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STUDY OF MUTAGENIC EFFECT OF UV-C RADIATIONS ON SEED GERMINATION AND SEEDLING GROWTH OF *BRASSICA CAMPESTRIS* L.

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ABSTRACT

In the present work effect of UV-C radiation was observed on the seed germination and seedling growth of *Brassica campestris* L. An exposure of *B. campestris* seeds to enhanced levels of UV-C radiation (05, 10 and 20 minutes) altered the germination and growth pattern. Indeed, high UV-C doses in this experiment (20 min.) determined irreversible growth at morphological levels. The UV-C exposure to dry seeds of *B. campestris* was found to be stimulatory. The higher doses of UV-C not only increased the seed germination percentage but also root shoot ratio, total length of seedling, seedling vigour index and seedling tolerance index. The R/S of control set was found to be 0.6 in control, 0.9 in dose 05 min., 0.7 in dose 10 min., and 1.1 in dose 20 min. treatment. The total length of the seedling on 5^{th} day was found to be 2.5, 4.0, 3.9 and 4.1cms in control, 05 min., 10 min. and 20 min. respectively. The SVI was found to be 235, 399, 381 and 410 in control, 05 min. 10 min. and 20 min. respectively. The STI values were observed to be 162, 157 and 166 in treatments 05 min., 10 min. and 20 min. respectively.

Keywords: **UV C effect, seed germination, seedling growth, *Brassica campestris*.**

INTRODUCTION

Plants use sunlight for photosynthesis and, as a consequence, are exposed to the ultraviolet (UV) radiation that is present in sunlight. Irradiation is a method of exposing substances or plants or plant materials with radiation. During irradiation the high energy radiation pass through the matter and cause ionizing or electromagnetic disturbances that affect the internal structure or matter of plants. During the past few decades, the ozone reduction problem has stimulated considerable research on higher plant responses to UV-radiation (Caldwell and Flint, 1994). Ultraviolet radiation (UV-R) is a component of the solar light and a part of the non-ionizing region of the electromagnetic spectrum, which comprises approximately 8-9% of the total solar radiation that naturally reaches the Earth. Depending on its wavelength, it can be divided in three different ranges: UV-A (315-390 nm), UV-B (280-315 nm) and UV-C (100-280 nm). Among these, UV-C is the radiation with the lower wavelength, or rather with the higher associated energy (Katerova *et al*., 2009).

It is known that UV-C has an acute germicidal action on microorganisms in water, on surfaces and in air (Siddiqui *et al*., 2011). Moreover, it can induce oxidative results and genetic mutations in plants that in turn have strong negative effects on plant morphology, flowering, pollination, transpiration and photosynthesis (Murali & Saxe, 1984; Booij-James *et al.*, 2000). Fortunately, UV-C is strongly affected by the ozone layer in the stratosphere, so that the amount of this radiation reaching the Earth's surface, except for high mountains, is extremely low (Häder *et al.*, 2007). Nevertheless, in the last decades, human activities have produced dangerous chemicals, such as chlorofluorocarbons (CFC), which have been released into the atmosphere and have contributed to the depletion of ozone protective layer (McFarland and Kaye, 1992).

UV-C radiation has been used as a mutagenic agent in plants, and it is known to reactivate the maize Mutator transposable element (Walbot, 1992). To prevent mutation and/or cell death, UV radiation-induced DNA damage must be repaired before DNA replication. Repair of UV radiation-induced lesions may be of particular importance in plant pollen, especially in wind pollinated species (Jackson, 1987). DNA damage must also be repaired to allow transcription (Sauerbler and Hercules, 1978). Although UV-C damage is not physiologically relevant for plants growing in the sun, short-wavelength (UV-C) radiation from germicidal lamps has often been used to study DNA damage in animals and bacteria, as well as in plants. UV-C has been used because DNA has a strong absorption maximum in the UV-C range (at 260 nm).

UV-C photons are highly energetic, and high levels of damage can thus be created quickly. Also, high-output UV-B radiation sources and spectroradiometers are expensive. The best-studied UV radiation-induced DNA lesion is the cyclobutane pyrimidine dimer (CPD). Other types of DNA damage are the pyrimidine (6,4) pyrimidone dimer, diverse rare DNA photoproducts, and indirect types such as DNA-protein cross links and singlet oxygen damage (Peak and Peak, 1986). Severa1 investigators have measured CPD DNA damage directly in plants or in plant cell cultures (McLennan, 1987; Pang and Hays, 1991; Quaite *et al*., 1992), but no other types of UV radiation-induced DNA damage have been reported in plants.

Some UV radiation responses have been measured in both the UV-C and UV-B regions of the spectrum, including tissue damage (Bornman *et al*., 1986), induction of carotenoids and polyamines (Tevini and Teramura, 1989; Kramer *et al*., 1991), damage to the photosynthetic apparatus (Kulandaivelu and Noorudeen, 1983), phototropism (Baskin and Lino, 1987), ATPase destruction (Murphy, 1983), unscheduled DNA synthesis in pollen (Jackson, 1987), and anthocyanin and flavonoid induction in parsley, sorghum, and peanut callus (Wellman, 1971; Fritzemeier *et al*., 1983; Hashimoto *et al*., 1991). In each case, the response to UV-C differs from the UV-B effect. In the case of tissue and photosynthetic damage, qualitatively different responses to the two types of UV radiation were observed. In the studies of phototropism and ATPase destruction, two separate activities were found, one responsive to UV-C and one to UV-B. Furthermore, UV-C and UV-B have opposite effects on carotenoid levels (Tevini *et al*., 1981; Campos *et al*., 1991) and anthocyanin levels (Hashimoto *et al.*, 1991). Therefore, as a general rule, UV-C is not a useful model for physiological responses induced by UV-6.

The present study was conducted to observe the effect of UV-C irradiation treatment on water soaked seeds on germination and seedling growth of *Brassica campestris* L. commonly called as mustard.

MATERIALS AND METHODS

Collection of study materials:

The seeds of *Brassica campestris* L. were obtained from Pantnagar Agricultural University, Pantnagar. For the UV-C radiation treatments, the UV irradiation lamp of horizontal laminar air flow (PCR UV^3 HEPA Workstation, Analytik Jena AG; ST51G/W51UV tube with a wavelength of about 280nm, UV-C intensity was 54 mJ cm^{-2}) in Botany department of S.B.S. Govt. PG college was used. The distance of seeds from the UV-C radiation lamps was approximately 50 cm. For experiment, healthy, dry and uniform size *Brassica campestris* L. seeds

were irradiated separately with UV-C at different period of exposure (5, 10 and 20 minutes). Dry seeds not treated with UV-C irradiation were maintained as control. All the treatments were done in 5 replications. The seed germination and seedling growth of *Brassica campestris* was observed up to 05 Days.

Sampling and data

The seed germination characteristics and seedling growth patterns were estimated with the help of parameters listed below:

(a) Survival of seedling,
(b) Temporal and total (percent) seed germination,
(c) Germination rate,
(d) Length of root and shoot of the seedlings,
(e) Root/Shoot Ratio,
(f) Seedling Vigour Index, and
(g) Seedling Tolerance Index

For estimating temporal pattern (i.e., relative frequency of seed germination after every 24 hours) of seed germination per day and total seed germination, 5 replicates of 20 seeds each were analyzed. The seeds of each accession of *B. campestris* (B-1) were first surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for 3-4 minutes and washed thoroughly and then with distilled water. Finally the seeds were exposed to various doses of UV-C (5, 10 and 20 minutes). After treatment the control and treated seeds were placed on double layered filter papers (3 mm, Whattman, filter papers) with cotton pads 'sandwiched' between them equidistantly in various treatments in petri dishes and incubated under white light at 22^{o}C.

The control and UV treated sets were raised in distilled water, only a fixed amount (about 50 ml) of distilled water was added in petri dishes. Lengths of the seedling parts were measured after every 24 hours of the start of seed germination experiment. A precise ruler was used to measure the radicle length and plumule length of the seeds. On fifth day roots of the seedlings were removed to measure root and shoot lengths and then fixed in fixative for mitotic analyses. The rate or speed of seed germination for each replicate was calculated as:

$$\mathbf{R = \sum n / \sum Dn}$$

Where n is the number of seeds germinated on day D, and

D is the number of days from the beginning of the test.

Seedling Vigour Index (SVI) was also calculated by using the formula

SVI = Seedling length x Germination %

(Proposed by Abdul-Baki and Anderson (1973))

Seedling Tolerance Index was calculated by using the formula

$$STI = \frac{\text{Mean length of the longest root in treatment}}{\text{Mean length of the longest root in control}} \times 100$$

(Proposed by Turner and Marshal (1972))

All the data, collected from experiments, were analyzed statistically by calculating mean and standard deviation following standard methods. Standard error (S.E.) was calculated whenever required in order to access the deviation of the sample mean from the population mean.

$$S.E. = \frac{S.D.}{\sqrt{n}}$$

Where S.D. = Standard deviation and n = number of samples.

OBSERVATIONS

The seed germination was observed after 24 hours in both control and UV –C treated sets. The data is shown in table 2. The germination data is graphically shown in Figure 1. The 5 and 20 minutes treatments showed 100% survival rate where as control and 10 minutes treated seeds showed only 90% survival rates. The control showed 95 percent germination on the fifth day, the 10 minute treated seeds showed about 98 percent germination. The 5 and 20 minutes treatments showed 100 percent germination.

The germination rates were found to be 2 in control and UV-C treated sets. The shoot emerged on the fourth day. The data showing temporal root and shoot growth has been shown in table 3. The length of root and shoot has also been shown in the same table. The data related to seedling growth is graphically shown in figure 2. The root shoot ratio, total length of the seedling, seedling vigour index and seedling tolerance index is given in table 5. The data related to root shoot ratio, total length of the seedling, seedling vigour index and seedling tolerance index is graphically shown in figures 1, 2, 3 4 and 5.

The R/S of control set was found to be 0.6 in control, in dose 5 min. to be 0.9, in dose 10 M to be 0.7 and in dose 20 M it was found to be 1.1. The total length of the seedling on fifth day in control, 5, 10 and 20 min. was found to be 2.5, 4.0, 3.9 and 4.1 cms respectively. The SVI was found to be 235, 399, 381 and 410 in control, 5 M, 10 M and 20 M respectively. The STI values were observed to be 162, 157 and 166 in treatments 5 M, 10 M and 20 M respectively.

RESULTS AND DISCUSSIONS

UV-C irradiation treatment on *B. campestris* L. Seeds generally promoted the seed germination. The increasing duration of UV-C irradiation up to 20 min. increased the promoter effect on the seed germination of *B. campestris* L. as compared to dry seeds. Maximum

germination was of 100% was recorded at 5 min. and 20 min. UV-C irradiation, while it was low at control and 10 min. UV-C treatments.

The UV-C treated *B. campestris* L. seeds generally increased the seedling (root and shoot) growth at all exposure periods of UV-C irradiation as compared to control at all sampling days (Table 4, Fig. 3) and was found maximum at 20 min. UV-C treatment.

When compared to the root growth, the increase of shoot growth was found to be more in more UV-C treatments at all sampling days and the root/shoot ratio of *B. campestris* L. seedlings increased with the UV-C treatments. The total length of the *B. campestris* L. seedlings was (root and shoot length) was found to be more in seeds treated with UV-C irradiation than control (Table 5, Fig. 3).

In general UV-C irradiation seed treatment progressively increased seedling vigour index (SVI) of *B. campestris* L. was more in UV-C irradiated dry seeds as compared to control seeds. UV-C generally promoted the SVI of *B. campestris* L. at all sampling days as compared to controls (Table 5, Fig. 4). The tolerance index of *B. campestris* L. seedling towards the UV-C treatment was increased at all sampling days than dry seeds of control (Table 5, Fig. 5).

Siddiqui et al. (2011) reported that in ground nut seedlings showed increment in shoot weight, root length and root weight, leaf area and number of nodules when seeds of groundnut were treated with UV-C for 10, 15, 30 and 60 minutes period as observed in the present study which shows an increase in seed germination, seedling growth of *B. campestris* L. with increasing exposure period upto 20 min. of UV-C irradiation on dry seeds as compared to control. Many researchers observed that pre-sowing treatment of seeds with UV was effectively used to increase crop productivity (Jdanova, 1962; Dubrov, 1977; Ghallab and Omar, 1998; Shiozaki *et al.*, 1999). Thus dry UV-C irradiated seeds of *B. campestris* L., generally showed an increase of seed germination and all seedling growth parameters with increasing period of UV-C exposure upto 20 M as compared to dry seed controls at all sampling days.

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Table 1. Table showing detail of *Brassica campestris* seeds obtained

S.No.	Accession no.	Variety name/number	Source
01	B -1		GBPUAT, Pantnagar

Table 2. Data showing temporal and total percent seed germination of control and UV-C treated seeds of *B. campestris*.

	I Day	II Day	III Day	IV Day	V Day
Co.	0	90	90	100	100
5 M	0	100	100	100	100
10 M	0	90	100	100	100
20 M	0	100	100	100	100

(Co. = control, M = minutes)

Table 3. Data showing total percent seed germination of control and UV-C treated seeds of *B. campestris*.

	Mean	±	SE	Range		
Co.	95	±	2.89	90	-	100
5 M	100	±	0	100	-	100
10 M	97.5	±	2.5	90	-	100
20 M	100	±	0	100	-	100

Table 4. Mean data showing temporal root and shoot growth of control and UV-C treated seeds of *B. campestris*.

			Mean	±	SE	Range		
II DAY	Co.	R	1.0	±	0.2	0.0	-	2.0
		S	0.0	±	0.0	0.0	-	0.0
	5 M	R	1.0	±	0.3	0.5	-	3.0
		S	0.0	±	0.0	0.0	-	0.0
	10 M	R	0.7	±	0.4	0.0	-	5.0
		S	0.0	±	0.0	0.0	-	0.0
	20 M	R	0.8	±	0.1	0.5	-	1.0
		S	0.0	±	0.0	0.0	-	0.0
III DAY	Co.	R	0.4	±	0.6	0	-	6
		S	0.0	±	0.0	0.0	-	0.0
	5 M	R	1.1	±	0.4	2	-	6
		S	0.0	±	0.0	0.0	-	0.0
	10 M	R	0.94	±	0.64	1.4	-	7
		S	0.0	±	0.0	0.0	-	0.0
	20 M	R	0.9	±	0.1	5	-	6
		S	0.0	±	0.0	0.0	-	0.0
IV DAY	Co.	R	0.57	±	0.04	0.4	-	0.7
		S	1.1	±	0.14	0	-	1.5
	5 M	R	1.33	±	0.09	0.6	-	1.5
		S	1.35	±	0.19	0.7	-	2
	10 M	R	1.61	±	0.14	1	-	2.2
		S	1.42	±	0.1	0.9	-	1.9
	20 M	R	1.69	±	0.16	0.4	-	2.1
		S	1.76	±	0.11	1	-	2.2
V DAY	Co.	R	0.95	±	0.13	0.5	-	1.7
		S	1.52	±	0.18	0	-	2
	5 M	R	1.98	±	0.23	1	-	3.5
		S	2.01	±	0.08	1.8	-	2.5
	10 M	R	1.69	±	0.16	0.5	-	2.5
		S	2.2	±	0.14	1.3	-	2.9
	20 M	R	2.12	±	0.42	0.6	-	4
		S	1.98	±	0.12	1	-	2.5

Table 5. Mean data showing percent germination (% germ), root shoot ratio (R/S), Seedling Vigour Index (SVI), Seedling Tolerance Index (STI)

		% GERM	R/S	TL	SVI	STI
II DAY	Co.	95	0	1.0	95.0	
	5 min	100	0		100.0	
	10 min	98	0	1.0	140.0	100
	20 min	100	0		133.3	
III DAY	Co.	95	0	0.7	237.5	70
	5 min	100	0		90.9	
	10 min	98	0	0.8	104.3	75
	20 min	100	0		111.1	
IV DAY	Co.	95	0.518182	1.2	56.9	
	5 min	100	0.985185		37.3	
	10 min	98	1.133803	1.1	32.3	91.67
	20 min	100	0.960227		29.0	
V DAY	Co.	95	0.625	0.9	38.5	78.33
	5 min	100	0.985075		25.1	
	10 min	98	0.768182	0.9	25.2	75
	20 min	100	1.070707		24.4	

Fig. 1. Graph showing temporal and total seed germination of control and UV-C treated seeds of *B. campestris.*

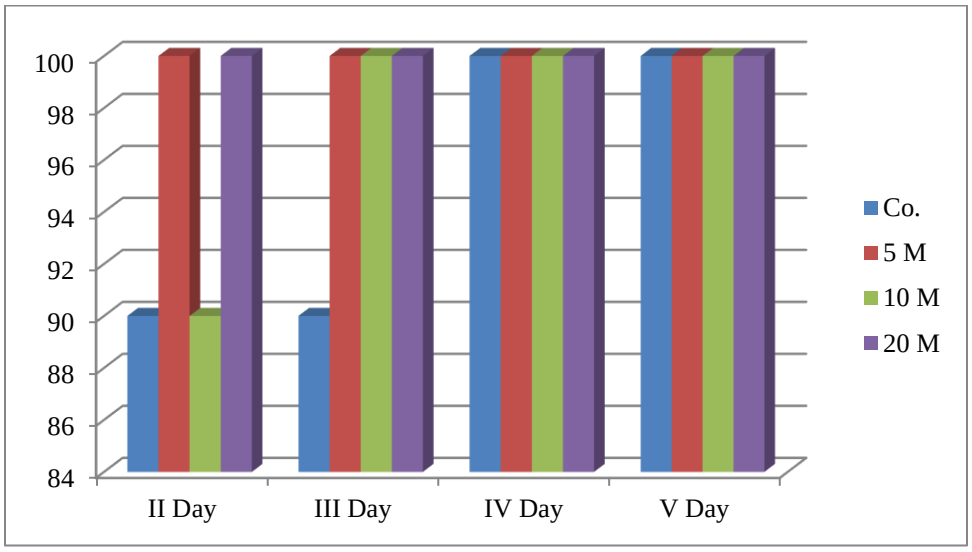

Fig. 2. Figure showing R/S of control and UV-C treated seeds of *B. campestris.*

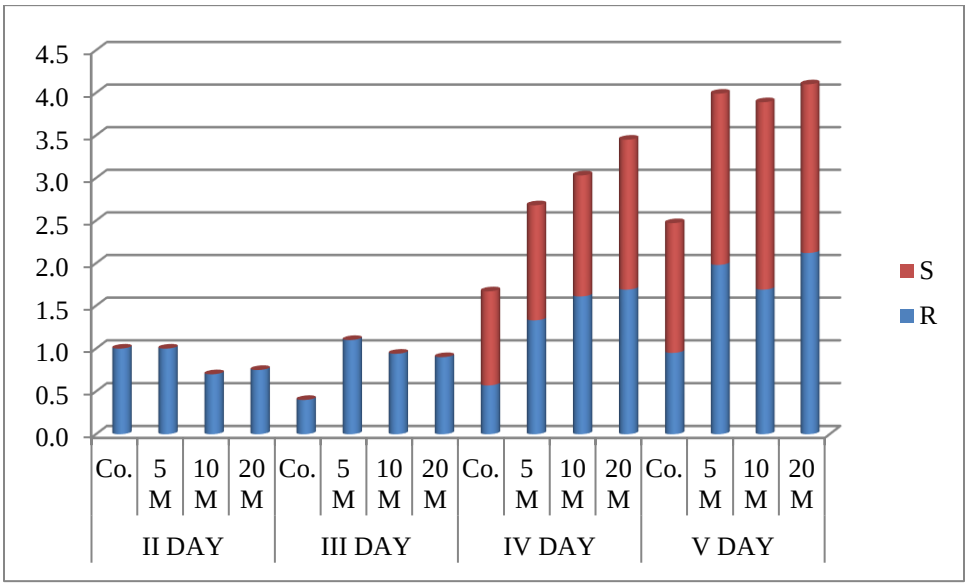

Fig. 3. Figure showing TL (total length in cms), of control and UV-C treated seedlings of *B. campestris.*

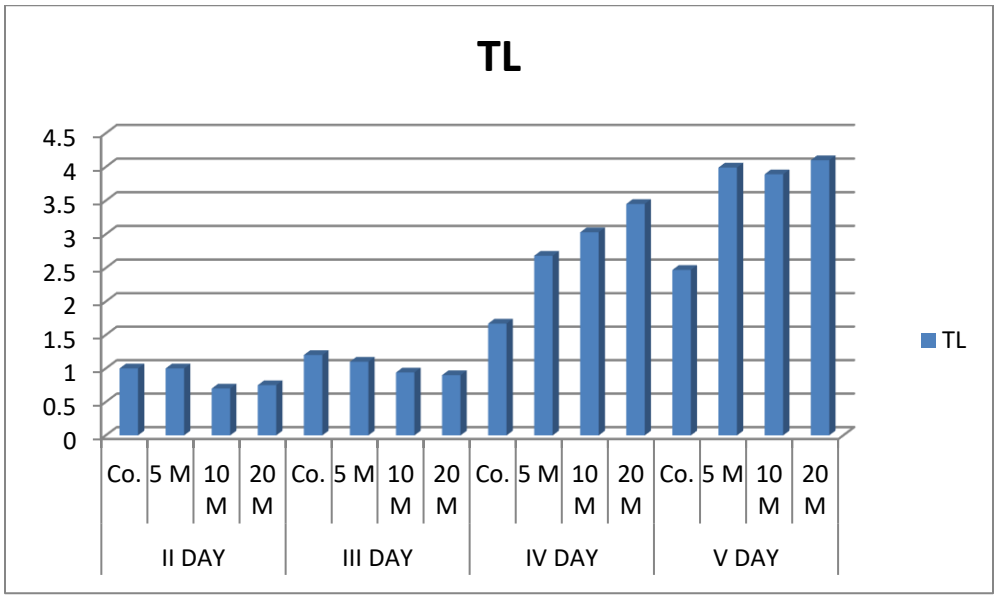

Fig. 4. Figure showing SVI (seedling vigour index) of control and UV-C treated seedlings of *B. campestris.*

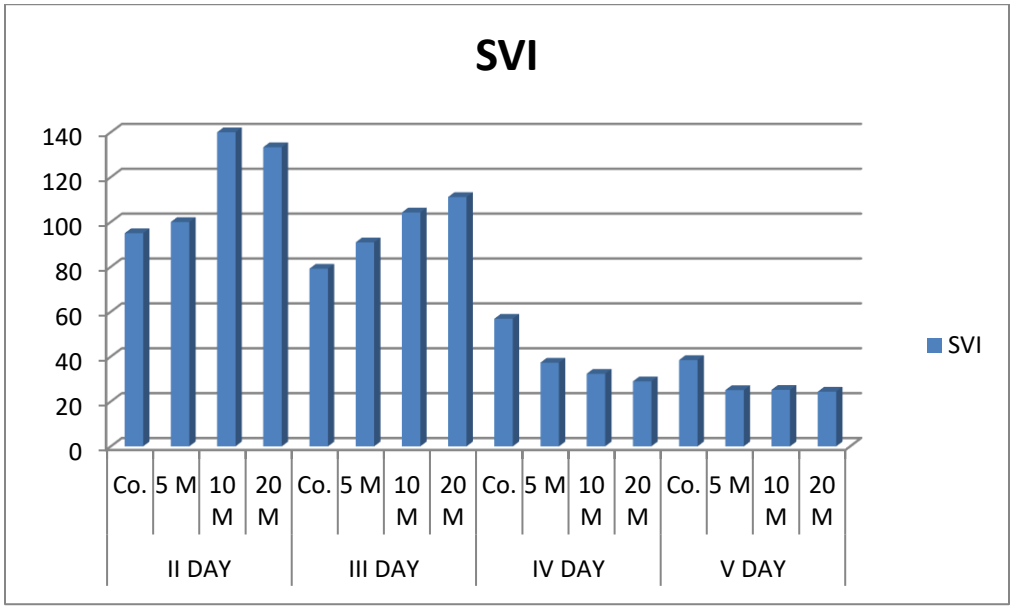

Fig. 5. Figure showing STI (seedling tolerance index) of UV-C treated seedlings of *B. campestris.*