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Chlorophyll and morphological mutants of *andrographis paniculata* derived by physical and chemical mutagens

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Abstract
A wide range of morphological mutants were identified in *Andrographis paniculata.* Micro and macro-mutants play an essential role to assess the dose/concentration of mutagens. In the present study, some of the chlorophyll mutants were observed in the different dose/concentrations of gamma rays they were chlorine, albino, Xantha, and viridis. Amongst the mutagens, Morphological mutants were observed in M_2 generation with effect of dose/concentration of mutagens and such mutants were, dwarf, tall, leaves, brown seed, early, maturing, bushy type, mutants were observed in M_2 generation. Mutant and its derivatives when used in cross breeding have found to be more productive in the development of improved *A. paniculata* EMS provided more number of chlorophyll and morphological mutants followed by gamma rays in this study.

Keywords: *andrographis paniculata*, chemical mutagens, physical mutagens and morphological mutants

Introduction
Kalmegh (*A. paniculata*) belonging to family of Acanthaceae. It is a hardy and erect herb and the plant which grows 30-110 cm mainly is an under shrub in moist and deciduous forests. Every part of this herb is tropical moist and deciduous forests. Mutations as a mechanism of creating variability were first identified by Hugo de Vries in the late nineteenth century, while experimenting on the 'rediscovery' of Mendel's laws of inheritance. Khar kwal 2012.

Mutagenesis is the process whereby sudden heritable changes occur in the genetic information of an organism not caused by genetic segregation or genetic recombination but induced by physical, chemical and biological agents. Populations of Kalmegh are spread throughout south India (Kerala, Karnataka and Tamil Nadu) and Sri Lanka. (Hooker 1885) Baht and Nana vatic (1978).

Mutation breeding has become increasingly popular in recent times as an effective tool crop improvement Acharya (2007) [1]. The chlorophyll mutants in M_2 seedlings were screened from 45th -50th day recorded. The various chlorophyll mutants were occurred in the *kalmegh*. Identified the chlorophyll mutants was done based on the nomenclature adopted Gustafesson (1940). The mutation breeding has become a proven way since the beginning of this century as one of the driving force for evolution.

The present study the effects of EMS and Gamma rays were studies on the frequency and spectrum of morphological mutants in M_2 generation of *A. paniculata.*

Materials and Methods
The seeds of *Andrographis paniculata* variety CIM Megha were obtained from Research form of Central Institute of Medicinal and Aromatic Plants Resource Centre, Hydrabad, India. The Experinmental material were divided in two sets. The first sets of seeds were treated with gamma rays (05 kr, 10kr, 15kr, 20kr, 25kr, 30, 35kr, 40kr, 45kr and 50kr) by irradiated seeds Indira Gandhi Centre for Aromatic Research (IGCAR) at Kalpakkam, India. Another set of seeds were treated with Ethylmethane Sulphonate at various concentrations such as (05mM, 10mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50mM) for the Ethyl Methane Sulphonate treatment the seeds were pre- soacked in water for four hours and various concentrations of EMS solution four hour and washed through running tap water before sowing.

The treated seeds alongside controls were directly sown after treatment. Chlorophyll mutations, as described by Gustaffson (1940) were scored throughout the plant growing period in M_2 generation. The data of chlorophyll mutants were collected and recorded at the time of maturing and expressed in percentage of control.

Experimental design
Mutually physical gamma rays irradiated and chemically treated (EMS) seeds were grown laterally with control (Untreated seeds) by randomized block design (RBD) with three replications at the Breeding field, Department of Botany, Annamalai University, AnnamalaiNagar, Tamil Nadu, India. The plots consisted of seven rows. The dry and dormant seeds of the *A.paniculata* were obtained from the present study on induced mutagenesis in *A.paniculata* was carried out in the Botanical Garden, Department of Botany, AnnamalaiUniversity during 2018th August. The Chemical was obtained from HI-MEDIA Laboratories, Mumbai having a half-life period of 30 hours with a molecular weight of 124.16 and density of 1.20.

Experimental design
Both physical gamma rays irradiated and chemically treated (EMS) seeds were grown along with control (Untreated seeds) by randomized block design (RBD) with three replications at the Breeding field, Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu, India. The plots consisted of seven rows including control at 20 cm spacing, 4 m long and 1.5 m wide. The field was

fertilized with organic fertilizer. Along with all the cultural practices such as irrigation, weeding and protection measures were taken throughout the growth period.

Growth Condition

After rising M_1 generation, seeds were collected from respective dose/concentration of mutagens. Form M_1 generation, M_2 generation was raised and the chlorophyll 45th day morphological mutants were isolated.

Isolation of mutants

In M_2 generation form both gamma rays and EMS at 45th day the following chlorophyll mutants such as Chlorino, Albino, Xantha, Variegata and Viridis and anthocyanin up to growth period morphological mutants such as, Dwarf, Tall, Mono stem, Tiny leaves, Hairy leaves, Male sterility, Brown seed, Early maturity, Long pod, Bottom branching, Top branching, Trailing, Spreading, and Bushy type.

Result and Discussion

In M_2 generation four different types of chlorophyll mutants were isolated in the field in M_2 generation. When seedlings were 30-40 days old. The spectrum of different of different M_2 Chlorophyll mutants included such as Albino, Chlorine, Xantha and Anthocyanin. A brief description of the isolated chlorophyll mutants was given in Table. (Fig.1). The frequency of chlorophyll and viable mutants observed in M_2 generation is mainly used as a dependable measures of genetic effects of mutagens Gautham 1998 [3]. The maximum frequency of chlorophyll mutations was observed mutations was noted mutation frequency was observed at 6.25 in 25 KR of gamma rays treatment and In EMS was noted mutation frequency were observed at 4.16 in 20KR.

Table 1: Frequency of Chlorophyll and Viable mutants in M_2 generation.

Mutagens (Dose/Conc.)	Gamma Rays			EMS		
	20kr	25kr	30kr	15mM	20mM	25mM
No of plant Studied	545	625	520	568	486	516
Albino	1	1	1	-	2	2
Xantha	-	2	1	1	2	-
Chlorina	1	1	-	1	1	2
Viridis	-	2	-	-	2	2
Anthocyanin	2	1	1	1	3	1
Tall	3	2	1	2	1	1
Dwarf	4	2	1	3	1	2
Bushy	3	1	2	4	3	2
Round shape leaf	1	2	1	1	1	2
Heart shape leaf	1	2	1	1	2	-
Needle shape	1	2	4	2	3	2
Bifid leaf	2	1	4	3	4	2
Early maturity	1	4	2	2	3	1
Late maturity	2	3	1	1	4	3
Total	21	26	20	22	32	22
Frequency	3.85	4.16	3.84	3.87	6.25	4.26

Bushy Mutant

Tall Mutant

Early Maturity

Fig 1: Morphological and Chlorophyll mutants of *Andrographis paniculata*

Fig 2: Different shapes seed

Fig 3: Various chlorophyll mutation

Spectrum of chlorophyll mutation

The spectrum of chlorophyll mutation obtained in the plant. The mutagens induced different types *viz*, albino, chlorine, Xantho, Anythocyanin, Viridis was observed in M_2 generation.

Albino

These mutant leaves were white in colour appears and due to absence of all pigments such chlorophyll mutant identified in the plants at 30-45 days after the germination.

Viridis

The seedlings *of A. paniculata* were characterized by dark green in the early stages of developmental and the normal green in the later stages.

Xantha

The seedlings were vary from deep yellow to yellowish White appears in the stage 45-55 days after the germination.

Chlorina

The mutant normally chlorine do not survive the seedling have light yellowish green leaves.

Anthocyanine

Anthocycanine are natural plant pigments and its sources of anthocyanine are generally easy to identify due to their red, blue, purple colour appeared. Generally frequency of

chlorophyll mutations exposed decrease with increase in the concentration of various mutagens. The morphological mutants induced through the physical and chemical mutagens in *A. paniculata*. Hence forward a wide spectrum of viable mutant experiments. The progenies of tall dwarf bushy early maturity, late maturity, mutants bred true for the altered traits in M_2 generation. Such mutant's types capable under the influence of polygenes. Konzak 1969 [9]. High frequency and broad spectrum of morphological mutants through the induced physical and chemical mutagens has been reported in *Vignamungo* Arulbalachandran and Mullainathan 2009 [10] and Goyal and Khan (2010) [11]. Morphological mutants induced physical and chemical mutagens induced by *Ciserariticum* Khan *et al*., 2004 [12]. Relative differences in the mutability of the genes to different traits have been observed as some mutagens for instantly. Dwarf mutants appeared more frequently with gamma rays and EMS. It is also induced comparatively more mutation affecting growth habit identified characters like bushy and prostrate than Gamma rays and EMS. In the present investigation tall and dwarf mutants were observed in different mutagenic treatment. Among the concentration maximum number of mutants was recorded at 20mM of EMS treatment. Leaf mutants isolated that is called progressive in term of Gottschalk 1972 [4] and Nerker 1973 [7]. Phylogenies significance of leaf mutant. The gamma radiation and chemical mutagen induced cellular damage results in the altered of metabolism which ultimately leads to leaf abnormalities the leaf abnormalities were attributed to the chromosomal breakage, disturbed auxin synthesis and accumulation of free Amino acids Gunkel and Sparrow 1961. Mutations in these chlorophyll genes are reflected in the M2 and subsequent generations in the form of different types of mutants Wani, A.A. and M. Anis, 2004] [13]. EMS was to be higher superior to gamma rays including a higher frequency and wider spectrum of chlorophyll mutations in M2 generation Swaminathan, M.S.*et al*., 1970] [14]. A number of chemical mutagens have been Practical Applications. Cambridge, University. found to be equally and even many times more 11. Ahmed John, S., 1997. Effect of gamma irradiationeffective and efficient mutagens [17-19].

Conclusion

Chlorophyll mutants incidental in this investigation, how chlorophyll gene response to mutagen gamma rays and EMS. Mutations in these chlorophyll genes are reflected in the M_2 and successive generations in the form of different types of mutants. While, morphological mutants are viable and useful to breeding approach to obtain suitable ideotype in *Andrographis paniculata.* Hence, mutant and its derivatives when used in cross breeding have found to be more productive in the development of improved varieties of *Andrographis paniculata.*

Appendix

EMS: Ethyl Methane Sulphonate
K: Kilo radiation
mM: milli Molar
IGCAR: Indira Gandhi Centre for Atomic Research

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