

INVESTIGATION ON INDUCING GENETIC VARIABILITY IN CHILLI (*Capsicum annum* L.) THROUGH MUTATION BREEDING

K.D.A. PERERA, S.J. ARASAKESARY AND W.M.C.J.K. CHANDRARATNE
Field Crops Research and Development Institute, Maha Illuppallama

ABSTRACT

The recommended varieties of chilli do not possess a high level of resistance to many pests and diseases. Genetic variability of these characters in the available germplasm is insufficient and induced mutation breeding is one strategy to overcome this problem. Therefore, the feasibility of creating genetic variability for these traits through induced mutation was investigated at the FCRDI, Maha Illuppallama during the period 1997 - 1999. ^{60}Co gamma rays were used as the mutagenic source and a series of experiments was conducted to find out the effective dose range, lethal dose of ^{60}Co gamma rays for chilli seeds and to identify genetic variability in chilli due to such inductions. Chilli seeds at 13% moisture level after exposure to different levels of ^{60}Co gamma radiation produced variations in growth and flower characteristics, but with a lethal effect on seeds at the dose range of 80 kr and more. Thirty to fifty percent reduction in seedling growth was noticed when chilli seeds were exposed to 29 - 44 kr of ^{60}Co gamma radiation on chilli variety MI-2 and 31-36 kr on KA-2. Hence a dose range of 30 - 45 kr could be considered as the effective dose range to induce mutations in chilli seeds. When seeds of chilli variety KA-2 were exposed to 35 kr of ^{60}Co gamma rays genetic variability was observed in the M-2 generation with respect to plant height, fruit colour and floral structure. Two types of stable mutants were found; one with yellow green fruits and the other having flowers with undehiscent anthers with non-functional inserted stigma. The latter may be desirable and have a great value for hybrid seed production of chilli as the anthers kept undehiscent throughout the functional period of the flowers. Investigation of the feasibility of maintaining this character and incorporating it into other varieties with normal flowers and stigma is under way.

KEY WORDS: Mutants, Irradiation, Desirable dose.

INTRODUCTION

Chilli is one of the most important spice crops grown in Sri Lanka. The average annual extent cultivated to chilli is around 32,000 ha and the national average yield is around 1 t/ha. The low national average yields are mainly due to the moisture stress, pests and diseases, and use of poor quality seeds. The currently recommended varieties do not possess high levels of resistance to biotic and abiotic stresses. Emphasis has been given over the past several years to develop chilli varieties resistant to pests and diseases, drought and

earliness (3-4 month maturity group). A large number of germplasm accessions, both local and introduced, was screened and sources with different degrees of resistance to these factors have been found. However, the level of resistance possessed by these sources is not very high over the recommended varieties (Perera *et al.*, 1996). In such situations mutation breeding has been used in other crops to induce genetic variability in the available germplasm.

Application of mutagens to induce genetic variability in chilli has been attempted

by several workers in the past. The first mutation experiment conducted on chilli dates back to 1940 (Raghavan and Venkatasubhan, 1940) and since then mutagenic effects of chemical mutagens, x-rays and gamma rays have been investigated, and several useful mutants have been obtained (Daskalov, 1968, 1971, 1974, 1981; Sotirova and Daskalov, 1983; Karasz, 1974; Subramanya and Ozaki, 1983). However, the information on most effective mutagens, dose or concentration, treatment condition and plant part treated is not fully understood and most of the published data are of a preliminary nature. Daskalov (1986) reviewed the literature on mutation breeding of chilli pepper and listed the doses and treatment conditions used in these studies as a general guidance for future studies. However, due to the wide range of mutagen sensitivity, he suggested that it is appropriate to conduct a pilot test to determine reduction, survival and sterility of the M-1 generation before starting mutation breeding. The objective of this study is to determine desirable dose range, and lethal doses of ^{60}Co gamma rays on chilli (*Capsicum annuum*), and to study the genetic variability due to mutations in two chilli varieties.

MATERIALS AND METHODS

Effective dose range and lethal dose of ^{60}Co gamma rays on chilli

Two experiments were conducted at the Field Crops Research and Development Institute (FCRDI) in 1997 to determine the effective dose range and lethal dose of ^{60}Co gamma rays on chilli. The first experiment was carried out in March 1997 with two varieties of chilli, MI-2 and KA-2. Seeds with 13% moisture were exposed to 7 doses of ^{60}Co gamma rays (10, 15, 20, 25, 30, 35 and

40 kr) and were sown in a nursery bed with non-irradiated seeds. Each treatment had 50 seeds and 3 replications. Data were recorded on the number of seeds germinated at 15 days after sowing, seedling height and number of leaves at weekly intervals on 10 plants randomly selected beginning 3 weeks after sowing (WAS) up to 5 WAS.

Since the dose range used in the first experiment (10-40 kr) was not lethal, another experiment was conducted one month later with higher doses. In this experiment only the variety MI-2 was used and the seeds were exposed to 30, 40, 50, 60, 70, 80, 90 and 100 kr of ^{60}Co gamma rays. Seeds were sown in nursery beds along with non-irradiated seeds. Each treatment had 50 seeds and 3 replications. Number of seeds germinated at 15 days after sowing (DAS) was recorded.

Induction and evaluation of genetic variability in chilli

The variety KA-2 was used in this experiment. Twenty five grams of dry seeds at 13% moisture level were exposed to the appropriate dose of 35 kr of ^{60}Co gamma rays and on the following day, seeds were sown in nursery beds. Thirty five-day old seedlings were transplanted in the field in isolation to avoid any cross-pollination and periodically observed for genetic variations. The first ten fruits developed in each plant were collected separately for use in M - 2 evaluation.

Two fruits were selected randomly out of 10 fruits harvested from individual plants in M-1 generation (the first generation of the mutagen-treated seeds) and seeds of these pods were sown in nursery beds as progenies during Maha 1998/99 season. Each progeny

had 9 - 29 seedlings and these seedlings were transplanted in the field. The crop was managed without any application of agro-chemicals as one of the objectives was to identify pest and disease resistance in mutants. Some mutants showing genetic variability were identified and selfed. M - 3 generation seeds were tested in the following Yala 1999 season to evaluate uniformity and stability of the mutants. In some instances the pollen viability was tested using acetocarmine.

RESULTS AND DISCUSSION

Effective dose range and lethal dose of ^{60}Co gamma rays on chilli

In the first experiment, the number of germinated seeds of the two varieties (MI-2 and KA-2) varied. However, exposure of seeds to 0 - 40 kr range of ^{60}Co gamma radiations did not affect the viability of seeds but some variation in growth between dose levels was observed. Also none of the tested doses

Table 1. Effect of different doses of ^{60}Co gamma rays on germination of seeds of 2 varieties of chilli in two experiments.

Dose (kr)	Number of seeds germinated/50 seeds		
	1 st Experiment		2 nd Experiment
	MI-2	KA-2	MI-2
0	43(86)	39(78)	41(82)
10	37(74)	39(78)	NT
15	42(84)	35(70)	NT
20	44(88)	39(78)	NT
25	42(84)	34(68)	NT
30	39(78)	36(72)	37(74)
35	40(80)	32(64)	NT
40	45(90)	35(70)	34(68)
50	NT	NT	17(34)
60	NT	NT	15(30)
70	NT	NT	12(24)
80	NT	NT	0(0)
90	NT	NT	0(0)
100	NT	NT	0(0)
Dose(LSD) (0.05)	NS		9.01
Variety (LSD) (0.05)	1.95		-
CV%	8.5		23

*Percentages are given in the parenthesis

NT - Not tested

inhibited the seed germination (Table 1). However, in the second experiment, with higher doses, a significant decline in germination at 50 kr and a complete lethality of seeds were recorded thereafter at 80-100 kr.

With the increase in dose of ^{60}Co gamma radiation, a declining trend in seedling height and the number of leaves per plant were also observed. However, the seedling height was found to be the most affected growth parameter. Hence, seedling height measured in the first experiment at weekly intervals from 3 WAS upto 6 WAS was statistically analyzed (Table 2). In both varieties, significant height reductions were observed with time when the seeds were exposed to 25-40 kr range. The dose range at which 30-50% reduction of the normal growth occurs is considered as the desirable dose range for ionizing radiation in mutagenic treatments of cereals (Sigurbjörnson, 1983), but literature on this aspect for other crops is

not available. Therefore, in this study, too, the dose range that retards growth by 30-50% was considered as the desirable dose range.

A regression analysis with seedling height measured at 5 WAS and dose range was carried out. The selection of data of 5 WAS was done because the recommended seedling age for transplanting of chilli is 30 - 35 DAS. Seedling height decreased with increase in dose of ^{60}Co gamma radiation for both varieties and showed a very high linear correlation for the variety MI-2 ($R^2 = 0.95$) and polynomial relationship for KA-2 giving ($R^2 = 0.94$) (Fig. 1). Using the regression formula for the varieties it was found that 30 - 50% growth reduction occurs in chilli variety MI-2 at the dose level of 29 - 44 kr of ^{60}Co gamma rays and for the variety KA-2 at 31 - 36 kr. Therefore, when ^{60}Co gamma rays are used for inducing mutation in chilli, 29 - 44 kr range can be identified as the effective dose range for treating seeds at 13% moisture level.

Table 2. Effect of different mutagenic doses on seedling height of 2 chilli varieties at different stages of growth.

Dose (kr)	Plant height (cm)							
	3 WAS		4 WAS		5 WAS		6 WAS	
	MI-2	KA-2	MI-2	KA-2	MI-2	KA-2	MI-2	KA-2
0	6.4	5.4	10.2	8.7	14.7	11.6	15.8	15.5
10	6.0	5.7	9.9	9.1	14.1	13.9	16.8	17.5
15	5.9	5.0	10.3	7.9	13.1	11.8	16.3	13.8
20	5.3	4.7	7.8	7.7	11.6	12.6	14.8	14.8
25	4.7	3.6	7.3	6.5	11.3	10.5	13.7	14.0
30	3.5	2.6	5.6	4.3	9.2	8.5	12.3	12.3
35	3.9	2.1	6.5	3.9	9.1	6.4	11.9	10.1
40	3.0	2.9	5.3	4.8	8.2	8.9	11.9	12.5
LSD (0.05)	0.8		1.2		2.7		3.6	
CV (%)	11.5		10.1		14.9		15.1	

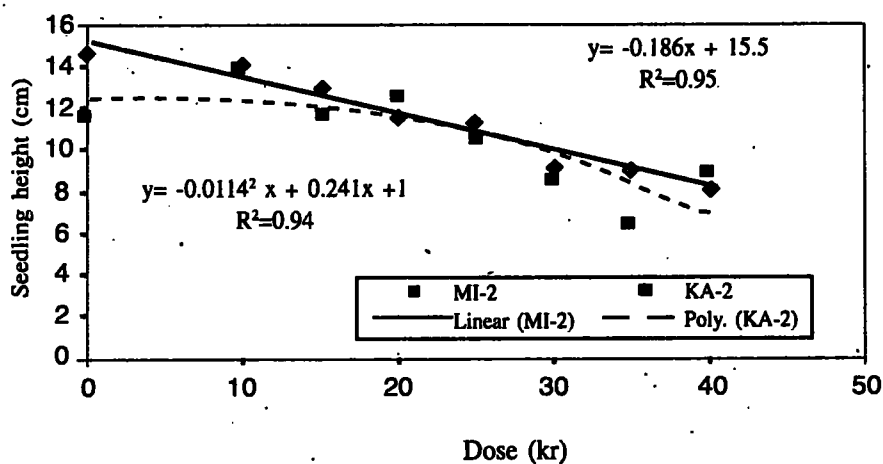


Fig. 1. Effect of different mutagenic doses of ^{60}Co gamma radiation on seedling height of chilli.

Table 3. Mutants identified in M-2 generation of KA-2.

Characters of Mutants	Number of progenies	Number of individuals
1. Deformed flowers with no Fruit setting (small greenish-white corolla tightly attached to the ovary, with deformed ovary and anthers).	01	07
2. Flowers with inserted stigma and undehiscent anthers and with parthenocarpic fruits.	01	06
3. Normal plants with short plant stature and yellow-green-fruits.	03	11
4. More number of pods with tolerance to leaf curl complex	4	07
5. Pod with tolerance to anthracnose.	1	4

Induction and evaluation of genetic variability in chilli

An M-1 population of variety KA-2 had 1335 established individuals in the field. No significant genetic variation was found in this M-1 population except meager differences in number of fruits and reaction to leaf curl complex. M-2 population had 956 progenies with 15,935 individuals. At the seedling stage, 23 progenies produced 386 chlorophyll deficient (albino) plants and after transplanting in the field these plants did not show any progress in growth. The rest of the M-2 plants (15549) showed normal growth. At flowering and fruiting stages, a few mutants with changes in plant height, floral morphology, fruit colour, fruit size, and tolerance to leaf curl complex (LCC) and anthracnose disease were identified (Table 3). Of these, mutants with undehisced anthers, deformed flowers and yellow-green-fruits were easily recognisable whereas identifying plants resistant/tolerant to LCC and anthracnose was done with difficulty as all the plants were affected by this pest and disease at varying levels (0 – 75%) of infection. The plants which had 1 – 10% leaf curl infection were identified as tolerant to LCC and the plants having all the pods free from anthracnose spots as resistant to anthracnose. However, these plants identified as tolerant/ resistant did not maintain these levels of resistance in M-3 and M-4 generations.

In the M-2 generation, 6 mutated plants with undehisced anthers were identified. They produced flowers with normal colour and size as in normal KA-2 but their anthers remained undehisced throughout the functional period of flowers (until withering) and all flowers in a plant showed a similar character (Fig. 2). These plants produced many flowers for a long period and produced small parthenocarpic fruits in large numbers. This

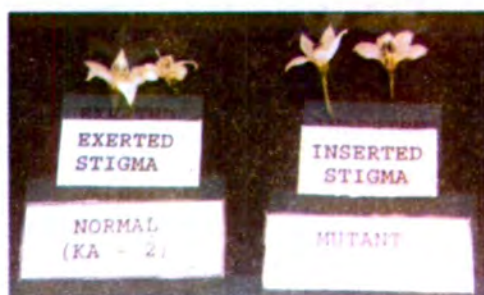


Fig. 2. KA-2 mutant with undehisced anthers.

makes them easily identified. The undehiscent anthers of these plants were dissected and pollen viability was examined by staining with acetocarmine. All the pollen was stained red and resembled well developed viable pollen in appearance.

In another test, anthers were dried and pollen was extracted and transferred to the stigma of the same plant and onto the flowers of normal chilli plants (variety MI-2). Also pollen from MI-2 was transferred to the stigma of the mutant (reciprocal cross). Selfing did not produce any fruits, but of the 50 crosses made with the MI-2 variety, six fruits were formed, but reciprocal crosses were unsuccessful. This confirms the viability of the pollen of these undehiscent anthers.

It was found that the stigma of these flowers is dissected at the tip into threads. Failure of selfing and reciprocal crosses confirms that the stigmas of these flowers are deformed and non-functional. However the undehiscent anther character of this mutant may have great value in hybrid seed production. Therefore, investigations are under way to study the feasibility of maintaining and transferring this character to obtain a variety with normal stigma and undehiscent anthers.



Fig. 3. KA-2 mutant with yellow green fruits.

The M-2 generation also had 11 mutated plants with yellow-green fruits whereas the normal KA-2 has dark green pods (Fig. 3). These plants also had short plant stature and had only 6 – 10% leaves affected by LCC under natural infection. This could be considered as having a high level of tolerance to LCC. When the M-3 generation was raised by selfing M-2 individuals of these 11 mutants, 9 progenies were uniform with respect to the yellow-green-fruit character while 2 progenies segregated to green colour and yellow-green-colour pods. Uniform progenies were selfed and the M-4 were found to be stable and uniform for fruit colour and other characteristics. Seeds of this KA-2 mutant (yellow-green fruited) were collected and sent to the Plant Genetic Resources Centre, Department of Agriculture for storage and future use.

CONCLUSIONS

Treating seeds with ^{60}Co gamma radiation could be a means of creating significant genetic variation in growth and flower characteristics in chilli. An increase in dose level up to 70 kr will induce changes in seed viability and any further increase in dose level is lethal. A dose level of 30 - 45 kr can be considered as the effective dose range to induce mutation in chilli varieties MI-2 and KA-2. The variety KA-2 exposed to 35 kr ^{60}Co gamma rays produced two stable mutants with respect to floral biology, pod characters and plant height. Even though the expected objectives of getting resistance to pest and disease and earliness were not achieved in these experiments, the dose range found can be utilized to generate genetic variability in chilli in the future.

ACKNOWLEDGEMENTS

Authors express their gratitude to Dr. K.D.S.M. Joseph, the former Director of the Field Crops Research and Development Institute, Maha Illuppallama for the guidance provided in preparation and reviewing this manuscript. Sincere thanks are also extended to Mr. R.U.B. Shantha Bandara, Technical Assistant in the chilli breeding programme at the Field Crops Research and Development Institute, Maha Illuppallama for assisting in conducting these experiments and collecting data.

REFERENCES

- Daskalov, S. 1968. A male sterile pepper (*C. annuum* L.) mutant. Theor. Appl. Genet. 38: 370 - 372.
- Daskalov, S. 1971. Two new male sterile pepper (*Capsicum annuum* L.) mutants. C.R. Acad. Sci. Agric. Bulg. 4: 291 - 294.
- Daskalov, S. 1974. Investigations on induced mutations in sweet pepper (*Capsicum annuum* L.). pp. 81-90. Proceedings of the EUCARPIA meeting on Genetics and Breeding of *Capsicum*, 1-4 July, 1974. Budapest, Hungary.
- Daskalov, S. 1981. Value of dwarf or compact-type mutants as a tool in breeding programmes, exemplified with *Capsicum annuum* L. In Induced Mutations a Tool in Plant Research. pp. 502-503. International Atomic Energy Agency, Vienna.
- Daskalov, S. 1986. Mutation breeding in Pepper. In Mutation Breeding Review. No.4. pp. 1-26. International Atomic Energy Agency, Vienna.
- Karasz, I. 1974. Breeding of spice pepper variety H.S.-X-3 from irradiated material. pp. 20-25. Proceedings of the EUCARPIA meeting on Genetics and Breeding of *Capsicum*. 1-4 July, 1974. Budapest, Hungary.
- Perera, K.D.A., S.J. Arasakesari and G.D.S.N. Chandrasena. 1996. Evaluation of chilli (*Capsicum* spp.) germplasm for resistance to thrips. pp. 131 - 138. Proceedings of an International Seminar on The Present Status and Future Prospects of Plant Genetic Resources Conservation and Utilization. 9 - 12 December, 1996, Kandy, Sri Lanka.
- Raghavan, T. and K. Venkatasubban. 1940. Studies in the South Indian chillies. I. A description of the varieties, chromosome numbers and the cytology of some x-ray derivatives in *Capsicum annuum* L. Proceedings of Indian Academy of Science. 12: 29 - 46.
- Sigurbjörnsson, B. 1983. Induced mutations: In Crop Breeding. Copyright 1983. pp. 153 - 176. American Society of Agronomy and Crop Science Society of America. 677 S, Secoge road, Madison, WI 53711.
- Sotirova, V. and S. Daskalov. 1983. Use of induced mutations in developing pepper forms resistant to *Phytophthora capsici* Leonian. pp. 123 - 126. Proceedings of the 5th EUCARPIA Meeting of the *Capsicum* and Eggplant Working Group. 4-7 July, 1983. Plovdiv.
- Subramanya, R. and H. Ozaki. 1983. Cleistogamy in pepper (*Capsicum annuum* L.) and its inheritance. pp. 53 - 56. Proceedings of the 5th EUCARPIA Meeting of the *Capsicum* and Eggplant Working Group, 4 - 7 July, 1983. Plovdiv.