AGING EFFECT OF WHEAT AND BARLEY SEEDS UPON GERMINATION MITOTIC INDEX AND CHROMOSOMAL DAMAGE

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SUMMARY: The experiment was carried out to ascertain the effects of age on wheat and barley seeds. Germination percentage of different years old wheat and barley seeds were found to decrease gradually with an increase of the storage time. The germination percentages of wheat seeds were much lower than that of barley seeds. Mitotic index and chromosomal irregularities from root tip cells were also studied. Most of the irregularities were characterized by precocious separation of chromosomes and inactivation of spindle mechanism, chromosome fragment, laggard, bridge, condensed and sticky chromosome, ring chromosomes etc. In both the materials, the frequencies of dividing cells were found to decrease with the increase of the age of seeds. Frequency of abnormal cells were also found to increase with the increase of the age of seeds. The frequency of chromosomal aberrations with increased storage time was closely related to the loss of germinability.

Key Words: Aging germination mitotic index, chromosomal damage.

INTRODUCTION

Aging heat, irradiation and hybridity bring about an increase in the frequency of structural chromosomal alterations as well as an increase in the frequency of gene mutations. How these factors are related to one another and how they cause changes in the chromosome and gene mutation rates are not well understood. However, the fact that all these external agents cause similar changes and indicate a broad fundamental process as the primary cause of mutations (22).

Crepis tectourm L. plants from old seeds (6 and 7 years old) presented very much greater percentage of chromosomal mutations than those from young seeds, suggesting a hypothesis that the aging had an important effect on the mutation process under natural conditions (20). In Maize (24), in *Datura* (4, 5) and in *Crepis* (10) likewise showed a high mutation rates in plants from old seeds, which suggested that the phenomenon is of general character. The effects of age, moisture and x-ray on the seed of barley exhibited a of disturbed cells in the mitotic division after germination (11). A much higher rate of chromosomal mutations in root tip cells of *Pisum sativum* from four years old seeds were reported than from fresh seeds (7).

Dry seeds have many advantages as material for experimental studies on chromosome behavior. Seeds are in a relatively dehydrated condition and physiological activity is at a minimum. Seeds of many plants can be kept viable for a number of years (22) and thus the effect of aging may be investigated. The present study was intended with different years old wheat and barley seeds to observe the effect of aging on seed germination and mitotic index, and on the frequency and nature of chromosomal mutations from root tip cells along with the determination of the relationship between chromosomal anomalies and seed germinability.

MATERIALS AND METHODS

This experiment was conducted with the moisture free old seeds of hexaploid wheat (*Triticum aestivum*, var. Sonalika) and diploid barley (*Hordeum vulgare*). Those seeds were stored in desiccator containing calcium chloride for 1, 7 and 10 years in the Cytogenetics Laboratory of the Department of Botany, University of Rajshahi, Bangladesh.

Different years of old seeds of wheat and barley were presoaked in distilled water and 100 seeds for each class

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were allowed to germinate on moist filter paper at room temperature (20-23°C). The germination percentage was recorded every day for 7 days. When the roots grew up to 1-1.5 cm in length they were fixed in 1:3 aceto-alcohol for 48 hours and then transferred to 70% alcohol.

Root tip cells were stained by squash method (14) using hematoxylin as stain. Photomicrographs were taken from the desired preparations.

RESULTS

Germination tests of different years old wheat and barley seeds were made soon after removal from storage. There were significant differences in germination percentages (Table 1) among the different years old wheat and barley seeds. Germination frequency of both the materials were found to decrease with the increase of the storage time. Germination percentage of wheat was much lower than that of barley although they were stored for the same period of time.

Mitotic observation from the root tip cells showed mitotic depression and various type of chromosomal irregularities (Tables 2 and 3). Most of the abnormalities were characterized by precocious separation of chromosomes and inactivation of spindle mechanism, chromosome fragment (Figure 2), lagging chromosome (Figures 5 and 6), chromosome bridge (Figures 4 and 5), condensed and sticky chromosome (Figure 3) and ring chromosome. In both wheat and barley root tips, the frequency of dividing cells were found to decrease with the increase of the age of seeds. Similarly frequency of abnormal cells were found to increase with the increase of the age of seeds. In addition to above mentioned abnormalities few cells were found with multi-polar and unequal separation of chromosomes and disorganized prophase chromosomes (Figure 1).

Table 1: Germination percentages of different years old wheat and barley seeds.

Seeds	Years old	Germination percentages $(\bar{x} \pm S.E)$
Wheat	1	88.08±0.50
	7	27.01±0.18
	10	16.70±0.14
Barley	1	92.65±0.70
	7	38.88±0.55
	10	21.50±0.14

The pattern of increase of the frequency of aberrations with increased storage time was closely related to the loss of germinability. The correlation co-efficient in the data for wheat (r=-1) and barley (r=-1) indicated that the frequency of chromosomal mutations were more closely related with loss of germinability (Figure 7).

DISCUSSION

Studies on germination percentage of different years old wheat and barley seeds indicated a decrease of seed viability with the increase of age. The results obtained with aged seeds of wheat and barley demonstrated the deteriorative effects of age. Storage conditions, particularly temperature and moisture have been indicated as the main factors influencing seed

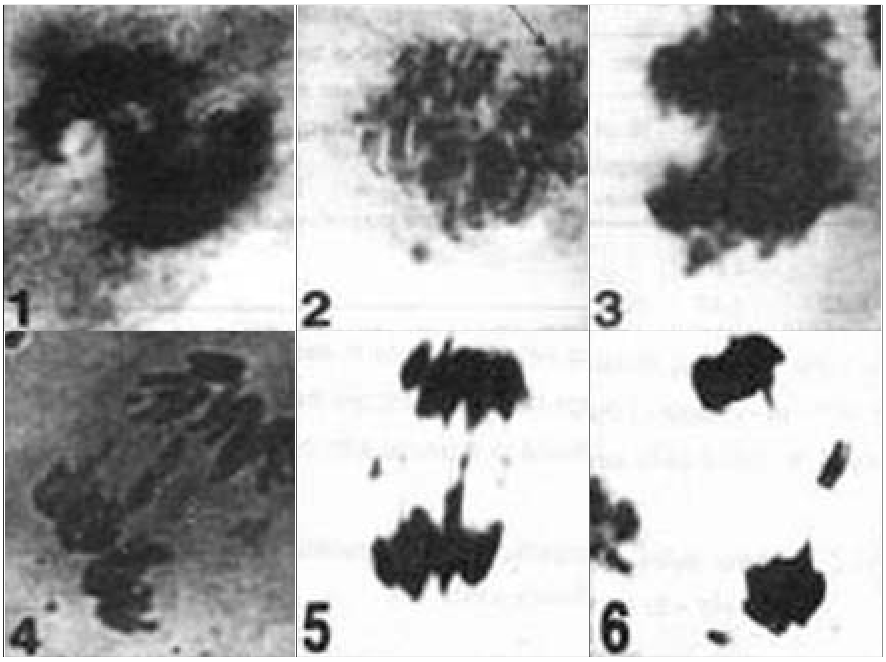
Table 2: Mitotic index and frequency of chromosomal irregularities at different stages of root tip cells of different years old wheat and barley seeds.

Seeds	Years old	Number of cells examined	Mean % of dividing cells	* Mean % of chromosomal irregularities	Percentages of dividing cells along with the chromosomal irregularities at different stages							
					Prophase		Metaphase		Anaphase		Telophase	
					% of cells	% of irregularities	% of cells	% of irregularities	% of cells	% of irregularities	% of cells	% of irregularities
Wheat	1	8160	8.71	1.92	3.05	-	2.57	1.92	1.42	-	1.67	-
	7	8968	3.49	10.08	1.02	0.08	0.75	6.82	0.28	1.92	1.44	1.26
	10	6867	3.07	11.15	0.76	-	0.70	7.21	0.56	2.57	1.05	1.37
Barley	1	8570	10.21	1.77	3.55	-	2.62	1.77	1.28	-	2.76	-
	7	10201	3.33	9.77	0.93	-	1.17	6.35	0.42	2.40	0.81	1.02
	10	9720	3.15	11.16	1.06	0.12	0.95	8.10	0.51	2.36	0.63	0.58

* Percentages of cells with irregularities are on the basis of total number of dividing cells.

Table 3: Frequency and nature of different types of abnormalities in root tip cells of different years old wheat and barley seeds.

Seeds	Years old	Mean % of abnormal cells	Percentage of cells with different abnormalities							
			Precocious separation of chromosomes and inactivation of spindle mechanism	Chromosome fragment	Laggard chromosome	Chromosome bridge	Condensed and sticky chromosome	Ring chromosome	Multipolar and unequal separation	Disorganized prophase chromosome
Wheat	1	1.92	1.65	0.27	-	-	-	-	-	-
	7	10.08	2.39	0.80	-	2.38	4.16	-	0.27	0.08
	10	11.15	1.38	1.37	0.34	2.92	4.46	0.34	0.34	-
Barley	1	1.77	0.80	0.97	-	-	-	-	-	-
	7	9.77	1.33	1.33	0.66	2.01	3.78	0.45	0.21	-
	10	12.28	2.04	2.74	0.89	2.29	3.05	0.86	0.29	0.12



Figures 1-6: Different types of abnormalities in root tip cells of wheat and barley. 1. Disorganized interphase nucleus of wheat. 2. Metaphase of wheat with fragment. 3. Disturbed anaphase of wheat. 4. Anaphase of barley with bridge, laggard fragments. 5. Early telophase of barley with bridge laggard and fragments. 6. Telophase of barley with lagging chromosome.

longevity. High temperature and moisture accelerate loss of viability in most species (3, 23, 25). However, in the present study the germination percentages were recorded from those old seeds which were kept moisture free in the desicator containing calcium chloride. Both wheat and barley seeds lost germinability with the increase of age. It might be due to the consumption of respiratory substances which led to exhaustion of reserve substances.

The present findings indicated a remarkable variation of germination percentage of wheat and barley seeds, although they were stored for the same period of time, subsequently. However, this type of variation would have been among species and within species. Since seed longevity is known to vary greatly among species and within species (19). Twelve percent germination was reported in barley seeds stored for 123 years in a glass vial embedded in the foundation stone of a building in

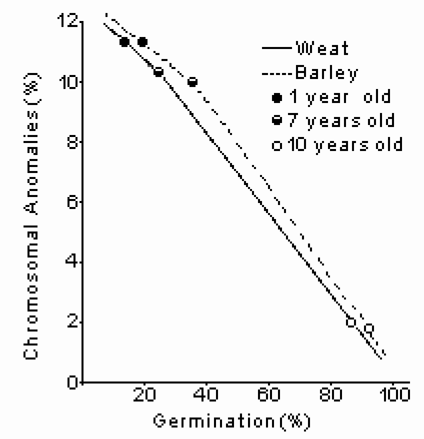


Figure 7: Relationship between the chromosomal anomalies and seed germinability of wheat and barley.

Nuremberg (2). Likewise 96% germination of barley seeds was reported after 32 years storage in an unsealed container at air temperature at the Dry Land Experiment Station, Lind, Washington (13). Germination of barley seeds dropped to 46.2% of the initial germination after 21 years in a dry unheated room in Colorado, indicating a much shorter longevity for barley seeds (27).

In the cytological studies at mitosis it was observed that with the increase of the age of wheat and barley seeds, percentage of dividing cells decreased. Depression of cell division was seen obviously in the root tip of 10 years old wheat and barley seeds where the mitotic index reached not more than 3.07% and 3.15% compared to 8.71% and 10.21% in the root tips of one year old wheat and barley seeds, respectively. The rate of mitosis is closely related to resultant level of ATP (8). Hence, it might be that cell division is energy dependent process and thereby the movement of chromosomes mainly depend upon the energy generating system. In the present findings any toxic material in the aged seeds may disturb the respiratory pathways, resulting in the low production of energy containing and other essential compounds-ATP, sugars and protein molecules.

Most of the abnormal cells observed in the present study were precocious separation of chromosomes and inactivation of spindle mechanism, chromosome fragment, laggard chromosome, chromosome bridge, condensed and sticky chromosome, ring chromosomes, multipolar and unequal separation of chromosomes etc. The formation of chromosome fragments might be due to the stickiness of chromosomes and consequent failure of separation. Breakage and reunion of the broken ends cause the chromosome bridges and this type of bridges continue its existence up to early telophase. It was found to bring about condensation and stickiness of chromosomes ultimately resulting in the formation of ring chromosomes. The probable cause of condensed and sticky chromosomes might be due to the fact that the chromosomes start contraction at metaphase/anaphase stages while as a result of any toxic material the chromosomes can not reach the poles and remain scattered in the cytoplasm. However, chromosome stickiness arises due to improper folding of chromosome fibres into single chromatid and chromosome (17). A plausive explanation of the multipolar and unequal separation of chromosomes might be due to the formation of more than two poles followed by the development of more than one spindle. The multipolar condition is determined by position and number of poles (18). The number of poles in a cell depends on the position of the assemblage of RNA and polysaccharides which remain disturbed in the from of sol or gel.

The present findings suggest that seed aging has effects similar to those of ionizing radiation and chemical mutagens. Furthermore, it might be suspected that the mitotic inhibition caused by seed aging in this study as well as that of ionizing radiation (12) and chemical mutagens (9) is related to the induction of chromosomal aberrations, because all these treatments have the effect of inducing chromosomal aberrations.

The frequency of chromosomal aberrations was found to increase with the increase of the age of wheat and barley seeds. This might be due to the acceleration of optimum temperature inside the desiccator containing calcium chloride. The mutagenic effects of high temperature have been reported (6, 21, 24, 28-30). In contrast to these results on cytological changes were found in the root tip cells of wheat and barley seeds treated by high temperature (31, 32). However, some works have confirmed that more genetic changes are observed in treatment of high moisture content seeds with high temperatures (1,15, 26, 33-35).

A close relationship between the loss of seed germinability and frequencies of chromosomal mutations were found in this study. This agrees with the results in barley, broad bean and pea seeds stored at 25°C to 45°C in combination with 12% and 18% moisture content of seeds (1). The increase in number of anaphase per root in five years old lettuce seeds were closely associated with the loss germinability (15, 16) and it indicated that the increase of chromosomal damage with decreasing germination was quite rapid between 90% and 75% germination.

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