

Morpho-Anatomical Variations in *Sisymbrium irio* L. Plants Raised from Seeds Treated with γ Radiation

Anish Mohammad, Sarita Verma, Mahmooduzzafar,* and Muhammad Iqbal



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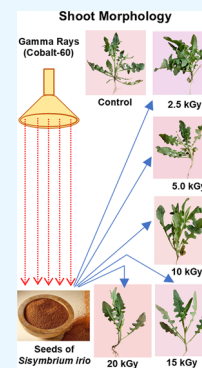
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ABSTRACT: This study determines the effect of γ irradiation on seed germination, growth, and morpho-anatomical traits of the *Sisymbrium irio* L. (London rocket) plant. Seeds irradiated with 2.5, 5, 10, 15, and 20 kGy of γ radiation showed a reduced germination percentage (13.68–56.84%) with reference to the control, showing an inverse relationship with radiation dose. Observations recorded at preflowering, flowering, and postflowering stages of plant growth showed a significant ($P < 0.05\%$) dose-dependent decline in many growth parameters, such as root length, shoot length, shoot dry weight, number of leaves, and pods per plant, due to the radiation effect. However, root dry weight, leaf length, leaf dry weight, number of branches, and number of flowers per plant increased at the lowest dose (2.5 kGy) and then declined steadily with the increasing level of radiation. Likewise, several anatomical features (length of fibers and vessel elements, diameter of vessel elements, proportion of cortex, and vasculature in the stem) showed a consistent decrease with increasing γ irradiation in treated plants compared with the control. However, the pith area and the number of vessels per microscopic field decreased significantly with the lowest radiation dose (2.5 kGy) and then increased gradually with higher doses at each ontogenetic stage. The vulnerability factor in the control as well as treated plants increased with increasing plant age. In treated plants, vulnerability was higher under the effect of low-level radiation than in the control, but it showed an inverse relationship with the increasing level of radiation, thus being the lowest at 20 kGy radiation dose. Mesomorphy also showed an almost similar variation pattern with reference to the radiation dose.



1. INTRODUCTION

Environmental stresses, natural or anthropogenic, affect the overall performance of plants by causing changes in their functional as well as morpho-anatomical characteristics,^{1–3} which result in altered growth and yield.^{4–7} Plants develop various internal mechanisms to resist these external pressures in order to ensure their health and survival.^{8,9} Efforts are also made to undo the undesirable impact of environmental harshness and improve the quality and quantity of plant products by using a variety of physical and chemical means.^{10–12} In recent years, radiation has been used effectively in various agricultural processes such as disinfection of grains, sterilization of substrate, degradation and cross-linking of natural polymers, mutation breeding, food protection, among others.¹³ One of the physical methods to improve sowing material is seed treatment with γ radiation.^{14,15} Radiations urge the atomic bonds of molecules to break down and release electrons, causing change in the molecule and generating ions.¹⁶ Irradiation of seeds may cause genetic variability that helps plant breeders in their selection.

γ Radiation can alter the physiological phenomena in plants. Their biological impact stems from their interaction with atoms or molecules, particularly water molecules, present in the cells, giving rise to free radicals.¹⁷ These radicals can damage or modify important compounds in plant cells and differentially affect the morphology, anatomy, biochemistry,¹⁸

Normally, low doses of radiation improve the metabolic activities of plants, leaving a positive effect on their growth and productivity, but higher doses prove to be injurious to plant health.^{14,15,19,20} In *Lathyrus chrysanthus*, for example, γ irradiation of seeds resulted in increased seed-germination percentage, seedling growth, and chlorophyll production at a relatively low (100–150 Gy) radiation treatment, but higher radiation doses (200–250 Gy) had significant negative impacts compared to the controls. Moreover, the extent of stimulatory effect of low radiation doses varied with the age of seedling.²¹

The genus *Sisymbrium* of the family Brassicaceae comprises about 90 species and *Sisymbrium irio*, an annual, stiffly erect, tap-rooted herb, is one of its most common invasive species, now found widely in many countries of Eurasia, southern Africa, the Andes (countries around the long Andean mountain range in South America), and North America.^{22,23} This species came to prominence as an invasive species, and people started calling it “London rocket” when it became abundant after the Great Fire of London in 1666 AD.²⁴ It possesses important

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medicinal properties and, under its vernacular name *Khaksi* or *Khubkala*, this herb is used frequently in the Indian systems of medicine, especially in *Tibb-e-Unani*, for some broad-spectrum therapeutic applications such as various inflammatory conditions and rheumatism.^{25,26} It works as an expectorant, febrifuge, and diuretic and also helps in treating voice disorders, chest congestion, and liver or spleen problems. Seeds are used for the treatment of inflammatory conditions, boils, pimples, cough, cholera, and nonspecific fever.²⁶

The crude and ethanolic extracts of seeds have shown antipyretic, analgesic, and antimicrobial effects, showing marked antibacterial action against both Gram-positive and Gram-negative organisms.²⁷ The polarity-based extract of this plant could inhibit the growth of major disease-causing bacterial strains.²⁸ The plant synthesizes secondary metabolites, such as derivatives of terpenes that act as hormones, stimulate enzymatic activity, and exhibit anticancer and antimicrobial activity as well as a capacity to scavenge free radicals.²⁹ Numerous flavonoids, alkaloids, steroids, tannins, saponins, phenolic compounds, fatty acids, steroids, amino acids, and proteins of immense chemotherapeutic interest have been isolated from various parts of the plant.^{26,30} The oil obtained from this plant has been shown to comprise 2 esters and 7 acids (38.80%), 11 sulfur- and 11 nitrogen-containing compounds (36.41%), 15 terpene derivatives (8.19%), 5 aromatic compounds (3.53%), 6 aliphatic hydrocarbons (6.29%), 4 fatty alcohols (2.49%), and 3 additional chemical compounds (1.17%).²⁹

It is a general understanding that low-level γ radiation is often beneficial for the metabolic activities of plants, but after a threshold level, it becomes harmful to their overall performance. However, it is still unclear what sort of changes it causes to the growth and structural features of plants. Despite the tremendous therapeutic potential of *S. irio*, little information exists on seed germination, seed longevity, and growth rate of seedlings/plants under harsh environmental conditions. Almost nothing is known about the impact of exogenous stresses, including radiation stress, on the morpho-anatomical traits of this species.

The present study is an attempt to fill this gap by determining the harsh impact of high-level γ irradiation on seed germination and on the growth and structural traits of the resultant plants and exploring the extent by which these radiation doses modify the normal features and threaten the health and survival of this valuable plant species.

2. MATERIALS AND METHODS

Healthy and authentically identified seeds of *S. irio* L. were obtained from the Herbal Garden of Jamia Hamdard in New Delhi. In line with some earlier works,^{31,32} these seeds were treated with γ radiation for the absorbed 2.5, 5, 10, 15, and 20 kGy, using a γ cell (GC-5000), at a dose rate of 1.65 kGy/h under ambient room temperature in the Institute of Nuclear Medicine and Applied Science (INMAS), New Delhi. The control and the irradiated seeds were germinated in field conditions at Jamia Hamdard, New Delhi. Germination percentage (GP) was calculated for five replicates using the formula

$$GP = \frac{\text{number of seeds germinated}}{\text{total number of seeds sown}} \times 100 \quad (\text{A})$$

The treated and untreated seeds were sown during the first week of November 2020 in the Herbal Garden of Jamia

Hamdard, having sandy loam soil (pH 7.3) under 70–80% relative humidity. The soil contained 51 and 7.9 ppm concentrations of nitrogen and sulfur, respectively. Each sample of seeds was sown in six rows (4 m long and 0.6 m wide), covering an area of 14.4 m², keeping hills 30 cm apart. Only 5 g seeds per hill were sown to avoid root intermingling. Plants were sampled at three developmental stages, namely, preflowering (45 days after sowing), flowering (80 DAS), and postflowering (130 DAS) stages, in order to analyze the effect of γ irradiation on plant morphology and stem anatomy. Field experiments were repeated three times with each of the five replicates. The data were collected according to the complete randomized block design (CRBD).

The root and shoot lengths were measured in centimeters, and the area of individual leaves was measured (in cm²) with a leaf-area meter (Modal 3000A, LICOR). For the biomass of root, leaves, and stem, the samples were oven-dried separately at 80 °C for 48 h. Dry weight was determined (in g) on a digital balance, while the percent dry weight of samples was calculated as

$$\% \text{dry weight} = \frac{\text{dry weight}}{\text{fresh weight}} \times 100 \quad (\text{B})$$

For anatomical studies, the fixed and preserved samples from the third internode of the stem were sectioned in the transverse plane at a thickness of 5–10 μm on the Reichert's sliding microtome, duly dehydrated in ethanol series, stained with Heidenhain's hematoxylin-safranin combination as per the standard procedure, and mounted in Canada balsam on glass slides for microscopic examination.³³ Microphotography was done using an Olympus (VenoxAH2) microscope.

In order to measure the individual cells of fibers and vessel elements accurately, the vascular tissue was macerated, following Ghouse and Yunus.³⁴ Tissue samples were cut into thin tangential slices of 1 mm thickness, which were treated with 60% HNO₃ until the elements got separated. The macerated cells (fibers and vessel elements) were neutralized with NaOH pellets for a few minutes. After washing with water, the macerated material was stained with safranin and mounted in 50% glycerol. From each sample, 50 elements were measured at random and analyzed statistically. The vulnerability index and the mesomorphy index of plants, as proposed by Carlquist,³⁵ were calculated by the following formulas, using the measurements of vessel elements, respectively

$$\text{vulnerability index (VI)} = \frac{\text{mean diameter of vessel } (\mu\text{m})}{\text{mean vessel density per mm}^2} \quad (\text{C})$$

mesomorphy index

$$\begin{aligned} &= \text{vulnerability index} \times \text{vessel} - \text{element length (MI)} \\ &= VI \times L_{VE} \end{aligned} \quad (\text{D})$$

Statistical analysis was done in five replicates ($n = 5$). The data obtained for various parameters, including seed germination and different morphological and anatomical attributes, were analyzed in five replicates using Duncan's multiple-range test. Analysis of variance (ANOVA) was also performed at significance difference ($P \leq 0.05$) for all measurements, mean separation, and standard error (SE) and compared with Duncan's multiple-range test at $P \leq 0.05$ for each replicate.

Table 1. Germination Percentage of *S. irio* L. Seeds, Control, and Seeds Treated with Various Doses of γ Radiation^a

seed germination%	control	2.5 kGy	5 kGy	10 kGy	15 kGy	20 kGy
field condition	95.00 (0.00)	82.00 (13.68)	70.00 (26.31)	62.00 (34.73)	58.00 (38.94)	41.00 (56.84)

^aMean \pm SD of five replicates. Values in parentheses display percent variation.

3. RESULTS

The data presented in Table 1 show a consistent and significant ($P < 0.05\%$) decrease in the germination percentage of irradiated seeds of *S. irio* L. in comparison to the control. The maximum decline was observed at the highest dose (20 kGy) of radiation.

Although the apparent look of the plant does not change gradually in accordance with the level of radiation stress, several growth and anatomical features, such as the length and biomass of root and shoot and the number of branches, leaves, flowers, and pods, undergo significant changes under high radiation doses. Root length increases significantly ($P < 0.05\%$) with growing plant age in the control plants. However, the application of radiation invariably reduces the root growth compared to the control. A significant and gradual decrease was recorded with the increase in the dose of γ irradiation in treated plants. The maximum decline was observed at the highest dose (20 kGy), the effect being maximum at the postflowering stage (71.96%), followed by the flowering (52.31%) and preflowering (34.49%) stages (Table 2). In the case of root dry weight, a slight increase over the control was observed with the lowest radiation dose (2.5 kGy) at the postflowering stage only. However, the higher doses caused a decline in a dose-dependent manner, with the maximum decline occurring with 20 kGy at the preflowering stage (85.29%), followed by the flowering (80.64%) and postflowering (60.02%) stages (Table 2).

Likewise, shoot length, which increased with plant age in the control population, depicted a significant ($P < 0.05\%$) dose-dependent decrease in treated plants. The maximum decline (58.85%) was observed with the highest (20 kGy) dose at the preflowering stage (Table 2). Shoot dry weight also increased with the plant age in the control population and decreased significantly in treated plants with the increasing dose of γ irradiation, showing the maximum decline (92.75%) with the highest dose at the postflowering stage (Table 2).

The number of branches per plant increased with increasing plant age. Compared to the control, their number in treated plants declined at 2.5 kGy radiation dose in samples from pre- and postflowering stages. However, the conditions improved with higher doses. At the flowering stage, there was a regular dose-dependent increase in the number of treated plants, reaching a maximum of 44.11%. At 20 kGy, percent variation varied from 10.20 to 44.11% during the different stages of plant development (Table 2).

Similarly, the number of leaves per plant in the control plants increased with the plant age. It increased with the lowest dose of radiation (2.5 kGy) with reference to the control, but higher doses (15 and 20 kGy) had a negative impact, causing a significant ($P < 0.05$) decline. The maximum effect (64.75%) with the highest dose was seen at the postflowering stage, followed by the flowering (60.21%) and preflowering (36.87%) stages (Table 2). Leaf length also showed a similar variation pattern in relation to the plant age in the control and to the radiation dose in the treated plants. The maximum decline appeared at the highest dose, showing the maximum (37.94%) impact at the preflowering stage (Table 2). Leaf dry weight

(mg/plant) also varied the same way in relation to the plant age and the radiation dose. It increased over the control with the lowest radiation dose but declined significantly and consistently with the higher ones. With the highest dose, the maximum (136.36%) decline occurred at the postflowering stage, which was followed by the flowering (134.27%) and preflowering (102.7%) stages (Table 2).

In the case of flowers per plant, a significant and the highest (104.73%) increase was noted at the lowest dose of radiation in comparison to the control; then, a consistent dose-dependent decline was caused by higher doses. Thus, the maximum decline occurred at 20 kGy (Table 2). Regarding the production of pods, the control population had the highest number of pods per plant, which showed a significant ($P < 0.05\%$) and dose-dependent decrease with the increasing dose of γ irradiation in the treated plants. Thus, the maximum decline occurred at the highest radiation dose (Table 2).

In the stem axis of the control plants, the cortex area increases gradually with plant age, showing only a little variation. In treated plants, a significant ($P < 0.05\%$) increase in cortex area was noticed with the lowest radiation dose, showing a 9.64% (postflowering stage) to 53.38% (preflowering stage) variation from the control. However, this increase was reduced gradually at higher doses, almost in a dose-dependent manner (Table 3 and Figures 1 and 2).

The area occupied by vasculature is somewhat larger during the flowering phase than in the pre- or postflowering stage, both in the control and treated plants. Irradiation invariably reduces the vascular zone at each stage of plant growth, with the reduction being inversely proportional to the radiation dose. The percent area at the highest (20 kGy) dose varied from 16.39% (flowering stage) to 21.99% (preflowering stage) (Table 3 and Figures 1 and 2).

In contrast, the pith area decreased from the preflowering to flowering stage and then increased at the postflowering stage in the control. In treated plants, a low dose of radiation had a significant impact up to the flowering stage, but higher doses caused a significant increase in pith area at each ontogenetic stage compared to the control. The maximum effect occurred with the highest dose at the postflowering stage. The percent area at this dose varied from 42.76% to 173.45% during the different stages of plant development (Table 3 and Figures 1 and 2).

The data presented in Table 3 show a significant ($P < 0.05\%$) increase in the fiber length in the stem with plant age in the control plants. In treated plants, the lowest dose of radiation (2.5 kGy) seems to have a hormetic effect during the preflowering and flowering phases, as evident from a large increase in the fiber length in comparison with the control, but there was a steep fall in the postflowering phase. However, the higher doses showed a consistent negative effect in a dose-dependent manner throughout the plant life. The decline in length was maximum at the postflowering (53.51%) stage, followed by the flowering (38.83%) and preflowering stages (34.29%).

On the contrary, the length of vessel elements in the stem of the control as well as γ -irradiated plants underwent a

Table 2. Effect of Different Doses (2.5, 5, 10, 15, and 20 kGy) of γ Irradiation on Growth Parameters of *S. irio* Plants at Different Stages of Plant Development^{ab}

parameters	control	2.5 kGy	5 kGy	10 kGy	15 kGy	20 kGy
preflowering	7.77 ± 0.68 (00.00)d	7.23 ± 0.64 (6.94)ef	6.36 ± 0.51 (18.14)def	5.88 ± 0.33 (24.32)efg	5.65 ± 0.27 (27.28)fg	5.09 ± 0.48 (34.49)g
flowering	9.94 ± 0.73 (00.00)e	7.56 ± 0.46 (23.94)d	6.90 ± 0.35 (30.58)def	5.78 ± 0.30 (41.85)efg	5.42 ± 0.34 (45.47)b	4.74 ± 0.45 (52.31)b
postflowering	15.16 ± 0.89 (00.00)a	7.72 ± 0.36 (49.07)d	7.03 ± 0.47 (53.62)de	4.98 ± 0.33 (67.15)g	5.45 ± 0.46 (64.05)c	4.25 ± 0.61 (71.96)d
preflowering	0.034 ± 0.017 (00.00)ef	0.034 ± 0.010 (00.00)l	0.025 ± 0.007 (26.47)ghi	0.014 ± 0.001 (58.82)t	0.006 ± 0.001 (82.35)ef	0.005 ± 0.002 (85.29)efgh
flowering	0.062 ± 0.001 (00.00)d	0.062 ± 0.003 (00.00)e	0.040 ± 0.001 (35.48)efgh	0.029 ± 0.001 (53.22)hi	0.022 ± 0.001 (64.51)ghi	0.012 ± 0.001 (80.64)d
postflowering	0.079 ± 0.004 (00.00)c	0.141 ± 0.003 (78.48)ab	0.128 ± 0.005 (62.02)a	0.128 ± 0.003 (62.02)efg	0.124 ± 0.003 (56.96)b	0.030 ± 0.002 (62.02)ab
preflowering	10.28 ± 0.45 (00.00)de	8.39 ± 0.57 (18.38)f	7.48 ± 0.45 (27.23)gh	6.41 ± 0.71 (37.64)f	5.80 ± 0.66 (43.57)ef	4.23 ± 0.62 (58.85)f
flowering	15.50 ± 0.96 (00.00)c	13.62 ± 1.34 (12.12)b	12.66 ± 1.56 (18.32)b	12.09 ± 2.26 (22.00)c	11.59 ± 1.12 (25.22)c	11.56 ± 1.57 (25.41)b
postflowering	25.01 ± 1.14 (00.00)d	15.28 ± 1.29 (38.90)c	14.65 ± 1.18 (41.42)d	14.09 ± 0.43 (43.66)d	13.15 ± 1.86 (47.42)b	12.77 ± 2.30 (48.94)d
preflowering	0.105 ± 0.001 (00.00)gh	0.070 ± 0.005 (33.33)jk	0.039 ± 0.006 (62.85)kl	0.021 ± 0.002 (80.00)k	0.018 ± 0.001 (82.85)hl	0.011 ± 0.002 (89.52)ijk
flowering	0.200 ± 0.002 (00.00)e	0.174 ± 0.002 (13.00)fg	0.161 ± 0.001 (19.5)ef	0.135 ± 0.002 (32.50)jk	0.051 ± 0.001 (74.5)ij	0.027 ± 0.001 (86.5)ef
postflowering	0.690 ± 0.053 (00.00)a	0.610 ± 0.002 (11.59)d	0.543 ± 0.009 (21.30)d	0.44 ± 0.008 (36.23)	0.421 ± 0.06 (38.98)b	0.050 ± 0.005 (92.75)c
preflowering	5.80 ± 0.37 (00.00)cde	5.40 ± 0.245 (6.89)cde	5.60 ± 0.245 (3.44)ab	5.80 ± 0.374 (00.00)cde	6.00 ± 0.315 (3.44)abc	6.80 ± 0.735 (17.24)a
flowering	6.80 ± 0.663 (00.00)ef	7.20 ± 0.86 (5.88)cde	7.80 ± 0.735 (14.70)de	8.00 ± 0.316 (17.64)f	9.00 ± 1.924 (32.35)bcd	9.80 ± 0.374 (44.11)cde
postflowering	9.80 ± 0.280 (00.00)a	6.80 ± 0.374 (30.61)bcd	8.00 ± 0.894 (18.36)def	8.40 ± 0.60 (14.28)f	8.60 ± 1.503 (12.24)ab	10.80 ± 1.158 (10.20)cd
preflowering	10.93 ± 0.50 (00.00)	16.40 ± 1.09 (50.04)	15.20 ± 1.20 (39.06)	11.70 ± 0.60 (7.04)	7.50 ± 0.65 (31.38)	6.90 ± 0.59 (36.87)
flowering	18.60 ± 1.05 (00.00)	16.70 ± 1.01 (10.21)	15.70 ± 0.75 (15.59)	12.50 ± 0.64 (32.79)	8.30 ± 0.70 (55.37)	7.40 ± 0.48 (60.21)
postflowering	34.05 ± 1.40 (00.00)	28.10 ± 2.24 (17.47)	21.40 ± 1.00 (37.15)	19.20 ± 0.93 (43.61)	18.72 ± 0.56 (45.02)	12.00 ± 0.67 (64.75)
preflowering	7.38 ± 0.648 (00.00)abc	8.54 ± 0.48 (15.71)	6.74 ± 0.46 (8.67)ab	5.80 ± 0.28 (21.40)abc	5.54 ± 0.098 (24.93)def	4.58 ± 0.116 (37.94)a
flowering	8.90 ± 0.502 (00.00)bc	8.88 ± 0.745 (0.22)def	7.80 ± 0.88 (12.35)def	7.68 ± 1.50 (13.70)f	7.38 ± 0.546 (17.07)de	7.12 ± 0.365 (20.00)cd
postflowering	9.62 ± 0.166 (00.00)ef	9.90 ± 8.401 (2.91)ef	9.58 ± 0.52 (0.41)ef	8.58 ± 0.32 (10.81)f	8.10 ± 0.308 (15.80)ef	7.36 ± 0.36 (23.49)ef
preflowering	0.181 ± 0.002 (00.00)ef	0.367 ± 0.008 (102.7)ef	0.214 ± 0.002 (18.23)fg	0.184 ± 0.01 (1.65)c	0.065 ± 0.002 (64.08)c	0.014 ± 0.001 (92.26)df
flowering	0.256 ± 0.019 (00.00)de	0.600 ± 0.114 (134.37)ef	0.240 ± 0.051 (6.25)ef	0.195 ± 0.13 (23.82)g	0.077 ± 0.002 (69.92)fg	0.031 ± 0.003 (87.89)d
postflowering	0.330 ± 0.054 (00.00)de	0.780 ± 0.002 (136.36)ef	0.241 ± 0.039 (26.96)fg	0.202 ± 0.002 (38.78)g	0.085 ± 0.035 (74.24)a	0.078 ± 0.013 (76.36)b
flowers per plant	38.67 ± 10.14 (00.00)	79.17 ± 05.02 (104.73)	66.33 ± 0.33 (71.52)	55.83 ± 4.00 (44.37)	38.67 ± 4.00 (00.00)	33.50 ± 2.1 (13.36)
Pods per plant	34.10 ± 01.04 (00.00)	27.40 ± 0.92 (19.64)	25.50 ± 0.56 (25.21)	25.40 ± 0.83 (25.51)	24.10 ± 0.41 (29.32)	22.50 ± 0.40 (34.01)

^aMean ± SD on five replicates. Values in parentheses represent the percent variation ^bValues with different superscripts are significantly ($P < 0.05$) different from each other (Duncan's multiple-range test). * $P \leq 0.05$. The values represent mean ± SD. CD at 5%; treatment 0.077*; developmental stages 0.055*; treatment developmental stages: 0.173*.

Table 3. Effect of Different Doses (2.5, 5, 10, 15, and 20 kGy) of γ Irradiation on the Growth Parameters of the Stem of *S. irio* L. Plants at Different Stages of Plant Development^{a,b}

parameters	control	2.5 kGy	5 kGy	10 kGy	15 kGy	20 kGy
preflowering	24.65 ± 1.13 (00.00)	37.81 ± 0.32 (53.38)	Cortex Area in Stem (mm ²) 34.37 ± 0.97 (39.43)	32.41 ± 0.99 (31.48)	31.33 ± 1.38 (27.09)	29.71 ± 1.42 (20.52)
flowering	28.24 ± 0.70 (00.00)	32.87 ± 0.95 (16.39)	28.98 ± 1.77 (2.62)	28.66 ± 2.11 (1.48)	25.55 ± 1.54 (9.52)	25.12 ± 1.60 (11.04)
postflowering	29.75 ± 0.58 (00.00)	32.62 ± 0.92 (9.64)	32.03 ± 1.70 (7.66)	31.52 ± 2.52 (5.94)	30.39 ± 1.34 (2.15)	30.31 ± 0.41 (1.88)
preflowering	65.66 ± 3.17 (00.00)	61.82 ± 3.89 (5.84)	Vasculature Area in Stem (mm ²) 61.31 ± 3.70 (6.62)	59.25 ± 2.49 (9.76)	54.69 ± 1.44 (16.70)	51.22 ± 5.85 (21.99)
flowering	66.79 ± 1.17 (00.00)	62.74 ± 2.56 (6.06)	62.06 ± 0.99 (7.08)	61.17 ± 1.44 (8.41)	60.89 ± 2.21 (8.83)	55.84 ± 2.54 (16.39)
postflowering	63.07 ± 2.19 (00.00)	57.89 ± 3.19 (8.21)	56.01 ± 4.40 (11.19)	54.28 ± 2.86 (13.94)	53.13 ± 3.57 (15.76)	51.24 ± 4.18 (18.76)
preflowering	9.80 ± 0.79 (00.00)	4.53 ± 0.41 (53.78)	Pith Area in Stem (mm ²) 6.97 ± 0.45 (28.88)	7.82 ± 1.01 (20.20)	9.61 ± 0.68 (1.94)	13.99 ± 0.96 (42.76)
flowering	4.97 ± 1.14 (00.00)	3.22 ± 0.23 (35.21)	8.60 ± 0.89 (73.04)	9.85 ± 0.55 (98.19)	11.28 ± 1.74 (126.96)	13.59 ± 1.04 (173.44)
postflowering	7.18 ± 1.08 (00.00)	7.08 ± 1.05 (1.39)	12.19 ± 1.03 (69.78)	13.65 ± 1.25 (90.12)	14.25 ± 0.75 (98.47)	18.45 ± 1.39 (156.97)
preflowering	511.40 ± 17.3 (00.00)	566.60 ± 21.56 (10.79)	Fiber Length (μm) 464.40 ± 18.42 (9.19)	419.00 ± 15.11 (18.06)	396.00 ± 13.15 (22.56)	336.00 ± 10.88 (34.29)
flowering	547.40 ± 21.76 (00.00)	569.60 ± 15.33 (4.05)	484.80 ± 11.34 (11.43)	404.40 ± 25.11 (26.12)	388.80 ± 10.91 (28.97)	334.80 ± 18.54 (38.83)
postflowering	617.00 ± 32.20 (00.00)	513.60 ± 20.85 (16.76)	381.60 ± 12.62 (38.15)	341.10 ± 14.44 (44.71)	309.60 ± 11.70 (49.82)	286.80 ± 6.56 (53.51)
preflowering	128.40 ± 5.67 (00.00)	102.00 ± 8.05 (20.56)	Length of Vessel Element (μm) 99.60 ± 4.40 (22.42)	99.60 ± 5.95 (22.42)	96.00 ± 5.66 (25.23)	96.00 ± 5.06 (25.23)
flowering	91.20 ± 4.08 (00.00)	86.40 ± 5.88 (5.26)	81.60 ± 5.88 (10.52)	78.00 ± 6.26 (14.47)	72.00 ± 2.53 (21.05)	68.40 ± 4.75 (25.00)
postflowering	88.80 ± 4.45 (00.00)	81.60 ± 3.92 (8.10)	79.20 ± 4.45 (10.81)	72.00 ± 4.38 (18.91)	67.20 ± 4.80 (24.32)	66.20 ± 5.52 (25.45)
preflowering	25.24 ± 1.02 (00.00)	23.88 ± 1.94 (05.39)	Vessel Diameter (μm) 22.60 ± 1.29 (10.46)	22.04 ± 1.92 (12.68)	21.09 ± 1.19 (16.44)	21.04 ± 1.11 (16.64)
flowering	27.48 ± 0.71 (00.00)	25.30 ± 0.90 (7.93)	25.20 ± 1.67 (08.30)	23.66 ± 1.75 (13.90)	22.10 ± 1.50 (19.58)	21.84 ± 1.06 (20.52)
postflowering	29.80 ± 1.48 (00.00)	26.10 ± 1.22 (12.42)	25.50 ± 0.81 (14.43)	23.76 ± 1.19 (20.27)	23.22 ± 1.02 (22.08)	22.30 ± 2.30 (25.17)
preflowering	10.79 ± 0.33 (00.00)	7.49 ± 0.64 (30.58)	Number of Vessels Per Microscopic Field (mm ²) 8.20 ± 0.46 (24.00)	9.69 ± 0.57 (10.19)	10.27 ± 0.61 (4.81)	10.73 ± 1.15 (0.56)
flowering	9.98 ± 0.60 (00.00)	6.14 ± 0.52 (38.47)	7.49 ± 0.31 (24.94)	7.97 ± 0.84 (20.14)	10.09 ± 0.67 (1.10)	10.09 ± 0.67 (1.10)
postflowering	7.10 ± 0.82 (00.00)	5.47 ± 0.43 (22.95)	5.93 ± 1.04 (16.47)	6.15 ± 0.56 (13.38)	7.04 ± 0.95 (0.84)	7.80 ± 0.74 (9.85)
preflowering	2.34 ± 0.49 (00.00)	3.19 ± 0.27 (36.32)	Vulnerability Factor 2.75 ± 0.14 (17.52)	2.27 ± 0.31 (2.99)	2.05 ± 0.28 (12.39)	1.96 ± 0.38 (16.24)
flowering	2.75 ± 0.34 (00.00)	4.12 ± 0.19 (49.82)	3.36 ± 0.36 (22.18)	2.96 ± 0.20 (7.64)	2.19 ± 0.27 (20.36)	2.16 ± 0.31 (21.45)
postflowering	4.19 ± 0.16 (00.00)	4.77 ± 0.23 (13.84)	4.30 ± 0.23 (2.63)	3.86 ± 0.31 (7.87)	3.29 ± 0.18 (21.47)	2.85 ± 0.28 (31.98)
preflowering	300.46 ± 0.47 (00.00)	325.38 ± 0.24 (8.29)	Mesomorphy 273.90 ± 0.23 (8.84)	226.09 ± 0.060 (24.75)	196.80 ± 0.24 (34.50)	188.16 ± 0.30 (37.38)
flowering	250.80 ± 0.41 (00.00)	355.96 ± 0.24 (41.93)	274.18 ± 0.34 (9.32)	230.88 ± 0.35 (7.94)	157.68 ± 0.32 (37.13)	147.74 ± 0.31 (41.09)
postflowering	372.07 ± 0.25 (00.00)	389.23 ± 0.25 (4.61)	340.56 ± 0.26 (8.47)	277.92 ± 0.19 (25.30)	221.09 ± 0.15 (40.58)	188.67 ± 0.20 (49.29)

^aMean ± SD on five replicates and percent variation in parentheses ^bValues with different superscripts are significantly ($P < 0.05$) different from each other. * $P \leq 0.05$. The values represent mean ± SD. CD at 5%; treatment 0.077*; developmental stages 0.055*; treatment developmental stages: 0.173*.

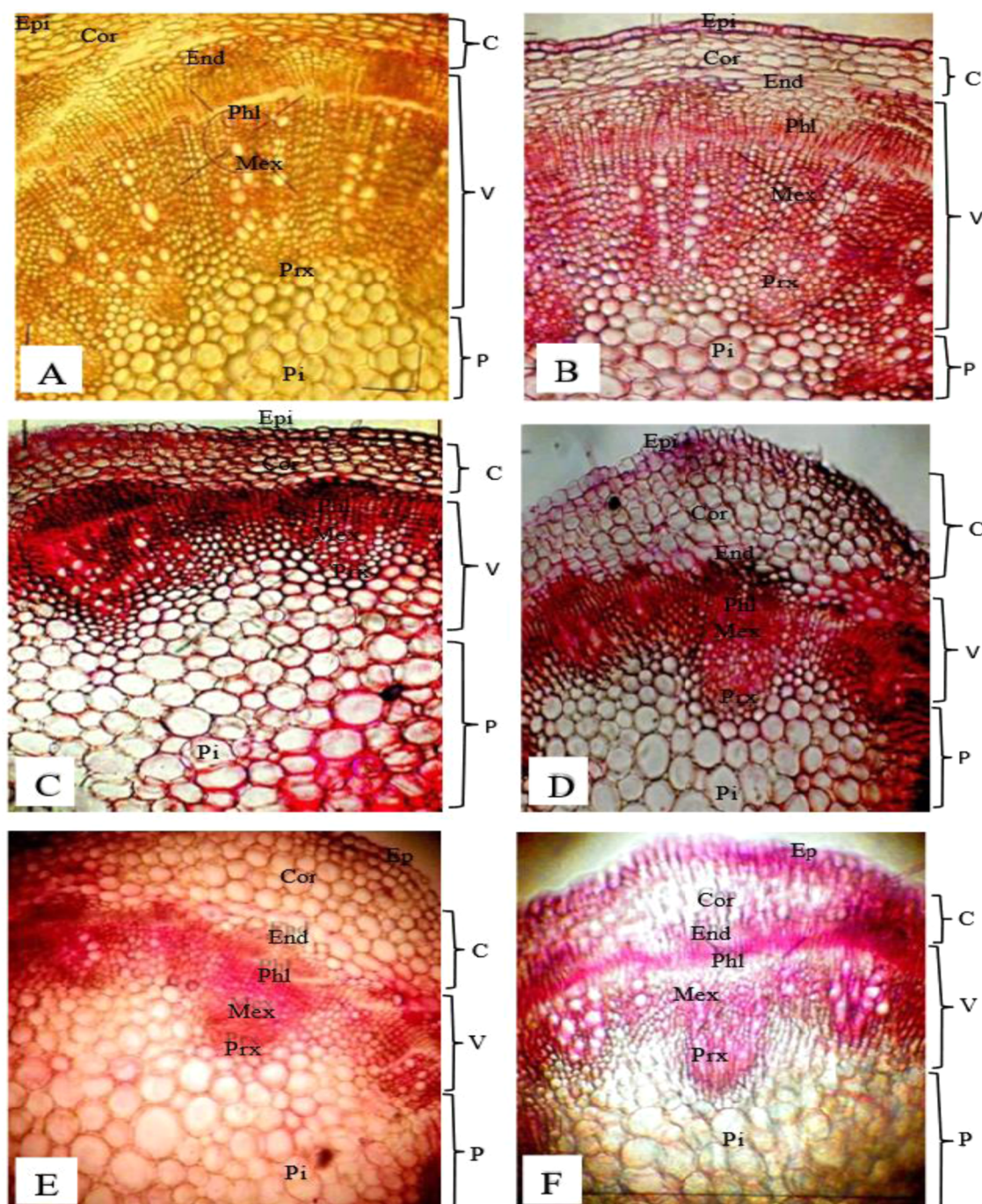


Figure 1. Transverse sections of *S. irio* L. plant stems showing the effect of γ radiation on cortex area, vasculature area, and pith area in stem during the flowering stage (magnification: $\times 100$). (Epi, epidermis; Cor, cortex; End, Endodermis; Phl, Phloem; Mex, Metaxylem; Prx, Protoxylem; Pi, Pith; C, Cortex; V, Vasculature; P, Pith). (A) Transverse section of the stem of the control plant, (B) transverse section of the stem of the γ -irradiated plant (2.5 kGy), (C) transverse section of the stem of the γ -irradiated plant (5 kGy), (D) transverse section of the stem of the γ -irradiated plant (10 kGy), (E) transverse section of the stem of the γ -irradiated plant (15 kGy), and (F) transverse section of the stem of the γ -irradiated plant (20 kGy).

significant decrease with increasing plant age. The increasing doses of γ irradiation also had a similar impact in treated plants, causing the maximum decline (up to 25.45%) at the highest dose (Table 3).

The average diameter of vessel elements increased with the growing plant age both in the control and γ -irradiated plants, with the rate of increase relatively lower in the treated lot. The impact of radiation depended on the dose, causing the maximum decline with the highest dose. When seen in relation

to plant ontogeny, the maximum (25.17%) effect of radiation was found at the postflowering stage, followed by the flowering (20.52%) and preflowering (16.64%) stages (Table 3).

In control as well as treated plants, vessel density, i.e., the number of vessels per microscopic field in transverse view, decreased significantly with plant age. Under the impact of radiation, it was relatively low in comparison with the control. The lowest radiation dose had the maximum impact, with a significant decline in vessel density, particularly at the flowering

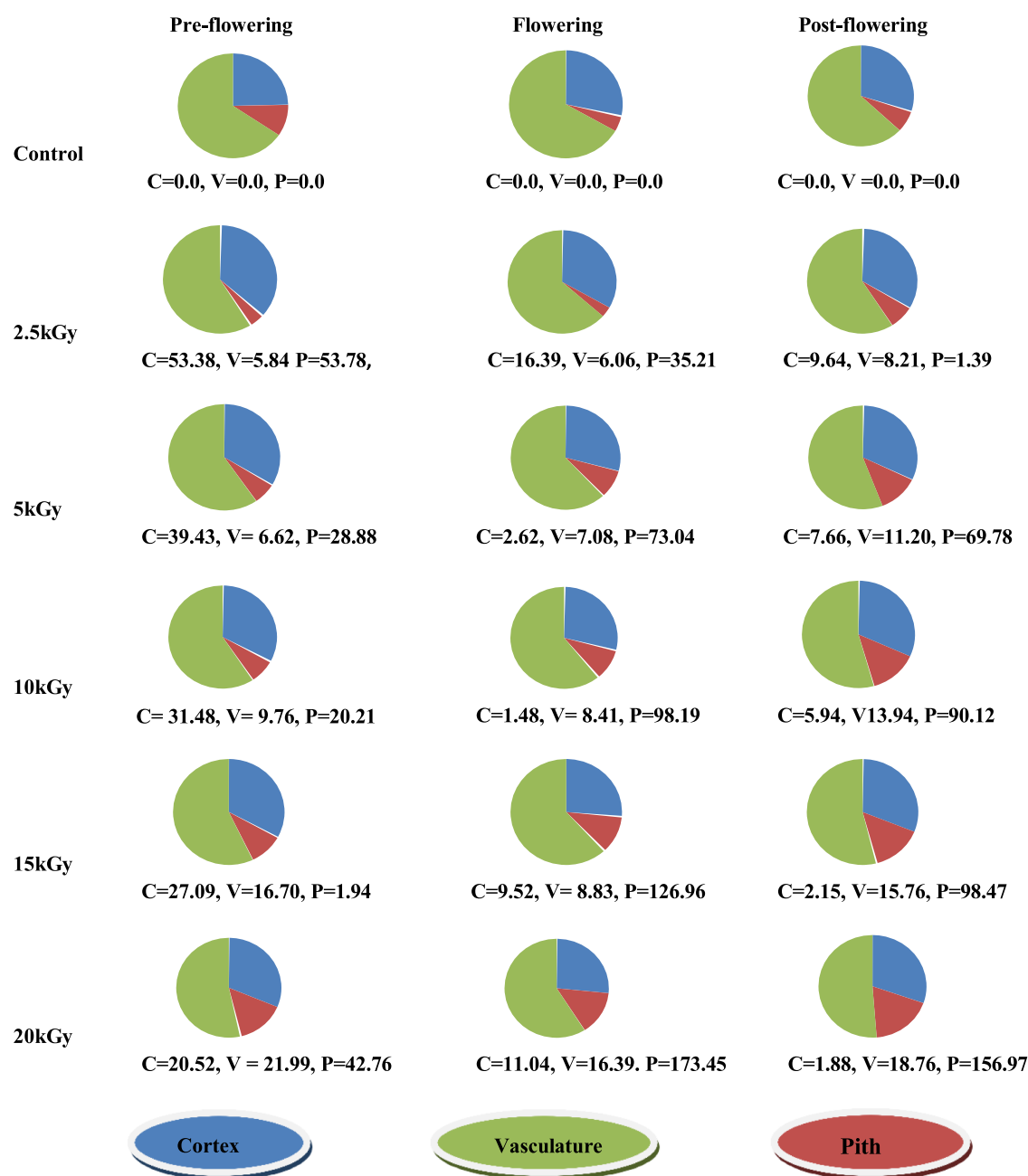


Figure 2. Pie diagram showing the relative proportion and percentage variation of the cortex, vasculature, and pith tissue in the stem of the *S. irio* L. plant.

stage (38.47%). The increase in the dose of radiation then improved the vessel density almost consistently, deepening the impact in treated plants. Thus, the extent of the decline in vessel density with reference to the control was the minimum at the highest dose (Table 3).

The vulnerability factor (mean vessel diameter/mean vessel density) was found to increase significantly with the growing plant age in both the control and treated plants. In the treated plants, it increased significantly at the lower radiation doses but decreased at higher doses compared with the control (Table 3). Mesomorphy (vulnerability \times mean vessel-element length) showed a significant fall from the preflowering to flowering stage and then a sudden rise during the postflowering stage in the control plants. Under the effect of low radiation, it showed a sudden increase, but higher doses of radiation caused a

gradual decline in the M value, showing the maximum fall at the highest (20 kGy) dose. The percent variation at this dose was maximum (49.19%) at the postflowering stage, followed by that at the flowering (41.2%) and preflowering (37.33%) stages. We noticed a significant reduction in germination percentage (Table 3).

4. DISCUSSION

The consistent decline observed in the seed-germination percentage in *S. irio* with increasing levels of γ radiation is in agreement with many earlier reports for various plant species.^{15,31,32,36–39} This is ascribed normally to the formation of free radicals and reactive oxygen species in irradiated seeds.^{17,39} On the other hand, stimulation of seed germination at low doses of γ irradiation, as noted in some other species, is

possibly due to the activation of synthesis of RNA, or protein or enzyme involved in auxins formation for germination.^{40,41} Bonde et al.⁴¹ reported that the percent seed germination in mung bean (*Vigna radiata*) increased progressively with the increasing dose of γ radiation up to a high level and then declined.

Unlike the apparent morphology of the root and shoot, their internal features showed a remarkable variation under the impact of γ radiation. The increase in shoot length of the growing plant underwent a significant ($P < 0.05\%$) dose-dependent decline in treated plants compared to the control, as reported earlier for *Abelmoschus moschatus* L.,³⁷ *Macrotyloma uniflorum* (Lam.) Verdc.,⁴³ *Trigonella foenum-graecum* L.,⁴² among others. A reduction in seedling height may be attributed to growth inhibition due to the low rate of cell division, decreased amylase activity, and increased peroxide activity.³⁸ However, some reports indicate a significant improvement in plant height by γ irradiation, as observed by Amir et al.⁴⁴ in *Abelmoschus esculentus*, for instance. Similar to the shoot length, the growing root length in *S. irio* was also retarded significantly ($P < 0.05\%$) with the increasing dose of γ irradiation (Table 2), as reported earlier for *T. foenum-graecum* L.,⁴² *Foeniculum vulgare* Mill.,⁴⁵ etc.

As expected, the number of branches increased with plant age in the control, and this improved further in γ -irradiated plants except at the initial dose (2.5 kGy). Opposite results were reported for *Dendranthema grandiflora*.⁴⁶ Also, the number of leaves per plant increased significantly ($P < 0.05\%$) with plant age in the control plants of *S. irio*; however, contrary to the number of branches, leaf number per plant was reduced significantly ($P < 0.05$) with increasing dose of γ radiation, except at the lowest dose (2.5 kGy), which showed favorable effect (Table 2). Almost similar results (decrease in number of leaves with the increase in radiation dose) are on record for species like *A. moschatus* L.³⁷ and *F. vulgare* Mill.⁴⁵ Leaf length in the *S. irio* plants also showed a similar trend as the leaf number, showing a consistent decrease with the increase in the radiation dose, although the lowest dose (2.5 kGy) was beneficial. These results coincide with the findings on the leaf length in *Solanum aethiopicum* L.⁴⁷

In the control plants, the number of flowers per plant showed a significant initial increase at the lowest dose of γ radiation and then a consistent dose-dependent decline with increasing radiation levels. An opposite condition was observed in *F. vulgare* Mill.⁴⁵ Likewise, the number of pods per plant was higher in the control population of *S. irio* than in the treated one, which showed a significant and consistent dose-dependent decrease in the number of pods with the increasing dose of γ irradiation. On the contrary, however, Hanafy and Akladios,⁴² noted that the increase in radiation intensity (25–200 Gy) was associated with the increase in the number of pods per plant in *Trigonella foenum-graecum*.

In the present study, the cortex area in the stem axis of the control *S. irio* plants showed very little variation with reference to plant age, being the least at the flowering stage. However, radiation doses caused a gradual decline in the cortex area almost in a dose-dependent manner. Thus, the maximum decline occurred with the highest dose (20 kGy). As opposed to the cortex area, the vasculature area was somewhat larger during the flowering phase than in the pre- or postflowering stage both in the control and treated plants. Irradiation invariably reduced the vascular zone at each stage of plant growth; the effect kept increasing with the increase in radiation

dose. The maximum decline thus occurred with the highest dose (20 kGy). In contrast, the pith area was significantly smaller at the flowering stage than at the pre- or postflowering stage in the control plants. The lowest dose of γ radiation significantly reduced the pith area at all stages of plant development, but higher doses caused a significant ($P < 0.05\%$) increase at each ontogenetic stage compared to the control (Table 3). Comparable information about other crop plants seems to be lacking.

A significant increase in fiber length with plant age was noticed in the control population of *S. irio*. High doses of radiation in treated plants had a consistent negative effect in a dose-dependent manner. As to the size of vessel elements in the stem, their diameter significantly increased with the growing plant age in the control as well as treated plants. However, higher doses of γ radiation had a negative impact. The number of vessels per microscopic field showed a reverse trend of variation (a consistent decrease) with plant age both in the control and the treated plants. With the increasing dose of radiation, the number of vessels per microscopic field increased. Such studies on the relative proportion of tissues or the size and density of their component cells in some other plant species facing different environmental stresses have shown diverse variation patterns.^{7,48–51}

Carlquist³⁵ introduced xylem-vessel indices, like vulnerability and mesomorphy, to quantify the xeromorphic/mesomorphic capacity of wood in terms of anatomical parameters such as vessel diameter, vessel-element length, and vessel density (vessels per mm²). He defined vulnerability (V) as “vessel diameter divided by vessel density”. Also, considering the shorter vessel elements to be more suitable to resist tensions, he introduced a more comprehensive index, viz., mesomorphy (M), which is “vulnerability multiplied by vessel-element length”. A higher ‘M’ suggests a greater efficiency, while a lower one indicates greater safety or xeromorphy. These indices have since been used frequently to determine the functional-cum-ecological status of plants. Vessel density, apparently related to hydraulic safety via redundancy and embolism resistance, is of special importance in xeromorphic woods, which often have narrow vessels in abundance. According to Carlquist,^{35,52} narrow vessels are hydraulically “safe,” while the wider ones are more vulnerable to embolism.

Emphasizing the hydraulic significance of wider vessels, Ewers et al.⁵³ held that from a functional perspective, the “mean vessel diameter” can preferably be termed as “mean hydraulic diameter”. Further, Jacobsen and Pratt,⁵⁴ based on their study of vulnerability-to-embolism curves, endorsed Carlquist’s functional interpretations of vessel diameter, as they found a strong correlation of vessel-diameter polymorphism (occurrence of diverse vessel-diameter classes within a wood) with the shape of vulnerability curves. However, no relationship could be detected between the vessel-element length and the curve shape, and it remains unclear how the shorter vessel elements may confer safety to the tissue. In the present study, vulnerability was somewhat high with low radiation doses and relatively low with higher doses in comparison to the control. Variation in mesomorphy also showed a more or less similar trend with reference to the radiation dose, with the lowest value occurring at 20 kGy (Table 3).

5. CONCLUSIONS

An analysis of the results indicates that the percentage germination of irradiated *S. irio* seeds was adversely affected, especially with higher doses (15 and 20 kGy) of γ radiation. High doses of ionizing radiation have an inhibitory effect on the growth and developmental processes of seedlings, suppressing the overall growth of plants and reducing the size and/or number of different plant organs. Certain anatomical features such as the proportion of cortex and vasculature in the stem, fiber length, and length and diameter of vessel elements declined on the whole, while the pith area increased, after an initial decline, with the increasing dose of γ irradiation. However, the number of branches, flowers per plant, and vessels per microscopic field (mm^2) in the transverse view increased under the effect of radiation. Lower radiation doses increased the level of vulnerability or mesomorphy, but higher doses caused a decline. Further research must explore, in particular, whether the high-level γ -radiation stress disturbs also the production and quality of the secondary metabolites of this species known for their therapeutic properties.

AUTHOR INFORMATION

Corresponding Author

Mahmooduzzafar – Department of Botany, School of Chemical and Life Sciences, Jamia Hamdard, New Delhi 110062, India; orcid.org/0009-0006-3879-9809; Email: m_zafar@jamiahamdard.ac.in

Authors

Anish Mohammad – Department of Botany, School of Chemical and Life Sciences, Jamia Hamdard, New Delhi 110062, India

Sarita Verma – Department of Botany, School of Chemical and Life Sciences, Jamia Hamdard, New Delhi 110062, India

Muhammad Iqbal – Department of Botany, School of Chemical and Life Sciences, Jamia Hamdard, New Delhi 110062, India

Complete contact information is available at:
<https://pubs.acs.org/10.1021/acsomega.4c04781>

Notes

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