Chapter IV

GERMINATION STUDY

A. RATE OF GERMINATION AND LD_{50} :

1. Introduction

Induction of mutations using chemical and physical agents such as EMS, γ - radiation is a potent method of creating new genetic variability which enhances the scope of selection. As explained earlier these agents directly bring about breakage and point mutations in DNA no matter whether plant organs are storage types, as seeds with low moisture content, or tissues with high moisture content. Such changes induced are readily visible in the seedlings when seeds germinate. If the dosage is too high i.e. lethal the seeds die, sublethal doses nevertheless, bring about number of phenotypic changes menifested as changes in morphology, physiology etc. in M_1 itself. Since chloroplasts and mitochondria are also known to have DNA, such change is also possible at their functional level.

Not all types of seeds respond in similar way. Depending upon their nature of storage material (oil, protein, starch), responses vary. Although mutagenic effect such as high energy radiation and chemical mutogens have been tried to increase genetic variability, their effect on physiological parameters especially in oil seeds is scanty. It is more so in the case of Carthomus tinctorius the safflower.

2. Material and methods

The seeds of safflower (Carthamus tinctorius L.) var. N 62-8 were used for the comparative study of EMS and γ-radiations on the physiological parameters. The seeds were obtained from Nimbkar Agriculture Research Institute, Phaltan (Maharashtra).

Five lots of about 100 g seed were γ - irradiated with 1, 2.5, 5, 7.5 and 10 Kr. doses at Bhabha Atomic Research Centre, Bombay. Prior to irradiation moisture percentage of the seeds was determined by loss in weight method and it was found to be 9.8 %. Control was maintained in the laboratory.

For the seed treatment with EMS method followed is of Hollaender (1971). For the treatment the seeds were initially flooded with water at 0°C for 12 h, to remove growth inhibitors and to completely hydrate the system. The water was changed periodically to ensure complete elimination of inhibitors if existed intrace. weighed amount of EMS was slowly dissolved in .1 M phosphate buffer (pH 7) by vigorous shaking so as to make stock solution. From the stock solution of 0.4 M EMS, 0.35, 0.3, 0.25, 0.2 M dilutions were made with phosphate buffer.

The presoaked seeds were divided in to six lots of 200 seeds each, including control and were soaked in respective

mutagen solutions of different concentrations for 6 h at 20°C. While doing so care was taken to permit, on an average, 0.5 to 1 ml of mutagen solution per seed. The treatment with mutagen was once again repeated but for only 2 h second time in order to ensure the effect. The seeds were rinsed 4-5 times with distilled water and kept in it for 12 h at 0°C. This would have enabled leaching out of any unreacted mutagen which would otherwise increase the physiological damage. 50 seeds of each of the treatment were sown in different pots for further investigation of seedlings and 150 seeds of each treatment were kept for germination in germination papers (in three replicates of 50 seeds each). Control was also sown and kept for germination. Same procedure was followed even for γ-irradiated seeds. This was carried out immediately after it was received. The rate of germination was scored both in irradiated as well as EMS treated seeds. LD_{50} was determined based on 96 h of germination.

3. Results and discussion

Irradiation effect and EMS effect on the rate of germination as well as overall germination as observed upto 96 h is depicted in Table 1.

It is clear from the result that the EMS effect is registered in two ways, firstly by damping down the germination rate and percentage and second by delayed germination. This

Table 4.1 : Effect of EMS and γ - radiation on the seed germination of Carthamus tinctorius L. var. N 62.8

Treatment	Hours after germination				Percentage of germination
	24	48	72	96	
Control	-	14	19	22	88
0.2 M EMS	-	-	9	16	80
0.25 M EMS	-	-	12	16	80
0.3 M EMS	-	-	-	15	60
0.35 M EMS	-	-	-	12	48
0.4 M EMS	-	-	-	11	44
Control	-	15	19	22	88
1 Kr	-	10	12	20	80
2.5 Kr	-	16	18	19	76
5 Kr.	-	13	18	18	72
7.5 Kr	-	11	15	17	68
10 Kr	-	9	13	17	68

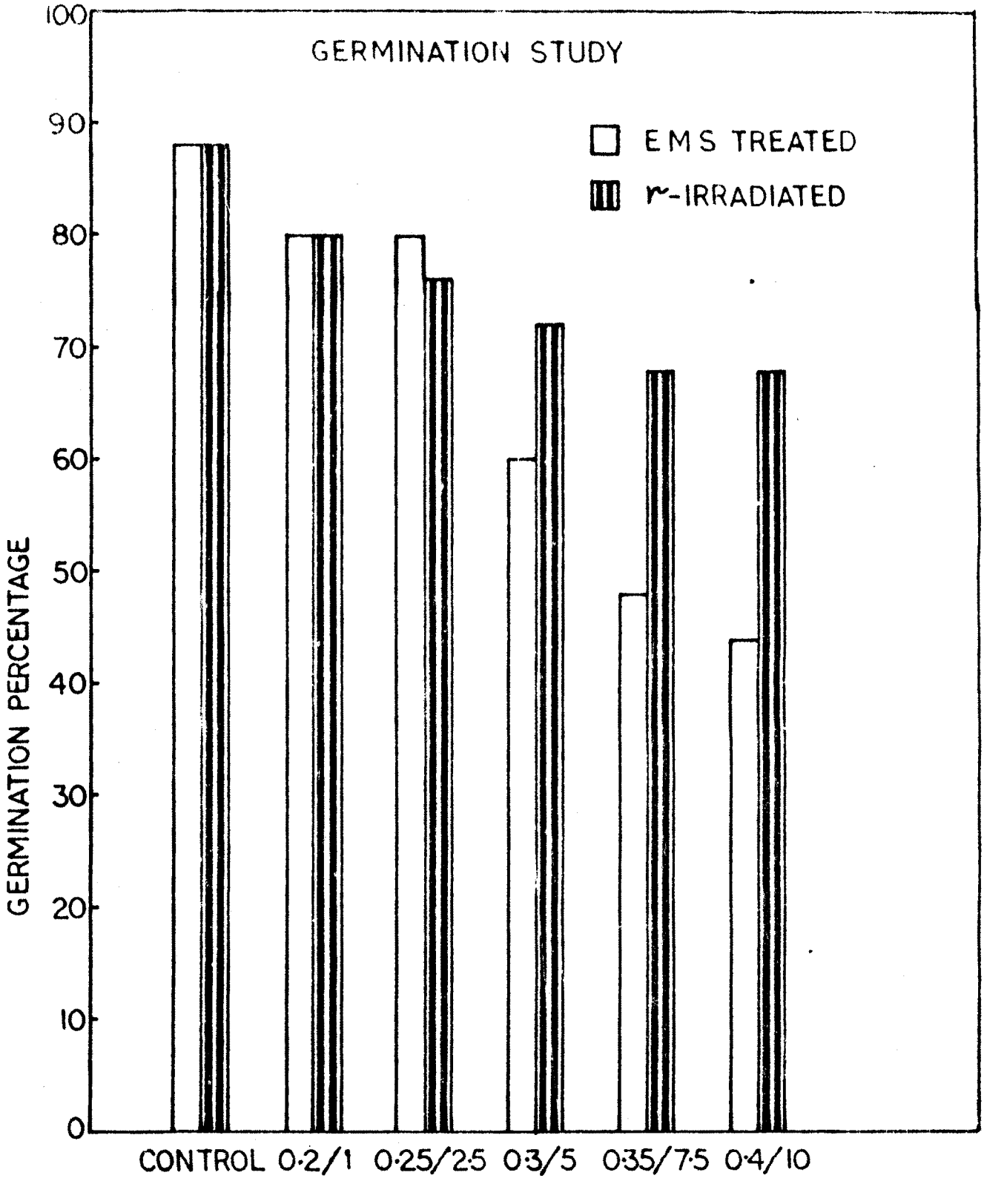

Fig. 4·1 CONCENTRATION OF EMS IN MOLARITY AND RADIATION IN K_r

effect went on increasing as the concentration of EMS increased. At 0.4 M EMS, the germination is not only delayed by 96 h but the total germination is reduced to 50% of the control. This reflects on the sublethal effect of the concentration; which may be taken as LD_{50} for this variety.

The result of germination study carried out in irradiated seeds is also given in Table 4.1. Unlike that in EMS treated seeds, germination in the irradiated seeds, started on the same day as that of control. In other owords no delay in the germination could be noticed. However, the rate of germination and overall percentage of germination slowly decreased as the dosage increased from 1 Kr to 10 Kr. At 10 Kr which is the highest dose chosen in the present experiment, the total percentage of germination as counted after 96 h is 77.5 of the control. In other words the dosage of irradiation required to reach LD_{50} in this variety of Carthamus appears to be much higher. However, higher doses are known to cause larger damages than lower ones, and there are always chances of getting more beneficial mutations in the lower dose than the higher ones.

In the present investigation EMS has a much pronounced effect on the overall germination at higher dose than the highest dose of 10 Kr irradiation. EMS has been known to cause greater effect on the seed germination in Carthamus var. Annigeri-1 and Nagpur-7 which have been studied both individually,

as well as in combination with - rays by Mallikarjunaradhya and Channabyregowda (1981). Similar observations were made by Shivraj and Ramana Rao (1963) in castor, Singh (1974) in safflower. However, Mallikarjunaradhya and Channabyregowda (1981) have scored in both the varieties 'Annigeri' and 'Nagpur 7' of safflower, 65.5 and 63.04 percent of germination of the control respectively in .4 % EMS treated ones. The combination of 48 Kr - rays with 0.3 % EMS on the contrary seem to have stimulated germination in their experiment. This reflects on the fact that the variety taken in present investigation seem to be more vulnerable to the EMS effect. Number of damaging effects caused by - irradiation on germination have been recorded by various workers. It is known to cause disruption and disorganization of tunica layer and thereby inhibit germination (Chauhan and Singh, 1975). High dose of irradiation has been shown to be impairing mitosis or sometimes virtual elimination of cell division in meristematic zone of germinating seeds occurs, (Cherry and Hageman, 1961). It is also known to cause respiratory inhibition (Woodstock and Justice, 1967). However, in the present investigation the dosage is low, there is apparently better recovery of germination although with increasing dose, the percentage is lowered. This has better advantage of showing the marginal effect of irradiation which may possibly provide some useful mutations that can possibly be sustained in the process of selection.

Since EMS is known to stimulate the formation of acids after hydrolysis which in turn reduces the pH of the medium

making it toxic (Froese - Gertzen et al., 1963) apparently the damage is not as severe at tissue level as that caused by irradiation.

In flax (Linum usitatissimum) cv. 'Mukta' seeds treated with caffein, EMS and DES, reveals that these chemical mutagens affect the rate of germination mainly acting as suppressors to various degrees, depending upon their chemical constitution and concentration (Maharaj et al., 1981). Sodium azide affects the germination rate in rice var. 'japonica' (Hiroshi and Inoue, 1981). Similar observations were also recorded in soybean by Wei-Mao and Chuanlu (1981). Usmanov and Sokhibnazarov (1976) have noted that NMU (N-nitrosomethyl urea) treated seeds of Arabidopsis thaliana showed decreasing capacity of germination with increasing concentration of NMU. Thus in the present comparative study of the effect of EMS and irradiation on Carthamus seeds of variety chosen, it is clear that EMS causes greater damage with increasing concentration and hence the inhibition of germination while irradiation does not.

B. ENZYMES OF GERMINATION :

1. Introduction :

Enzymes play an important role in germination of seeds. Enzyme activity is influenced by various factors as environmental, genetic etc. The activity of a particular enzyme is peculiar

in a perticular seed e.g., in starchy seeds the activity of enzyme amylase is more as compared to those in fatty seeds, the activity of protease is more in pulses and the activity of enzyme lipase is more in oily seeds. Biochemistry of fatty seed germination reveals that various enzymes are involved in the germination.

Dry seeds do not inherently pussess or store enzymes. In response to the imbibition of water by seeds, the genetic blue prints stored for the enzymes are stimulated and de novo synthesis of enzymes taken place. This has been extensively demonstrated by Peleg (1960). When seeds are exposed to mutagenic agents whether physical or chemical, target of action obviously is the genetic material. This effect possibly, either disables the genes to synthesize respective enzyme or may bring about some modification, if the type of mutation caused is hypomorphic. Based on this fact, it was thought interesting to study the enzymes involved in germination for their activities which by and large govern the overall seedling growth.

It is necessary to mention here by studying the EMS effect on germinating enzymes 0.2 M EMS treated seeds only were taken in to consideration and the enzymes studied are peroxidase, lipase and amylase. It has already been pointed out that lethal doses always cause greater damages. At sublethal level there are many benificial mutations. Since in this variety of

Carthamus the LD_{50} is at 0.4 M, it was thought to study the effect at 0.2 M EMS, so that at that juncture there may have many beneficial mutations.

2. Material and methods :

The seed of safflower (Carthamus tinctorius L.) var. N 62-8 was treated with 0.2 M EMS concentration by the method described earlier. It was kept for germination in moist germination papers. After 24 h the germinating seed was used for studying the activity of enzyme peroxidase, lipase and amylase.

For the estimation of peroxidase activity .5 g of germinating seeds were taken. The material was crushed in chilled morter with pestle in 10 ml of cold distilled water. It was filtered through two layers of cheese cloth. The filtrate was centrifuged. The supernatant served as enzyme source and enzyme activity was estimated colorimetrically by the method of Muehley (1954), and expressed as O.D. min^{-1}, g^{-1} of fresh tissue.

For asseying lipase seeds were taken and seed coats were removed. Decorticated seeds were crushed in mortar with pestle. The oil was removed by repeated extraction with cold petroleum ether. The seed residue free from oil was used as

as the source of enzyme lipase. Activity of enzyme was estimated by titration method of Wolf (1968). The enzyme activity was calculated as ml of N/50, aqu. KOH h^{-1}, g^{-1} of enzyme lipase.

For the estimation of activity of enzyme amylase .5 g of seeds were taken, crushed in chilled mortar with pestle in 20 ml of cold acetate buffer (pH 5). It was filtered through 4 layered cheese cloth. The filtrate was centrifuged for 5 min. at 3000 r.p.m. and the supernatant was used as enzyme source. The activity of enzyme was estimated calorimetrically by the method of Katsumi and Fukuhara (1969). Enzyme activity was calculated by following formula :

$$D.B. = 2 \times \frac{d - D}{d} \times \frac{100}{10}$$

d = Absorbance at zero minute

D = Absorbance at 1 h

From this enzyme activity was calculated to h^{-1}, g^{-1} of fresh tissue.

3. Result and discussion :

The activities of enzymes peroxidase, lipase and amylase are assayed from germinating seeds of control and 0.2 M EMS treated ones. It is clear from the table that there is overall increase in the activities of all the three enzymes due

Table 4.2 : EMS EFFECT ON THE ACTIVITIES OF PEROXIDASE, LIPASE AND AMYLASE OF CARTHAMUS TINCTORIUS (L) VAR N 62-8 SEEDS 24 H AFTER TREATMENT :

Name	Treatment	Enzyme activities		
		Peroxidase[1]	Lipase[2]	Amylase[3]
Carthamus tinctorius Var N 62-8	Control	1.6	0.6	13.336
	0.2 M EMS	4.8	2.2	20.952

1. Activity expressed as O.D. min^{-1}, g^{-1} of fresh tissue.
2. Activity expressed as ml. of N/50 aqu. KOH h^{-1}, g^{-1} of enzyme.
3. Activity expressed as D.B. h^{-1}, g^{-1} of fresh tissue.

to the mutagen treatment. If the peroxidase and lipase activities have increased three times in the treated ones, that of amylase increased one and half times.

Monselise and Kahan (1966) have shown that there is a change in composition and enzyme activity of juice of shamouti orange following γ - irradiation. Such a stimulatory results was also recorded in the leaves of leafy mutants of tomato, Subhash et al., (1981) studying peroxidase isozyme pattern in leafy variants of L. esculentum var. HS 110 produced after treatment with 0.1 % hydroxylamine showed the stimulatory effect. The stimulatory activity of the enzyme peroxidase has been recorded in Saintpaulia ionantha Wendl. in response to the mutagen ethylene gas and X-ray treatment both in combination as well as individually (Warfield et al., 1971). Singh (1974) while working with irradiation effect on safflower showed that 20 Kr dose of irradiation stimulated the activities of catalase, lipase and the ascorbic acid content of the germinating seeds.

Woodstock and Justice (1967) recorded the marked and significant increase in catalase and lipase activity with the onset of germination of the irradiated seeds. The work on the enzymes of germinating seeds treated with mutagen as EMS appears to be scanty; the present investigation indicates (Table 4.2) that like radiations, 0.2 M EMS has a stimulatory effect on the enzymes of seed germination in C.tinctorius L. var N 62-8.