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Artificial Aging Effect on Germination, Chromosomal Changes and Yield in Chickpea (Cicer arietinum L.)

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University of Rajshahi

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ARTIFICIAL AGING EFFECT ON GERMINATION, CHROMOSOMAL CHANGES AND YIELD IN CHICKPEA (*Cicer arietinum* L.).

A THESIS

SUBMITTED TO THE DEPARTMENT OF BOTANY,

UNIVERSITY OF RAJSHAHI

IN FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

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IN

BOTANY

BY

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DEPARTMENT OF BOTANY

UNIVERSITY OF RAJSHAHI

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CERTIFICATE

I have the pleasure in certifying the thesis entitled " Artificial aging effect on germination, chromosomal changes and yield in chickpea (***Cicer arietinum*** L.)" submitted by Mst. Jakeya Sultana to the University of Rajshahi for the degree of Master of Philosophy in Botany.

I do hereby certify that i) the candidate has fulfilled the residential requirement, ii) the works embodied in the thesis were carried out by the candidate, and iii) to the best of my knowledge the data are genuine and original. No part of the work has been submitted in substance for any degree.

Golam Kabir
Supervisor 12.4.95

DEDICATED

TO

MY PARENTS

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(Mst. Jakeya Sultana)

THE AUTHORESS

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ABSTRACT

This study includes the artificial aging effect on seed germination, chromosomal changes and yield in chickpea (Cicer arietinum L. var. Nobin). Aging of seeds was made artificially by constant temperature at 30°, 32°, 35° and 40°C for 7, 10 and 15 days treatment durations. Control seeds were not treated.

It was found that both in laboratory and in the field, germination percentage decreased gradually with the increase of temperature as well as treatment durations. Untreated seeds showed the highest percentage of germination.

Maximum number of anaphase cells were observed in longer ones compared to short sized roots. In most of the treatments minimum root length with maximum anaphase cells was found to be 1.5 mm. Seeds with delayed germination began mitosis at root length below than 1.0 mm. Aberrant anaphase cells were studied at different germinating root lengths. In root lengths above 4.0 mm frequency of aberrant anaphase was found to increase. With increase of temperature it was also found to increase . With treatment durations aberrant anaphase showed positive and negetive relationship. In some cases their relationship was not found.

Mean percentage of dividing cells determined at the root length of 1.0-1.5 cm was found to decrease with the increase of temperature as well as treatment durations. Significant effect of temperature, treatment duration and their interaction was found also to cause the chromosomal anomalies. Laggards and bridges were highly significant at 40°C for all the treatment durations.

Interphase chromosome volume (ICV) in root tip cells of untreated seeds was found to be $1.68m^3$ *at the root length of 1.0-2.0 mm and it was found to decrease with the increase of root length. In root tip cells of treated seeds similar results were obtained. But it was found to*

increase remarkably with the increase of temperature compared to that of treatment durations. Among four different temperature regimes and three treatment durations 40°C for 15 days treatment time was found to be more effective to increase the interphase chromosome volume.

The pollen mother cells (PMCs) of the plants raised from treated and untreated seeds showed various types of chromosomal aberrations. These were fragments, lagging chromosomes, bridges and micro-nuclei. It was also observed that the percentage of abnormalities increased with the increase of temperature and as well as treatment durations. In PMCs of the plants raised from the treated seeds chromosome association, chiasma frequency per PMC and per bivalents, and terminalization co-efficients were found to vary. Percentage of PMCs with 8 ring bivalents were found to range from 78 to 48 and those with 1 rod and 7 ring bivalents ranged from 46 to 22%. In none of the cases uni, tri or quadrivalent were found. Significant effect of temperature was found to vary the chromosome association.

Data on yield and yield components were recorded from the plants at maturity raised from the treated and untreated seeds. In case of plant height and primary branches length, temperature and treatment duration showed non-significant effect individually, while significant effect of their interaction was observed. In case of secondary branches, pods/plant, pod volume and 100 seed weight effect of temperature, treatment duration and their interaction were non-significant. But in case of seeds/pod both temperature and treatment duration showed significant effect.

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INTRODUCTION

Maintenance of seed germplasm implies that the genetic integrity of a sample or collection is preserved (Murata *et al.* 1981). Several problems associated with maintenance of genetic integrity have been identified including differential survival in storage, selection during rejuvenation, outcrossing with other accessions, and genetic drift (Allard, 1970). In addition to these problems, the occurrence of genetic changes such as chromosomal aberrations and genetic mutations during seed storage has been suggested a serious problem in the preservation of seed germplasm (Roberts, 1973, 1975, 1978).

To guard against the possibility of genetic changes during storage, the International Board for Plant Genetic Resources (1976) recommended that for long term storage of germplasm not more than 5 to 10% deterioration should be allowed before regenerating a sample. In practice, however, 5 to 10% deterioration is not realistic to use if the number of stocks is extensive. At the USDA National seed storage laboratory in Colorado, 35 to 40% deterioration were used to determine when an accession was regrown (Murata *et al.* 1981). Moreover, frequent regeneration of seed stocks leads to genetic changes through the natural evolutionary process as mentioned by Allard and Roos (1979). Seed longevity is known to vary greatly among and within species. Storage conditions, particularly temperature and moisture content are the main factors influencing seed longevity.

High temperature and seed moisture conditions accelerate loss of viability of seeds in most species (Owen, 1956; Barton, 1961; Roberts, 1972). Because of the importance of germplasm

preservation at constant temperature, information is needed on the nature and frequency of chromosomal and genetic changes occurring during seed storage in order to develop the procedure to minimize the alteration and loss of genetic information. Investigations have shown that temperature is the most influential environmental factor affecting the induction of seed dormancy during seed formation and expression of dormancy during germination (Vegis, 1964; George, 1967; Belderok, 1968; Weisner and Grabe, 1972; Olsson and Mattsson, 1976; Strand, 1980).

In general, low temperature (10°C) during seed development induces deep and prolonged dormancy while that low temperature during germination breaks dormancy of freshly harvest seeds. Intermediate temperature(20°C) allows different cereal crops to express their degree of dormancy, and high temperature (30°C) allows seeds with a very low, or no dormancy to germinate.

A good number of works has confirmed that more genetic changes are observed in the treatment of high moisture and high temperature (Harison, 1966; Roberts *et al.* 1967; Abdalla and Roberts 1968 and Villers, 1974). Delay in the occurrence of first mitosis of roots and shoots is clearly associated with the induction of genetic changes by various factors. Mitotic inhibition caused by seed aging, as well as that of ionizing radiation and chemical mutagens, is related to the induction of chromosomal aberrations (Murata *et al.* 1981).

However, aging plays an important role in inducing mutation under natural and artificial condition (Haferkamp *et al.* 1953; Aufhammer and Simon, 1957; Barton, 1961; Roberts, 1972; Orlva *et al.* 1976; Murata *et al*, 1980; Murata *et al*, 1981; Akhter *et al.* 1992). This type of mutation might be due to the presence of any toxic materials present in the aged seeds produced due to exhaustion of respiratory reserve substances. Peto (1933) in maize, Cartiledge and Blackeslee (1934, 1935) in *Datura*, Stubbe (1934, 1935a,b) in *Antirrhinum*, and Gerassimova (1935) in *Ceriops* verified a high mutation rate from old seeds, which suggested that the phenomenon is of a general character. It has also been stated in different experiments that with the increase of the age of seeds, percentage of dividing cells decreased and chromosomal abnormalities increased. Gustafsson (1937) studied the effects of age, moisture and X-ray on the seed of barley, and reported on the number of disturbed cells in the mitotic division after germination. Nichols(1941) reported on spontaneous aberrations and the effects of aging on *Allium* seed and also stated that the percentage of chromosomal aberrations increased with the age of seeds. D. Amato(1948a) found greater frequency of chromosomal aberrations in plants from one year old seeds than in fresh ones in the primary division of root tip cells in seedlings of *Northoscorduns fragans* kunth. D. Amato (1951) also verified a much higher rate of chromosomal mutations in root tip cells of the seedling of *Pisum sativum* L. from 4 years old seeds than from fresh seeds. Nilan and Gunthard (1956) found that the frequency of spontaneous chromosomal aberrations in root tip cells of wheat increased with the age of the seed from 0.02% in 1 year old seed to 0.43% in 17 year old seed.

The effect of temperature on the somatic and pollen mother cells of different species have been considered in relation to characters like degeneration of chromatin material, stickiness of chromosome formation of micronuclei and induced sterility (Takagi, 1929; Sax, 1937; Emsweller and Brierley, 1943; Pao and Li, 1948; Jain and Rana, 1963; Rana, 1965). High temperature treatment in a wide variety of organisms has been known to induce failure of pairing as well as desynapsis resulting the occurrence of univalents. Thus, it does not need only to establish whether asynapsis or desynapsis is largely responsible for the occurrence of univalents. It has, however, been considered that in respect of the percentage of cells affected in root tip cells and PMCs; as well as the number of univalents found in the affected cells influencing chiasma frequency.

Aging, heat, irradiation and hybridity bring about an increase in the frequency of structural chromosomal alterations as well as increase in the frequency of gene mutations (Nichols,1942). How these factors are related to one another and how they cause changes in the chromosome and mutation rates are not well understood. However, the fact that all these external agents cause similar changes and indicates a broad fundamental process as the primary cause of mutations.

Roberts and Abdalla (1967) reported that temperature and moisture are major factors which control the viability period of seeds. Consequently, it might be summarised that there are some sort of connection between the accumulation of chromosome damage and loss of viability.

Temperature plays an important role in all aspects of plants growth and development (Went, 1953). Okusanya (1978) found that high temperature and low light intensity contributed the increase of plant height and high shoot weight ratio, both of which are manifested in seedling etiolation. Relative to the 30°C temperature treatment, a three fold increase in duration of grain filling at 15°C was not adequate to compensate for the 90% reduction in the kernel growth rate that occurred. Data on the temperature effects on yield and yield components of pulse crops are not very adequate. Some works on the temperature effects on yield and yield components of legumes (Balhuis *et al.* 1959; Cox, 1979; Ono *et al.* 1974) have been done. Ketring (1979) observed that the highest temperature treatment significantly reduced the subterranean pegs and mature seed weight. His study with a temperature of 35°C showed an inhibitory effect on peanut development even when plants were grown under well watered conditions.

In Bangladesh most growers produce their own seeds and store them at ambient temperatures. Thus, it is important to know how at any particular temperature germination, pattern of growth, chromosomal mutations and yield response in any particular crop can be determined.

However, it gradually becomes apparent that chronological age of the seed is not only the factor involved in the production of chromosome aberrations. It is evidenced that factors such as temperature and moisture are also important. It appears not only from cytological work on *Crepis* (Navashin and Gerassimove,1936 a,b) but also from investigations on pollen abortion in *Datura* (Cartiledge *et al.* 1936).

In view of the aforesaid research attributes the present study was intended with *Cicer arietinum* L. to determine the artificial aging effect on

a) germinability of the seeds and its relationship with aberrant anaphase,

b) time of first mitosis,

c) relationship of aberrant anaphase with treatment duration at different root lengths,

d) mitotic index along with different abnormalities,

e) interphase chromosome volume,

f) meiotic behavior along with chromosome association and chiasma frequency, and

g) yield and yield components.

REVIEW OF LITERATURE

A good number of works on the effect of temperature on germination, seedling growth and development have been done in different pulses. But most of the works were confined with natural aging and field temperature. Information on the effect of artificial aging on germination, seedling growth, development, chromosomal anomalies, and yield and yield components are very scanty. Thus, the literature regarding the effect of natural and artificial aging on germination, chromosomal aberrations and yield in chickpea and some other crops are reviewed below.

Wilson and Hottes (1927) investigated the effects of temperature and moisture stress on final germination percentage of spring wheat cultivation, but did not compare other characteristics of the germination responses.

Navashin and Shkvarnikov (1933a, b), Shkvarnikov and Navashin (1934, 1935) and Shkvarnikov (1936, 1939) studied temperature effect on wheat and Crepis and they observed that treatment of fresh seeds with temperatures of 50°-60°C for 20 days had a comparable effect on the production of chromosome aberrations, particularly at room temperatures for 6-7 years.

Induced condition of univalence due to high temperature treatment has been quite consistent. High temperature treatment in a wide variety of organism has been known, in the past,to induce failure of pairing as well as desynapsis, resulting in either case in the occurrence off univalents (Sax, 1937; Emsweller and Brierley, 1943; Dowrick, 1957; Rana, 1965).

Nichols (1942) studied the effects of age and irradiation on chromosomal aberration in *Allium* seed. He stated that the aging of seed increased the number of aberrant cells. He found irradiation of the dry seeds also to cause an increase in the number of aberrations. He found no correlation between the age of seed and its radiosensitivity, indicating that possibly two different mechanism may be involved. He found also the number of aberrations to be greater when the moisture content of the seeds was increased during the period of the delayed germination.

Swanson (1943) reported the behaviour of meiotic prophase chromosomes as revealed through the use of high temperatures. Elliot (1955) studied the effect of temperature of chiasma frequency.

Smith (1943, 1946) reported that exposing seeds of cereals to temperatures of 50°-70°C for 5-15 days or 80°C for 45-80 minutes had little effects upon the frequency of chromosome aberrations.

Emsweller and Brierley (1943) reported about the regular ty of nuclear and cell divisions in plants affecting by many agents, such as X-rays, ultra violet light, various chemicals, disease, and temperatures. Both low and high temperatures as well as sudden changes from one to the other were found to show the particular effect on plant of the Creole and Giganteum varieties of *Lilium longiflorum* with young flower buds which were removed from a cool greenhouse (10°-16°C) and exposed for 30 minutes to a temperature of 45°-46°C. They were then returned to a greenhouse

with temperature of 13°-19°C. As a result of this treatment, the frequency of all the irregularities found in creole plants was greatly increased. These included (a) so called 'Blown-up' cells, in which all or practically all the chromosomes were broken into numerous fragments; (b) formation of restitution nuclei; and (c) increase in the degree of asynapsis from partial to complete. In Giganteum, partial to complete asynapsis was induced, and in the buds on different plants enucleate pollen mother cells were found as well as others with only a fragment or with bivalents ranging in number from 1 to 15. The treatment evidently affected both the last pre-meiotic mitosis and the early stage of the meiotic cycle.

Gunthardt *et al.* (1953) observed that chromosomal aberrations and genetic mutations arose from storing seeds of several economic crop species. Decreased viability and increased cytogenetic changes were parallel effects of aging. Both chromosomal aberrations and genetic mutations had arisen in the seeds and that the frequencies of these cytogenetic changes increased with age. The types of chromosomal aberrations appeared to be identical to those arising from ionizing radiations.

Went (1953) observed the effect of temperature on plant growth. He observed that temperature played an important role in all aspects of plant growth and development.

Dowrick (1956) studied the cytological effects of high temperatures in *Tradescantia bracteata* and *Uvlaria perfoliata.* He found that increase in temperature resulted an increase in the

frequency of interstitial chiasmata. This increase as followed at higher temperatures by a fall in both terminal and interstitial chiasmata; asynapsis resulted. At high temperatures prophase was much clearer than was usual in *T. bracteata.* Spiralisation was also abnormal and major coils opened out and finally disappeared. In *T. bracteata* the major coils began to open out at specific points. When major coils were absent, constrictions not normally visible, spiralisation was seen on some chromosomes of *T. bracteata.* These were probably in heterochromatic segments.

Owen (1956), Barton (1961) and Roberts (1972) stated that in storage conditions, temperature is the main factor for influencing seed longevity. They observed that the high temperature accelerated loss of viability in most species. More works have confirmed that more genetic changes are observed in the treatment of high m.c. seeds with high temperature (Roberts *et al.* 1967; Abdalla and Roberts, 1968; Villiers, 1974, 1975; Villiers and Edgeumbe, 1975).

Read and Beaton (1963) studied the effects of fertilizer, temperature and moisture on germination of wheat. Moisture level and temperature had little effect on total germination. Total germination was reduced more when seeds were placed after use of the fertilizer than when it was 1.5 or 2.8 cm distant. The rate of germination was significantly affected by all the factors but to a different extent for different fertilizers. Decreasing soil moisture increased the time required for germination but here also fertilizers extended to the seeds 2.8 cm away, whereas other showed no effect at 1.5 cm even though they did reduce germination when close to the seed.

Abdalla and Roberts (1968) reported effects of temperature, moisture and oxygen on the induction of chromosome damage in seeds of barley, broad beans and peas during storage. It had been found that an increase in any of these factors increased the rate of loss of seed viability and that any treatment which led to a loss of viability also led to an accumulation of aberrant cells in the embryo . Under most storage condition, irrespective of the combination of factors which led to loss of viability or the rate at which viability was lost, the relationship between percentage viability and mean frequency of aberrant cells in the surviving seed population was always the same.

Utkhede and Jain (1970) observed the effect of high temperature on the pollen mother cells of the different species in relation to characters like degeneration of chromatin material, stickiness of the chromosome, formation of micronuclei and induced sterility. Utkhede and Jain (1974) subjected again a number of diploid and polyploid species of wheat to the cdition of high temperature treatment. They found various temperature treatments to induce formation of univalents in a number of pollen mother cells.

Townsed and Mc Ginnies (1972) made a study which compared the temperature requirements for seed germination of several native and introduced legume species with those of commonly used species. Seed germination of 17 legumes species was studied under dark conditions at alternating temperatures of 5°C, 20°C, 15°-25°C, 20°-35°C, and constant 20°C. Duration of the alternating temperatures was 12 hr. Seedling counts were taken on the 7th, 14th, 21st and 28th days of germination. Species differed significantly in germination response to

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temperature. Half of these species, however, were at least moderately sensitive to temperature for rate of germination. There was considerable interaction among some species and temperatures for both total germination and rate of germination. In comparison to other treatments, rate of germination at constant 20°C was especially high for three species. Rate of germination and total germination increased with increase in temperature through 15°-25°C. At 20°-35°C, total germination dropped substantially, but rate of germination remained high. Temperature requirements for seed germination of some species suggest why they are relatively easy or difficult to establish in the field. The species that germinated well at most temperatures but are difficult to establish in the field are very small seeded.

Gramshow (1972) studied the germination of *Lillium rigidum* seeds, in the light (12 hr day length) and in the dark, at constant temperatures of 12°, 18° and 24°C and an alternating temperature of 24/12°C (12/2hr) in freshly harvested seeds and in seeds stored for 18 weeks. In freshly harvested seeds the highest germinability (80%) was recorded at 12° in either light or dark and at 24/12°C in the light. After 18 weeks storage, a germinability between 95 and 100% was observed at 12°C and 24/12°C in the dark and at 24°C and 24/12°C in the light. In another experiment in which seeds from a different source were used; seeds were kept in six different environments and recovered at 3-weekly intervals during a 21 weeks post harvest period and were examined for germinability and germination rate. The six environments were storage in a room, storage in a 60/15°C temperature cabinet, and four field treatments in which seeds were buried 0.2 and 1.0 cm under both a bare and mulched soil surface. Major increases in seed germinability with

age occurred during the first 9 weeks after harvest. The different environments influenced the relationship between seed age and germinability only during the first 9 weeks.

Yfolis and Fasoulas (1973) studied interactions of genotype and temperature on cotton boll period and their implication in breeding. They observed effects of environmental temperature on boll maturation period of cotton and the genotype response in a 3-year field experiment. This experiment showed a negative correlation between period and mean temperature and revealed genotypic difference regarding degree of temperature influence and sensibility to temperature changes. The observed genotype X temperature interactions may help cotton breeders in establishing breeding centres in creating desirable gene combinations for wider adaptability to temperature.

Riaz *et al.* (1973) carried out an experiment with the seeds of American cotton, which were stressed at 50°, 60°, 70°, 80° and 90°C for 24 or 48 hours before seedling emergence. Stresses at 50° to 70°C had a stimulatory effect on seedling emergence and subsequent performance of the cotton plants, but higher temperatures caused thermal injury or killed the seed.

Roberts (1973) observed that seeds suffered damage to their nuclear material during storage. This was expressed as chromosome breakage, and the induction of recessive mutations which were manifested by increased pollen abortion in the plants produced from the stored seeds, and by segregation of mutant phenotypes in the subsequent germinations. These studies have

shown that for any species there was a predictable relationship between percentage viability and the amount of chromosome damage in the surviving seeds. This was so irrespective of the combination of conditions which led to the loss of viability, or how rapidly the viability was lost.

Marshall *et al.*(1974) studied temperature and day length effect, to a major and minor degree, respectively for the expression of male sterility in *G. hirsutum* stocks carrying *G. anomalium* or *G. arboreum* cytoplasm. Generally, sterility increased with increasing temperature and day length. Day temperature above 33°C were required for the consistent expression of male sterility in the sterile A lines tested, while the maintainer, or B lines became completely sterile at day temperatures above 36°C. It was concluded that while the production of hybrid cotton seed by means of the sterile A lines currently available may be feasible in some tropical areas of Australia. It would be desirable to develop genotypes in which the cytoplasmic male sterile character was stable under a much set of temperature regimes.

Hunter *et al.* (1974) observed photoperiod and temperature effects on corn. Coligada and Brown (1975) studied the response of corn (*Zea mays* L.) in the pretassel initiation period to temperature and photoperiod.

Paterson *et al.*(1976) collected seeds from different genotypes of *A. fatua* and *A. barbata* and tested for ecotypic variation under different temperatures of summer storage and subsequent germination. The predominance of *A. fatua* in cereal crops was considered to be due largely to its

well-developed seed dormancy. This characteristic was virtually absent in *A. barbata*, and this species did not normally survive. The level of seed dormancy and its rate of dissipation in both species was related to the genotypic origin of seed. That from the south of temperate zone was more dormant than that from the north. Dissipation of dormancy in both species appeared to be a function of time, temperature during storage or at germination having only limited effect.

Adam and Hayes (1978) studied the effect of temperature on the germination of seven cultivars of *Zea mays* L. Significant differences were found between the cultivars. The cultivars pioneer 131 and Inra-200 showed the most rapid germination and radicle extension at low temperature. The cultivars Anjou 210 and Kelvedon 33 germinated slowly and showed poor seedling growth at low temperature. As the gradient plate proved to be capable of elucidating differences between cultivars with respect of germination characteristics, it was suggested that gradient plate and field experiments be carried out in parallel to determine if the gradient plate could provide a simple means of finding those cultivars most suitable for growing in northern regions.

Sholberg Muir (1979) found the viability of faba bean seed heated in sealed containers decreased as moisture content (14.7-19.4%), temperature (40°-70°C) and exposure time (0.5-96h) were increased. To decrease viability by 10% at 70°C, faba beans with 19.4 and 14.7% moisture content required an exposure time of 0.5 and 1h, respectively. At 40°C faba beans at 19.4% moisture content required 72h while these with 14.7% moisture required more than 96h exposure

time. An equation to estimate the exposure time required to reduce viability to 50% was fitted to the data.

Salehuzzaman *et al.* (1979) reported the effects of exposures to different durations of high (35°C) and low (10°C) temperatures on the germination of the seeds of flax (*Linum usitatissimum* Linn.) and sesame (*Sesamum indicum* DC). Their study was to find out a technique to screen seeds before sowing for obtaining optimum germination. In flax, both high and low temperatures delayed germination. In sesame, exposure to 10-24 hr at high temperature enhanced the rate of germination, but low temperature delayed it.

Herrero and Johnson (1980) found high temperatures during maize (*Zea mays* L.) pollination to result in poor kernel set, but little is known of the direct effects of temperature on pollen germination. The purpose of their research was to determine how in vitro pollen germination of different maize genotypes is affected by high temperature stress during anthesis. Tassels from field grown plants were excised at beginning anthesis, placed in water and transferred to growth chambers maintained at day time temperature of 27°, 32° and 38°C. Night time temperatures were maintained at 6°C cooler. In vitro germination was measured after 24 and 48 hours in the growth chamber as well as on pollen collected directly in the field. Genotypes differed in their response to temperature. In some genotypes pollen germination steadily decreased as temperature increased. Others either germinated equally well at 27° and 32°C or germinated better at 32°C than at 27°C. All genotypes had a lower germination at 38°C than at 32° or 27°C and

several genotypes exhibited no germination after 48 hours at 38°C. After 24 hours in the 38°C chamber, six inbreds widely used in the 1970's germinated significantly better. Growth environment affected absolutely in vitro germination percentage, but in general genotypes retained similar relative responses to increasing temperature.

Young (1980) observed effects of temperature and preflooding on rice yields in Kenya. Yields over an 11-year period were examined in relation to meteorological data and cultivation activities. It was found that the period during which the units were flooded prior to transplanting, correlated with the data of transplanting, with longer preflooding periods causing higher yields and not the transplanting data. A yield/temperature relationship was also established.

Murata *et al.* (1980) studied mitotic delay in root-tips of peas induced by artificial seed aging. They observed that the delay was associated with a low activation of cell division following the start of the first mitosis.

Murata *et al.* (1981) studied chromosome damage induced by artificial seed aging in barley. They found delayed and reduced germination of the seed with the increasing in time of storage. They also found high temperature and seed moisture content to induce rapid loss of germinability. The frequency of aberrations were found to increase with the increased storage time. Their findings indicated that the frequency of chromosomal aberrations induced by seed aging might be estimated from the germination percentages.

Jones *et al.* (1981) studied the effect of temperature extremes on kernel characteristics, respiration, starch synthesis, soluble sugar and protein content during grain filling in maize (*Zea mays* L.). Kernels cultured in vitro on a defined media at 15°, 30°and 35°C were compared to kernels from ears developed in the field. Kernels cultured for 7 days at 35°C had higher dry weights than kernels from other treatments; however, their growth ceased by the 14th day. High soluble sugar content of aborted kernels suggested that inhibition of starch synthesis might have caused kernel abortion at 35°C. Respiration was higher for kernels from field-grown ears. During the linear phase of dry matter accumulation, kernel growth rates were 0.65, 6.26, and 5.26 mg kernel^{-1} day^{-1} for 15°C, 30°C and field kernel growth at 30°C was negated by pronounced shortening of the duration of the grain filling period. Relative to the 30°C treatment, a three-fold increase in duration of grain filling at 15°C was not adequate to compensate for the 90% reduction in kernel growth rate that occurred. Cool temperatures that occurred in the field or that were maintained in vitro caused soluble sugar levels to remain high and deposition of starch in the endosperm to lag several days behind that observed at 30°C. Cool temperatures did not affect the synthesis of proteins in general or caused differential synthesis of an individual or specific class of protein in the zein fraction.

Kamizyo and Nobunori (1982) studied the effect of aging of pollen grains on the behaviour of generative nucleus in *Tradescantia paludosa*. Pollen grains collected at 8 a.m.were allowed to stand for 7hr at room temperature, and at 3 p.m. In both the culture series conducted with the medium supplemented with one of either concentration of 10% or 20% sucrose, the

elongation of pollen tubes was only slightly depressed by the aging, but the meiotic activity was greatly depressed; namely, a great majority of nuclei remained in prophase. At the same time, the frequency of necrotic nuclei greatly increased and at the end of 8hr cultivation, almost of all nuclei rushed into necrotic state before anaphase. Two different kinds of abnormal mitosis, i.e., unravelled twisted chromosomes and asynchronous condensation of chromosomal arms in the same nucleus increased in the frequency.

The response of tea shoots to temperature was studied in the field and in controlled environments (Tanton, 1982). Shoot extension stopped below the temperature about 12.5°C. Shoots of mature tea took 491 day degrees above the base temperature of 12.8°C to grow to a harvestable size of 15 cm. Temperature is the major environmental variable affecting the yield of tea, but within the framework of a temperature model shoot extension is severely depressed when daily maximum vapour pressure deficits rise above 2.3 kpa. Day length did not affect shoot extension when the night were cool (10°C), but growth rate was depressed by short days (11h) when night were warm (20°C). Soil temperatures between 18°-25°C did not affect shoot extension.

Rinne (1984) found the temperature index as presented by Coligado and Brown in the case of a particular corn hybrid, has been extended to predict the occurrence of the maturity of sweet corn in Finland. The growing experiments showed that the index performed well and could be used to explain quantitatively why sweet corn is a rare plant in Finland. The index showed to be

nothing but a group of degree-day indices, computed as a difference between the daily temperature and a reference value. This in turn depended on the photoperiod and the daily temperature amplitude but was nearly independent of the daily mean temperature.

Mares (1984) observed germinability in mature wheat grain which showed a market dependence on temperature. The optimum temperature for the complete germination of all grains ranged from 20°C for the non-dormant variety, Timgalen, to 10°C for the strongly dormant red wheat RL 4137, where as the optimum in terms of the shortest lag period ranged from 25° to 15°C for the same varieties. Germinability gradually increased during post-harvest storage and for after ripened grain, the optimum temperature for both complete germination and shortest lag period were greater than 30°C. Germinability could also be increased by pre-treating imbibing grains at temperatures of 5°, 10° or in some cases 15°C. This treatment was only effective for grain at moisture contents >25% (dry weight) and the effect was not reversed by redesiccation. The pre-treatment temperature required for maximum germinability decreased with increasing levels of grain dormancy. Complete removal of dormancy required a pre-treatment period of c.48h; however, lesser periods gave the shortest lag period in the case of the dormant varieties.

Summerfield *et al.* (1984) conducted an experiment with plants of two genotypes of chickpea (*Cicer arietinum* L.), which were transferred between cabinets at various times so that they experienced either warm (30°C) or hot (35°C) day (both in combination with a typical night temperature of 10°C) for different durations of reproductive growth. After growing in average

(30°C day - 10°C night) or warmer than average (30°-18°C) temperatures for the first 28 days form sowing and then average temperatures were transferred into the hot regime. Diurnal vapour pressure deficits were adjusted so that plants experienced a constant atmospheric relative humidity (70%) in all thermal regimes. The greater the proportion of the reproductive period spent in hot days the smaller the seed yield produced; plants transferred at 50% flowering were almost barren.

Silsbury *et al.* (1984) measured effect of constant temperature of 10°, 15°, 20°, 25° and 30°C on the germination, emergence and early vegetative growth of *Trifolium subterraneum* L. in temperature controlled glass houses and in a growth cabinet. Percentage of germination and emergence were hardly affected by temperatures of 10°-20°C, but at 25°C were reduced to 50%, and at 30°C to about 10%. The rates of germination and emergence were slowest at 10°C, but showed little change with temperature over the range 15°-30°C. Time to closed canopy and to a dry weight of 133 gm were shorter where plants were supplied NO_3^- than where mineral nitrogen was withheld and a symbiotic system established. Rates of N_2-fixation, as measured by acetylene reduction assay, were not markedly affected by temperature over the range 10°-25°C. Relative efficiency ranged from about 0.55 at 10°, 15° and 20°C to about 0.66 at 25°C. At 30°C nodulation still occurred, but nitrogenase activity was very slight.

Hughes *et al.* (1984) studied the effects of temperature and moisture stress on germination and seedling growth of four tropical plant species. When night/day temperatures ranging from 5°/10°C to 25°/30°C were imposed, none of the species germinated at 5°/10°C within 14d. At

10°/15°C or above, at least 50% of seed of all species germinated in 14d although germination of *P. americanum* was more depressed by 10°/15°C than the other three species. In the second experiment, seeds were exposed to a range of osmotic potentials (0 1.5 MPa) at night/day temperatures of 10/15, 15/20 and 20/25°C. Decreasing osmotic potentials had only small effects on germination at 15°/20°Cor 20°/25°C but had marked effects on all species at 10°/15°C. The field experiment treatments comprised a number of sowing dates and irrigation treatment to give a range of soil temperatures and moisture potentials. Considerable differences between *Z.mays* and the other three species were recorded.

Hur and Nelson (1985) reported the temperature effects on germination of birdsfoot trefoil and seombadi. It was important to know the temperature range of germination and the time course of seed germination of `MO-20' birdsfoot trefoil (*Lotus corniculatus* L.) and seambadi (*Dystaenia takesimana kitagwa*), a member of the umbellifera family, using germination chambers at constant temperatures of 3°, 6°, 9°, 12°, 15°, 20°, 25° and 30°C. Final germination of both species was highest at 20°C. Time to radicle emergence of birdsfoot trefoil decreased as temperature increased. Seombadi seeds germinated only between 12° and 25°C with little temperature influence on time to radicle emergence. Birdsfoot trefoil showed a linear relationship between germination temperature and the reciprocal of time to reach 50% of final germination, but there was no relationship for seambadi, suggesting factors other than temperature were controlling the rspon e.Base temperature for germination of birdsfoot trefoil was calculated to be 4.7°C. Germination

rate of birdsfoot trefoil showed a marked increase between 9° to 12°C, suggesting an arrhenius response, i.e., an abrupt change in effect of temperature.

Delaney *et al.* (1986) observed temperature and water stress effects on germination of Ruby valley pointvetch (*Oxytropis riparia* Litv.). There objectives were to evaluate the sensitivity of pointvetch germination to temperature and osmotic stress levels. Seven constant germination temperatures ranged from 5 to 35°C. The NaCl and PEG-imposed stress levels ranged 0.0 to -3.0 MPa with intervals of -0.5 MPa. Hard seed content ranged from 87 to 96%, depending on seed lost. Germination of pointvetch was maximal between 10° and 30°C compared to 5° to 30°C for alfalfa (*Medicago sativa* L.). Pointvetch had the capability for over 90% germination in -2.0 MPa Nacl solutions, while alfalfa germination responded similarly to NaCl and PEG imposed osmotic stress, while pointvetch germination was less affected by NaCl than by PEG imposed by Nacl than by PEG-imposed stress. The germination tolerance of pointvetch to osmotic and salt stress was unique and warrants further study.

Bhattacharyya *et al.* (1985) studied the embryos of aged non-germinating wheat seeds, when placed on sucrose/glucose, germinated well and grew in to normal plantlets, while on agar they remained ungerminated as in the intact seed. From the results of amylase assays in seeds of different ages it appeared that failure of amylolytic activity in aged seeds could be a cause of unavailability of utilizable substrate to the embryonic axis of aged seeds. This would bring about a

limitation in the capacity of germination and growth of the embryonic axis even before the embryo became non-viable.

Scully and Waines (1987) conducted an investigation to identify accessions of two species of *Phaseolus* that could potentially germinate and emerge under cool soil conditions, and to determine an optimum germination temperature for tepary beans. Germination of 129 common and 18 tepary bean accession was initially evaluated at 10°C. The 10 earliest common and the five earliest tepary beans to germinate were entered into an emergence test at three temperatures (12°, 14° and 16°C). The common bean emerged 1 day earlier than the teparies at 12° and 14°C, while the teparies emerged 1 day earlier at 16°C. The most cold tolerant common bean at emergence was PI 165-426, while accessions G40034 and PI 319-443 displayed the highest cold tolerance among the tepary beans. In general, tepary and common beans took approximately the same time to germinate except at 35°C where teparies germinated faster. Scarification resulted in an earlier germination response, but the effect was small. Both germination experiments indicated that the optimum temperature ranged for teparies is 25° to 35°C, where as it was lower, 20° to 30°C, for common beans.

Brown (1987) found germination of *Aristida armata* to be compared at different temperatures on a thermogradient plate. Temperatures ranged from 10°C to 50°C with day/night differentials of 0, 5, 10 and 15°C. Alternating temperatures improved overall germination, particularly at the extremes of temperature. Average temperatures of 35°C and higher were fatal to

many seeds. Day temperatures of 17.5°C and lower inhibited germination but did not prevent subsequent germination under warmer conditions. There was little variation in the rate of germination with incubation under constant temperatures. Under alternating temperatures, maximum germination occurred at lower temperatures than those under which germination rate was greatest.

Mutters *et al.* (1989) found that high temperature caused reductions in grain yield of cowpea [*Vigna unguiculata* (L.) walp.] that are associated with low pollen viability and pod set. Preliminary controlled environment studies showed differences in proline accumulation in anthers and pollen of heat-tolerant and heat-sensitive genotypes under hot and optimal temperatures,but insufficient tissue was available to, if the differences were significant. The objective was to determine whether heat injury under field conditions was associated with specific patterns of proline accumulation in leaves and reproductive tissue using heat sensitive on heat tolerant cowpea genotypes. Under moderate and hot temperatures, proline was the most abundant free amino acid in the anthers of both heat-sensitive and heat-tolerant cowpea genotype. Under moderate and hot temperatures, proline was the most abundant free amino acid in the anthers of both heat- sensitive and heat-tolerant cowpea genotype. No differences in leaf proline concentrations were observed. Under ot conditions, proline levels in anthers decreased faster as pollen matured in heat tolerant genotypes was compared with heat sensitive genotypes. At pollen maturity, heat-sensitive genotypes contained more proline in anther and had lower levels in pollen than the heat-tolerant genotypes under hot conditions but similar levels under more optimal temperatures. The results

suggested that heat injury during floral development of sensitive cowpea genotypes might be due to inhibition of proline translocation from anther walls to pollen.

Kundu *et al.* (1989) investigated germination and seedling growth of eleven cultivars of rapeseed under different levels of soil moisture, temperature and nitrogen fertilizer. In general, the highest percentage of seedling emergence was observed between 40 and 60% of field capacity whereas at 80 and 100% of field capacity, there was a marked reduction with a few exceptions. Hypocotyle and root lengths were also highest between 40 and 60% of field capacity. The optimum temperature for germination was found to be 20° to 30°C. At 35°C seeds of few cultivars germinated but at 40°C no germination took place with the gradual increase of nitrogen concentrations, there was gradual decrease in germination, and hypocotyle and root lengths in all the cultivars.

Ganguli and Sen-Mondi (1990) reported that the growth performance of deteriorated wheat embryos in cultural conditions depended largely on the method of aging treatment. Low viability embryos obtained by different aging methods behaved widely differently when grown on sucrose media. The ability of badly deteriorated embryos, which did not germinate when attached to the endosperm, to utilize sucrose for rejuvenation was found to be much lower in naturally stored seed stocks than in artificially deteriorated ones. Studies on chromatin associated proteins in embryos showed a similar increase in acid soluble protein in both naturally aged and artificially aged embryos and acid insoluble (non-histone) proteins was particularly marked in the hot water

dip artificially aged embryos. They concluded that different conditions that caused deterioration of intact seeds might bring about completely different effects in cellular event of the embryonic axis.

Evers (1991) studied annual clover (Trifolium spp.) which stands sometimes poor because of low and slow germination due to unfavorable temperatures. The study was conducted to identify optimum temperature for germination of relatively new clover species to the U.S. southeast to improve stand establishment. High temperature germination was also examined to estimate summer germination which usually results in seedling mortality and decrease of the soil seed-bank. Seed of 10 cultivars of different species were subjected to alternating 12h day/12h night temperatures of 15°/5°, 20°/10°, 25°/15°, 30°/20° and 35°/25°C. Germination counts were made at 3, 6, 9 and 12d to determine percent germination and germination rate index (GRI). Germination was >80% for all entries at the three lowest temperature treatments except `Larisa' subclover at 25°/15°C. The 35°/25°C treatment suppressed germination to <25%. The optimum temperature for GRI was 25°5°Cfollowed by 20°/10°, 15°/5°, 30°/20° and 35°/25°C.

Akhter *et al.* (1992) carried out an experiment 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 wheat 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

fragments, 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.

Wilson *et al.* (1992) studied seed germination response for eleven forage cultivars of *Brassica* to temperature. They observed that slow germination and poor seedling vigor limit stand establishment. Temperature had effects on germination of 11 forage *Brassica* cultivars, comprised of three species plus one hybrid, were studied in an incubator. Germination studies were conducted in the dark at 10 constant temperatures (0°, 2°, 5°, 15°, 20°, 30°, 35°, 40°and 50°C). Number of seeds germinated were counted on day 4 and 14 after planting. Cultivars differed significantly ($P \leq 0.001$) for percentage germination on both day 4 and 14 at all temperature treatments except 2° and 40°C. Under the cool temperatures (5° and 10°C), Civastro-R, Polaris, Purple Top, and Stubble were the earliest germinating cultivars. At the 40°C temperature regime, Maris vestral initiated germination earlier than that of the other cultivars, but total germinatiion was highest for polaris. The cultivars Civastro-R, Polaris, Purple top and stubble could serve as potential parents of a new cultivar because of their ability to germinate rapidly over a wide range of temperature.

Paul (1992) observed that great variability existed for germination percentage at the lowest (8.5° C) and highest (27°C) temperatures for twelve cultivars of *Brassica*. Higher yields were

party dependent upon obtaining a rapid and uniform emergence of seedlings in the soil. Although the germination tests in the laboratory did not always correspond to the seedling emergence in the field. This experiment was designed to study the effect of a range of high and low temperatures on germination of the same twelve cultivars of *Brassica.*

Islam *et al.* (1992) made an experiment on the effect of temperature on germination of seeds and post seedling growth of rice, wheat and corn, and they reported that application of a temperature of 20°C at different range of treatment time had no such effect on days to germination of the seeds of rice, wheat and corn. At constant temperatures for certain time 30° and 40°C temperature appeared to suppress the percentage of germination. Some seeds of all the crops which did not germinate at 30° and 40°C, were transferred to 20°C, but there was no further germination. It indicates that high temperature might be lethal to the seeds.

MATERIALS AND METHODS

MATERIALS

The experiment was conducted with the seeds of the chickpea (*Cicer arietinum* L.), var. Nobin. The seeds were procured from the Regional Agricultural Research Station, Ishurdi, Pabna.

METHODS

Seed treatment :

Fresh dry seeds were spread over in petridishes and treated at constant temperatures of 30°, 32°, 35° and 40°C in hot air oven for 7, 10 and 15 days prior to their germination. Controlled seeds were spred over in petridishes and kept in the room temperature (20°-25°) for fifteen days. Moisture condition of the seeds were recorded by electronic moisture meter, HE 50.

Germination in laboratory and in field:

One part of the treated seeds were presoaked with distilled water for one hour in beakers and 150 seeds for each treatment were allowed to germinate on moist filter paper in petridishes in the laboratory at room temperature (20-25°C). The untreated seeds (control) were also allowed to germinate under same environmental condition. Germination percentage was recorded after seven

days. Other part of the treated seeds along with control were sown in the experimental field with three replications of each treatment. Germination percentage was recorded after fifteen days on the basis of the seedling emergence.

Collection and fixation of root tips:

For each treatment roots of 1.0-2.0, 2.1-3.0, 3.1-4.0, 4.1-5.0, 5.1-6.0 mm and 1.0-1.5 cm in length were fixed in 1:3 aceto-alcohol. After 48 hours of fixation root tips were transfered to 70% alcohol and stored in the refrigerator untill they were used.

Staining of root tips and preparation of slides:

In order to record the mitotic behavior and interphase chromosome volume of root tip cells temporary slides were prepared by squash method of Haque et al.(1976) using haematoxyline as stain with certain modifications. The staining schedule was as follows:

a) Removing from 70% alcohol the materials were washed with distilled water for 5-6 minutes.

b) The root tips were than treated with 50% HCl for about 15 minutes at room temperature to dissolve the middle lamella of cells.

c) The root tips were washed again with distilled water for 5 minutes.

d) After washing, the materials were mordanted by 2% aqueous solution of iron alum (Ferric ammonium sulfate) for 5 minutes.

e) The root tips were washed again with frequent changes of distilled water for 7 minutes in order to complete removal of the mordanting fluid from the tissues.

f) Then the root tips were stained with 0.5% hematoxylin for 15 minutes.

g) The stained root tips were washed in distilled water for 5 minutes.

Stained root tips were then squashed by plastic tapper on to a clean slide with a drop of 0.5% acetocarmine. The cells were then covered with a coverglass and pressure was applied by a rubber tipped pencil. The slide was warmed over an alcohol flame and again a slight pressure was applied by a finger tip over the cover glass kepping the slide duly wrapt in blotting paper. Slides were then observed under microcoscope and data were recorded. Photomicrographs were taken from the desired preperations.

Determination of interphase chromosome volume (ICV):

In order to calculate the interphase chromosome volume (ICV), occular values of nuclear volume were converted into micron (m) by a stage micro-meter. The nuclear volume (NV) was calculated using the formulla for a sphare ($NV=4/3pr^3$) as suggested by Nayar *et al.*(1971). The mean nuclear volume divided by the somatic chromosome number gave the interphase chromosome volume.

Preparation and design of experimental field:

The same experimental field prepeard for germination study in Regional Agricultural Research Institute of Ishurdi, Pabna on November 23, 1992; was also used for the collection of flower buds and to record the data on yield and yield components. The experiment was laid out in split plot design with three replications of each treatment along with control. Mannuring, weeding and irrigation were done as and when necessary.

Collection and preservation of flower buds:

For meiotic study, young flower buds for each treatment along with control were fixed in aceto-alcohol (1:3) to which a small amount of ferric chloride (1 gm in 500 ml of aceto-alcohol) was added imparting a straw colour. After 48 hours of fixation they were transfered to 70% ethanol and kept in a refrigerator till used.

Preparation of slides for meiotic study:

Temporary slides were prepared from suitable anthers by acetocarmine smear technique as follows:-

i) Young anther was placed on a clean slide and a drop of 2% acetocarmine was added.

ii) The anther wall was then ruptured by a curved dissecting needle and the anther wall was removed.

iii) Thereafter the pollen mother cells were coverd with a cover glass, warmed gently over an alcohol flame and a slight pressure was exerted by thumb or fingur tip to spread out the pollen mother cells as well as the chromosome.

Cytological screening was made from metaphase-I to telophase-II. Data on chromosome association and chiasma frequency was recorded at diakinesis. Photomicrographs were taken from the desired preparations.

Measurment of yield and its components:

The data were recorded from ten plants of each replication at the time of harvest raised from the treated and untreated seeds. The different characters were measured as follows:-

i) Plant height: Plant height was measured from the ground to the top of the shoots for each plant seperately.

ii) Primary branches per plant: It was counted considering those branches which originated more or less from the base.

iii) Secondary branches per plant: Branches originating from the primary branches only were counted for this character.

iv) Pods per plant: Number of seed bearing pods per plant were counted at maturity.

v) Pod volume: It was recorded in cubic centimeter (cc) with the help of measuring cylinder containing water on the basis of five random pods per plants.

vi) Seed per pods: Seeds from five random pods were counted and the mean seed number per pod was estimated.

vii) Hundred seeds weight: Weight of 100 random seeds was determined using an electric balance.

Analysis of data:

Data recorded from different experiment were analysed statistically as follows:

Mean, standard error, standard deviation, regression, analysis of variance (two way and three way), angular transformation and LSD were calculated using the following formulae.

Mean:

$$\overline{X} = \Sigma X/N$$

where, $\overline{X}$ = Arithmetic mean.

Σ = Sumation.

X = Value per variable.

N = Number of variable.

Standard error,

$$S.E. = sd/N$$

Where, S.E. = Standard error.

Sd = Standard deviation.

N = Number of variables.

$$Sd = \{\sum X^2 - (\sum X)^2/N\}/(N-1)$$

Standard deviation,

$$r = (SPXY)/(SSX)(SSY)$$

Co-relation co-efficient,

Where, r = Co-rrelation co-efficient.

SPXY = Sum of product of XY.

SSX = Sum squrre of X.

SSY = Sum square of Y.

Here, $SPXY = \sum XY - (\sum X)(\sum Y)/N$

$SSX = \sum X^2-(\sum X)^2/N$

$SSY = \sum Y^2-(\sum Y)^2/N$

Where, SPXY = Sum of product of XY.

X = Value per variable from data-1.

Y = Value per variable from data-2.

Σ = Summation.

N = Number of variable.

SSX = Sum square of X.

SSY = Sum square of Y.

Analysis of variance (2-way):

The effect of temperature treatment,treatment duration and their inter-action were detrmined according to the following formula:

Source	SS	df
Total	$\sum X^2$-CF	N-1
Temperature treatment(T)	$1/rdl\sum T^2$-CF	T-1
Treatment duration(D)	$1/rtl\sum D^2$-CF	D-1
T X D	$1/rtl\sum (TXD)^2$-CF-TSS-DSS	(T-1)(D-1)

Error	Total SS-(TSS+DSS+T.DSS)	(Tdf-all df)

'T' is the sum of all readings of each temperature treatment over replications and treatment duration 'D' is the sum of all readings of each treatments of each treatment duration over replications and temperature treatment.

Where as, TSS and DSS are the sum of squares of temperature treatment(T) and treatment duration(D). And TXDSS represent the sum of squares of their interactions and CF (correction factor) is equal to $(\sum X^2)/n$.

Mean squares were determined by dividing the individual sum of square (SS) values by their respective degrees of freedom (df) and the mean square values were tested against the error mean square value (Error MS).

Analysis of variance (3-way):

The effect of temperature treatment, treatment duration and root length and their interactions were determined according to the following:

Source	SS	df
Total	$\sum X^2$-CF	N-1

Temperature treatment(T)	$1/rdl\sum T^2$-CF	T-1
Treatment duration(D)	$1/rtl\sum D^2$-CF	D-1
Root leangth (L)	$1/rtd\sum L^2$-CF	L-1
TXD	$1/rl\sum(TXD)^2$-CF-TSS-DSS	(T-1)(D-1)
TXL	$1/rd\sum(TXL)^2$-CF-TSS-LSS	(T-1)(L-1)
DXL	$1/rt\sum(DXL)^2$-CF-DSS-LSS	(D-1)(L-1)
TXDXL	$1/r\sum(TXDXL)^2$-CF-TSS-DSS	
	-LSS-T.DSS-TXLSS-D.LSS	(T-1)(D-1)(L-1)
Pooled Error	TotalSS- (TSS+DSS+LSS+	(Tdf-all df)
	T.DSS+T.LSS+D.LSS+T.D.LSS)	

`T' is the sum of all readings of each temperature treatment over replications, treatment duration and root length. `D' is the sum of all readings of each treatment duration over replications, temperature treatment and root length. `L' is the sum of all readings of each root length over replication, temperature treatment and treatment duration.

Whereas, TSS, DSS and LSS are the sum of squares of temperature treatment (T), treatment duration (D) and root length (L), respectively. TXDS, TXLSS, DXLSS, TXDXLSS represent the sum of squares of different interactions and CF (correction factor) is equal to $(SX)^2/n$.

Mean squares were determined by dividing the individual sum of squares (SS) values by their respective degrees of freedom (df) and the mean square values were tested against the error mean square values (Error MS).

L. S. D. = Least significant difference.

$= \sqrt{(}$ 2 Error MS/r) x t (t at 5% and 1% level)

where, r = replication.

RESULTS

The experimental findings obtained in the present study are presented under the following heads:

1. Seed germination.
2. Time of first mitosis.
3. Frequency of aberrant anaphase.
4. Relationship between aberrant anaphase and seed germination.
5. Relationship between aberrant anaphase and treatment durations.
6. Mitotic index and chromosomal abnormalities.
7. Interphase chromosome volume (ICV).
8. Meiotic irregularities.
9. Chromosome association and chiasma frequency.
10. Yield and yield components.

1. Seed germination :

Test on germination was made after removal of seeds from the temperature treatment at 30, 32, 35 and 40°C for 7, 10 and 15 days durations. The treated seeds along with control were allowed to germinate on moist filter paper in petridishes in the laboratory at room temperature (20 - 25°C).

Results obtained on the effect of artificial aging on germination of the seeds are given in Table-1. In general, all the treatments including control had relatively a low percentage germination in the field than in the laboratory.

At 30°C for 7, 10 and 15 days treatment durations germination percentages were 91.32, 90.10, 88.90% in laboratory and 86.09, 84.11, 82.95% in field, respectively. At 32°C, for 7, 10 and 15 days treatment durations, percentage of seed germination were 86.85, 85.96, 84.00% in laboratory and in field those were 82.08, 81.05, 80.10%, respectively. At 35°C temperature for same treatment durations, in laboratory and field germination were respectively, 82.85, 81.09, 80.48% and 70.00, 77.78, 77.39%. At 40°C temperature for same treatment durations, germination percentages in laboratory and in field were 79.96, 78.05, 75.88% and 75.00 74.13, 73.21%, were respectively. Germination of untreated seeds were found to be 97.27 and 92.91% in laboratory and in the field, respectively.

It was observed that both in laboratory and in the field, germination percentage decreased gradually with the increase of temperature as well as treatment durations. Lowest germination percentage (73.21%) was found in field from the seeds treated at 40°C for 15 days treatment time.

Relationship between germination percentage and temperature (Fig. 1) indicated progressive decrease in germination percentage with the increase of temperature. It was observed

Table 1 : Effect of artificial aging on germination of the seeds of *Cicer arietinum*

Treatment conditions			Seed germination(%)	
Temperature (°C)	Treatment time (days)	Moisture (%)	In laboratory $(\bar{X} \pm S.E)$	In field $(\bar{X} \pm S.E)$
Control (20-25)	15	21.67	97.27 ± 0.52	92.91 ± 1.42
	7	12.30	91.32 ± 0.31	86.09 ± 1.42
30	10	12.27	90.10 ± 0.79	84.11 ± 1.11
	15	12.20	88.90 ± 0.30	82.95 ± 1.67
	7	12.20	86.85 ± 1.12	82.08 ± 2.42
32	10	12.20	85.96 ± 0.52	81.05 ± 2.14
	15	12.20	84.00 ± 0.56	80.10 ± 1.18
	7	12.19	82.85 ± 0.70	79.00 ± 1.27
35	10	12.12	81.09 ± 0.61	77.78 ± 2.01
	15	12.11	80.48 ± 0.50	77.39 ± 1.91
	7	12.10	79.96 ± 0.49	75.00 ± 1.27
40	10	12.04	78.05 ± 0.29	74.13 ± 1.33
	15	12.00	75.88 ± 0.30	73.21 ± 1.19

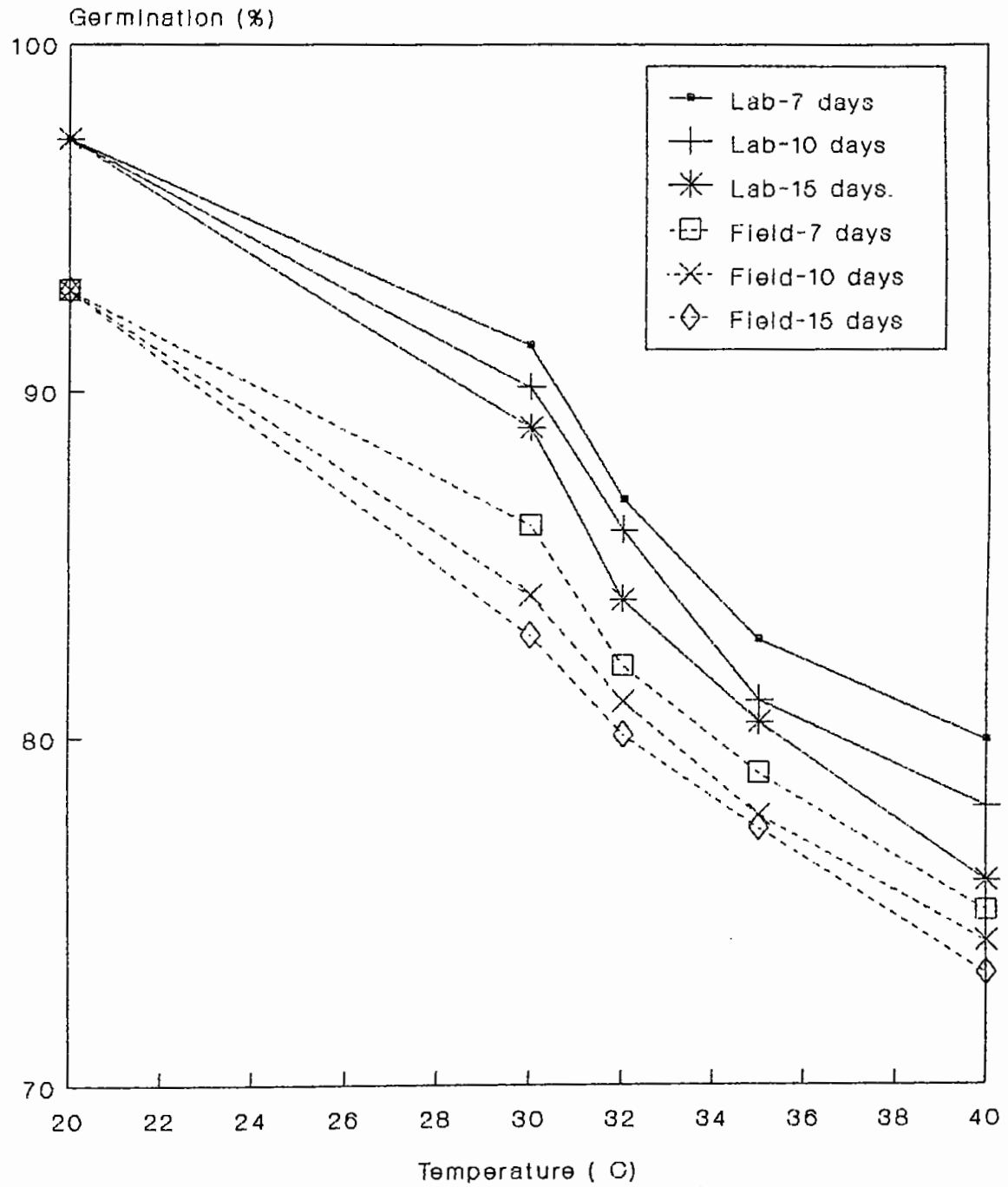


Fig. 1 Progressive decrease in germination(%) with the increase of temperature.

that the root emergence at almost all the treatments were delayed and germination percentage decreased linearly with the increase of temperature.

2. Time of first mitosis:

To determine the time of first mitosis number of anaphase cells at different root lengths and the minimum root length showing anaphase cells were studied following the schedule of Murata *et al.* (1977, 1978). Number of anaphase cells at different germinating root length and the minimum root length for first mitosis are given in **Table-2.** Number of anaphase cells were observed first in roots of 1.0-2.0 mm length and maximum numbers were noted in roots of 5.1-6.0 mm long (**Plate-1**). The minimum root length showing any anaphase cell was 1.5 mm. In artificially aged seeds, maximum number of anaphase cell was observed progressively in longer roots. Maximum number of anaphase cells (1.9) was found at the root length of 5.1-6.0 mm emerged from the seeds treated at 40°C for 15 days. In almost all the treatments minimum root length with anaphase cells was found to be 1.5 mm. In seeds germinating more than 70%, first mitosis was found to begin in roots of 1.5 mm length. Seeds with delayed germination began mitosis at root length below than 1.00 mm. This delay was associated with loss in germination and indicated a relationship of first mitosis with aging.

Table 2: Number of anaphase cells at different germinating root lengths and the minimum root length for first mitosis.

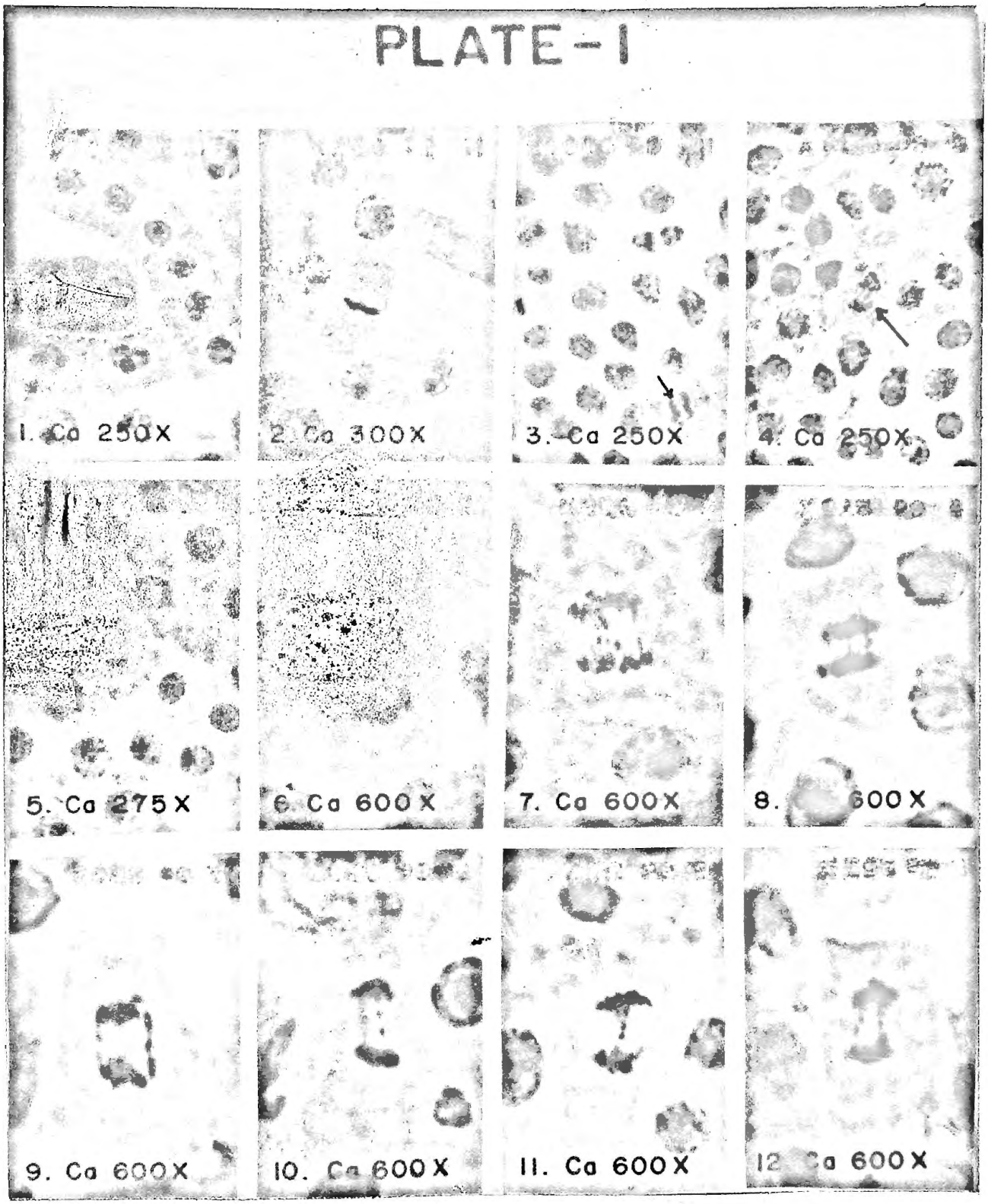
Temperature treatment (°C)	Treatment duration (Days)	Number of anaphase cells at germinating root lengths					Minimum root length with anaphase
		1.0 - 2.0 (mm)	2.1 - 3.0 (mm)	3.1 - 4.0 (mm)	4.1 - 5.0 (mm)	5.1 - 6.0 (mm)	
Control (20-25)	15	0.5 ± 0.1	0.9 ± 0.31	0.9 ± 0.4	1.5 ± 0.47	1.6 ± 0.52	1.5
	7	0.2 ± 0.13	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.3	0.5 ± 0.3	1.5
30	10	0.1 ± 0.1	0.1 ± 0.1	1.0 ± 0.0	0.6 ± 0.16	0.9 ± 0.23	1.5
	15	0.0	0.4 ± 0.2	0.4 ± 0.12	0.4 ± 0.3	0.3 ± 0.15	2.5
	7	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.21	0.9 ± 0.3	0.7 ± 0.26	1.5
32	10	0.5 ± 0.22	0.3 ± 0.1	0.5 ± 0.17	0.3 ± 0.14	1.1 ± 0.09	1.5
	15	0.1 ± 0.1	0.1 ± 0.01	0.3 ± 0.21	0.2 ± 0.2	0.6 ± 0.2	1.5
	7	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.13	0.4 ± 0.16	1.2 ± 0.29	1.5
35	10	0.0	0.3 ± 0.15	0.3 ± 0.15	0.1 ± 0.1	0.5 ± 0.3	2.5
	15	0.1 ± 0.1	0.5 ± 0.16	0.3 ± 0.15	0.0	0.9 ± 0.27	1.5
	7	0.1 ± 0.1	0.0	0.3 ± 0.14	0.4 ± 0.16	0.7 ± 0.15	1.5
40	10	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.13	0.4 ± 0.3	0.5 ± 0.3	1.5
	15	0.0	0.4 ± 0.1	0.2 ± 0.13	0.6 ± 0.21	1.9 ± 0.18	2.5

Plate-1

Photomicrographs showing dividing and nondividing root tip cells with a few of the chromosomal irregularities.

1. Nondividing cells with nuclei at the root length < 1.00mm.
2. A single metaphase cell at the root length of 1.00mm.
3. Anaphase (arrow headed) as an indicator of the first mitosis (average root length 1.50mm).
4. Aberrant anaphase (arrow headed) at the root length of 1.00 - 2.00mm.
5. Telophase at the root length of 2.10 - 3.00mm.
6. Anaphase (Normal).
7. Anaphase with chromosome fragment.
8. Anaphase with chromatid bridges.
9. Telophase (early) with chromatid bridge.
10. Telophase with broken chromatid bridge.
11. Telophase with single chromatid bridge.
12. Telophase with double chromatid bridge.

PLATE-I


1. Ca 250X
2. Ca 300X
3. Ca 250X
4. Ca 250X
5. Ca 275X
6. Ca 600X
7. Ca 600X
8. 600X
9. Ca 600X
10. Ca 600X
11. Ca 600X
12. Ca 600X

Analysis of variance (Table-3) indicated the significant effect of root length, interaction of temperature and root length, for number of anaphase cells at different germinating root length. Least significant difference (Table-4) on the contrary indicated non-significant effect to increase the number of anaphase cells from that of control.

Table 3: Analysis of variance for number of anaphase cells at different germinating root length from the artificially aged seeds.

Source	df	SS	MS	F
1. Temperature(T)	3	8.25	2.75	2.27^{NS}
2. Treatment duration(D)	2	5.12	2.55	2.11^{NS}
3. T X D	6	16.20	2.70	2.23^{NS}
4. Root length(L)	4	148.78	37.19	30.76^{**}
5. T X L	12	32.93	2.74	2.27^{*}
6. D X L	8	24.82	3.10	2.57^{*}
7. T X D X L	24	21.41	0.89	0.74^{NS}
8. Pooled error	60	72.55	1.21	
9. Total	119	330.06	2.77	

NS = Nonsignificant at 5% level
* = Significant at 1% level
** = Significant at 0.1% level

Table 4: Least significant difference(LSD) valus for the number of anaphase cells at different root length.

Temperature (°C)	Treatment duration (Days)	Number of anaphase cells (X) in the root length of					LSD	
		1.0-2.0 (mm)	2.1-3.0 (mm)	3.1-4.0 (mm)	4.1-5.0 (mm)	5.1-6.0 (mm)	5%	1%
Control (20-25)	15	0.5	0.9	0.9	1.5	1.6		
	7	0.2^{NS}	0.1^{NS}	0.1^{NS}	0.4^{NS}	0.5^{NS}		
30	10	0.1^{NS}	0.1^{NS}	1.0^{NS}	0.6^{NS}	0.9^{NS}		
	15	0.0	0.4^{NS}	0.4^{NS}	0.4^{NS}	0.3^{NS}		
	7	0.1^{NS}	0.2^{NS}	0.3^{NS}	0.9^{NS}	0.7^{NS}		
32	10	0.5^{NS}	0.3^{NS}	0.5^{NS}	0.3^{NS}	1.1^{NS}	2.20	2.95
	15	0.1^{NS}	0.1^{NS}	0.3^{NS}	0.2^{NS}	0.6^{NS}		
	7	0.1^{NS}	0.1^{NS}	0.2^{NS}	0.4^{NS}	1.2^{NS}		
35	10	0.0	0.3^{NS}	0.3^{NS}	0.1^{NS}	0.5^{NS}		
	15	0.1^{NS}	0.5^{NS}	0.3^{NS}	0.0	0.9^{NS}		
	7	0.1^{NS}	0.0	0.3^{NS}	0.4^{NS}	0.7^{NS}		
40	10	0.1^{NS}	0.1^{NS}	0.2^{NS}	0.4^{NS}	0.5^{NS}		
	15	0.0	0.1^{NS}	0.2^{NS}	0.3^{NS}	1.3^{NS}		

NS = Nonsignificant at 5% level

3. Frequency of aberrant anaphase:

Aberrant anaphase cells were observed at different germinating root lengths. Frequency of aberrant anaphase was found to increase with the increase of temperature and these are given in Table-5. In control, percentage of aberrant anaphase was found to be 9.26%. At 30°C for 7, 10 and 15 days treatment time, the percentages of aberrant anaphase were 30.77, 37.04 and 33.33%, respectively. At 32°C temperature for 7, 10 and 15 days durations, percentages of aberrant anaphase were 36.36, 37.04 and 38.46, successively. At 35°C temperature for 7, 10 and 15 days durations the frequency of aberrant anaphase were 40.00, 41.67 and 44.44%. At 40°C temperature for the same treatment time the percentages of aberrant anaphase were 46.67, 46.15 and 47.37%.

An interesting result was obtained in this study that in roots of 1.0-4.0 mm length emerged from control as well as from aged seed the frequency of aberrant anaphase was very less. But with the further increase of the root length frequency of aberrant anaphase was found to increase.

Analysis of variance (Table-6) indicated significant effect of root length and the inter action of TXD, TXL, DXL and TXDXL to accelerate aberrant anaphase. On the contrary LSD values (Table-7) indicated non-significant effect to accelerate the number of aberrant anaphase from that of control.

Table 5: Mean percentage of aberrant anaphase at different germinating root lengths from the artificially aged seeds.

Temp. (°C)	Treatment duration (days)	* Mean percentage of aberrant anaphase at different root lengths					Number of anaphase cells obtained	Number of aberrant anaphase cells	Total percentage of aberrant anaphase
		1.0–2.0 (mm)	2.1–3.0 (mm)	3.1–4.0 (mm)	4.1–5.0 (mm)	5.1–6.0 (mm)			
Control (20-25)	15	0.0	0.0	0.1±0.08	0.1±0.01	0.3±0.1	54	5	9.26
	7	0.0	0.0	0.0	0.0	0.4±0.1	13	4	30.77
30	10	0.1 ± 0	0.1±0.0	0.0	0.2±0.1	0.6±0.1	27	10	37.04
	15	0.0	0.0	0.3±0.1	0.1±0.07	0.1±0.06	15	5	33.33
	7	0.2±0.1	0.2±0.1	0.0	0.1±0.05	0.3±0.1	22	8	36.36
32	10	0.0	0.0	0.2±0.1	0.2±0.0	0.6±0.2	27	10	37.04
	15	0.3±0.1	0.0	0.0	0.2±0.1	0.0	13	5	38.46
	7	0.0	0.0	0.1±0.08	0.2±0.04	0.5±0.3	20	8	40.00
35	10	0.0	0.1±0.04	0.1±0.09	0.0	0.3±0.2	12	5	41.67
	15	0.0	0.3±0.1	0.2±0.1	0.1±0.1	0.2±0.1	18	8	44.44
	7	0.0	0.0	0.1±0.0	0.1±0.03	0.5±0.1	15	7	46.67
40	10	0.0	0.1±0.02	0.0	0.0	0.5±0.0	13	6	46.15
	15	0.0	0.0	0.6±0.1	0.1±0.05	0.2±0.1	19	9	47.37

* = Mean values obtained from 10 roots in each case

Table 6: Analysis of variance for the mean percentage of aberrant anaphase.

Source	df	SS	MS	F
1. Temperature(T)	3	1.90	0.63	1.62^{NS}
2. Treatment duration(D)	2	1.44	0.72	1.84^{NS}
3. T X D	6	15.51	2.58	6.63^{**}
4. Root length(L)	4	92.07	23.02	59.02^{**}
5. T X L	12	31.57	2.63	6.74^{**}
6. D X L	8	41.79	5.22	13.38^{**}
7. T X D X L	24	69.79	2.91	7.46^{**}
8. Pooled error	60	23.73	0.39	
9. Total	119	277.80	2.33	

NS = Nonsignificant at 5% level
** = Significant at 0.1% level

4. Relationship between aberrant anaphase and seed germination:

The pattern of increase of the frequency of aberrant anaphase with increase of temperature treatment was closely related to loss of germinability. Linear regression (Fig. 2) line for pooled between the germination percentage and the frequency of aberrant anaphase. The frequency of roots with aberrations was found to increase linearly as the germination percentage decreased.

Table 7: Least significant difference (LSD) values for aberrent anaphase at different root length.

Temperature (°C)	Treatment duration (days)	Mean percentage of aberrant anaphase at the root length of 1.0-2.0 (mm)	2.1-3.0 (mm)	3.1-4.0 (mm)	4.1-5.0 (mm)	5.1-6.0 (mm)	LSD 5%	LSD 1%
Control (20-25)	15	0.0	0.0	0.1	0.1	0.3		
	7	0.0	0.0	0.0	0.0	0.4^{NS}		
30	10	0.1^{NS}	0.1^{NS}	0.0	0.2^{NS}	0.6^{NS}		
	15	0.0	0.0	0.3^{NS}	0.1^{NS}	0.1^{NS}		
	7	0.2^{NS}	0.2^{NS}	0.0	0.1^{NS}	0.3^{NS}		
32	10	0.0	0.0	0.2^{NS}	0.2^{NS}	0.6^{NS}	1.26	1.67
	15	0.3^{NS}	0.0	0.0	0.2^{NS}	0.0		
	7	0.0	0.0	0.1^{NS}	0.2^{NS}	0.5^{NS}		
35	10	0.0	0.1^{NS}	0.1^{NS}	0.0	0.3^{NS}		
	15	0.0	0.3^{NS}	0.2^{NS}	0.1^{NS}	0.2^{NS}		
	7	0.0	0.0	0.1^{NS}	0.1^{NS}	0.5^{NS}		
40	10	0.0	0.1^{NS}	0.0	0.0	0.5^{NS}		
	15	0.0	0.0	0.6^{NS}	0.1^{NS}	0.2^{NS}		

NS = Nonsignificant at 5% level

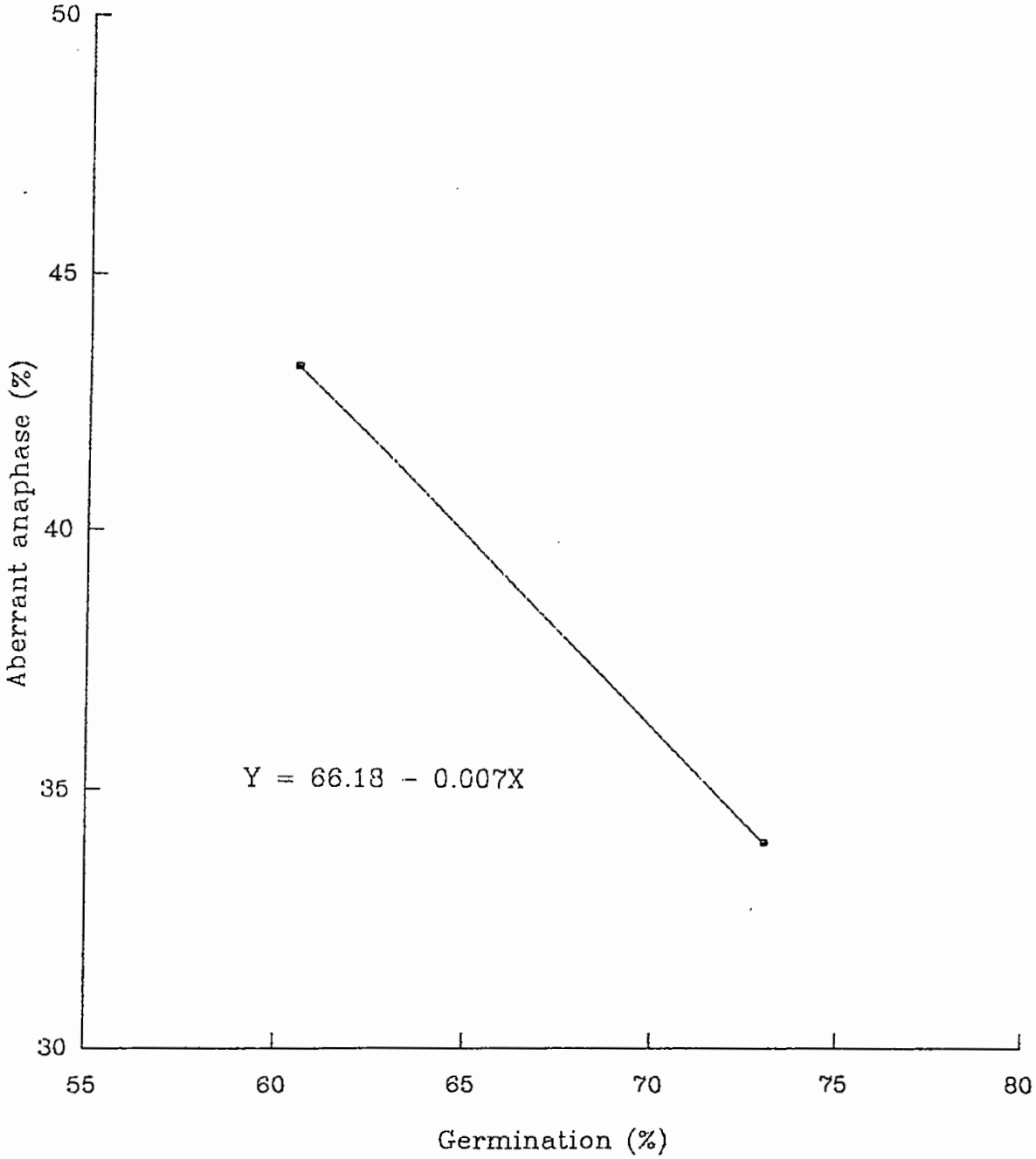


Fig. 2 Relationship between the frequency of aberrant anaphase in root tips and germination percentage.

5. Relationship between aberrant anaphase and treatment durations :

The relationships between aberrant anaphase and treatment durations were studied at different root lengths and are shown in **Fig. 3 through Fig. 20**. It indicated negative relation for all treatment durations in root length of 1.0-2.0, 2.1-3.0 and 5.1-6.0 mm at 30°C. But at same temperature, treatment durations and aberrant anaphase indicated positive relation in root length of 3.1-4.0 mm and 4.1-5.0 mm.

At 32°C temperature, in the root length of 2.1-3.0 and 5.1-6.0 mm negative relation was found. But in root length of 1.0-2.0, 3.1-4.0 and 4.1-5.0 mm the relationship was positive. At 35°C temperature, relationship between aberrant anaphase and treatment duration was zero in root length of 1.0-2.0 mm, but in 2.1-3.0 3.1-4.0 mm it was positive and in 4.1-5.0 and 5.1-6.0 mm relationship was negative. Similar result was obtained at 40°C in root length of 1.0-2.0 mm as it was found in case of 2.1-3.0 mm, and 5.1-6.0 mm the relation was negative. The relation between aberrant anaphase and treatment duration at the root length of 3.1-4.0 mm and 4.1-5.0 mm was positive.

6. Mitotic index and chromosomal abnormalities:

Mean percentage of dividing cells and different abnormalities were determined at root length of 1.0-1.5 cm and these are shown in the **Table-8**. It was observed that the percentage of

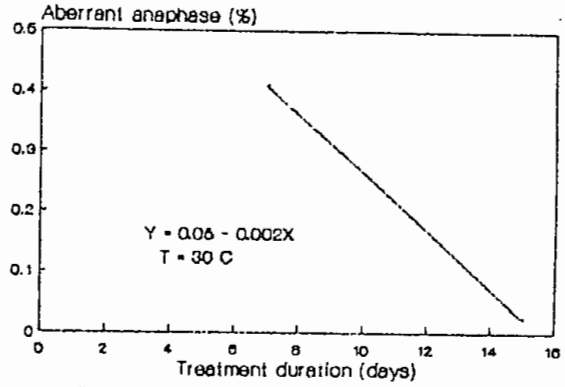


Fig. 3 At the root length of 1.0-2.0 mm

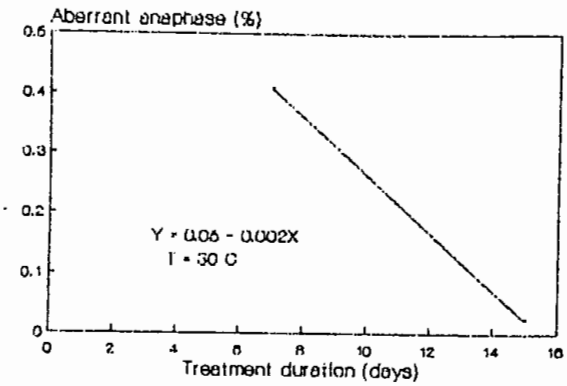


Fig. 4 At the root length of 2.1-3.0 mm

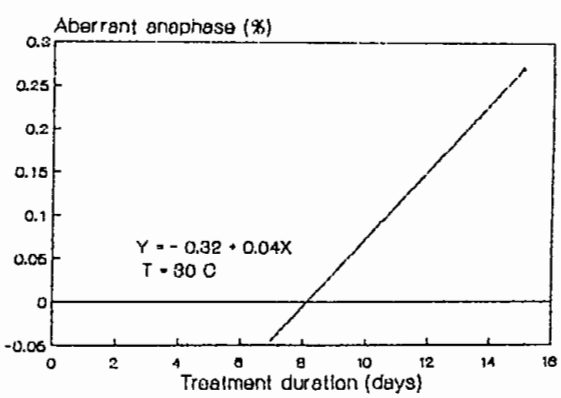


Fig. 5 At the root length of 3.1-4.0 mm

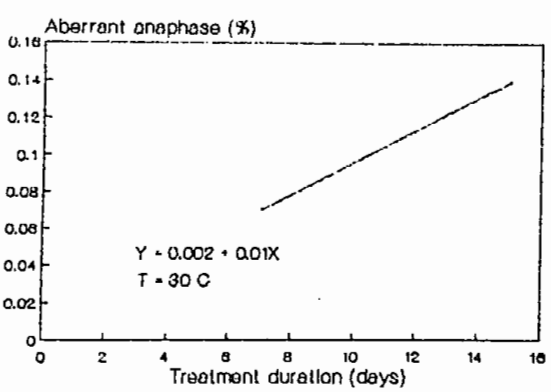


Fig. 6 At the root length of 4.1-5.0 mm

Figs. 3–6 Relationship between the frequency of aberrant anaphase and treatment duration at different root lengths.

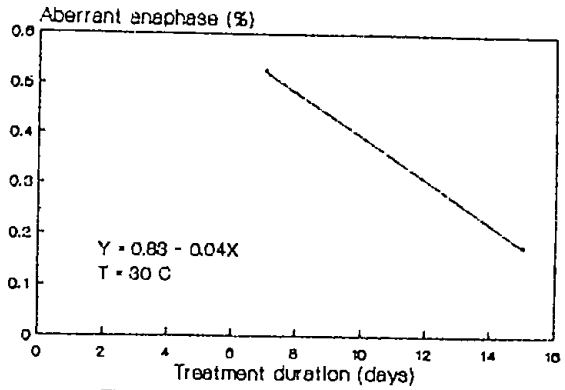


Fig. 7 At the root length of 5.1-6.0 mm

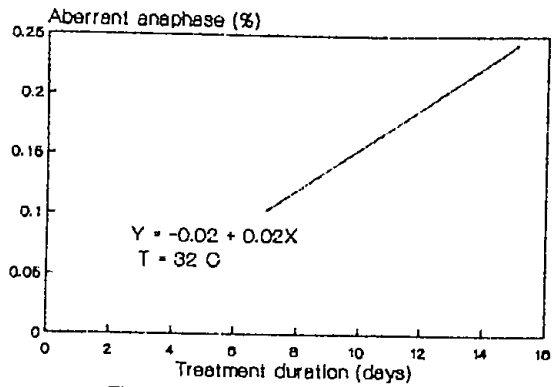


Fig. 8. At the root length of 1.0-2.0 mm

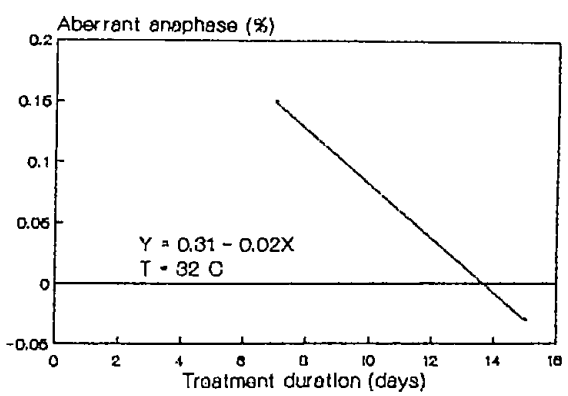


Fig. 9 At the root length of 2.1-3.0 mm

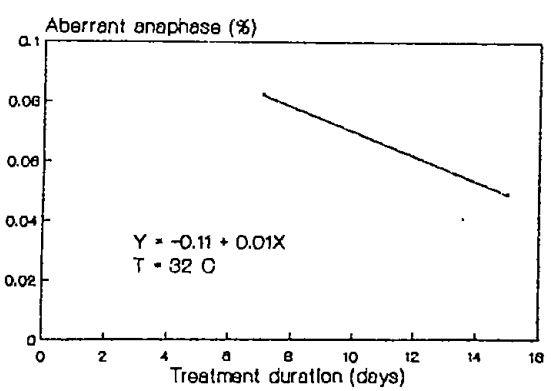


Fig. 10 At the root length of 3.1-4.0 mm

Figs. 7–10 Relationship between the frequency of aberrant anaphase and treatment duration at different root lengths.

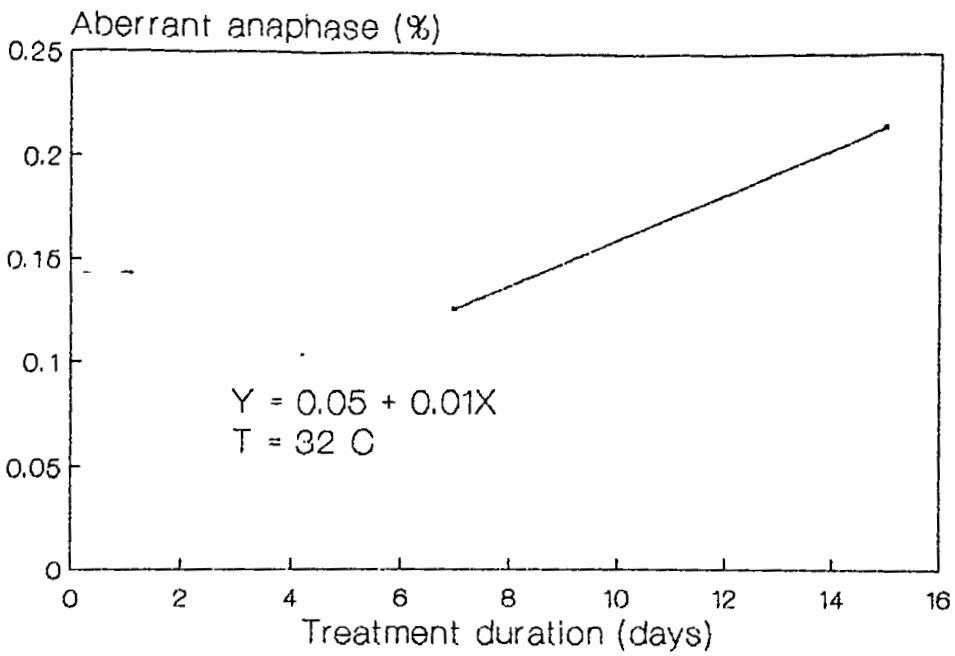


Fig. 11 At the root length of 4.1-5.0 mm

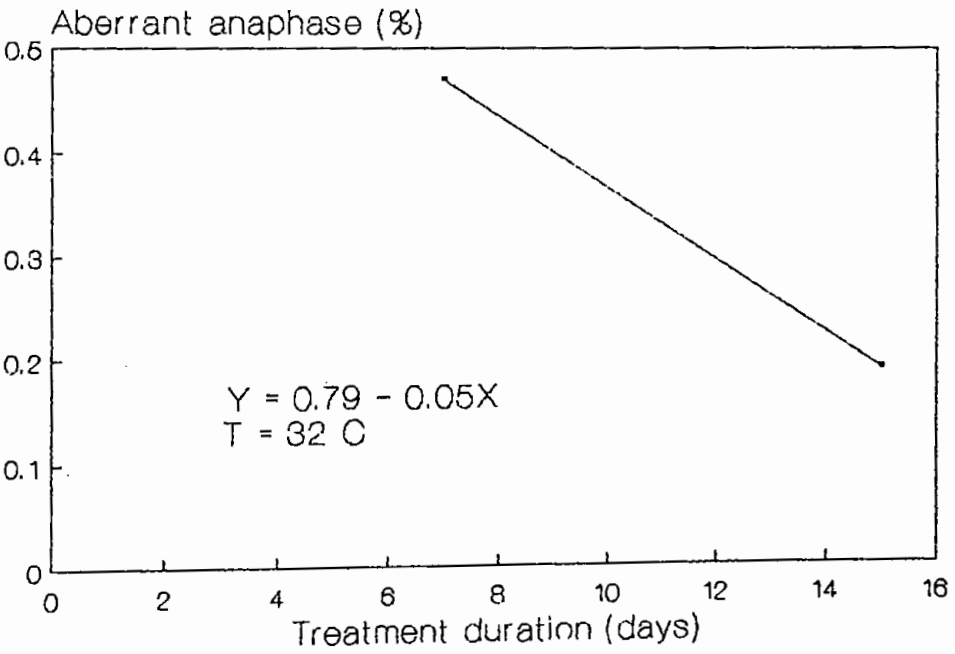


Fig. 12 At the root length of 5.1-6.0 mm

Figs. 11–12 Relationship between the frequency of aberrant anaphase and treatment duration at different root lengths.

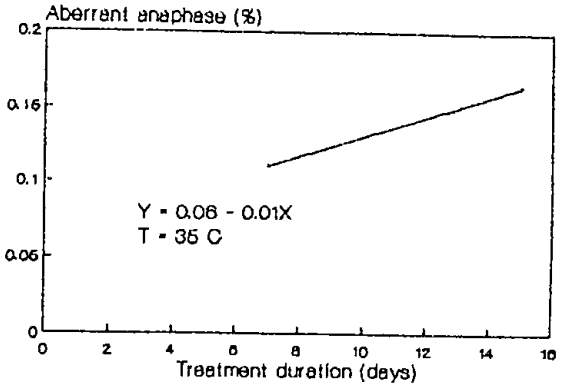


Fig. 13 At the root length of 2.1-3.0 mm

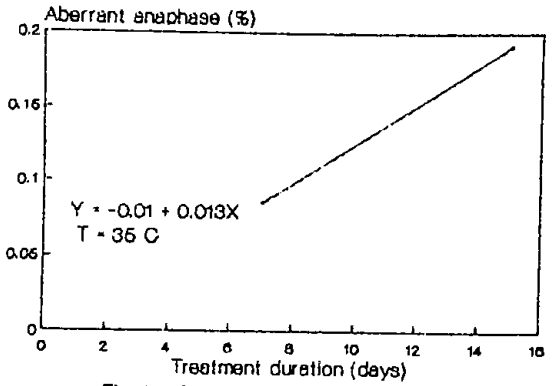


Fig. 14 At the root length of 3.1-4.0 mm

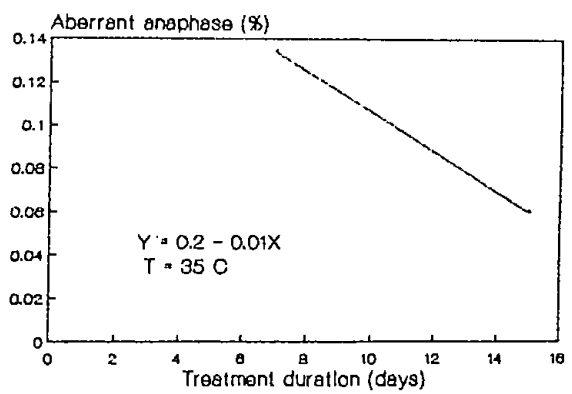


Fig. 15 At the root length of 4.1-5.0 mm

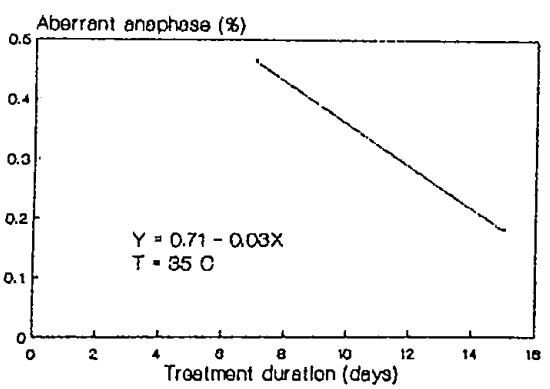


Fig. 16 At the root length of 5.1-6.0 mm

Figs. 13–16 Relationship between the frequency of aberrant anaphase and treatment duration at different root lengths.

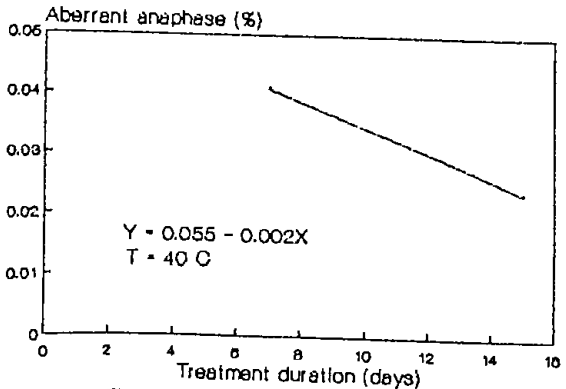


Fig. 17 At the root length of 2.1-3.0 mm

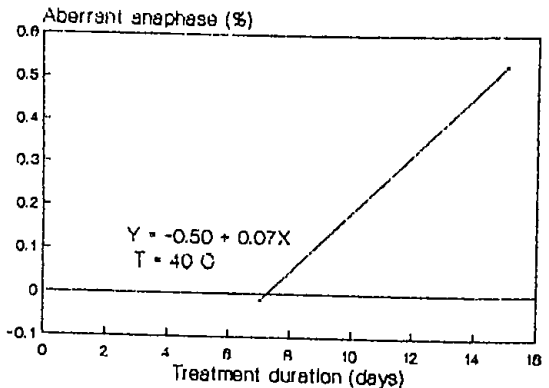


Fig. 18 At the root length of 3.1-4.0 mm

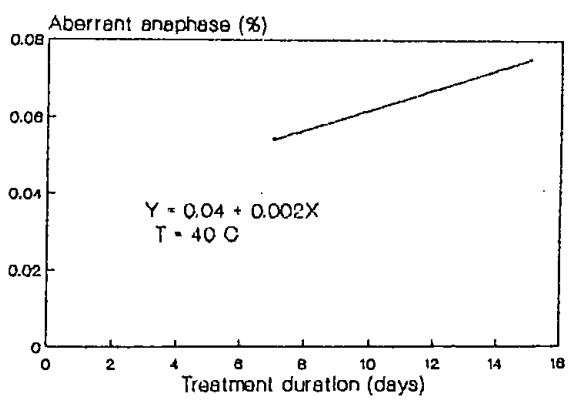


Fig. 19 At the root length of 4.1-5.0 mm

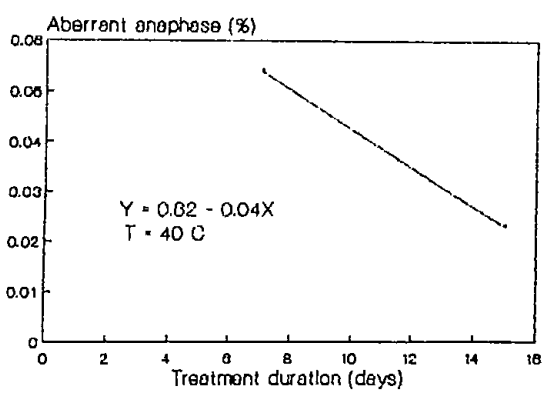


Fig. 20 At the root length of 5.1-6.0 mm

Figs. 17–20 Relationship between the frequency of aberrant anaphase and treatment duration at different root lengths.

Table 8: Mean percentage of dividing cells and different abnormalities at root length of 1.0 - 1.5 cm emerged from the artificially aged seeds.

Temperature (°C)	Treatment duration (days)	Total No.of cells studied	Percentage of diving cells ($\bar{X}$ ± S.E.)	Percentage of abnormalities ($\bar{X}$ ± S.E.)	Percentage of different abnormalities ($\bar{X}$ ± S.E.)			
					Fragment	Laggard	Bridge	Micronuclei
Control (20-25)	15	989	22.1 ± 9.49	0.4 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.0
30	7	1000	12.0 ± 0.44	0.5 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.25 ± 0.16	0.05 ± 0.01
	10	1009	11.4 ± 0.98	1.0 ± 0.2	0.2 ± 0.1	0.4 ± 0.1	0.4 ± 0.15	0.0
	15	979	10.5 ± 0.98	0.8 ± 0.4	0.1 ± 0.1	0.4 ± 0.1	0.3 ± 0.14	0.0
32	7	987	9.3 ± 2.04	0.85 ± 0.3	0.5 ± 0.01	0.1 ± 0.1	0.2 ± 0.1	0.05 ± 0.1
	10	987	8.3 ± 0.91	1.0 ± 0.2	0.1 ± 0.1	0.5 ± 0.51	0.4 ± 0.22	0.0
	15	1006	8.0 ± 0.33	1.9 ± 0.1	0.2 ± 0.13	1.0 ± 0.31	0.7 ± 0.26	0.0
35	7	989	7.8 ± 0.33	1.3 ± 0.7	0.2 ± 0.13	0.4 ± 0.27	0.7 ± 0.26	0.0
	10	991	7.3 ± 0.21	1.4 ± 0.0	0.2 ± 0.13	0.5 ± 0.21	0.5 ± 0.27	0.2 ± 0.0
	15	977	7.1 ± 0.82	2.0 ± 0.0	0.2 ± 0.15	0.6 ± 0.30	1.0 ± 0.23	0.2 ± 0.1
40	7	1003	7.0 ± 0.65	3.0 ± 0.2	0.2 ± 0.13	1.4 ± 0.34	1.4 ± 0.26	0.0
	10	1000	6.9 ± 0.16	3.1 ± 0.3	0.5 ± 0.31	1.2 ± 0.29	1.0 ± 0.41	0.4 ± 0.1
	15	1091	6.7 ± 0.49	6.0 ± 0.0	0.8 ± 0.25	2.2 ± 0.34	2.3 ± 0.29	0.7 ± 0.26

dividing cells were gradually decreased with increase of temperature s well as treatment duration. In control percentage of dividing cells was 22.10%, but it was more or less half at 30°C and decreased gradually up to one third from that of control. On the other hand various types of anomalies viz, fragments, laggards, bridges and micronuclei were found at different stages. Photomicrographs of different abnormalities are shown in Plate 1. In control, mean percentage of abnormalities (0.40%) was very less. But with increase of temperature anomalies were found to increase. At 30°C for 7, 10 and 15 days of treatment durations percentage of anomalies were 0.50, 1.00 and 0.80%. At 32°C for 7, 10 and 15 days treatment time percentages were 0.85, 1.00 and 1.90%. At 35°C for the same treatment durations percentages of abnormalities were 1.3, 1.4 and 2.0%. At 40°C, percentage of abnormalities were 3.00, 3.10 and 6.00% for 7, 10 and 15 days of treatment durations, respectively.

Analysis of variance (Table-9) indicated significant effect of temperature, treatment duration and their interaction to cause the chromosomal abnormalities.

LSD values (Table-10) indicated that laggards and bridges were highly significant at 40°C for all the treatment durations. Laggards and bridges were found significant at 32 and 35°C for 15 days treatment durations, respectively. In rest of the treatments none were found significant.

Table 9: Analysis of variance for percentage of different abnormalities in root length of 1.0-1.5 cm.

Source	df	SS	MS	F
1. Temperature(T)	3	43.06	14.35	88.60**
2. Treatment duration (D)	2	8.49	4.25	26.21*
3. T X D	6	3.27	0.55	3.42NS
4. Error	12	1.94	0.16	
5. Total	23	56.78	2.47	

NS = Nonsignificant at 5% level

* = Significant at 1% level

** = Significant at 0.1% level

7. Interphase chromosome volume (ICV):

Root tips from the treated seeds at different degrees of temperature for different treatment durations (7, 10 and 15 days) were studied for interphase chromosome volume. It was found to increase with the increase of temperature as well as treatment durations except in some cases. On

Table 10: Least significant difference (LSD) values for the percentage of different abnormalities.

Temperature (°C)	Treatment duration (days)	Percentage of different abnormalities				LSD	
		Fragment	Laggard	Bridge	Micro-nuclei	5%	1%
Control (20-25)	15	0.1	0.1	0.2	0.0		
	7	0.1^{NS}	0.1^{NS}	0.3^{NS}	0.5		
30	10	0.2^{NS}	0.4^{NS}	0.4^{NS}	0.0		
	15	0.1^{NS}	0.4^{NS}	0.3^{NS}	0.0		
	7	0.1^{NS}	0.1^{NS}	0.1^{NS}	0.5		
32	10	0.1^{NS}	0.5^{NS}	0.4^{NS}	0.0	0.716	1.004
	15	0.2^{NS}	1.0^{*}	0.7^{NS}	0.0		
	7	0.2^{NS}	0.4^{NS}	0.7^{NS}	0.0		
35	10	0.2^{NS}	0.7^{NS}	0.5^{NS}	0.2		
	15	0.3^{NS}	0.7^{NS}	1.0^{*}	0.1		
	7	0.2^{NS}	1.4^{**}	1.4^{*}	0.0		
40	10	0.5^{NS}	1.2^{**}	1.4^{*}	0.4		
	15	0.8^{NS}	2.4^{**}	2.8^{**}	0.7		

NS = Nonsignificant at 5% level
* = Significant at 1% level
** = Highly significant at 0.1% level

the other hand ICV were found to decrease with the increase of root length and these are shown in Table-11.

In control, interphase chromosome volume was found to be 1.68m^3 at the root length of 1.0-2.0 mm and it was found to decrease at 1.55m^3 in the root length of 5.1-6.0 mm. In root tip cells of treated seeds it was found also to decrease. Increase of ICV was found remarkably with the increase of temperature in comparison to that of treatment durations. At different degrees of temperature ICV were found to increase very slightly. The highest ICV was found at 40°C and lowest at 30°C temperature. Among four different temperatures and three treatment durations 40°C for 15 days treatment durations was found to be more effective to increase the interphase chromosome volume.

Analysis of variance (Table-12) indicated significant effect of temperature (T), treatment durations(D), root length (L) and the interaction of TXD and TXL on ICV. Interaction of DXL and TXDXL showed non-significant effect. LSD values (Table 13) indicated that all the treatments significantly influenced the interphase chromosome volume, except at 30°C for 7 days.

8. Meiotic irregularities:

The pollen mother cells (PMCs) of the plants raised from the treated and untreated seeds were studied. Various types of chromosomal aberrations were found in both the types of plants.

Table 11: Interphase chromosome volume (ICV) at different germinating root lengths from artificially aged seeds.

Temperature (°C)	Treatment duration (days)	Interphase chromosome volume (ICV) at different root length (μ^3 ± S.E.)				
		1.0-2.0 (mm)	2.1-3.0 (mm)	3.1-4.0 (mm)	4.1-5.0 (mm)	5.1-6.0 (mm)
Control (20-25)	15	1.68±0.07	1.65±0.02	1.62±0.01	1.59±0.01	1.55±0.05
30	7	1.73±0.01	1.73±0.001	1.72±0.001	1.71±0.01	1.67±0.02
	10	1.74±0.01	1.74±0.003	1.73±0.0	1.72±0.005	1.72±0.01
	15	1.75±0.0	1.74±0.005	1.74±0.01	1.75±0.005	1.74±0.01
32	7	1.76±0.001	1.75±0.001	1.75±0.005	1.76±0.01	1.75±0.02
	10	1.77±0.01	1.75±0.01	1.74±0.02	1.77±0.01	1.75±0.01
	15	1.78±0.02	1.76±0.01	1.75±0.005	1.79±0.005	1.78±0.02
35	7	1.79±0.001	1.77±0.01	1.76±0.02	1.77±0.005	1.78±0.01
	10	1.81±0.005	1.81±0.001	1.77±0.02	1.78±0.01	1.75±0.05
	15	1.88±0.001	1.87±0.0	1.78±0.005	1.79±0.0	1.80±0.1
40	7	1.91±0.01	1.890.001	1.87±0.005	1.81±0.01	1.81±0.01
	10	1.95±0.01	1.940.01	1.90±0.004	1.85±0.03	1.85±0.04
	15	1.98±0.01	1.970.01	1.96±0.005	1.93±0.03	1.91±0.01

The different types of abnormalities were fragments, lagging chromosomes, bridges and micronuclei, and these are shown in **Plate 2.**

Table 12: Analysis of variance for the interphase chromosome volume(ICV) at different germinates root length from the artificially aged seeds.

Source	df	SS	MS	F
1. Temperature(T)	3	0.53	0.17	170.71^{**}
2. Treatment duration(D)	2	0.05	0.02	24.32^{**}
3. T X D	6	0.02	0.003	2.81^{*}
4. Root length(L)	4	0.03	0.008	8.25^{**}
5. T X L	12	0.03	0.002	2.46^{*}
6. D X L	8	0.002	0.0002	0.21^{NS}
7. T X D X L	24	0.01	0.0005	0.52^{NS}
8. Pooled error	60	0.04	0.001	
9. Total	119	0.70	0.006	

NS = Nonsignificant at 5% level
* = Significant at 1% level
** = Significant at 0.1% level

Mean percentage of different abnormalities are given in the Table-14. In control mean percentage of abnormal PMCs was 0.97. At 30°C for 7, 10 and 15 days treatment durations,

Table 13: Least significant difference (LSD) values for the interphase chromosome volume at different germination root length.

Temperature (°C)	Treatment durations (days)	Interphase Chromosome volume ($\bar{X}$) in the root lengths of					LSD	
		1.0-2.0 (mm)	2.1-3.0 (mm)	3.1-4.0 (mm)	4.1-5.0 (mm)	5.1-6.0 (mm)	5%	1%
Control (20-25)	15	1.68	1.65	1.62	1.59	1.55		
30	7	1.73^{NS}	$1.73^{\dagger}$	$1.72^{\ddagger\ddagger}$	$1.71^{\ddagger\ddagger}$	$1.65^{\ddagger\ddagger}$		
	10	$1.74^{\dagger}$	$1.74^{\ddagger\ddagger}$	$1.73^{\ddagger\ddagger}$	$1.72^{\ddagger\ddagger}$	$1.73^{\ddagger\ddagger}$		
	15	$1.75^{\dagger}$	$1.74^{\ddagger\ddagger}$	$1.74^{\ddagger\ddagger}$	$1.75^{\ddagger\ddagger}$	$1.74^{\ddagger\ddagger}$		
32	7	$1.76^{\dagger}$	$1.75^{\ddagger\ddagger}$	$1.75^{\ddagger\ddagger}$	$1.76^{\ddagger\ddagger}$	$1.75^{\ddagger\ddagger}$		
	10	$1.76^{\ddagger\ddagger}$	$1.75^{\ddagger\ddagger}$	$1.74^{\ddagger\ddagger}$	$1.77^{\ddagger\ddagger}$	$1.75^{\ddagger\ddagger}$	0.063	0.084
	15	$1.78^{\ddagger\ddagger}$	$1.76^{\ddagger\ddagger}$	$1.75^{\ddagger\ddagger}$	$1.79^{\ddagger\ddagger}$	$1.78^{\ddagger\ddagger}$		
35	7	$1.79^{\ddagger\ddagger}$	$1.77^{\ddagger\ddagger}$	$1.76^{\ddagger\ddagger}$	$1.77^{\ddagger\ddagger}$	$1.78^{\ddagger\ddagger}$		
	10	$1.80^{\ddagger\ddagger}$	$1.81^{\ddagger\ddagger}$	$1.77^{\ddagger\ddagger}$	$1.78^{\ddagger\ddagger}$	$1.75^{\ddagger\ddagger}$		
	15	$1.88^{\ddagger\ddagger}$	$1.87^{\ddagger\ddagger}$	$1.78^{\ddagger\ddagger}$	$1.79^{\ddagger\ddagger}$	$1.80^{\ddagger\ddagger}$		
40	7	$1.91^{\ddagger\ddagger}$	$1.89^{\ddagger\ddagger}$	$1.87^{\ddagger\ddagger}$	$1.81^{\ddagger\ddagger}$	$1.81^{\ddagger\ddagger}$		
	10	$1.95^{\ddagger\ddagger}$	$1.94^{\ddagger\ddagger}$	$1.90^{\ddagger\ddagger}$	$1.85^{\ddagger\ddagger}$	$1.85^{\ddagger\ddagger}$		
	15	$1.98^{\ddagger\ddagger}$	$1.97^{\ddagger\ddagger}$	$1.96^{\ddagger\ddagger}$	$1.93^{\ddagger\ddagger}$	$1.91^{\ddagger\ddagger}$		

NS = Nonsignificant at 5% level

$\dagger$ = Significant at 1% level

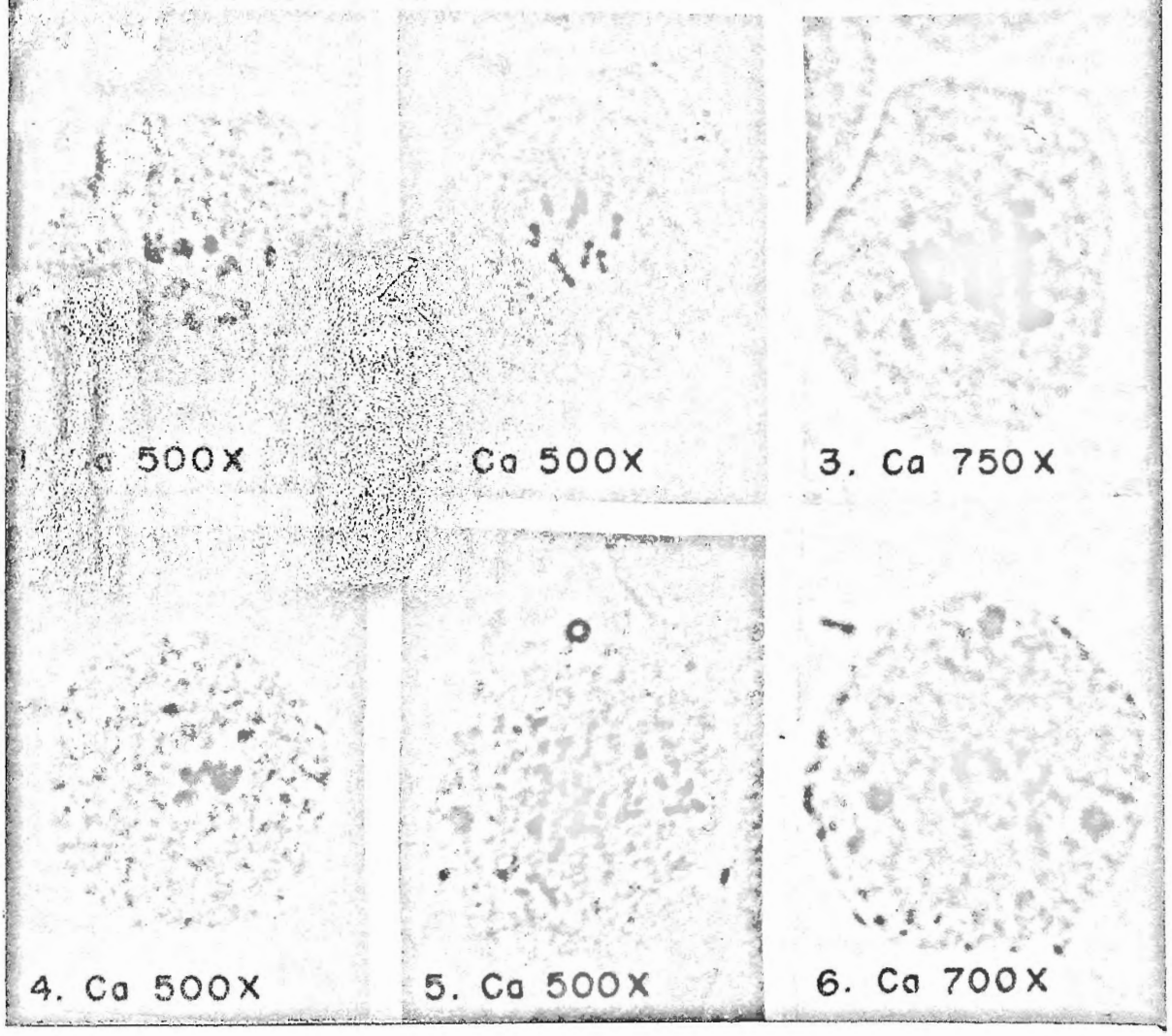
$\ddagger\ddagger$ = Significant at 0.1% level

Plate 2

Photomicrographs showing abnormalities in some of the pollen mother cells

1. Diakinasis (Normal)
2. Pro-metaphase-I (Normal)
3. Metaphase-I with chromosome fragment (arrow headed)
4. Condensed metaphase-I chromosome alongwith chromosome fragment.
5. Irregular distribution of anaphase-II chromosome
6. Tetrahedral arrangement of microspore.

PLATE-2



Ca 500X

Ca 500X

3. Ca 750X

4. Ca 500X

5. Ca 500X

6. Ca 700X

Table 14: Mean percentage of different abnormalities in pollen mother cells (PMCs) of the plants raised from artificially aged seeds.

Temperature (°C)	Treatment duration (Days)	Total PMCs studied	Mean percentage of abnormalites PMCs ($\bar{X}$±S.E)	Mean percentage of different abnormalities. ($\bar{X}$±S.E)			
				Fragments	Laggards	Bridge	Micronuclei
Control (20-25)	15	2969	0.97±0.03	0.21±0.05	0.31±0.01	0.45±0.41	0.0
30	7	3095	1.12±0.18	0.22±0.04	0.62±0.21	0.28±0.19	0.0
	10	2897	1.22±0.08	0.31±0.02	0.35±0.05	0.56±0.41	0.0
	15	2900	1.28±0.03	0.30±0.19	0.37±0.14	0.54±0.40	0.07±0.01
32	7	2379	1.35±0.30	0.21±0.01	0.72±0.21	0.42±0.21	0.0
	10	2698	1.37±0.05	0.21±0.001	0.71±0.20	0.45±0.19	0.0
	15	2984	1.38±0.24	0.19±0.01	0.72±0.18	0.46±0.12	0.01±0.001
35	7	2996	1.40±0.09	0.34±0.02	0.48±0.15	0.48±0.19	0.1±0.01
	10	2705	1.49±0.06	0.36±0.01	0.45±0.11	0.16±0.09	0.07±0.001
	15	2859	1058±0.02	0.41±0.21	0.42±0.20	0.67±0.10	0.08±0.01
40	7	2536	1.75±0.05	0.50±0.30	0.47±0.31	0.78±0.15	0.0
	10	2649	2.21±0.15	0.68±0.64	0.56±0.12	0.85±0.22	0.12±0.01
	15	2798	3.08±0.26	0.53±0.21	1.17±0.23	1.17±0.03	0.21±0.001

percentage of abnormalities were 1.12, 1.22 and 1.28, respectively. At 32°C for 7, 10 and 15 days, abnormalities were 1.35, 1.37 and 1.38%, respectively. At 35°C for same treatment durations percentages of abnormalities were 1.40, 1.49 and 1.58. At 40°C for three different treatment durations abnormalities were 1.75, 2.21 and 3.08%. It was also observed that the percentage of abnormalities increased with the increase of temperature and as well as treatment durations.

Analysis of variance (Table-15) indicated significant effect of both temperature and treatment durations to cause the chromosomal anomalies in pollen mother cells.

Table 15: Analysis of variance for the percentage of different abnormalities in PMCs of the plants raised from artificially aged seeds.

Source	df	SS	MS	F
1. Temperature (T)	3	32.09	10.70	267.50^{**}
2. Treatment duration (D)	2	3.75	1087.00	425.00^{*}
3. T X D	6	5.93	0.99	247.5^{NS}
4. Error	24	0.09	0.004	
5. Total	35	41.85	1.19	

* = Significant at 1% level

** = Significant at 0.1% level.

NS = Non-significant at 5% level

On the other hand, LSD values (Table-16) indicated significant increase of different abnormalities from that of control except in some cases of 30, 32 and 35°C temperature.

9. Chromosome association and chiasma frequency:

In order to study the effect of temperature on chromosome association and chiasma frequency at diakinesis same preparations of PMCs made for meiotic study were used . The PMCs containing only bivalents were observed and the data on chromosome association and chiasma frequency were tabulated which are shown in Table -17. Pattern of chromosome association, percentage of rod and ring bivalents, mean chiasma frequency per PMCs and per bivalents, terminal and interstitial chiasma and terminalization co-efficient were calculated.

In control 78% PMCs were found with 8 ring bivalents and 22% with 7 ring and one rod bivalents. Chiasma frequency per PMCs and per bivalents were 19.62 and 2.45, respectively. Terminalization co-efficient was found to be 0.80.

In PMCs of the plants raised from the treated seeds chromosome association, chiasma frequency per PMC and per bivalents and terminalization co-efficients were found to vary. Percentage of PMCs with 8 ring bivalents were found to range from 78 to 48 and those with 1 rod and 7 ring bivalents ranged from 46 to 22%. In none of the cases uni, tri, or quadrivalent were

Table 16: Least significant difference (LSD) valus for the percentage of different abnormalities in pollen mother cells.

Temperature (°C)	Treatment duration (days)	Mean percentage of different abnormalities				LSD	
		Fragments	Laggards	Bridge	Micro-nuclei	5%	1%
Control (20-25)	15	0.21	0.31	0.45	0.0		
	7	0.22^{NS}	0.62^{**}	0.28^{**}	0.0		
30	10	0.31^{NS}	0.35^{NS}	0.56^{*}	0.0		
	15	0.30^{NS}	0.37^{NS}	0.54^{NS}	0.07^{NS}		
	7	0.21^{NS}	0.72^{**}	0.42^{NS}	0.0		
32	10	0.21^{NS}	0.71^{**}	0.45^{NS}	0.0	0.11	0.14
	15	0.19^{NS}	0.72^{**}	0.46^{NS}	0.01^{NS}		
	7	0.34^{*}	0.48^{*}	0.48^{NS}	0.1^{NS}		
35	10	0.36^{**}	0.45^{*}	0.61^{**}	0.07^{NS}		
	15	0.41^{**}	0.42^{*}	0.67^{**}	0.08^{NS}		
	7	0.50^{**}	0.47^{*}	0.78^{**}	0.0		
40	10	0.68^{**}	0.56^{**}	0.85^{**}	0.12^{**}		
	15	0.53^{**}	1.17^{**}	1.17^{**}	0.12^{**}		

NS = Nonsignificant at 5% level
* = Significant at 1% level
** = Highly significant at 0.1% level

Table 17: Chrosmosome association and chiasma frerquency in plants raised from the artificially aged seeds.

Temp. (°C)	Treatment duration	Total PMCs studied	Chrosmosome association (Number and percentage) Rod	Ring	Rod	Ring	Total No. of chiasmata in 50 PMCs	Range of distribution of chiasmata Terminal (X ± S.E.)	Interstitial (X ± S.E.)	Total	Chiasma frequency per PMC (X ± S.E.)	Chiasma frequency per bivalent (X ± S.E.)	Terminalization co-efficient
			0	8	1	7							
Control (20–25)	15	50	39 (78)		11 (22)		981	156.2 ± 1.51	37.6 ± 2.27	193.8	19.62 ± 0.17	2.45 ± 2.24	0.80
	7	50	31 (62)		19 (38)		947	156.6 ± 1.11	33.2 ± 1.96	189.8	18.94 ± 0.27	2.38 ± 0.04	0.82
30	10	50	34 (68)		16 (32)		944	154.8 ± 0.26	31.2 ± 0.73	186.0	18.88 ± 0.07	2.35 ± 0.01	0.83
	15	50	34 (68)		16 (32)		942	158.4 ± 0.48	30.8 ± 1.65	189.2	18.84 ± 0.06	2.35 ± 0.001	0.84
	7	50	37 (74)		13 (26)		941	157.4 ± 0.17	29.6 ± 2.64	187.0	18.82 ± 0.24	2.35 ± 0.03	0.84
32	10	50	37 (74)		13 (26)		941	157.0 ± 0.95	31.2 ± 0.97	188.2	18.82 ± 0.07	2.34 ± 0.01	0.83
	15	50	31 (62)		19 (38)		941	155.8 ± 0.56	25.6 ± 1.47	181.4	18.82 ± 0.49	2.33 ± 0.05	0.86
	7	50	39 (78)		11 (22)		940	157.6 ± 0.28	28.4 ± 0.68	186.0	18.80 ± 0.05	2.35 ± 0.01	0.85
35	10	50	33 (66)		17 (34)		924	155.2 ± 0.19	29.4 ± 1.12	184.6	18.48 ± 0.05	2.31 ± 0.01	0.84
	15	50	29 (58)		21 (42)		924	155.0 ± 0	30.8 ± 0.49	185.8	18.48 ± 0.07	2.31 ± 0.01	0.83
	7	50	24 (48)		26 (52)		923	153.3 ± 0	27.2 ± 0.74	180.5	18.46 ± 0.09	2.31 ± 0.01	0.85
40	10	50	27 (54)		23 (46)		916	154.8 ± 0.35	28.0 ± 1.23	182.8	18.32 ± 0.05	2.29 ± 0.01	0.85
	15	50	34 (68)		16 (32)		894	155.0 ± 0.34	27.0 ± 1.12	182.0	18.88 ± 0.22	2.29 ± 0.02	0.85

Note: Values within the parenthesis indicate the percentage of two different chromosome association.

found. Chiasma frequency per PMC and per bivalent were somewhat less compared to that of control, and ranged from 18.94 to 17.88 and 2.35 to 2.29, respectively. Terminalization co-efficient was also somewhat less than that of control and ranged from 0.86 to 0.82.

Analysis of variance (Table-18) indicated significant effect of temperature independently. Treatment duration and inter action of temperature and treatment duration were found to show non significant effect in changing the chromosome association, chiasma frequency and terminalization co-efficient.

Table 18: Analysis of variance for the chiasma frequency per PMC in plants raised for the artificially aged seeds.

Source	df	SS	MS	F
1. Temperature (°C)	3	4.08	1.36	7.16^{**}
2. Treatment duration	2	0.62	0.31	1.63^{NS}
3. T X D	6	0.66	0.11	0.59^{NS}
4. Error	48	9.36	0.19	
5. Total	59	14.72	0.25	

** = Significant at 0.1% level

NS = Non-significant at 5% level

10. Yield and yield components:

Data on yield and yield components were recorded from the plants at maturity raised from the treated and untreated seeds. Mean values for yield and yield components are given in Table-19. The data were analyzed statistically and presented in Table-20.

In case of plant height and primary branches length, temperature and treatment duration showed non significant effect, while their interaction was observed. In case of secondary branches, pods/plant, pod volume and 100 seed weight effect of temperature, treatment duration and their interaction were non-significant. In case of seeds/pod both temperature and treatment duration showed significant effect statistically while their interaction was found to be non significant.

Table 19: Mean values for yield and yield components in plants raised from the artificially aged seeds.

Temp. (°C)	Treatment duration (days)	Mean values of different characters (X ± S.E.)						
		Plant height (cm)	Primary branch length (cm)	Secondary branch length (cm)	Pods per plant	Pod volume (cc)	Seeds per pod	100 seed weight (gm)
Control (20–25)	15	67.27 ± 2.27	65.36 ± 4.77	44.01 ± 3.21	124.90 ± 5.62	5.16 ± 0.05	2.18 ± 0.01	17.75 ± 1.48
30	7	61.34 ± 0.33	60.33 ± 0.35	43.01 ± 4.04	86.53 ± 6.00	4.16 ± 0.02	1.30 ± 0.01	16.08 ± 0.14
	10	53.20 ± 1.61	52.89 ± 2.33	28.36 ± 1.58	90.90 ± 5.98	4.34 ± 0.16	1.28 ± 0.03	14.00 ± 1.39
	15	56.47 ± 3.78	52.88 ± 4.51	32.67 ± 4.66	79.27 ± 0.33	4.08 ± 0.08	1.23 ± 0.01	15.43 ± 0.26
32	7	59.34 ± 2.45	55.78 ± 4.31	33.70 ± 1.85	85.07 ± 11.12	4.05 ± 0.03	1.19 ± 0.02	15.31 ± 0.62
	10	52.67 ± 0.64	48.45 ± 0.86	28.78 ± 1.09	109.60 ± 14.41	4.15 ± 0.19	1.17 ± 0.003	15.46 ± 0.41
	15	55.90 ± 4.07	57.06 ± 6.08	34.66 ± 5.73	93.63 ± 9.93	3.91 ± 0.37	1.16 ± 0.01	13.68 ± 1.64
35	7	47.53 ± 3.88	43.32 ± 3.65	24.26 ± 0.20	72.07 ± 14.26	4.21 ± 0.18	1.15 ± 0.01	16.75 ± 1.07
	10	60.17 ± 4.00	56.14 ± 3.65	39.34 ± 3.26	112.00 ± 5.72	4.08 ± 0.08	1.14 ± 0.001	14.58 ± 1.11
	15	69.43 ± 6.64	60.28 ± 7.53	44.83 ± 1.27	88.80 ± 1.47	3.94 ± 0.20	1.14 ± 0.003	14.04 ± 1.06
40	7	62.17 ± 0.29	63.21 ± 3.78	40.67 ± 3.00	103.74 ± 22.99	3.92 ± 0.18	1.13 ± 0.002	14.45 ± 1.78
	10	53.63 ± 5.35	49.00 ± 5.07	32.34 ± 5.07	77.47 ± 11.64	4.20 ± 0.11	1.11 ± 0.001	14.77 ± 1.92
	15	54.33 ± 8.37	52.25 ± 9.21	33.46 ± 5.10	68.33 ± 0.72	4.57 ± 0.24	1.08 ± 0.002	14.35 ± 1.54

Table 20: Analysis of variance for yield and yield components in plants raised from artifically aged seeds.

Source		Temperature	Treatment duration	T X D	Error	Total
df		3	2	6	24	35
Plant height	SS	35.24	128.95	905.19	1366.65	2436.03
	MS	11.75	64.47	150.86	56.94	69.60
	F	0.21^{NS}	1.13^{NS}	2.65^{*}		
Primary branch	SS	72.72	115.73	1087.86	1677.82	2954.12
	MS	24.24	57.86	181.31	69.91	84.40
	F	0.35^{NS}	0.83^{NS}	2.59^{*}		
Secondary branch	SS	25.26	129.03	912.85	1738.87	2806.01
	MS	8.42	64.51	152.14	72.45	80.17
	F	0.12^{NS}	0.89^{NS}	2.10^{NS}		
Pods/ plant	SS	893.21	1400.72	4113.57	8425.13	14832.62
	MS	297.74	700.36	685.59	351.05	423.79
	F	0.85^{NS}	1.99^{NS}	1.95^{NS}		
Pod volume	SS	0.22	0.07	0.88	2.30	3.48
	MS	0.07	0.03	0.15	0.09	0.10
	F	0.77^{NS}	0.33^{NS}	1.67^{NS}		
Seeds/ Pod	SS	0.13	0.01	0.004	0.01	0.155
	MS	0.04	0.005	0.001	0.0004	0.004
	F	96.00^{**}	12.00^{**}	1.60^{NS}		
100 seed weight	SS	2.32	10.54	15.16	107.90	135.93
	MS	0.77	5.27	2.56	4.49	3.88
	F	0.17^{NS}	1.17^{NS}	0.56^{NS}		

NS = Nonsignificant at 5% level
* = Significant at 1% level
** = Significant at 0.1% level

DISCUSSION

The results obtained in the present study are discussed under the following heads:

1. Seed germination,
2. Time of first mitosis,
3. Frequency of aberrant anaphase,
4. Relationship between aberrant anaphase and seed germination,
5. Relationship between aberrant anaphase and treatment durations,
6. Mitotic index and chromosomal abnormalities,
7. Interphase chromosome volume,
8. Meiotic irregularities,
9. Chromosome association and chiasma frequency, and
10. Yield and yield components.

1. Seed germination

Studies on germination of artificially aged seed in laboratory and in the field indicated a decrease of seed viability with the increase of both temperature and treatment duration. Murata *et al.* (1981) stated that temperature and moisture content are the main factors influencing seed longevity. High temperature and moisture accelerate loss of viability in most species (Owen 1956, Barton 1961, Abdalla and Roberts 1968, Roberts 1972, Okusanya 1977,1978). The results

obtained in the present study with artificially aged seeds also demonstrated the deteriorative effects of high temperature. Germination percentage of the seeds treated at 40°C for 15 days indicated that this temperature is not encountered constantly in natural habit, and it may be stated that the seeds of chickpea could respond better to alternating temperature than that of control. However, Frisen and Shebiski (1962) found the greatest number of oat seeds germination at temperature of 60 and 70°F. At 50°F, emergence was slow and lagged well behind that obtained at higher temperature.

In the present study, treated seeds lost germinability significantly and it might be due to the consumption of respiratory substances which leads exhaustion of that reserve materials. Akhter *et al.* (1992), Islam *et al.* (1992), Islam (1993) and Chowdhury (1994) reported similar results.

Seed longevity is found to vary among and within species. Aufhammer and Simon (1957) found 12% germination in barely seeds stored for 123 years in a glass vial embedded in the foundation stone of a building in Nuremberg. Haferkamp *et al.* (1953) reported that 96% of barely seed germinated after 32 years storage in unsealed containers at air temperature. Robertson *et al.* (1943) found that germination of barely seeds dropped to 46.2% of the initial germination after 21 years in a dry and unsealed room, indicating much shorter longevity of the barely seeds.

In the present study, seeds were treated at 30, 32, 35 and 40°C and germination percentage decreased progressively. Riaz *et al.* (1973) treated seeds of American cotton at 50-90°C for 24

and 48 hours and found stimulatory effect of 50-70°C on emergence of seedling. Above 70°C, temperature killed the seeds. Gramshow (1972) reported relationship between seed age and germinability.

Some seedlings emerged from the artificially aged seeds in field showed some sort of abnormalities and they were not found survive. Necrotic abnormalities in roots and shoots of some seedlings of *Pisum sativum* (Murata *et al.* 1980) and of *Crepis.* (Navashin 1933, Navashin and Gerassimova 1936a, b.) were reported. Some seeds treated at 40°C in the present study showed delayed germination. This delayed germination might be related to increased temperature. It indicated that the deteriorative effects of seed aging inhibited the metabolic process to some extent. Murata *et al.* (1981) suggested that this type of inhibition might have occurred during the early stage and might be related to genetic changes induced by seed aging.

2. Time of first mitosis:

The time first mitosis was estimated from the number of anaphase cells per root in the control and artificially aged seeds under different temperature regimes following the schedule of Murata *et al.* (1977, 1978). They used different root lengths as an indicator of the first mitosis considering anaphase stage as a prime condition of the nuclear division. The time of first mitosis in root tip cells induced by artificially aged seed was found to delay. However, in seeds with less than 70% germination, roots were 1.5 mm longer before first mitosis occurred as compared with the

control seeds. This delay was associated with slow activation of cell division following the start of the first mitosis and it was taken into consideration in scoring chromosomal aberrations at first mitosis.

In this study, it was observed that the frequency of aberrant anaphase and of roots with aberrant anaphase were higher as the temperature increased. Similar results were also found by Murata *et al.* (1981), in case of barely, with the increase of storage time. In this study, it was also observed that germination percentages declined in all temperature treatments. Similar results were obtained with two to four years old onion seeds (Orlova *et al.* 1976), and artificially aged seeds (Murata *et al.* 1980). Barber and Callen (1942) observed that the progress of cell division might be modified or suppressed by a great variety of agents. Subnormal temperature is known to interfere with the mitotic spindle in plant cells (Darlington and La Cour 1940). Any physical factor may disturb the respiratory pathways of plants which results abnormalities. It is reported that the rate of mitosis is closely related to the resultant level of ATP. Rosch (1950) observed inhibition of mitosis in root tip cells of *Allium cepa* when irradiated with X-rays.

3. Frequency of aberrant anaphase:

It was clearly observed that the frequency of aberrant anaphase and of roots with aberrant anaphase were higher as the treatment time increased and the germination percentages declined in all the treatment conditions. The increasing rate of both the frequencies with increased treatment

time was accelerated by the higher temperature. The mutagenic effects of higher temperature and moisture content on chromosomes have been reported by Peto (1933), Navashin and Shkvarnikov (1933), Shkvarnikov and Navashin (1934), Shkvarnikov (1936 a, b) and Carteledge *et al.* (1936). In contrast to these results, Smith (1943, 1946) found no cytological changes in root tip cells of wheat and barley seeds treated by high temperature (60 to 80°C) alone. However, more genetic changes were observed in the treatment of high moisture content seeds with high temperature (Harrison 1966, Roberts *et al.* 1967, Abdalla and Roberts 1968, Villiers 1974, 1975, Villiers and Edgumbe 1975).

At anaphase, bridges were most frequent type and they were found in all the treatments. The separation of the chromatid at mid prophase of mitosis is a strong deviation from the normal one. Generally, the chromatid separate only at late metaphase after some biochemical reactions take place between the two sister chromatids (Sybenga, 1974). When a pair of fragments were found in the cell at anaphase apart from those which were single (chromatid) may have arisen due to breakage of chromosome rather than chromatids (Caldercott and Smith 1952). Yagyn and Morris (1957), Prasad (1972), Kalloo (1972), Shaikh and Godward (1974) and Singh and Godward (1972,a) reported that the paired bridges at anaphase were found and it was due to the fusion between broken chromosomes rather than broken chromatids. Goplan and Njagi (1984) reported the formation of similar anaphase bridges in *Vicia faba* root tip cells with a variety of agents.

4. Relationship between aberrant anaphase and germination:

The pattern of increase of the frequency of aberrant anaphase with increase of temperature treatment was closely related to loss of germinability in chickpea seeds. This agreed well 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 (Abdalla and Roberts, 1968, Harrison and Mc Leish 1954) and Harrison (1966) also mentioned that the increase in number of abnormal anaphase per root in five years old lettuce seeds was closely associated with loss of germinability. They indicated that the increase of chromosome damage with decreasing germination was quite rapid between 90% and 70% germination. In the present investigation, rapid increase in the frequency of abnormal anaphase was related with the loss of germinability. Murata *et al.* (1981) clearly observed that the frequencies of aberrant anaphase and of roots with aberrant anaphase were higher as the storage time increased, and the germination percentages declined in all storage conditions.

Akhter (1990) found a close relationship between the loss of seed germinability and frequencies of chromosome mutations from root tip cells of different years old wheat and barley seeds.

In this study, however, a good correlation between the increase of aberrant anaphase and decrease of seed germination was observed. This result indicated that the chromosomal anomalies might be gradually taking place before the loss of seed germination.

5. Relationship between aberrant anaphase and treatment durations:

Aberrant anaphase and treatment durations studied at different root lengths indicated both positive and negative relationship. In some cases, no relation was observed between aberrant anaphase and treatment durations. Negative relationships were observed at the root length of 1.0-2.0 mm, 2.1-3.0 mm and 5.1-6.0 mm at 30°C, 2.1-3.0 mm and 5.1-6.0 mm at 32°c, 4.1-5.0 mm and 5.1-6.0 mm at 35°C, and at the root length of 2.1-3.0 mm and 5.1-6.0 mm at 40°C. However, the relationship between the frequency of aberrant anaphase and root length could not be found, but some increase of aberrant anaphase were noticed with the increase of root length. It was also noticed that more than 5 mm long roots showed increased abnormalities at all temperatures. Murata *et al.* (1980) reported the similar results. In the present study, it was obviously observed that treatment duration increased somewhat the aberrant anaphase. However, in most of the cases negative relation indicated that the rate of increase of aberrant anaphase with increasing treatment duration was not directly related. On the contrary, this was not entirely unexpected because there are two main sources of aberrant anaphase. One is the regime of temperature and the other is treatment duration. Although, the latter source showed negative attitude, but it played somewhat positive role in this study. At the same time root length was considered for causing this irregularity.

But it showed no such significant role below the length of 2.5 mm . Rather, it was observed that increase of temperature increased abnormality at all ranges of root lengths. Murata *et al.* (1981) reported 6.00% aberrant anaphase at root lengths of 2.1-3.00 mm, dropped to 3.07 in roots of 3.1-4.0 mm long and increased again with root elongation up to 7 mm. In a vary few cases intermediate and positive relation was observed and it indicated a weak dependence of aberrant anaphase on treatment duration. In some cases relationship was nil. Thus from overall findings in the present study, it may be stated that increase of aberrant anaphase does not depend on treatment duration solely, rather depends on high temperature and root length. Only a few positive relation may be a variable result and might even be negative.

6. Mitotic index and chromosomal abnormalities:

In the study of mitosis root tips (1.0-1.5 cm) were found to show the mitodepressive effect with the increase of temperature as well as treatment time. Akhter (1990) found a strong mitodepressive effect on *Allium cepa* roots. They observed that with the increase of the age of seeds as well as with the increase of the concentration of wheat and barley seeds extracts percentage of cell division decreased. Depression of cell division was seen obviously in the root of chickpea emerged from the seeds treated at 40°C for 15 days where the mitotic index reached not more than 6.7% compared to 22% in the control. Shehab (1979) found similar results on mitotic index of onion. Jain and Sarbhoy (1987) found a considerable fall in the mitotic index of all *Lens*

and *Pisum* induced by chlorinated hydrocarbon. However, Murata *et al.* (1980) reported mitotic delay in roots of pea induced by artificial seed aging.

Barber and Callen (1942) observed that the progress of cell division might be modified or suppressed by a great variety of agents. Subnormal temperature is known to interfere with the mitotic spindle in plant cell (Darlington and La Cour 1940). The present findings might be due to high temperature treatment which caused exhaustion of respiratory reserve substances and caused ultimately mitotic inhibition. The rate of mitosis is closely related to the resultant level of ATP (Epel,1963) and it might be that cell division is energy dependent process and thereby, the movement of chromosomes depends mainly upon the energy generating system.

Mitotic studies revealed different types of abnormalities also in the root tip cells of chickpea. Most of the abnormalities were chromosome fragments, laggards, chromosome bridges and micro-nuclei. Murata *et al.* (1981) reported chromosomal damage in barley induced by artificial seed aging. Jain and Sarbhoy (1987) found the effect of chlorinated hydrocarbon as partial or entire inactivation of spindle mechanism followed by scattering of chromosome. Smith (1943,1946) reported that exposing seeds of cereals to temperatures of 50-70°C for 5-15 days or 80°C for 45-80 minutes had little effect upon the frequency of chromosome aberrations. Gunthardt *et al.* (1953) observed chromosome aberrations and genetic mutations in plants raised from storing seeds of several economic crop species. However, decreased viability and increased

cytogenetic changes were observed in the chickpea of the present study. Abdalla and Roberts (1968) reported similar results.

At anaphase and telophase stages bridges were most frequent type and they were found in all the treatments. The separation of the chromatid at mid prophase of mitosis is a strong deviation from the normal one generally, the chromatids separate only at late metaphase after some biochemical reaction place between the two sister chromatids (Sybenga 1974). When a pair of fragments were found in the cell at anaphase stage apart from those which were single (chromatid) and may have arisen due to breakage of chromosome rather than chromatids (Caldecott and Smith 1952). Yagyu and Morris (1957), Shaik and Godward (1974) reported that the paired bridges at anaphase stages were found and it was due to the fusion between broken chromosome rather than broken chromatids (Sax 1940, Caldecott and Smith 1952). The single bridge results from the fusion of two or more broken chromatids (Sax 1940). Goplan and Njagi (1984) reported the formation of similar anaphase bridge in *Vicia faba* root tip cells induced with a variety of agents.

Smith (1943, 1946) found cytological changes in root tip cells of wheat and barley seeds treated by high temperatures (60 to 80°C) alone. Peto (1933), Shkvarnikov and Navashin (1934), Shkvarnikov (1936 a, b) and Cartledge *et al.* (1936) reported also the mutagenic effects of high temperature. However, some works have confirmed that more genetic changes are observed due to combined treatment of high moisture and temperature (Harrison 1960, Roberts *et al.* 1967, Abdalla and Roberts 1968, Villiers 1974, 1975, Villiers and Edgeumbe 1975).

In the present study, other abnormalities like laggards, fragments and micronuclei were also found as radionimetic effects of temperature. This might be due to the exhaustion of respiratory reserve substance during the treatment of seeds by high temperature. Any physical factor may disturb respiratory pathways of any crop species which results abnormalities in mitotic cells (Islam 1991).

7. Interphase chromosome volume (ICV):

Interphase chromosome volume studied from the root tip cells of chickpea seeds treated by different constant temperature for different treatment time along with control were recorded. The relationship of ICV with different root length and constant temperatures for different treatment durations indicated that with increase and decrease of the treatment duration of constant temperature there was an increase and decrease of ICV. ICV in root tip cells of different root length emerged from the treated seeds were found to be higher than that of the control and almost all the differences between them were found to be statistically significant. The increase and decrease of ICV results with an increase and decrease of the alteration in cell membrane configuration and permeability, modification of chromosomal proteins and changes in sensitivity to chemicals. Yamakawa and Sparrow (1965, 1966) studied the ICV as a reliable index of radiosensitivity in plant. Their work established a positive linear correlation between radiation sensitivity and chromosome volume in plant cells. They reported that correlation of ICV and

reduction of viable seed set by chronic irradiation of 21 cultivated plants during reproductive stage in one hand and the correlation of ICV with pollen abortion induced by chronic gamma irradiation on the other hand. Sparrow *et al.* 1968 (Cited in Nayer *et al.*, 1970) found that increase in mutation rate per roentgen in plants of five higher plants was highly correlated with an increase in both ICV and DNA content per chromosome. Niger *et al.* (1970) also reported that radiosensitivity of plants measured in terms of meiotic abnormalities in pollen mother cells growing in high radiation area of Kerala coast and adjoining areas in South India was found to show significant positive correlation with ICV. Underbrink *et al.* (1973) found positive roles of interphase chromosome volume (ICV), nuclear volume (NV) and ploidy on the degree of pollen abortion induced by radiation. The maximum percentage of aborted grains was determined over a post irradiation period equivalent to an entire period of microsporogenesis. Dose response curve constructed for each materials was found to vary in slope.

Statistical analysis in the present study indicated significant role of temperature, treatment durations, root length and the interaction of temperature and treatment duration, temperature and root length in increasing interphase chromosome volume.

However, ICV is considered to be reliable index of mutagen sensitivity in plant cells. Such type of effect in plant cells due to the action of cold and heat treatment may also be help in predicting the mutation rate.

8. Meiotic irregularities:

Studies on pollen mother cells of the plants raised from the treated seeds revealed different chromosomal anomalies which are namely chromosome fragment, lagging chromosome, bridges, micronuclei etc. The percentages of bridges and lagging chromosomes were higher than fragments and micronuclei. However, their frequencies were found to vary from treatment to treatment. Highest percentage of chromosomal anomalies were found at 40°C for 15 days and lowest in control. It was also observed that with an increase of temperature percentage of the abnormalities increased gradually. Dowrick (1957) studied the influence of temperature on meiosis. In his paper he stated that many works (Plough 1917, 1921, White 1934, Barber 1941, 1942, Swanson 1941, 1942a,b, 1943 and Elliot, 1955) have shown that change in temperature can affect the general pattern of meiosis as well as form and behavior of the chromosomes. These workers frequently considered the influence of isolated temperature on a comparatively small range of temperature. However, Dowrick (1957) noticed that chromosomes may inhibit three major effect from changes in temperature. These are alternation in the frequency and changes in the position of chiasmata and variations in internal coiling. All these effects may occur simultaneously but for convenience chromosome coiling was considered separately. Other minor effects were also considered.

In the present study, it was observed that the percentages of abnormalities increased somewhat with the increase of temperature. Emsweller and Baierley (1943) studied the regularity of nuclear and cell divisions in plants considerably affected by many agents, such as X-rays,

ultraviolet light, various chemicals, disease and abnormal temperatures. Both low and high temperature, as well as a sudden changes from one to the other, have been shown to be particularly effective. They discussed some of the meiotic irregularities that arose in *L. longiflorum* as the result of a sudden change from low to high temperature. The meiotic irregularities observed in Cereal and Giganleum varieties of *L. longiflorum* exposed to 45-46°C for 30 minutes.

The suppression of chromosome pairing was also noticed by heat treatment. Sax (1937) studied the effect of sudden temperature changes on meiosis in *T. paludosa.* The treatments were more effective when plants were transferred from 8-38°C. Such chromosomal aberrations as interchanges, inversions, fusions and fragmentations were induced. Dermen (1938) found that fragmentation, fusion and chromatid bridges were induced in *Rhoeo discolor* by changing from either a low to high temperature for the reverse. Kamizyo and Nobunori (1982), Smith (1943, 1946), Dowrik (1957), Abdalla and Roberts (1968), Utkhede and Jain (1970) and Roberts (1973) also observed same result in their investigation.

Structural changes in both mitotic and meiotic cells of four species of legumes were reported by Shaikh *et al.* (1980). They observed bridges, fragments, micronuclei, laggards and regenerated cells, fragments of nucleus and univalents in the meiotic cells. Gamma rays induced chromosomal abnormalities in first meiosis of two tetraploid wheat varieties (Shaha *et al.*, 1980). The abnormalities observed by them were laggards, unequal distribution of chromosomes at anaphase-I and formation of micronuclei at telophase-I. Shaikh (1972) noticed increased

percentage of abnormal cells in *Lathyrus sativus* and *Vicia ervilia* treated with gamma rays. In the present study, mainly four types of abnormalities were observed of which laggard and bridges were of most frequent. Sudhakaran (1971) and Jayabalan and Rao (1987 a) reported that the aberrant behaviors of bivalents and multivalents which lag in different stages might be due to delayed terminalization or stickiness of the ends of the chromosome and chromosome bridges may arise by failure of terminalization in a few cells. Such bridges have been reported by Sax (1960). Micronuclei were found but with vary low frequency and this might be due to bivalent lagging chromosomes. The lagging univalents do not give rise to micronuclei but are lost in the cytoplasm (Iyengar 1939).

9. Chromosome association and chiasma frequency :

Effect of aging on chromosome association and chiasma frequency were recorded at diakinesis/first metaphase. The results indicated a preponderance of ring and rod bivalents in each treatment of chickpea. Univalent, trivalent and quardivalents were absent in all the treatments. Mean chiasma frequency per bivalent was maximum at control (2.45) and minimum (2.29) at 40°C for 10 and 15 days treatment durations. It was observed that the mean chiasma frequency increased and decreased with the increase and decrease of constant temperature along with treatment durations. There was no changes in mean chiasma frequency per bivalent in comparison to control. Similar results were observed by Marquardt (1951). Hayter and Riley (1967) attributed the failure of chromosome pairing and chiasma formation in 5D deficient plants to the inability of

the LTP alleles on chromosome 5A to stabilize chiasma frequency at low temperature in case of *T. aestivum*. It had been reported in *Delphinium* (Jain and Basak, 1965) that the chiasma frequency at nuclear level showed no proportionate decrease, even though there was univalent formation following mutagenic treatments, since rest of the bivalents formed more chiasmata in such cases than they normally do.

Chiasma frequency per PMC and per bivalent were somewhat less compared to that of control in the present study. Lowrence (1960) studied the effect of irradiation on development stages of microsporogenesis for chiasma frequency in *Lillium* and *Tradescantia* which indicated the sensitive periods restricted to late zygotene and early pachytene stages. Based on a detailed study, Lawrence (1960) concluded that the chiasma frequency following mutagenic treatments might possible occur at two stages namely during DNA synthesis and in the second sensitive period at or slightly before the stage during which chiasma formation is generally considered to occur. In the former case, the decrease in the frequency of chiasma may be due to the disturbance in chromosome coiling, failure or restricted pairing at pachytene and delay in DNA syntheses, while in the later it may affect the process leading to chiasma formation. In the present study, reduced chiasma frequency might be due to delay in DNA synthesis or some other responses occurred due to temperature treatment.

The variation in chiasma frequencies brought about in *T. bracteata* and *U. perfoliata* by changes in temperature are similar to those found by Plough (1917) in *Drosophila* after consideration has been given to the mistake pointed out by Smith (1936). They are also similar to

the results of Elliot (1955) with *Hyacinthus orientalies* and resemble those of White (1934) in species of *Locusta, Sohistocerea* and *Stenobothus*.

In this study, it was observed that from control to highest temperature treatment chiasma frequency decreased somewhat. Same result was observed by Darlington *et al.* (1937). Where the reduction in chiasma frequency, which occurred at high temperatures has not been demonstrated in animals nor in *H. orientaties*. While these temperatures would be lethal to the animals . It seems likely that a reduction in chiasma frequency would have occurred in *H. orientaties* had the experiments been continued above 20°C. In the absence of terminalization the number and position of chiasmata are directly related to the degree of chromosome pairing at pachytene (Wilson, 1959). However, in the present study terminalization was also found to vary due to temperature treatment.

The maximum number of chiasmata are evidently formed at different temperatures in different spices. This maximum is undoubtedly related to the conditions under which growth normally occurs. Most species are adapted to given an optimum amount of crossing over at normal temperature (Dowrick, 1957). At high temperature, pairing, if occur at all, is confined to the chromosome ends and centromere region of the long arms (Darlington, 1940).

High temperature may have similar effects like infra-red radiation, gamma radiation and numerous chemicals agents which affect chromosome movement and the chromosome association and chiasma frequency.

10. Yield and yield components:

When any plant materials are induced by external factors the structural and chemical changes take place and then the effects become visible morphologically and genetically. Heat, age and X-rays have been reported to produce a number of similar genetical, cytological and physiological effects in seed and in plants grown from those treated seeds. After appropriate action of these agents, germination has been reported to be lower and slower, and reduced viability of the seedlings (Shkvarnikov, 1936 and Crocker *et al.*, 1917)

In the present study, effect of different constant temperatures for different treatment durations on yield and yield components along with the control were studied.

In case of plant height and lengths of primary branches both temperature and treatment duration showed no statistical effects, while their interaction indicated significant effects. Islam (1991) reported similar results in three cereal crops. Non-significant effect of temperature and treatment duration were also found in case of the length of secondary branches, pods/plants, pods

volume and 100 seed weight. While they showed significant effect for seeds/pod in the presents study.

However, it is stated that high temperatures may shorten period of early stages of development and this effect can be seen in later stages of development. The early stages of plant growth are extremely critical since later development and hence crop productivity depends on germination and seedling emergence. Before a seed can germinate, it must be placed in favourable conditions (Kundu *et al.*, 1989). In case of chickpea, with delay in flowering, grains develop at increasing high temperatures and lower relative humidities, with the consequence that yield traits diminish progressively. However, in the present study, the materials were treated at high temperatures prior to seeding in the field. Thus, it may be stated here that both artificial and natural temperature minimized the yield and yield traits and caused no such deterioration.

Summerfield *et al.* (1984) conducted an experiment with two genotypes of chickpea, which were transferred between cabinets at various times so that they experienced either warm (30°C) or hot (35°C) for different durations. They observed that the greater the proportion of the reproductive period spent in hot days the smaller the seed yield produced. Multers *et al.* (1988) found high temperatures to cause reduction in grain in cowpea that were associated with low pollen viability and pod set. The present findings indicate that artificial aging prior to seeding might have little effect on yield and yield traits.

SUMMARY

This investigation was un ertaken to study the artificial aging effect on germination, chromosomal changes and yield in chickpea. The seeds of chick pea (*Cicer arietinum* L.) were used as plant material in this study and they were procured from Regional Agricultural Reseach Station, Ishurdi, Pabna.

Aging of seeds was made artificially by constant temperature at 30°, 32°, 35° and 40°C for 7, 10 and 15 days treatment durations. Control seeds were not treated.

Ten experiments were conducted in this study and they are- (1) Germination test, (2) Time of first mitosis, (3) Frequency of aberrant anaphase, (4) Relationship between aberrant anaphase and seed germination, (5) Relationship between aberrant anaphase and treatment duration, (6) Mitotic index and chromosomal abnormalities, (7) Interphase chromosome volume, (8) Meiotic irregularities, (9) Chromosome association and chiasma frequency, and (10) Yield and yield components.

It was observed that both in laboratory and in the field, germination percentage decreased gradually with the increase of temperature as well as treatment durations. Untreated seeds showed the highest percentage of germination (in laboratory 97.27% and 92.91% in field). Lowest germination percentage (73.21%) was found in field form the seeds treated at 40°C for 15 days.

Relationship between germination percentage and temperature indicated progressive decrease in germination with the increase of temperature.

For the study of first mitosis, germinating root tips of different lengths (1.0-2.0, 2.1-3.0, 3.1-4.0, 4.1-5.0, 5.1-6.0 mm and 1.0-1.5 cm) were fixed in 1:3 acetoalcohol and after 48 hours of fixation they were preserved in 70% ethanol. Root tip cells were stained with haematoxylin, and number of anaphase cells ere determined.

Maximum number of anaphase cells were observed in longer ones compared to short size roots. In most of the treatments minimum root length with maximum anaphase cells was found to be 1.5 mm. Seeds with delayed germination began mitosis at root length below than 1.0 mm. This delay was found to be associated with loss in germination and indicated a relationship of mitosis with aging.

Aberrant anaphase cells were studied at different germinating root lengths. Frequency of aberrant anaphase were found to increase with the increase of temperature. An interesting result was obtained that in roots of 1.0-4.4 mm the frequency of aberrant anaphase was very less. But in root lengths above 4.0 mm frequency of aberrant anaphase was found to increase.

The pattern of increase for the frequency of aberrant anaphase with the increase of temperature was closely related to the loss of seed germination.

Aberrant anaphase and treatment durations studied at different root lengths indicated both positive and negative relationship. In some of the cases, no relation was observed between aberrant anaphase and treatment durations.

Mean percentage of dividing cells and different abnormalities were determined at the root length of 1.0-1.5 cm. It was observed that the percentage of dividing cells decreased gradually with the increase of temperature as well as treatment durations. In control percentage of abnormalities was very less (0.40) but with the increase of temperature abnormality increased gradually. Statistical analysis indicated significant effect of temperature, treatment duration and their interaction to cause the chromosomal anomalies. LSD values indicated that laggards and bridges were highly significant at 40°C for all the treatment durations. Laggards and bridges were found to be significant at 32 and 35°C for the 15 days treatment durations, respectively. In rest of the treatments none was found significant.

Interphase chromosome volume (ICV) in root tip cells of control seeds was found to be $1.68m^3$ at the root length of 1.0-2.0 mm and it was found to decrease with the increase of root length. In root tip cells of treated seeds similar results were obtained. On the contrary, ICV was found to increase remarkably with the increase of temperature compared to that of treatment durations. Among four different temperature regimes and three treatment durations 40°C for 15 days treatment time was found to be more effective to increase the interphase chromosome volume. Analysis of variance indicated significant effect of temperature (T), treatment durations (D), root length(L) and the interaction of TXD and TXL on ICV. Interaction of DXL and

TXDXL showed non significant effect. LSD values indicated that all the treatments significantly influenced the interphase chromosome volume, except at 30°C for 7 days.

The pollen mother cells (PMCs) of the plants raised from treated and untreated seeds showed various types of chromosomal aberrations. These were fragments, lagging chromosomes, bridges and micro-nuclei. It was also observed that the percentage of abnormalities increased with the increase of temperature and as well as treatment durations. Analysis of variance indicated significant effect of temperature, treatment durations and their interaction to cause and increase the chromosomal anomalies. On the other hands, LSD values indicated significant increase of different abnormalities, except in some cases of 30°, 32° and 35°C temperature from that of control.

The PMCs were found with bivalents only. In control 78% PMCs were found with 8 ring bivalents and 62% with 7 ring and one rod bivalents. Chiasma frequency per PMCs and per bivalent were 19.62 and 2.45, respectively. Terminalization co-efficient was found to be 0.80. In PMCs of the plants raised from the treated seeds chromosome association, chiasma frequency perPMC and per bivalents and terminalization co-efficients were found to vary.Percentage of PMCs with 8 ring bivalents were found to range from 78 to 48 and those with 1 rod and 7 ring bivalents ranged from 46 to 22%. In none of the cases uni, tri or quadrivalent were found. Chiasma frequency per PMC and per bivalent were somewhat less compared to that of control, and ranged from 18.94 to 17.88 and 2.35 to 2.29, respectively. Terminalization co-efficient was

also somewhat less than that of control and ranged from 0.86 to 0.82. Significant effect of temperature was found to vary the chiasma frequency.

Data on yield and yield components were recorded from the plants at maturity raised from the treated and untreated seeds. In case of plant height and primary branches length, temperature and treatment duration showed non-significant effect individually, while significant effect of their interaction was observed. In case of secondary branches, pods/plant, pod volume and 100 seed weight, effect of temperature, treatment duration and their interaction were non-significant. In case of seeds/pod both temperature and treatment duration showed significant effect while their interaction was found to be non significant.

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