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Gamma rays impact on vegetative growth parameters in M_1 generation of *Swertia chirayita* (Roxb. Ex Fleming) H. Karst

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Abstract
Swertia chirayita is a critically endangered, highly valuable herb of temperate Himalayas which belongs to family Gentianaceae and occurs at an altitude of 1200-3000 m in India. The present studies focused on the impact of the gamma doses tested (2-30 kr at a dose interval of 2kr) on the growth performance of main shoot and beyond upto complete senescence. Some of the gamma ray doses had a promotory effect on plant height, no. of leaves per plant, leaf length, leaf width and no. of costae per lamina in comparison to control. Most of the M_1 plants died before reaching reproductive phase. Of the M_1 plants that did produce flowers (G_1, G_3, G_6, G_7, G_9, G_{11} and G_{13}), only G_7 and G_{11} produced viable seeds (48-72% germination). Light brown seed (48.33% germination) were observed in one plant impacted by 14 kr dose in addition to normal dark brown seed (70 to 71.67% germination).

Keywords: M_1 plants, mutation, gamma rays and doses

Introduction
Swertia chirayita is a critically endangered, highly valuable and highly prized herb in India which belongs to family Gentianaceae (Rai *et al*., 2000) [17]. It is well known for its medicinal properties (Joshi and Dhawan, 2005) [8]. The plant is native to temperate Himalayas and reported to occur at an altitude of 1200-3000 m, from Kashmir to Bhutan and 1200-1500 m in the Khasi hills (Kirtikar and Basu, 1984) [11]. It is reported to occur in regions of Dhauladhar, Mandi, Kullu, Kangra, Shimla etc. regions of Himachal Pradesh(Chauhan, 1999) [4].
The bitterness, antihelmintic, hypoglycemic and antipyretic properties are attributed to amarogentin, swerchirin, swertiamarin and other active principles of its herb. It is official in Indian Pharmacopoeia and was formerly also official in British and American Pharmacopeia as tincture and infusion (Joshi and Dhawan, 2005) [8]. In Indian medical systems, chirayita is used as a remedy for bronchial asthma, liver disorders, chronic fever, anaemia, stomachic and diarrhea. In Ayurveda, *Swertia chirayita* is used as antipyretic, anthelmintic, antiperiodic, laxative, in asthma and leucorrhea. Chirayita is also used as one of the ingredients in "Chandra Prabati" which is an ayurvedic drug for cancer. The plant is best known in India as the main ingredient in mahasudarshana churna, a remedy containing more than 50 herbs (Encyclopedia of medicinal plants). Herbal medicines such as Ayush-64, Diabecon, Mensturyl syrup and Melicon-V ointment contain chirayita extract in different concentrations for its antipyretic, hypoglycemic, antifungal and antibacterial properties. *Swertia chirayita* has an established domestic (India) and international market which is increasing at the rate of 10% annually (Joshi and Dhawan, 2005) [8]. Under National Ayush Mission (NAM) of NMPB (National Medicinal Plant Board), launched by Ministry of AYUSH, Govt of India during 12th plan period, *Swertia chirayita* is one of the prioritized 95 medicinal plant species for which central Government is providing 75% subsidy (@ of cost of cultivation). The demand for raw material of *Swertia chirayita* is increasing every year as sale of its raw material is providing a complimentary source of cash for many people, most of which is collected from wild. Availability of its natural stock is very low due to overexploitation and unscientific harvesting from its natural habitat. So the plant has a very narrow genetic base, as it is present in very few pockets of natural habitat. Induced mutations are an important complementary method for the creation of genetic variability for the creation of specific characters in a crop when the variability is completely exhausted preventing genetic improvement through conventional breeding techniques. As a source of variability, mutation breeding has been used successfully in several crops for improving agronomically important traits (Maluszynski *et al*., 1995) [15]. With this view, the present study was initiated to study the impact of gamma rays on growth

parameters of *Swertia chirayita*. This study was in continuation to earlier work which was recorded upto radicle stage growth of M_1 plants (Kumar, 2012) [13]. During the period of present study (2011-2013), these M_1 plants at radicle stage further developed main shoot as well as cauline leaves and impact of gamma radiation on main shoot was studied. The observations revealed the impact of gamma rays on the growth and development of M_1 plants on various parameters like height, leaf shape and size.

Materials and methods

The study was conducted on the M_1 generation plants of *Swertia chirayita* growing at Shilly farm of the Dr. Y.S. Parmar University of Horticulture and Forestry Solan. At the stage of commencement of the present study (November 2011), the plants of the M_1 generation were at radicle leaf stage ready for transplantation. This M_1 generation was raised by treating open pollinated seeds of *Swertia chirayita* with fifteen doses of gamma radiations (2 to 30 kr at a dose interval of 2 kr) from a $C0^{60}$ source using Gamma Chamber-900 (manufactured by Board of Radiation & Isotope Technology, Dept. of Atomic Energy, Govt. of India) at Dept. of Chemistry, Himachal Pradesh University, Summer Hill, Shimla and sown in nursery beds in April 2010. Besides untreated seeds were also sown in nursery beds to serve as control. The seedlings were pricked into polybags (containing sand +soil +FYM media in the ratio of 1:1:1) during November 2010. The seedlings of M_1 generation maintained in polythene bags (15 treatment and untreated plants) were transplanted to main field (at a spacing of 30×45 cm) in November 2011.

Results & Discussion

Some of the gamma ray doses had a promotory effect on quantitative parameters like plant height (78.66 to 81.72 cm in comparison to 68.22 cm in control), no. of leaves per plant (102.27 to 113.27 leaves per plant in comparison to 79.24 no. of leaves in control), leaf length (13.64 to 13.8 cm in 6 and 8 kr doses in comparison to 12.12 cm in control), leaf width (5.0 to 5.46 cm in 6, 8 and 14 kr doses in comparison to 4.31 cm in untreated plants) and no. of costae per lamina (in comparison to average no. of 7.28 in untreated plants, the plants treated by 12, 16, 22 and 24 kr doses exhibited 7.95 to 10).

Fig 1, 2 & 3: M_1 plants in pots, field and polyhouse

Fig 4 & 5: M_1 plants at flower initiation stage
Fig 6: Light and dark colored seeds of G_7 treatment (14kr)
Fig 7: Flowers of *Swertia chirayita* (M_1 progeny)

Table 1: Effect of different doses of gamma rays on different quantitative morphological parameters (at about to flowering stage)

Field Condition/ Gamma rays dose	Plant height				No. of leaves per plant				Leaf length				Leaf width				No. of costae per lamina			
	C_1 (cm)	C_2 (cm)	C_3 (cm)	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean
G_0(Control)	93.01	65.49	46.15	68.22	71.1	111.51	55.1	79.24	16.13	11.96	8.27	12.12	4.97	4.6	3.35	4.31	6.68	7.72	7.44	7.28
G_1(2Kr)	86.02	64.39	74.95	75.12	67.7	79.2	108.8	85.23	13.02	9.65	12.05	11.57	3.06	4.04	4.95	4.02	5.96	7.56	9.16	7.56
G_2(4Kr)	75.24	79.58	78.33	77.72	102.9	131.7	105.2	113.27	13.08	13.02	11.36	12.49	3.71	5.18	4.73	4.54	5.68	10	7.56	7.75
G_3(6Kr)	92.41	50.21	72.01	71.54	62.75	86.14	112.73	87.21	18.53	9.58	12.81	13.64	5.95	4.01	5.21	5.06	6.28	8.44	7.72	7.48
G_4(8Kr)	113.67	78.31	47.87	79.95	104.1	122.84	48.1	91.68	17.36	13.35	10.69	13.80	6.83	4.82	4.73	5.46	7.9	9	6.24	7.71
G_5(10Kr)	86.09	77.51	41.32	68.31	67.6	105.9	59.7	77.73	13.24	13.24	11.4	12.63	4.95	4.69	4.87	4.84	5.08	6.28	6.16	5.84
G_6(12Kr)	102.06	73.98	63.3	79.78	65	99.3	110.1	91.47	14.47	12.42	10.82	12.57	4.6	4.93	4.27	4.60	7.22	9	7.64	7.95
G_7(14Kr)	108.97	70.84	56.17	78.66	113	70.4	54	79.13	16.72	6.15	7.22	10.03	4.65	5.41	5.07	5.04	6.56	6.12	6.12	6.27
G_8(16Kr)	86.95	72.4	56.82	72.06	95.8	120	91	102.27	15.52	11.49	11.41	12.81	5.82	4.62	3.9	4.78	6.46	8.84	8.6	7.97
G_9(18Kr)	102.3	68.94	71.72	80.99	46.7	96	123.3	88.67	14.55	10.22	11.89	12.22	4.43	3.24	4.4	4.02	7.06	7.52	9.06	7.88
G_{10}(20Kr)	97.34	69.86	54.34	73.85	100.4	128.4	59.5	96.10	16.92	11.52	10.06	12.83	4.35	4.59	4.57	4.50	6.56	8.68	7.64	7.63
G_{11}(22Kr)	102.73	79.19	63.23	81.72	43.3	102.4	78.2	74.63	13.95	10.84	10.23	11.67	4.31	4.24	3.84	4.13	7.64	8.2	8.12	7.99
G_{12}(24Kr)	103.15	60.78	47.57	70.50	52.2	137.9	56.4	82.17	14.15	11.65	10.54	12.11	4.45	4.86	3.71	4.34	7.92	8.52	7.92	8.12
G_{13}(26Kr)	120.13	71.5	46.52	79.38	83.9	96.83	41.5	74.08	16.56	11.12	5.97	11.22	4.87	4.28	3.08	4.08	7.08	8.56	6.36	7.33
G_{14}(28Kr)	108.11	65.02	43.26	72.13	65.1	88.08	55	69.39	15.29	10.9	10.23	12.14	5.45	4.09	3.68	4.41	6.88	8.16	7.44	7.49
G_{15}(30Kr)	101.8	56.23	56.64	71.56	66.7	100.2	64.2	77.03	15.75	10.85	12.48	13.03	4.6	3.5	4.72	4.27	7.2	7.96	6.37	7.18
Mean	98.75	69.01	57.51		75.52	104.80	76.43		15.33	11.12	10.46		4.81	4.44	4.32		6.76	8.16	7.47	
CD $_{0.05}$ (G) =9.82					CD $_{0.05}$ (G) =17.67				CD $_{0.05}$ (G) = 1.28				CD $_{0.05}$ (G) = 0.57				CD $_{0.05}$ (G) = 0.63			
CD $_{0.05}$ (C)=4.25					CD $_{0.05}$ (C)=7.65				CD $_{0.05}$ (C)= 0.56				CD $_{0.05}$ (C)= 0.24				CD $_{0.05}$ (C)= 0.27			
CD $_{0.05}$ (G×C) = 17.01					CD $_{0.05}$ (G×C) = 30.61				CD $_{0.05}$ (G×C) = 2.23				CD $_{0.05}$ (G×C) = 0.96				CD $_{0.05}$ (G×C) = 1.09			

C_1- Polyhouse; C_2- Pots; C_3- Open Field

After the onset of spring in March-April 2012, the plants (about 21-22 months after seed sowing) of all the treatments under all the three conditions (C_1, C_2, and C_3) started developing main shoot. Simultaneously, the no. of radicle leaves started diminishing which totally disappeared by the time the main shoot attained full height. The maximum height attained by the main shoot ranged between 100-120cm (Table-1) upto the beginning of flowering phase.

Plant height was one of the major expressions impacted by the mutagen tried. As against 68.22 cm height observed in untreated plants, plant height of 78.66 cm and above was recorded in M_1 plants treated with 8, 12, 14, 18, 22 and 26 kr doses of gamma rays. In none of the treatments observed, the height was less which was either at par or exceeded the control. Increase in plant height of treated population over the control has also been observed in large seeded chickpea (Karim *et al*., 2008) [10]. Chowdhury *et al*. (1975) [5] observed an increase in height of guar by gamma irradiation. Reduction in seedling height following treatments with gamma rays has been observed in Barley (Sharma, 1970) [18] and in black gram (Charumathi *et al*., 1992) [3]. Seedling height is widely used as an index in determining the biological effects of various physical and chemical mutagens in M_1 progeny (Konzak *et al*., 1972) [12]. A significant variation in plant height, internode length, leaf morphology, no. of inflorescence, flower colour and phytosterols content has been observed in identified variants of *Asteracantha longifolia* treated by EMS (Behera *et al*., 2012) [2].

Kumar (2012) [13], who studied these M_1 plants (included in present study) of *Swertia chirayita* upto radicle stage growth has reported that maximum plant height at radicle leaf stage was observed in untreated plants and all the M_1 plants were negatively impacted by the gamma doses applied. This is an interesting feature observed in this species where some of the gamma doses have promotory effect on plant height during the reproductive phase but have negative effect during the vegetative phase (radicle stage).

No. of cauline leaves per plant was also impacted by gamma radiations applied. No. of leaves / plant were recorded at about to flowering stage. As against 79.24 leaves observed in untreated plants, 102.27 to 113.27 leaves per plant were observed in 4 and 8 kr treatment where as all other treatments either gave at par or were less than the untreated plants. Gupta *et al.* (1979) [7] has also reported reduction in number of leaves per plant in *Mentha citrata* following exposure to various doses of gamma rays. An increase in herbage yield of *cv* Mentolna-14 from *Mentha arvensis* var. *piperascence* has been obtained by treating its stolons with various doses of gamma radiations (Zheljazkov *et al*., 1996) [20]. Mital *et al*. (1972) [16] exposed the stolons of Japanese mint (*Mentha arvensis*) to gamma rays and obtained a significant improvement in herbage yield. Positive shifts in yield and yield parameters i.e., no. of leaves, plant spread, no. of tillers, leaf length, leaf breadth, rhizome no. biomass yield and crop duration were observed in Kochalam (*Kaempferia galanga*) using EMS as mutagen by Kanakamanay (2008) [9]. However, no increase in no. of radicle leaves/plant at the radicle leaf stage of these M_1 plants of *Swertia chirayita* as compared to untreated plants was observed by Kumar (2012) [13].

As far as cauline leaf length was concerned, 6 & 8 kr doses had a promotory effect on leaf length (13.64 to 13.8 cm) in comparison to other treatments which produced at par results in comparison to untreated ones (12.12 cm) except 14 kr dose which had a negative impact (10.03 cm). The radicle leaf length was negatively impacted in all these under study M_1 plants as per Kumar (2012) [13]. This again indicates that the changes in radicle and cauline leaf length in M_1 plants of *Swertia chirayita* are independent of each other and no correlation could be drawn.

Cauline leaf width was also impacted by various doses of gamma radiations. 6, 8 and 14 kr doses of gamma rays had a promotory effect on leaf width (5.04cm and above) in comparison to untreated plants (4.31 cm). None of the treatment had negative impact on leaf width of cauline leaves. For the same parameter at radicle leaf stage as per Kumar (2012) [13], none of the treatment had a promotory impact on leaf width while 8, 10 and 14 kr dose had a negative impact on leaf width. This shows that dimension of both radicle as well as cauline leaves have behaved independently of each other in M_1 generation of the species.

Gupta *et al.* (1979) [7] have reported promotory effect of 2, 4, 6 kr dose of gamma radiations on leaf length and breadth in *Mentha citrata*. Datta (2012) [6] have also reported gross leaf morphological changes (dark green, broad, narrow leaf and drooping leaf) in *Andrographis paniculata* as a result of EMS & DES treatment.

In all the treated as well as untreated plants, on both sides of the midrib, lateral prominent veins (costae) arise from its base. These costae ran almost parallel to each other as per the lamina outline. These costae gave rise to numerous fine lateral veins in different directions that formed a fine network on the entire lamina surface. Variation with regard to no. of these costae per lamina as impacted by different gamma rays doses is presented in table no.1. Against an average of 7.28 costae / lamina in untreated plants, M_1 plants impacted by 12, 16, 22 and 24 kr dose exhibited 7.95 (and above) no. of such costae. In terms of range, the no. of such costae ranged from 5 to 13 in treated plants and 5 to 9 in untreated plants. In Guar, treatment with 35 kr dose of gamma rays plus sodium azide reduced the no. of secondary and tertiary veins from 15 and 69 to 4 and 5, respectively (Badami and Bhalla, 1992) [1].

Table 2: Floral attributes of M_1 Plants

Gamma dose	Flower size		Fruit set	Seed colour	Seed Germination Studies		
	Length (cm)	Spread (cm)			Germination Percent (%)	Germination Initiation (No. of days after sowing)	Germination energy (%)
G_0 (control)	0.62	1.24	90%	Light brown	70	16	53.33
G_1(2Kr)	0.50	0.76	No				
G_3(6Kr)	0.50	0.73	No				
G_6(12Kr)	0.83	1.37	5%				
G_7(14 Kr)	0.59	1.15	10%	Light brown	48.33	17.33	46.67
				Dark brown	71.67	18.33	66.67
G_9(18Kr)	0.55	0.63	No				
G_{11}(22Kr)	0.58	0.90	10%	Dark brown	70	17	61.67
G_{13}(26Kr)	0.57	0.85	5%				
$CD_{0.05}$	0.07	0.20					

These M_1 plants showed gradual decline in terms of growth and vigour as the growing season advanced. Slowly, one by one individual plants showed signs of mortality and most of the plants across different treatments died. However, no such effect was noticed in untreated plants which grew normally completing reproductive phase successfully. This indicates the impact of mutagen (gamma rays) was getting pronounced at or after the emergence of main shoot followed by its progression towards the reproductive phase. These M_1 plants upto the radicle stage did not show any deleterious effects of mutagen and grew quite healthy as per the studies conducted by Kumar (2012) [13]. It appears that the genetic as well as physiological changes inflicted by the mutagen get expressed at stages during or immediately before entering the reproductive phase or during the same in *Swertia chirayita*.

Lata and Gupta (1971a) [14] subjecting the stem cuttings of Edward and Damask roses to gamma radiation observed that lower doses improved shoot/root growth and survival percentage whereas higher doses were found to be increasingly harmful.

Of the seven gamma dose treatments that succeeded in some of the M_1 plants producing flowers, M_1 plants of 12, 14, 22 and 26 kr dose only did mature upto fruit setting stage. However, in contrast to about 90% fruit set in untreated plants, such fruit set ranged between 5 to 10%. Further of the four treatments (12, 14, 22 and 26 kr dose) that succeeded in producing fruits, only plants of two treatments (14 & 22 kr dose) produced seed. Chowdhury *et al.* (1975) [5] has also reported similar ill effects of mutation in Guar with regard to no. of pods per cluster, no. of pods per plant and seed yield per plant.

As per literature and also observed in present studies the seed of *S. chirayita* is uniformly dark coloured. However, the seed produced by M_1 plant of 14 kr was of two types (dark and light coloured) whereas it was only dark coloured in 22 Kr treatment. Mutants with change in seed colour from normal violet to light grey or light brown were also observed in Guar by Swamy and Hashin (1979) [19]. The dark coloured seed set by untreated as well as 14 & 22 kr dose impacted plants did not show any variation with regard to germination percentage (70-71%). However, the light coloured seeds of 14 kr gave only 48.33% germination. The germination initiation ranged between 16-18.33 days in all the treatments including control. However the dark coloured seeds of 14 & 22 kr doses gave higher germination energy with light coloured seeds of 14 kr dose giving the least.

Conclusion

Mutations are known to produce deleterious effects expressing at any level from vegetative to reproductive stage. Some of the gamma ray doses had a promotory effect on quantitative parameters like plant height, no. of leaves per plant, leaf length, leaf width and no. of costae per lamina as compared to untreated plants. These M_1 plants showed gradual mortality with only few plants in each treatment reaching almost upto flowering phase with some entering flowering phase and still fewer ultimately producing seeds.

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