefficient of variation (CV %) were done using IBM SPSS statistics 20. Following parameters were studied from the M_1 generation.

Seed Germination

After recording germination counts, the percentage of seed germination was calculated on the basis of total number of seeds sown in the petridishes and in the field.

$$\text{Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds sown}} \times 100$$

Seedling Height

Seedling height was recorded after 14 days by measuring the root and shoot lengths for each treatment and control.

Seedling emergence reduction (%) = [1 - (Average number of seedlings in a treated combination/Average number of seedlings in the control) X 100

Seedling growth reduction (%) = [1 - (Average length of seedlings in a treated combination / Average length of seedlings in the control)] X 100

Seedling vigour index = Average seed germination (%) X Average seedling height

Results

Seed Germination Percentage

The comparative data recorded on seed germination are presented (Table 1) and (Figure 1).

Parameters	Treatments	Mean	Std. Error	Std. Deviation	CV%	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Seed germination (%)	C	93.33	6.67	11.55	12.37	64.65	122.02	80	100
	T_1	86.67	6.67	11.55	13.32	57.98	115.35	80	100
	T_2	60	11.55	20	33.33	10.32	109.68	40	80
	Total	80	6.67	20	25	64.63	95.37	40	100
Seedling shoot length (cm)	C	13.78	0.45	0.77	5.6	11.86	15.7	12.98	14.52
	T_1	16.26	0.34	0.58	3.59	14.81	17.71	15.76	16.9
	T_2	8.77	0.39	0.68	7.71	7.09	10.45	8.11	9.46
	Total	12.93	1.12	3.36	25.95	10.35	15.51	8.11	16.9
Seedling root Length (cm)	C	8.2	0.36	0.62	7.55	6.67	9.74	7.63	8.86
	T_1	10.57	0.36	0.63	5.95	9.01	12.13	9.98	11.23
	T_2	5.13	0.38	0.65	12.68	3.51	6.74	4.48	5.78
	Total	7.97	0.81	2.43	30.44	6.1	9.83	4.48	11.23
Seedling total length (cm)	C	21.98	0.09	0.16	0.71	21.6	22.37	21.84	22.15
	T_1	26.82	0.7	1.21	4.51	23.82	29.83	25.74	28.13
	T_2	13.89	0.03	0.05	0.32	13.78	14.01	13.85	13.94
	Total	20.9	1.9	5.69	27.23	16.53	25.27	13.85	28.13

 # C=Control, T_1=UV (30mins/day/7days) and T_2=UV (60mins/day/7days).

Table 1: Estimates of mean ($\overline{X}$), Standard Error (S.E.), Standard Deviation (S.D.) and coefficient of variance (CV %) of seed germination (%), seedling shoot, root and total height in UV treated M_1 generation of pea.

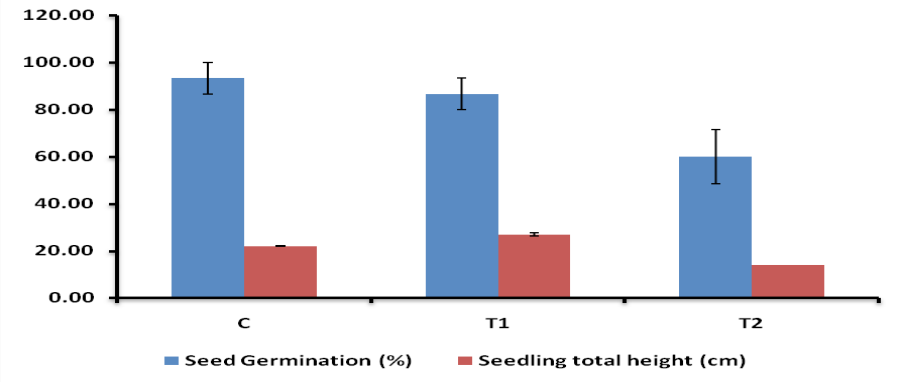

Figure 1: Comparative effect of UV irradiation doses on seed germination and seedling growth of pea (*Pisum sativum* L.)

The mean seed germination in control was 93.33 %. Seed germination observed in controlled laboratory condition gradually decreased with increasing duration of UV radiation exposure in the pea cultivars. It was observed to be 86.67 % and 60.00% in the UV treatment doses T_1 and T_2, respectively. From the day of sowing in the petriplates, it took one-two days to start seed germination and two-three days to attain 50% germination in controls; however, the emergence was delayed by two to four days in most of the UV treated.

Seedling Height (cm)

The estimated data on seedling height (length) showed distinct results (Table 1) and (Figure 1). The mean seedling height in control plants was 21.98 cm. It was observed that the seedling height increased in UV treatment T_1 (26.82 cm), while it decreased in UV treatment T_2 (13.89 cm) compare to control. The coefficient of variation (C.V.%) calculated for different parameters differed considerably from the control values, especially T_2 in seedling total height, which supports the possibilities of further improvements and selections. (Figure 2) showed the UV treatment of seeds and variable germination pattern, and seedling growth.

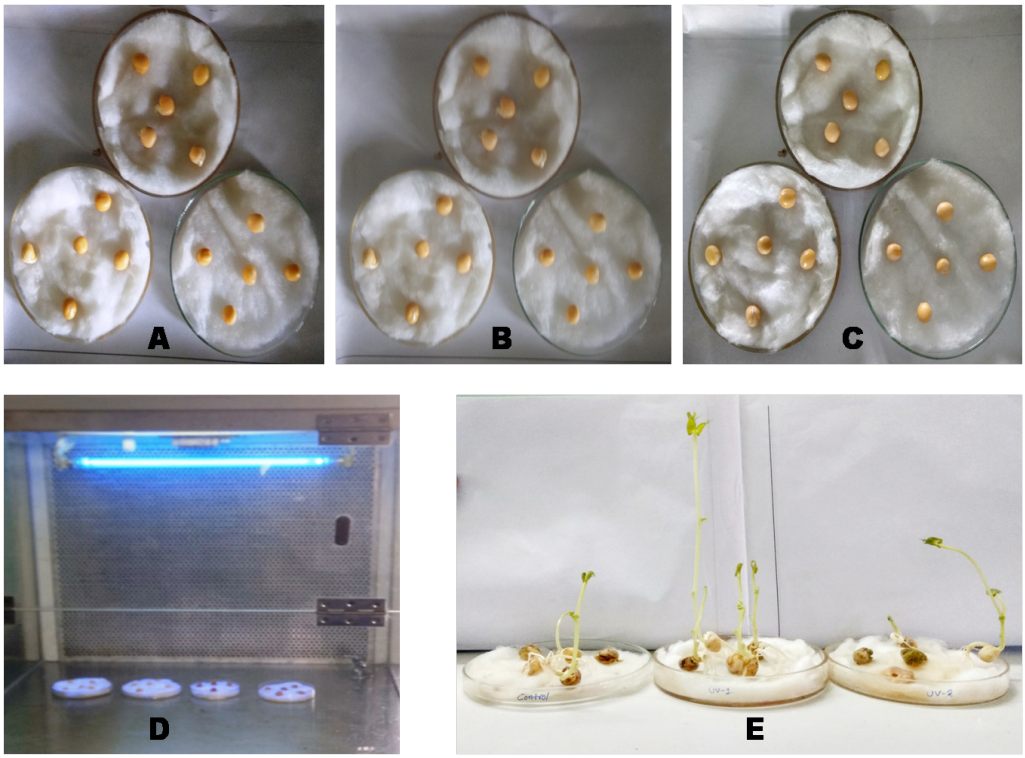

Figure 2: Seeds showing germination initiation after second day after first UV treatment doses A: Control; B: T1=UV (30mins/day/7days) and C: T2=UV (60mins/day/7days); D: Treatment chamber showing UV lamp; E: Seedlings showing differential growth on 8th day in C, T_1 and T_2 (right to left).

Seedling Vigour Index

The seedling vigour index was calculated by germination percentage multiplying with length of seedling. Maximum seedling vigour index was 2324.69 at UV T_1, followed by 2051.78 at control and 833.60 at UV T_2 (Table 2).

Treatments	Seedling vigour index (%)	Seedling emergence reduction (%)	Seedling growth reduction (%)
C	2051.78	--	--
T_1	2324.69	7.14	-22.04
T_2	833.60	35.71	+36.79
# C=Control, T_1=UV(30mins/day/7days) and T_2=UV(60mins/day/7days).			

Table 2: Effect of Ultraviolet Radiation (UV) on seed germination and seedling characters of pea.

Seedling Emergence Reduction (%)

The extent of germination inhibitions as compared with control was differed significantly in both the UV treatment condition. The seedling emergence reduction was maximum 35.71% at UV dose T_2, while in it was considerably low 7.14% at UV dose T_1 (Table 2).

Seedling Growth Reduction (%)

The estimation of seedling growth reduction resulted significant observations (Table 2). In UV radiation T_1, instead of reduction, the seedling growth was promoted by 22.04% due to which seedling height increased compared to control. However, seedling growth was found inhibited by 36.79% at UV radiation T_2.

Analysis of Variance (ANOVA)

The between-treatments One-Way ANOVA yielded a statistically significant effect with the p value less than 0.05 ($p<0.05$) in seedling shoot, root and total heght, except germination percentage for which the treatments effect was comparatively low. Thus, the null hypothesis of no differences between the parameter means of different treatment groups rejected and suggested that the applied UV radiation doses were successful in creating genetic variations through random micromutations (Table 3).

Parameters	Source	Sum of Squares	df	Mean Square	F	Sig.
Seed germination (%)	Between Groups	1866.67	2	933.33	4.2	0.072338ns
	Within Groups	1333.33	6	222.22		
	Total	3200	8			
Seedling shoot length (cm)	Between Groups	87.37	2	43.68	94.06	0.000030*
	Within Groups	2.79	6	0.46		
	Total	90.15	8			
Seedling root Length (cm)	Between Groups	44.64	2	22.32	55.76	0.000133*
	Within Groups	2.4	6	0.4		
	Total	47.05	8			
Seedling total length (cm)	Between Groups	256.06	2	128.03	257.45	0.000002*
	Within Groups	2.98	6	0.5		
	Total	259.04	8			
* Significant at 0.05 ($p<0.05$), ns: not significant						

Table 3: Significance in terms of probability (p) of F-test for the Analysis of Variance (ANOVA) for each of the parameters.

Discussion

[7] stated that the biological damage caused by mutation to germination, pollen sterility and survival at maturity may be considered as an indication of mutagenic sensitivity of the genotype. It helps in determining the biological sensitivity and mutagenic potency simultaneously, which is essential to select the mutagen concentrations at which mutation frequency is high and germination inhibition is low in the target crop. The seedling growth usually inhibited variably according to the UV dosage. The estimation of germination percentage of the treated seeds provides the primary idea about the genotypic sensitivity and mutagenic potency with the germination percentage below 50% as lethal or undesirable. Since, the purpose is to develop large mutagenized populations for effective screening of the mutants; therefore, generally the lethal treatments were discarded to ensure high frequency of mutation with lower growth inhibitions in the population. Physiological and biochemical processes in plants are significantly affected by UV irradiation stress. The irradiation of seeds with high doses of UV rays disturbs the synthesis of protein [8], hormone balance [9], leaf gas-exchange [10], water exchange and enzyme activity [10]. The diminution in the germination percentage of the treated seeds could be attributed to the disturbances in the genetically

controlled bio-physiological metabolic pathways necessary for seed germination that may include enzyme activity [11], hormonal imbalances [12] and inhibition of mitotic process [13]. It was also documented that the inhibition in the DNA synthesis due to the induced mutations by mutagenic treatments may be the reason for reducing seed germination [14].

The disruption and disorganization of the tunica layer due to radiation leads to the seed germination inhibition [15]. Interference of the UV irradiation with the enzyme synthesis as well as degradation of active enzymes required for plant auxin production may be another rationale for germination inhibition of the treated seeds. It has been reported that prolonged irradiation of wheat (*Triticum aestivum* L.) seeds with UV light (for 1-6 h) led to an increase in the level of lipid peroxidation in sprouts [16]. A dose dependent decrease in the triacylglycerol content and a concomitant increase in free fatty acids was observed. Delay in germination for more than three days in higher doses of mutagens could be due to the inhibition of the mitotic proliferation in root and shoot meristems. [17] viewed that the highest practicable mutants with lowest damage can be achieved by screening the mutagen doses. [18] suggested that induced mutagenesis through physical mutagens can create genetic divergence which can be useful for selection of high yielding mutants. Jayakumar and Selvaraj [19] also reported the effect of mutagenic radiation on the seed vigour, therefore, the implications of seed treatment with recommended doses of irradiation is high and can be useful if done under proper scientific investment [20]. On the other hand, it also suggests the detrimental effect of uncontrolled UV radiation on the crop productivity [21]; therefore, ozone layer must be protected from damaging activities of humans by curving the air pollution and global warming down to minimal level [22].

Conclusion

The present investigation was done to study the mutagenic effects of two durations of UV dosage on local pea (*Pisum sativum* L.) cultivar. The differential sensitivity of the pea cultivar towards the two different durations of UV dosage revealed the fact that the rate of mutations depends exclusively on the condition of interaction between the particular genotype and mutagen when the other variable factors were at constant. It was clear from the presented results that the UV irradiation has significant effect on the seed vigour, thereby directly on the total crop production. The pea genotype used in the present investigation showed positive sensitivity towards the UV irradiation while also confirms that excessive exposure can be lethal to the crop genotype. Therefore, it has been concluded that the UV irradiation is useful for inducing mutations in local pea genotypes, if exposure conditions and durations are favorably not lethal.

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