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Mutation Studies in Critically Endangered Temperate Medicinal Species *Swertia chirayita*- Evaluation of M_1 Mutants

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***Abstract*:** The present studies demonstrated the effect of gamma rays on morphology of *Swertia chirayita*. The 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 up to complete senescence. Eight induced variants based on morphological and growth features like (A) plants with suckering habit showing perennial nature, (B) plants without main shoot development, (C) lamina bifurcation, (D) leathery textured lamina, (E) whorl of 3 leaves at a node (as against a pair of such leaves under normal condition), (F) plants with more no. of costae per lamina, (G) flower colour change) and (H) vigorous plants were identified. These variants also showed variation in amaroswerin and amarogentin content with the levels either increasing or decreasing vis-a-vis untreated ones. The induced variant with suckering habit after vegetative splitting have again resprouted, produced main cauline shoots, flowers and also capsules (2nd reproductive cycle) This variant with suckering habit holds promise of developing a perennial type of *Swertia chirayita* with more than one reproductive cycle.

***Keywords*:** *Swertia chirayita,* Gamma rays, Variant, Amarogentin, Amaroswerin

Swertia chirayita (family Gentianaceae), commonly known as chirata or chirayita is a critically endangered, temperate Himalayan species occur at an altitude of 1200-3000 m, from Kashmir to Bhutan and 1200-1500 m in Khasi hills. It is a pluriannual plant (that remains in radicle leaf stage for a year and produce cauline shoot in next year; completing its life cycle in near about two and half years. The plant is well known for its bitterness, antihelmintic, and antipyretic properties due to the presence of amarogentin (the bitterest compound isolated from any plant till date) Keil et al (2000). The plant also contains compounds of xanthone derivatives like chiratanin, chiratol and iridoid glycosides like amaroswerin and tri-terpenoid alkaloids like swerchirin, gentianine, swertiamarin that have high medicinal value for curing diseases (Patil et al 2013). *S. chirayita* is a highly prized herb in India and used either alone or as one of constituents in some polyherbal formulations. It is official in Indian Pharmacopoeia and was formerly also official in British and American Pharmacopeia as tincture and infusion. (Joshi and Dhawan 2005). Whole plant is used in traditional medicine, however the root is mentioned to be the most powerful part. In Indian medical systems, chirayita is used as a remedy for bronchial asthma, liver disorders, chronic fever, anaemia, stomachic and diarrhea. In Ayurveda, *S. 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. *S. chirayita* has an established domestic (India) and international market(Joshi and Dhawan 2005). As per National Medicinal Plant Board (NMPB), New Delhi, the annual demand shortfall for the raw material of *S. chirayita* was 965.2 tonnes in 2001-2002 which has increased to 1284.7 tonnes in 2004-05 at the rate of 10% per year (http:/nmpb.nic.in). The plant is harvested from wild only for meeting the market requirements which is posing a great threat to its existence. So, due to unsustainable wild extraction as well as almost absent cultivation activity have contributed to its demand shortfall (Tabassum et al 2012). In addition, existing populations of *S. chirayita* have diminished considerably. Being a critically endangered as well as high commercial demand species, cultivation is the only option available for its sustainable utilization. However, for making its cultivation economical, there is need of improved varieties/strains with high biomass as well as active content. Persual of literature reveals that there exist no characterized varieties/strains in this species that hampers any genetic improvement work. Due to considerably small size of its population, the overall range of variability is also limited. To

increase genetic variation, induced mutations using gamma rays tried.

MATERIAL AND METHODS

Open pollinated healthy seeds of *S.chirayita* were irradiated with different doses of γ-rays i.e. 0 to 30 kr doses at a dose interval of 2 kr from a Co^{60} source using Gamma chamber- 900 (manufactured by Board of Radiation & Isotope Technology, Dept. of Atomic Energy, Govt. of India). The seeds treated with different doses of mutagens with 15 treatments, along with one control, were sown in a randomized (G_1 to G_{15}) block design with 3 (G_0) replications in the experimental at Medicinal and Aromatic Plants Research Farm Shilly (altitude 1550m amsl, latitude N 30° 54' 30'' and longitude E 77° 07'30'') in April 2010. Discriminating morphological features with respect to type, shape and size of various plants parts were determined as per standard literature (Robin et al 1964, Collett 1971, Weberling 1989, Nath 1996). The M_1 generation was evaluated and variants based on aberrant morphological features were identified. The M_2 generation was raised from the seeds obtained from selfing of M_1 plants.Leaf, stem and root samples from the identified variants of M_1 generation of Swertia chirayita were collected from the field after complete senescence and analysed for bitter compounds (Amarogentin and amaroswerin content) through standardized HPLC (High Performance Liquid Chromatography).

RESULTS AND DISCUSSION

The studies revealed the impact of gamma rays on the growth and development of M_1 plants on various parameters like plant habit, plant height, leaf shape and size, stomatal index and size, flower size and colour, phenological characters, pollen size and stainability, seed set and changes in active content. Based on aberrant morphological features, eight interesting induced variants were identified in M_1 generation namely (variant A)showing suckering habit plant, (variant B) with no main shoot development, (variant C) with some lamina bifurcation, (variant D) with leathery textured lamina, (variant E) with a whorl of three leaves at a node,, (variant F) with increased no. of costae, (variant G) showing altered floral colour and (variant H) showing more vigorous growth. The detailed features of these variants as follows:

Variant A (Suckering habit): After the normal development of aerial shoot, one plant each in treatments G_4, G_7, G_{10} and G_{13} were observed to have developed underground root suckers (after completing the reproductive phase), hence named as A_1, A_2, A_3 and A_4 respectively. In none of the untreated plants, this feature was noticed (Fig. 1-4).

These underground suckers were physically split and the splits were replanted. Almost all the splits developed aerial growth in next season. Some of these sprouts after developing cauline leaves slowly and gradually died. However three such plants have survived and they have successfully completed the reproductive phase.

Variant B (No main shoot development) - These types of plants did not grow beyond radicle leaf stage and failed to produce main shoot by the end of October when they died. Most of such plants were noticed in G_2 and G_3 treatment while few such plants were observed in G_6 and G_{10}also (Fig. 5).

Variant C (Lamina bifurcated) -This type of variant was observed in G_{10} treatment growing under pot condition. One or more leaves of this variant was bifurcated up to the lamina base. Initially the splitting of the lamina was observed at the tip region which gradually extended almost up to the base of the midrib. One of the branches of this variant also borethree leaves at a node arranged in a whorl (Fig. 6).

Variant D (Leathery textured lamina) - Some plants of G_4, G_6, G_8, G_{11}, G_{13}, G_{14} treatment (under polyhouse conditions); G2, G_4, G_8, G_{12} and G_{14} (under pot conditions); G_1, G_2, G_3, G_9

Fig. 1-2. Plants showing suckers at the end of 1st growth phase (October-2012); Variant A

Fig. 3-4. Suckers producing flowers in September/October 2013 (2nd cycle); Variant A

and G_{10} (under field conditions) were found with leathery textured lamina. These leathery textured leaves were bore on the main shoot only and were absent on the lateral branches which bore normal non- leathery textured lamina (Fig. 8).

Variant E (3 leaves at a node)- The variant E represents the plants having one of its branches with three leaves at a node whereas the other lateral branches on the plant were with normal single opposite pair of leaves. The plants showing this type of variation were in the treatments G_1, G_2, G_4, G_{10}, G_{13} and G_{14}. Besides this some untreated plants also exhibited this character which however did not set seed even after flowering (Fig. 10, 11 and 12).

Variant F (More no. of costae) – These types of plants exhibited a higher range of main costae/lamina (9-11) and were observed in all the treatments. The untreated plants were observed with the range of 5-7 no. of costae (Fig. 9).

Variant G (Flower colour change) - A single plant of G_6 treatment growing under polyhouse condition exhibited change in colour of streaks present on inner surface of petals. In untreated as well as other flowers of treated plants, the colour of streaks was dark purple which however was very light purple in this case (Fig. 13).

Variant H (Vigorous plants) - Vigorous plants were observed in G_4, G_6, G_7, G_9 and G_{11} treatments. These plants showed vigorous aerial growth in comparison to other treatments as well as untreated plants (growing under all the three conditions). Amongst three plants, plants of G_7 and G_{11} set viable seeds (Fig. 7). These eight induced variants were analyzed for active content (amarogentin and amaroswerin) in leaves, stems and roots and considerable enhancement in bitter content was observed in few variants.

Leaves: The maximum amarogentin content of 0.304% was f in leaves of variant H (Vigorous plants) and the minimum of 0.112% was in variant F (More no. of veins). The maximum amaroswerin content of 0.308% was in variant H(vigorous plants) and the minimum was observed in variant A (0.105%).

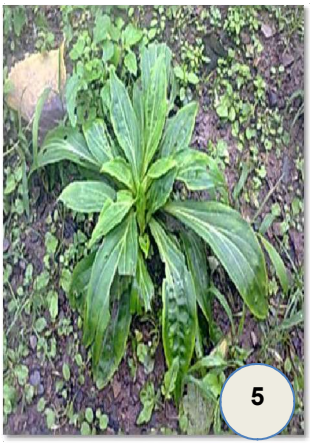

Fig. 5. Variant B (No main shoot development)

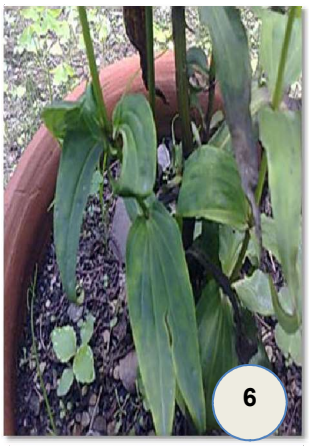

Fig. 6. Variant C (Lamina bifurcated)

Fig. 7. Variant H (Vigorous Plants)

Fig. 8. Variant D (Leathery textured lamina)

Table 1. Effect of gamma rays on amarogentin and amaroswerin content in leaves, stems and roots

Induced variants	Leaves		Stems		Roots	
	Amarogentin content (%)	Amaroswerin content (%)	Amarogentin content (%)	Amaroswerin content (%)	Amarogentin content (%)	Amaroswerin content (%)
A (Suckering habit)	0.237 (0.859)	0.105 (0.778)	0.269(0.877)	0.024 (0.724)	0.026 (0.725)	0.113 (0.783)
B (No main shoot development)	0.267 (0.876)	0.174 (0.821)	No stem	No stem	0.038 (0.733)	0.041 (0.736)
C (Lamina bifurcation)	0.206 (0.840)	0.134 (0.796)	0.275 (0.880)	0.021 (0.722)	0.041 (0.735)	0.018 (0.720)
D (Leathery textured lamina)	0.259 (0.871)	0.195 (0.833)	0.305 (0.897)	0.107 (0.779)	0.014 (0.717)	0.005 (0.711)
E (3 leaves at a node)	0.211 (0.843)	0.202 (0.838)	0.110 (0.781)	0.026 (0.725)	0.018 (0.720)	0.004 (0.710)
F (More no. of costae)	0.112 (0.782)	0.132 (0.795)	0.129 (0.793)	0.054 (0.744)	0.004 (0.710)	0.003 (0.709)
G (Flower colour change)	0.291 (0.890)	0.208 (0.841)	0.217 (0.847)	0.028 (0.727)	0.045 (0.738)	0.017 (0.719)
H (Vigorous plants)	0.304 (0.897)	0.308 (0.899)	0.208 (0.842)	0.025 (0.725)	0.060 (0.748)	0.022 (0.722)
Untreated plants	0.146 (0.804)	0.125 (0.790)	0.166 (0.816)	0.034 (0.731)	0.021 (0.722)	0.007 (0.712)
CD (p=0.05)	(0.046)	(0.057)	(0.037)	(0.016)	(0.013)	(0.004)

*Figures in parenthesis are transformed values

The untreated plants were with a amarogentin content of 0.146% and amaroswerin of 0.125% (Table 1).

Stems: Amongst the isolated variants, the variant B (no main shoot development) could not be analysed for amarogentin and amaroswerin content due to the fact that such plants did not produce main shoot. The maximum amarogentin content of 0.305% was observed in variant D (leathery textured lamina) and minimum of 0.110% in variant E (3 leaves at a node). The maximum amaroswerin content (0.107%) was observed in variant D (Leathery textured lamina) and the minimum (0.021%) in variant C (Lamina bifurcation). The untreated plants were observed with amarogentin content of 0.166% and the amaroswerin content of 0.034%(Table 1).

Roots: In roots, the maximum amarogentin content was in variant H (vigorous plants) (0.060%) and minimum in variant F (more no. of veins)(0.004%). The maximum amaroswerin content of 0.113% was in variant A (suckering habit) and the minimum of 0.003% was in variant F (more no. of veins). The untreated plants were observed with a amarogentin content of 0.021% and the amaroswerin content of 0.007% (Table 1).

In spite of great demand, supplies of *S. chirayita* still depend on wild sources which are becoming critical on account of over harvesting of its plants (generally before seed dispersal) leading to progressive clearance of their habitats). Low seed germination, long gestation period and delicate field handling requirements are some of the factors which discourage its commercial cultivation (Badola and Pal 2002 and Raina et al 2013). However, successful cultivation shall only be assured if better strains in terms of biomass/ active content but no improved strains are there in this species. Due to narrow genetic base and restricted distribution, any improvement programme is need relook. With this background, this work was started and its seeds were subjected to various doses of gamma rays and M_1 progeny raised. This species is a pluriannual (once flowering herb) (Clarke 1885) completing its life cycle in about 30 months (Shah 2008, Raina et al 2013). The growth and developmental stages in this species can be broadly sub-divided into two phases; i) radicle leaf stage (upto about 20 months after seed sowing) and ii) reproductive phase (20-30 months after seed sowing). Eight interesting types based on morphological variations were identified. Amongst these, four such variants were leaf based, three on the basis of plant type and one flower colour variant. Behera et al (2012) generated 142 genetic variants of *Asteracantha longifolia* (through EMS induced in vitro mutagenesis) of which 24 mutant lines including 4 dwarf mutants, 7 leaf mutants and 13 flower mutants were analysed at morphological level. Similarly remarkable variations in the leaf shape, plant height, leaf colour, tuber yield and forskolin content was observed in *Coleus forskholii* after subjecting to gamma irradiation (1 to 15 kr) followed by evaluation for three generation under field conditions (Srinivasappa et al 2010). Flower colour in the M_1 plants that progressed upto flowering stage was the least affected as most of these M_1 plants developed flowers that were similar to untreated plants in

Fig. 9. Variant F (More no. of costae)

Fig. 10-12. Variant E(3 leaves at a node)

Fig. 13. Variant G (Flower colour change)

terms of its colour. However one plant of 12 Kr treatment did produce flowers with altered colour pattern (light coloured purple streaks on inside surface of petals) as compared to untreated ones (dark coloured purple streaks on the under surface of petals). Similar changes have been observed in *Chrysanthemum morifolium* wherein due to gamma rays (0.5 to 1 Gy), the normal grey red flower colour was modified into yellow colour under culture conditions (Mishra et al 2003) and in *Pelargonium graveolens* 'Dark Mozart' cultivar with improved flower colour in contrast to 'Mozart' after X- ray treatment has been released (Maluszynski et al 2000).

CONCLUSIONS

The induced variant observed during the present studies were the plants showing suckering habit. The plants of *S. chirayita* are once flowering completing their life cycle in about 28-30 months. After seed setting these plants die without any scope of vegetative propagation. However, in case of plants showing suckering habit (Variant A), the observed plants developed suckers at the base which once split and replanted in 2012, sprouted and produced flowers again (2nd cycle) during 2013. Such plants can open a new opportunity of developing perennial strain of S. chirayita which can have immersed advantage in terms of substantial production of raw material avoiding repeated growing cycles after every seed setting stage. Breeding programmes aimed at developing perennial grain crops have been initiated in wheat, sorghum, sunflower, etc. as perennial store more carbon, maintain better soil/water quality and manage nutrients more conservatively than do annual plant communities besides having greater biomass as well as resource management capacity. Isolation and multiplication of plants of *S. chirayita* showing suckering habit (and thereby potential perennial habit) is an opportunity to circumvent repeated cycles of sowing and growing and may ultimately prove beneficial in successful domestication of *S. chirayita*.

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