COMPARATIVE EFFECT OF AGEING AND GAMMA IRRADIATION ON THE SOMATIC CELLS OF LATHYRUS SATIVUS L.

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ABSTRACT

Gamma irradiation is one of the most important physical mutagen used for inducing beneficial as well as harmful cytogenetic effects in many crop plants. The cytogenetic changes occurring due to the storage of Lathyrus sativus L. seeds after gamma irradiation were examined during the present study. Mitotic index showed a dose dependant decrease while the dose and duration of treatments increased. The effect of ageing accompanied with gamma irradiation was found to be more deleterious as compared to that of individual treatments of the two. Various chromosomal abnormalities viz. fragmentation, scattering, bridges, laggards, stickiness, etc. were found at all the three treatment sets. A significant reduction in moisture content, germination percentage and increase in the percentage of chromosomal anomalies was recorded maximum in the stored gamma irradiated seeds which ultimately ensures the fact that immediate sowing of the irradiated seeds is of prime importance and it may adversely affect the survivability of the seeds as well as plant when sowing is delayed.

Key words: Lathyrus sativus, ageing, gamma irradiation, mitotic index, chromosomal anomalies.

INTRODUCTION

Ageing is a physiological phenomenon occurring in living organisms and is of universal concern [16]. Seeds have the potential to reproduce new individuals and adapt to survive under a wide range of unfavorable conditions for growth while holding the record for cellular longevity. The development of new trends in agriculture requires ageing process in plants to be investigated, firstly in seeds for biological (germplasm conservation) and economic reason (production of commercial seeds).

Many previous studies [6, 20] have shown that in a wide range of species, there is a close relationship between loss of seed viability during storage and the accumulation of genetic damage in the surviving seeds. The main factors affecting this relationship are seed moisture content, temperature and storage period i.e. increasing either results in an increase in loss of seed viability or an increase in the frequency of chromosomal aberrations [21].

Studies on biological effects of ionizing radiations and their utility in inducing gene mutation have been a topic of immense interest for cytologists. A great deal of efforts has been made to explore the utility of ionizing radiation for genetic improvement in a number of crop plants like pearl millet [13] and soybean [11].

On contrary, grass pea has received a very little attention in this regard. Grass pea is cultivated for grain and fodder in several parts of India and sometimes becomes the only available food source for poor population in the times of drought and famine.

In view of the significance of seed longevity vis-à-vis ageing, mutational research has been planned to elucidate the cytological impact of ageing and gamma irradiation on the somatic cells of grass pea. This study is an attempt to investigate the effects of storage conditions on the loss of viability and accumulation of chromosomal aberrations in non-irradiated seeds and stored gamma irradiated seeds.

MATERIALS AND METHOD

Seeds of Lathyrus sativus L. var. Pusa-24 procured from National Bureau of Plant Genetics Resources (N.B.P.G.R.), New Delhi were irradiated at four different doses of gamma rays i.e. 200, 400, 600 and 800Gy at National Botanical Research Institute (N.B.R.I.), Lucknow. Seeds were divided into two lots after irradiation. First lot was studied immediately after irradiation and second lot after one year of storage.

After irradiation and storage (one year), seeds were germinated on wet filter paper in petridishes. The germination test was conducted using three replications of 100 seeds from each sample in rolled towel paper as per procedure described by ISTA [10].

The initial moisture content of the seeds before ageing and gamma irradiation was about 15.2% and 99.6% normal germination was recorded. Fresh seeds were considered as control.

After germination, excised root tips were fixed and placed in individual glass vials in a fresh solution of Carnoy's fixative (3:1 alcohol: acetic acid) for 24hours and then transferred in 70% alcohol. For slide preparation, squash technique with 2% acetocarmine was utilized.

RESULTS

Table-1 clearly depicts the lowering of moisture content as the doses and durations of treatments increased. The lowest moisture content value (5.8%) was noticed at 800Gy+A_1 treatment set which is even less than the half of the control's (15.2%). Similarly, the germination percentage showed a sharp decline along with the increase in the dose and duration. Stored γ irradiated seeds showed

Table 1: Comparative effect of storage (ageing) and combination treatments of stored gamma irradiated seeds of grass pea on the moisture content and germination percentage

Treatments (Dose/Duration)	Moisture content (%)	Germination (%)
Control	15.2	99.6
A_1	14.6	88.4
200Gy	12.3	80.8
200Gy + A_1	12.0	78.4
400Gy	11.1	68.6
400Gy + A_1	10.6	60.1
600Gy	8.4	52.9
600Gy + A_1	8.0	50.7
800Gy	6.6	42.4
800Gy + A_1	5.8	40.6

A_1- ageing of one year storage, Gy- Gray the reduction in germination percentage from 78.4% to 40.6% whereas freshly γ irradiated seeds displayed the reduction from 80.8% to 40.2% (Table-1).

In control sets, mitosis was almost normal (2n=14) with metaphase (Fig.1) and anaphase stages (Fig.2). Mitotic index was recorded to be 15.34% with negligible amount (0.31%) of chromosomal abnormalities. However, the M.I. of one year ageing treatment was recorded as 14.72% (Table-2).

In the root tips of gamma-irradiated set, M.I. showed a gradual decrease, i.e. 14.92% at 200Gy dose to 7.11% at 800Gy dose (Table-2). However, the M.I. of stored gamma irradiated seeds was recorded maximum as 15.17% (200Gy+A_1) and minimum as 6.90% (800Gy+A_1).

Table-2 and Graph-1 illustrates the effect of storage period and gamma irradiation on the frequency of chromosomal damages accumulated. The chromosomal aberrations observed during the study were classified under two categories: chromosome type and chromatid type. Chromatid type aberrations comprised of single fragment of unequal size whereas chromosome type aberrations comprised of one or more double fragments.

A number of other chromosomal abnormalities have been recorded in the treated sets viz. unorientation, stickiness at metaphase (Fig.3), precocious movement of chromosomes towards both poles (Fig.4), scattering with small fragments (Fig.5), multiple fragmentations (Fig.6), laggard (Fig.7), bridges at anaphase (Fig.8), stickiness at anaphase (Fig.9), etc. Among all the abnormalities registered, fragmentation was found to be the predominant abnormality followed by bridges. The pooled results confirmed that chromatid type aberrations were predominant in each set of treatments of ageing and gamma irradiation. However, stored γ irradiated seeds induced more abnormalities as compared to that of A_1 treatment set (Table-2).

An interesting observation explored during the present study was a sharp decline in moisture content, germination percentage and M.I. values in the stored gamma irradiated seeds. However, the individual treatments of A_1 and gamma irradiation were comparatively less damaging regarding the mitotic index values. On the other hand, the effect of individual treatment of gamma irradiation was comparatively more deleterious than ageing (A_1) treatments. The only negative aspect noticed during the study of individual ageing treatment was the induction of some chromosomal abnormalities but that too were lower in comparison to gamma irradiation and combination treatment of the two.

However, the maximum viability loss with maximum abnormality percentage (35.17%) was recorded at 800Gy + A_1 treatment set (Table-2). The effect of individual and

Table-2: Mitotic indices and chromosomal abnormalities in freshly gamma irradiated and stored (ageing) gamma irradiated seeds of grasspea

Treatments	Metaphasic abnormalities (%)					Anaphasic abnormalities (%)					TCO	T Ab (%)	M I (%) Mean±S.E.
	St	Pm	Un	Sc	Oth	St	Lg	Bg	Us	Oth			
Control	-	-	-	-	-	0.32	-	-	-	-	2034	0.32	15.34±0.13
Immediately after gamma irradiation (First Lot)													
200Gy	-	0.66	-	0.33	0.66	1.64	0.98	-	0.33	-	2044	4.59	14.92±0.33
400Gy	1.05	1.39	1.05	1.05	1.74	1.05	0.70	1.39	0.70	2.09	2045	12.20	14.03±0.23
600Gy	2.35	1.96	1.57	2.35	2.75	1.57	1.18	2.35	1.57	3.14	2156	20.78	11.83±0.57
800Gy	6.29	2.80	4.20	1.40	2.80	4.90	2.80	2.10	2.10	3.50	2071	32.87	7.11±0.73
After one year storage (ageing) of gamma irradiated seeds (Second Lot)													
A_1	0.31	0.31	0.63	-	-	-	-	-	-	-	2126	2.23	14.72±0.37
200Gy+ A_1	0.93	0.31	1.24	-	0.31	0.62	0.31	-	-	0.31	2122	4.04	15.17±0.08
400Gy+ A_1	1.35	1.01	1.01	0.34	1.35	2.02	1.01	0.67	0.67	1.01	2098	10.44	14.16±0.28
600Gy+A_1	3.15	1.57	2.36	0.79	1.57	2.36	1.57	1.18	1.18	1.97	2167	17.72	11.72±0.46
800Gy+ A_1	3.45	3.45	2.76	4.14	4.83	2.76	2.07	4.14	2.76	4.83	2039	35.17	6.90±0.87

TCO- Total cells observed, M.I. - Mitotic Index, Tab- Total abnormality percentage, Un- Unorientation, Pm- Precocious movement, Fg- Fragmentation, Sc- Scattering, St- Stickiness, Lg- Laggard, Bg- Bridge, Us- Unequal separation, Oth- Others

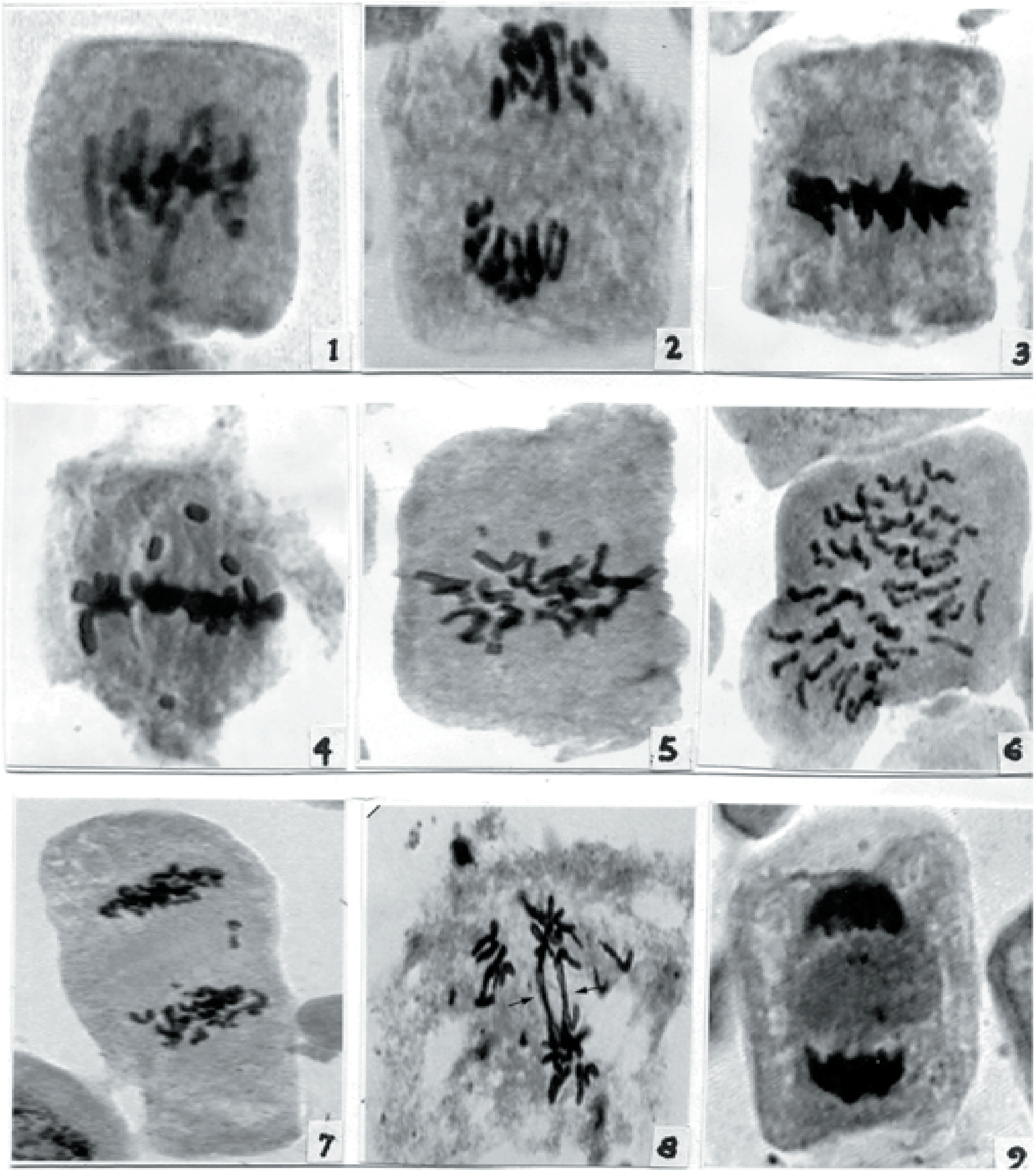

Fig.1- Normal metaphase (2n=14); Fig.2- Normal anaphase (14:14 separation); Fig.3- Stickiness at metaphase; Fig.4- Precocious movement of chromosomes towards both poles; Fig.5- Scattering with small fragments at metaphase; Fig.6- Multiple fragmentations at metaphase; Fig.7- Scattering at anaphase with laggard; Fig.8- Double bridge at anaphase; Fig.9- Stickiness at anaphase

Figures 1-9: Different types of chromosomal aberrations after ageing and gamma irradiation in grass pea.

combination treatments of ageing and gamma irradiation on the M.I. and seed viability can be summarized as follows:

(γ irradiation + A_1) < γ irradiation < A_1

A significant reduction in moisture content, germination percentage and enhancement in the percentage of chromosomal anomalies was recorded in stored gamma irradiated seeds which ultimately ensures the fact that immediate sowing of the irradiated seeds is of prime importance and it may adversely affect the survivability of the seeds as well as plant when sowing is delayed.

DISCUSSION

In modern agriculture, seed deterioration under storage is a problem [18]. Ageing process is affected by the genetic factor [3]. Data clearly reveal that effect of storage (ageing) decreased the mitotic index values as compared to controls. Mitotic inhibition due to ageing can be attributed to blocking of mitotic cycle during anaphase, which may result from prolonged G_2 period or defective DNA synthesis.

While working on barley, broad bean and peas, [1] concluded that the relationship between loss of seed viability and accumulation of aberrations during first mitosis was asymptotic, i.e. there was an initial rapid rise in aberrant cells with decrease in seed viability, but as the viability decreased below 50% further increase in aberrant cells was hardly detectable. However, in case of our study, it was found that there was a further considerable increase in the frequency of aberrant cells even below 50% viability [23, 17, 12]. Considering numerous studies of other authors such as Vesna D. Dragicevic et al [22], seed ageing is the foremost oxidative stress that leads to a decline in germination and loss of viability.

Although Abdalla and Roberts [1] have reported the preponderance of chromosome type aberrations over chromatid type in aged seeds of barley, broad bean and pea but recently it has been concluded that chromatid type aberrations predominated in wheat [9]; barley [4]. Similar results were in case of our study.

Fragmentation at metaphase as well as at anaphase is one of the most common chromatid type aberration observed at all the treatment sets. They may be the result of chromatin erosion and chromatid breaks. These changes represent an irreversible and toxic effect [7]. Occurrence of fragmentation at any stage of division is considered as one of the most significant abnormality that establishes the mutagenicity of a particular mutagen. Fragmentation leads to deletions, insertions that can alter the genetic architecture of the plant. Bridge formation could be attributed to chromosomal stickiness [5] and stickiness is considered to be a type of physical adhesion mainly involving the proteinaceous matrix of chromatin [19]. Kumar [14] also observed bridges, laggards and fragments in aged chilli seeds. Due to its easy identification, the use of bridges and fragments as indicators of the occurrence of chromosomal variations had been found to be an efficient method and favored the counting of a great numbers of cells. This criterion has been reported as being useful in detecting abnormalities in seeds stored for long periods of time [8].

Increase in chromosomal aberration frequency in mitosis along with the doses of gamma rays might perhaps be due to the interactions of ionizing particles with the protoplasm mediated by excitations either directly or indirectly that ultimately has increased aberration frequency. The results of the present study depict that one year storage, decreased the mitotic index gradually and also showed inhibitory effect on stored gamma irradiated seeds due to inhibitory effect on spindle apparatus. Our findings are contrary to those of Carlson et al [2] and Misra & Raghuvanshi [15] while working on Trigonella foenum-graecum.

The present investigations clearly reveal the fact that storage of gamma irradiated seeds may induce a number of anomalies and loss of viability to some extent. Hence, our study suggests that in order to retain the viability of seeds of grass pea, immediate sowing after gamma irradiation should be performed to reduce the chances of hampering of genetic structure via fragmentations and other such anomalies.

Another fact explored during the present study is that the seeds of grass pea stored for one year after irradiation exhibited higher percentage of chromosomal abnormalities as compared to the non radiated seeds stored for one year. Hence this combination of ageing and gamma irradiation may provide an additional tool in inducing variations without much loss in viability. Through mutation breeding in grass pea, we can exploit this trait to enhance the mutagenic potential of gamma-irradiated seeds stored for one year. It can be suggested that some genetic repairing mechanism must be acting in order to enhance the efficiency of gamma rays in this given time period. These results also depict the storage capability of the seeds of grass pea with a long-term viability. Certain varieties exhibit greater longevity hence, grass pea germplasm should be screened for this character and an attempt should be made to isolate this trait.

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