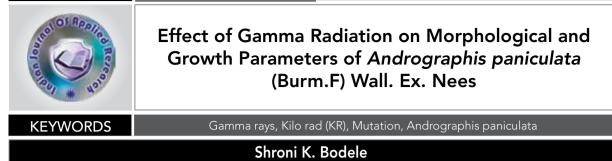
Botany



Department of Botany, Institute of science, civil lines, Nagpur

ABSTRACT Gamma (γ) -ray irradiation is a conventional technique to produce mutants in plant breeding. The current study investigated the effect of gamma rays on days to germination, germination percentage, survival percentage, shoot length /plant, root length /plant, number of branches /plant, number of leaves /plant, days to first flower and inter-nodal distance by treating the seeds of Andrographis paniculata with different dosages of gamma rays. Dry seeds of Andrographis paniculata were irradiated with 10, 20, 30, 40, 50, 60, 70, 80, 90 & 100 KR by a 60 Co. The study revealed that gamma radiation significantly affected on germination percentage. However, other parameters show declining tendency with increasing doses of gamma rays.

INTRODUCTION

Mutation is a sudden heritable change in organism generally the structural change in gene. The term mutation was first introduced by Hugo de Vries1 in 1900. Mutations can be induced either spontaneously or artificially both in seed and vegetative Propagated crops. Mutation breeding is one of the conventional breeding methods in plant breeding. It is relevant with various fields like morphology, cytogenetics, biotechnology and molecular biology etc. Induced mutations have recently become the subject of biotechnology and molecular investigation leading to description of the structure and function of related genes. Mutated genes have therefore; become valuable material to plant breeders and molecular biologists for understanding not only the function but also in shuffling and isolating the genes between varieties (Souframanian et al.2, 2002). Mutation induction techniques such as radiation or chemical mutagens are good tools for increasing variability in crop species because spontaneous mutations occur with an extremely low frequency. Mutation techniques have significantly contributed to plant improvement worldwide and have made an outstanding impact on the productivity and economic value of some crops.

Andrographis paniculata is a medicinal plant belonging to the family of Acanthaceae. Diterpenoids and flavanoids are the main chemical constituents of Andrographis paniculata and these compounds are believed to be responsible for the biological activities of the plant (Tang and Eisenbrand3, 1992; Saxena et al.4, 1998). It is widely used in Chinese and Ayurvedic medicine for the treatment of gastric disorders, infectious diseases and common colds. It has multiple pharmacological properties such as anti-protozoal, hepatoprotective, anti-HIV, anti-inflammatory (Sheeza et al.5, 2006), antipyretic, anticancer (Li et al.6, 2007), antitumor, hypoglycemic (Borhanuddin et al.7, 1994), hypotensive activities and has been used for the treatment of snake bites. Andrographis paniculata is also used as a folk medicinal remedy for fever, pain relief and disorders of the intestinal tract. The ability of Andrographis paniculata to lower fever has been demonstrated independently in several reports. Inflammation plays an important role in the pathogenesis of several neurodegenerative diseases including parkinson's disease. Main component of Andrographis paniculata is andrographolide has been reported to possess an anti-inflammatory effect in vitro by modulating macrophage and neutrophil activity (Chiou et al.8, 1998; Chiou et al.9, 2000; Shen et al.10, 2000; Shen et al.11, 2002). Andrographis paniculata is most important medicinal plant for human being and pharmaceutical use for making medicines.

The use of gamma radiation to induce mutation is a method that has been applied in plant breeding to increase genetic

variations (Brunner12, 1995). Gamma rays belong to ionizing radiation and interact with atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and have been reported to affect the morphology, anatomy, biochemistry and physiology of plants differentially depending on the irradiation level. These effects include changes in the cellular structure and metabolism of the plants e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system and accumulation of phenolic compounds (Kim et al.13, 2004; Kovacs and Keresztes14, 2002; Wi et al.15, 2005).

Due to its medicinaly important property, the aim of the current study is to use nuclear breeding techniques for search of some beneficial mutants in *Andrographis paniculata* and also to evaluate the effect of gamma rays on morphological and growth parameters of *Andrographis paniculata*.

MATERIALS AND METHODS:

The dry seeds of Andrographis paniculata were collected from Shail nursery, Nagpur, Maharashtra, India. The seeds with uniform size, color and weight were chosen for experimental purpose. The experiment was conducted during the year 2012 to 2013. Fifty seeds were packed in ten bags separately and treated in the gamma rays chamber at 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 KR respectively from 60Co gamma unit chamber installed at the department of chemistry, RTM Nagpur University, Nagpur. The 15 seeds of control and irradiated each were sown in earthen pots of equal size which were filled with dried pulverized garden soil. Eleven petridishes in which 15 seeds per treatment and control were planted and germination percentage for each treatment compared to control were subsequently determined. In field the morphological and growth parameters such as days to germination, germination percentage, survival percentage, shoot length /plant, root length /plant, number of branches / plant, number of leaves /plant, days to first flower and internodal distance were observed and recorded.

RESULTS AND DISCUSSIONS:

In respect of gamma rays treatment, there was a proportionate reduction in germination of *Andrographis paniculata* with increased doses of gamma rays and similar result were also reported in black-gram (Ramaswamy16, 1973), soybean (Balakrishnan17, 1991), rice (Ramesh et al.18, 2002) and Cowpea (Gnanamurthy et al.19, 2012). The seeds treated with higher gamma radiation i.e. 80KR, 90KR, 100KR show toxic effect on germination. The irradiated seeds of 80KR, 90KR, 100KR are not germinated.

Days to germination of Andrographis paniculata were sig-

RESEARCH PAPER

nificantly delayed by higher doses of gamma irradiation. The data in Table no.1 indicates that at higher doses of gamma rays increases days to germination. In the present experiment percentage of germination also show decreasing trend when compared to the control. The seedling survival reduced with increase in doses of gamma rays. Similar results have been obtained in soybean (Yamashita and Kawai20, 1987; Balakrishnan17, 1991). At 20KR very low survivability are seen. The irradiated seeds 30KR, 40KR, 50KR, 60KR and 70KR are not survived, after germination cotyledonary leaves are seen but, they get die within 1-2 week. According to (Kiong et al.21, 2008) study, results have shown that survival of plants to maturity depends on the nature and extent of chromosomal damage. Increasing frequency of chromosomal harm with increasing radiation dose may be responsible for less germ inability and reduction in plant growth and survival. Changes in the germination percentage were attributed to gamma rays treatments (Kiong et al 21, 2008).

If the dosages are too high, too many plants will be killed because mutagens can have direct negative effect on plant tissue and many mutations can be lethal. This is due to the fact that primary injuries are retardation or inhibition of cell division, cell death affects the growth habit and changes in plant morphology. If the dose is too low, there will not be enough mutation because of low mutation frequently and results in small mutated sector (Nazir et al.22, 1998). These effects are due to the cytological changes such as chromosomal damages, inhibited mitotic division, degeneration of nuclei, cell enlargement etc., have been reported (Sparrow et al.23,1952 ; Pollard24, 1964 ; Karpate and Chaudhuri25, 1997).



Fig.1: Seeds irradiated at 10KR shows plants growth



Fig.2: Seeds irradiated at 10 KR shows different types of leaf shape



Fig.3: Seeds irradiated at 10 KR shows different types of leaf shape



Fig.4: Seeds irradiated at 10 KR $% \left({{\rm{S}}_{\rm{R}}} \right)$ shows leaf color change to violet



Fig.5: Seeds irradiated at 10 KR $% \left({{\rm{S}}_{\rm{R}}} \right)$ shows leaf color change to violet



Fig.6: Seeds irradiated at 20KR shows plants growth



Fig.7: Seeds irradiated at 20KR shows plants growth



Fig.8: Seeds irradiated at 20 KR shows different types of leaf shape.

RESEARCH PAPER



Fig.9: Seeds irradiated at 20 KR shows different types of leaf shape

Table no.-1: Effect of Various Dosages (KR) of gamma irradiation on days to germination, germination percentage, survival percentage, shoot length /plant, root length /plant, number of branches /plant, number of leaves /plant, days to first flower and inter-nodal distance of Andrographis paniculata.

Yield <u>Parameters</u> Treatment dosages	tc q	ays ermi- ation	Gerr natic %		Survi val %		Shoot length / plant (Cm)	Root length /plant (Cm)
Control	6		93.33		92.85		16	8
10KR	8		86.66		84.61		12	7
20KR	9		80.00		33.33		10	5
30KR	15		66.66		0		-	-
40KR	17		53.33		0		-	-
50KR	1	7	33.33		0		-	-
60KR	1	8	26.66		0		-	-
70KR	19		20.00		0		-	-
80KR	-		-		-		-	-
90KR	-		-		-		-	-
100KR	-		-		-		-	-
Yield								Inter-
Paramotore		Number of		Nur	nber	Da		
Treatment dos- ages		branches / plant		of leaves /plant				distance (Cm)
Control		7		64		110		2.5
10KR		5		35		157		1.2
20KR		3		20		159		1.5

Volume : 3 | Issue : 6 | June 2013 | ISSN - 2249-555X

30KR	-	-	-	-
40KR	-	-	-	-
50KR	-	-	-	-
60KR	-	-	-	-
70KR	-	-	-	-
80KR	-	-	-	-
90KR	-	-	-	-
100KR	-	-	-	-

In the present investigation, shoot length /plant, root length /plant, number of branches /plant, number of leaves /plant (Fig. 1,6 & 7) showed decreasing trend with increasing doses of gamma rays compared to the control (Table no.1) and days to first flower increases with increasing doses of gamma rays. The inter-nodal distance of irradiated seeds of 20KR is less than the control but, more than the 10KR was observed. The colors of some leaves of 10KR irradiated plants are changes from green to violet when they get mature (Fig. 4 & 5). The different shapes of some leaves are also seen in 10KR and 20KR irradiated plants (Fig. 2, 3, 8 & 9). The abnormalities in leaves are due to disturbances by phytochromes, chromosomal aberrations, mitotic inhibition (Moh26, 1962; Mickaelsen et al.27, 1968; Abraham and Ninan28, 1968).

CONCLUSIONS:

Andrographis paniculata has been subjected to different doses of gamma irradiation (10KR-100KR) and various morphological and growth parameters were recorded. In the present investigation, it is observed that higher dosages i.e. 30KR-100KR shows highly toxic effect on germination of seeds and survivability of plants. Higher dosages of gamma rays show lethal effect on morphological and growth parameters of plants such as reduced germination percentage, survival percentage, shoot length, root length, number of branches, and number of leaves while days to first flower were increases. In irradiated plants, some leaves shows different shapes and in 10 KR plants some leaves show violet color. The effects of these higher doses are not statistically significant. The dosages that are suitable for mutation induction in Andrographis paniculata are in the range of 1-10KR. The information provide basic requirement for the use of gamma radiation for mutation induction in Andrographis paniculata.

REFERENCE [1] Hugo De Vries J. (1900), "Study on mutation and its use in various aspects". Journal of the Agricultural association of China, 140, 22-29. [2] Souframanian J., Pawar S.E. and Rucha A.G. (2002), "Genetic variation in gamma ray induced mutants in black gram as revealed by RAPD and ISSR markers". Indian J. Gent., 62(4), 291-295. [3] Tang W. and Eisenbrand G. (1992), "Chinese Drugs of Plant Origin, Chemistry, Pharmacology and use in Traditional and Modern Medicine". Springer Verlag, Berlin, 97-103. [4] Saxena S., Jain D.C., Bhakuni R.S. and Sharma R.P. (1998), Indian Drugs, 35, 458-467. [5] Sheeza K., Shihab P.K. and Kuttan G. (2006), "Antioxidant and antiinflammatory activities of the plant Andrographis paniculata Nees". Immunopharmacol. Immunotoxicol.,28, 129-140. [6] Li J., Cheung H.Y., Zhang Z., Chan G.K.L. and Fong W.F.(2007), "Andrographolide induces cell cycle arrest at G2/M Phase and cell death in HepG2 cells via alteration of reactive oxygen species", Eur. J. Pharmacol., 568, 31-44 [7] Borhanuddin M., Shamsuzzoha M. and Hussain A.H. (1904) "Investment" for the for the there the Neet Termes of the there in the Park Cover Dell 20. (2010) (Chem Dell 20. (2010) (Che (1994), "Hypoglycaemic effects of Andrographis paniculata Nees on non- diabetic rabbits". Bangladesh Med. Res. Counc. Bull, 20, 24-26. | [8] Chiou W.F., Chen C.F. (1974), hypoglycaemic effects of Andrographis paniculata Nees on hon-diabetic rabolis - bangladesh Med. Res. Counc. bull, 20, 24-20. [16] Chiou W.F., Chen C.F. and Lin J.J.(1998), "Andrographolide suppress the expression of inducible nitric oxide synthase in macrophage and restores the vasoconstriction in rat arota treated with lipopolysaccharide". Br. J. Pharmacol., 125,327-334. [19] Chiou W.F., Chen C.F. and Lin J.J. (2000), "Mechanism of suppression of inducible nitric oxide synthase (i NOS) expression in RAW 264.7 cells by Andrographolide". Br. J. Pharmacol., 129, 1553- 1560. [10] Shen Y.C., Chen C.F. and Chiou W.F. (2000), "Suppression of rat neutrophil reactive oxygen species production and adhesion by the diterpenoid lactone", Andrographolide. Plant Med., 66,314- 317. [11] Shen Y.C., Chen C.F. and neutrophil reactive oxygen species production and adhesion by the diterpenoid lactone", Andrographolide. Plant Med., 66,314-317. [11] Shen Y.C., Chen C.F. and Chiou W.F., (2002), "Andrographolide prevents oxygen radical production by human neutrophils; possible mechanism(s) involved in its anti-inflammatory effect". Br. J. Pharmacol., 135, 399-406. [12] Brunner H. (1995), "Radiation induced mutation for plant selection", 4PPL Radiat., 589-594. [13] Kim J.H., Baek M.H., Chung B.Y., Wi S.G. and Kim J.S. (2004), "Alterations in the photosynthetic pigments and antioxidant machineries of red pepper (Capsicum annuum L.) Seedlings from gamma-irradiated seeds". J. Plant Biology, 47,314-321. [14] Kovacs E. and Keresztes A.(2002), "Effect of gamma and UV-B/C radiation on plant cell". Micron, 33, 199-210. [[15] Wi S.G., Chung B.Y., Kim J.H., Baek M.H., Yang D.H., Lee J.W. and Kim J.S. (2005), "Ultrastructural changes of cell organelles in Arabidopsis stem after gamma irradiation". J. Plant Biol, 48(2), 195-200. [16] Ramaswamy N.M. (1973), "Investigation on induced mutagenesis in black gram (Phasiolus mungo L.)". Ph.D. Thesis, Tamil Nadu Agri. Univ., Coimbatore. [17] Balakrishnan P.C. (1991), "Induced mutagenesis in soybean (Glycine max (L.) Merill)", Ph.D. Thesis, Tamil Nadu Agri. Univ., Coimbatore. [18] Ramesh D.V. and Seetharami Reddi T.V.V. (2002), "Induced morphological variability among three genotypes of rice". J. Cytol. Genet., 3(2),115-120. [19] Gnanamurthy S., Mariyammal S., Dhanavel D. and Bharathi T. (2012), "Effect of gamma rays on yield and yield components characters R3 generation in cowpea by irradiation of Tanbakuro" Soy-Abstracts, 10, 1. [21] Kiong A.L.P., Lai A. G., Hussein S. and Harun A. R. (2008),"Physiological Responses of Orthosiphon stamineus Plantles to Gamma Irradiation". American Eurasian Journal of Sustainable Agriculture , 2(2), 135-149. [22] Nazir M. B., Mohamad O., Affida A. A. and Sakinah A. (1998), "Research highlights on the use of induced mutations for plant improvement in Malaysia". M [23] Sparrow A. H., Moses M. J. and Dubow R. J. "Relationship between ionizing radiation in plant: Marayala". Marayala "Marayala" indicative isotopes in agriculture. East Housing Mich. AEG Rep. T.D., No. 7521, 1952,125 – 139. [24] Pollard E. (1964), "Ionizing radiation: Effect on genetic transcription". Science, 146, 927-929. [25] Karpate R. R. and Choudhary A. D. (1997), "Induced mutation in Linum usitatissimum L.". Journal of Cytol. Genet. 32(1), 41 – 48. [26] Moh C. C. (1962), "The use of radiation induced mutations in crop breeding in Latin America and some biological effects of radiation in Coffee". Int. Jour. Appl. Radiation and Isotopes, 13,467 – 475. [27] Mickaelsen K., Ahnstrom G. and Li W. C. (1968), "Genetic effects of alkylating agent in barley. Influence of past-storage, metabolic state and pH of mutagen solution". Heriditas, 59, 353 – 374. | [28] Abraham A. and Ninan C. A. (1968), "Genetic improvement of the coconut palm: Some problems and possibilities". Indian J. Genetic and Plant Breeding, 28A, 142 - 153. |