POLYPLOIDY AND ITS APPLICATION IN FORESTRY

AND

A PRELIMINARY STUDY OF ABERRANT DOUGLAS-FIR SEEDLINGS

by

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ABSTRACT

The paper reviews the literature on polyploidy in respect to its possible application in forest tree breeding. The occurrence of spontaneous polyploidy in various species is enumerated and its qualities outlined. An account is presented of the success and failure thus far attained in the search for improved varieties of forest tree species through polyploidy. Finally, the potentialities of polyploidy in forestry are summarized and some recommendations concerning future lines of research are outlined.

The experimental work in connection with this thesis was the preliminary investigation of Douglas fir (Pseudotsuga menziesii (Mirob) Franco) aberrants found each year among the seedlings at the B.C. Forest Service nursery in Duncan. These abberrants have been thought to be spontaneous autopolyploids because they resemble such polyploids found among nursery stock of other coniferous species elsewhere. This study, however, indicated beyond any doubt that these abberrants are not polyploids. Other possibilities which might have caused the aberrant form are discussed.

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INTRODUCTION

During the past few decades considerable work has been carried out in the investigation of polyploidy in plants. Many superior polyploid strains have been developed either by selecting them from natural populations of diploids or by artificial induction. With the discovery of the effect of colchicine in the induction of polyploidy in plants, a new tool has been added by which many more polyploids have been produced. These polyploids have replaced the former diploids in cases where their superiority or usefulness has been demonstrated.

In forestry the investigation of polyploidy lags considerably behind other fields which deal with plant breeding. This lag is understandable because there is much less opportunity for introducing new strains of plants in forestry than in agriculture. Forestry is a less intensive form of plant culture than horticulture or the raising of field crops; the life span of the individual plant before harvest is much longer, and the practice of artificial methods of regeneration not as common. Another reason for the lack of intensive research on polyploidy in forest trees may be the fact that only a limited amount of success has been attained in finding superior polyploids.

The purpose of the present study is to survey the field of polyploidy research in plants in general and its

application in forestry, with particular emphasis on work carried out on coniferous trees. The practical work consisted of the study of abnormal Douglas-fir (Pseudotsuga menziesii (Mirob.) Franco) seedlings which were suspected to be autopolyploids. During the course of the study a schedule for squash technique in vegetative buds of Douglas-fir was developed and a solution to the problem of breaking dormancy in that species was undertaken.

MEANING OF POLYPLOIDY

Most animals and plants are diploids. A diploid organism carries two homologous sets of chromosomes in each cell nucleus, one set having come from each parent. Reproductive cells (gametes) are haploid i.e., they carry only one set of chromosomes. In rare instances the somatic nuclei of plants may contain only one set of chromosomes. Such monoploid plants are usually sterile and do not become established in nature. Living organisms with nuclei containing three or more sets of chromosomes are known as polyploids. A polyploid with three sets of chromosomes in the somatic nucleus is called a triploid, with four sets a tetraploid, and so on. An autopolyploid is a polyploid which originated from one parental species. An allopolyploid on the other hand, has its origin in the hybridization of two or more species. A euploid plant has one or more complete sets of chromosomes. An aneuploid plant has a normal complement (genome) plus some extra chromosomes or with some chromosomes missing.

Polyploids may be stabilized and capable of self-perpetuation, or unstabilized, in which cases vegetative reproduction is the only possible means of propagation. Particularly among unstabilized and artificially induced polyploids, several forms of retrogression to diploidy may occur. In mixoploids, diploid cells are encountered alongside polyploid cells. Chimeras are plants composed of tissues of different chromosomal count. In periclinal

chimeras several layers of tissue are polyploid whereas adjacent layers are diploid. In sectorial chimeras certain sectors of the plant are polyploid and other sectors are diploid. The wide distribution of mixoploids, chimeras, and aneuploids, particularly among artificially induced polyploids indicates that cytological examination of the various tissues of plants is necessary before these plants can be identified as true polyploids.

CAUSES OF POLYPLOIDY

The occurrence of spontaneous polyploidy has been attributed to extreme temperature changes and other sudden and severe changes in the environment. Experimental evidence indicates that polyploidy may be induced by rather moderate temperature changes in some genera, but that sudden changes are more effective. Extreme temperature changes encountered by diploid races that extend into unfavourable territory, or a change in the local climate, might cause polyploidy. A survey of the natural polyploids indicates that they have a more northerly distribution or a more alpine habitat than their diploid ancestors. In such habitats plants are subjected to greater and sharper temperature variations which might be the cause of polyploidy (Sax,1936).

Love and Love (1957) believe that the higher frequency of polyploids in arctic regions is an indication of the ability of polyploids to survive under adverse conditions. This theory, however, does not support the findings of Bowden (1940) who investigated the hardiness of polyploids as compared to that of diploids and did not find any greater hardiness in the polyploids.

Several abnormalities in meiosis, such as asyndesis (Andersson, 1947) have been observed to favour the formation of polyploidy in plants. Abnormalities in

the reproductive organs of plants might also favour the production of unreduced gametes and thus cause polyploidy. This suggestion finds support in a study made by Seitz (1954) on a hermaphrodite clone of grey poplar (Populus canescens Smith) with abnormalities in the development of the flowering parts. One per cent of the progeny produced by self pollination in such plants were triploid. Upon cytological examination of the anthers it was found that some of the pollen mother cells remained in the diploid stage due to failure in the reduction division, and in this way diploid pollen grains are believed to arise and produce polyploid progeny. It is possible also that selfing in plants increases the chances of the occurrence of polyploids since there is more chance for the accumulation of chromosome abnormalities due to a lower variability in the genetic make-up in such a method of fertilization. The fact that selfing is rare among the conifers (Allen,1942) might explain in part the rarity of polyploidy in those species.

Andersson (1947) considered polyploidy to arise in nature through the formation of unreduced gametes due to failure in the reduction division. Kiellander (1950), on the other hand, believed that, at least in the conifers, doubling of the chromosome number is more likely to take place after fertilization than before it. This was found in Datura stramonium by Blakeslee (Vide Muntzing,1936), and might also be true in the gymnosperms.

Several workers (Namikawa,1934) (Johnsson,1946) have reported a higher percentage of polyploids among twin seedlings of several genera than in the corresponding normal seedling populations.

It is also conceivable that, in nature, polyploids may arise through the spontaneous development of chimeras and the subsequent development of inflorescences on the polyploid sectors (Andersson,1947).

CHANGES CAUSED BY POLYPLOIDY

Polyploids may differ from diploids in the following ways:

a) vigor of vegetative growth (may be greater or lower),
b) size of cells and cell nuclei (may be larger),
c) fruit size and shape (may be shorter and stouter),
d) chemical and physical properties (may be quite different),
e) size and number of stomata (may be larger and fewer),
f) fertility of pollen grain and seed (may be lower in autopolyploids and higher in allopolyploids than in the corresponding parent species).
g) colour and shape of leaves (may be darker and thicker).

These changes are extremely variable however, and, when polyploidy is to be induced artificially, its effects cannot be predicted.

In forestry applications desirable changes would include greater rate of growth and an improvement in the chemical and physical properties of the woody parts. An increased rate of growth has been reported in such Angiosperm plants as poplar (Johnsson,1942,1953, and others), and birch (Johnsson,1946,1956, and others). Among the conifers, good results have been obtained with allotriploid larch which was obtained as the only progeny

from the cross of two species (Larsen and Westergaard, 1938), and a giant tetraploid form has been reported in seedlings of Cryptomeria japonica (Chiba,1952). All other instances of polyploidy reported in forest tree species resulted in retarded growth rates compared with those of the corresponding diploids.

As far as structural changes in the wood are concerned, Kanezawa (1951) reported an increase in fiber length of 30% and in fiber thickness of 40% in induced polyploids of Japanese cypress (Chamaecyparis obtusa Endl). Several reports have been made of an increased water content in autopolyploids as compared with diploids. Johnsson (1950) made a study of this problem in Alnus glutinosa and found that dry matter content of the wood is lower in the triploid than in the control and the specific weight of the wood in the dry state is lower. No difference in the shrinkage properties of the wood was observed between the two strains. On the basis of the measured data the pore volume was determined and found to be 70.13% of the total volume in the triploids as compared with 68.14% in the diploids. This difference in pore volume is explained by Johnsson as resulting from the larger elements in the wood of the triploids.

Several external features have been suggested by various workers as aids in identifying polyploids and

distinguishing them from diploids of the same species. None of these has proved to be so reliable as a direct chromosomal count.

Sax (1938) studied the number of stomata per square millimeter of leaf surface in herbarium material of a number of plant genera in the diploid and polyploid condition. He found, with some exceptions, a correlation between chromosomal counts and the size of stomata. In his opinion, stomatal size cannot be used as an absolute index of polyploidy, but in many cases it may be of use in preliminary surveys. Johnsson (1940) did not find a direct correlation between stomatal length and chromosome number in the genus Populus but, on the average, aneuploids seemed to have larger stomata than diploids, and tetraploids larger stomata than aneuploids and diploids.

The study of polyploidy in forest trees up to now dealt only with the Co (Co is the generation treated with colchicine) and C1 (C1 is the first progeny generation of plants treated with colchicine) generations and consequently little is known at present about the properties of subsequent generations in forest trees. The findings of Wettstein (1937) in polyploid races of mosses might throw light on the subject. Following the development of these mosses for several generations, he found that there was a decrease in size of cells and of stomata with the passage

of generations so that, after 11 years, the polyploids had the same cell and stomata sizes as the diploids. Fertility was also gradually restored yet the chromosome number remained the same and the polyploid races did not cross with the original races. Kanezawa (1948) considered that a similar development is likely to take place in forest trees and that with the passage of generations, as the polyploids become adapted to the environment, differences in cell and stomatal sizes will tend to be eliminated.

The size and fertility of the pollen grains have been suggested by some as aids in distinguishing between polyploidy and diploidy. The pollen grains usually tend to be considerably larger and to have a much lower fertility in polyploid races than in the corresponding diploids. Peto (1938) in his researches on spontaneous triploid poplars arrived at the conclusion that no correlation existed between chromosome number, the size of the pollen grain, and the quality of the pollen. Furthermore, in one of the polyploids (Populus alba var. auerointertexta) he found the fertility of the pollen to be as high as 94 per cent.

LIMITATIONS OF POLYPLOIDY

The findings of various workers in the field of polyploidy are summarized below as to its effect on the vigour of plants.

Plants show a varying degree of sensitivity to polyploidy. For every species there is an optimal number of chromosomes with which it exhibits its highest vigor and rate of growth. When this optimal number is exceeded, the growth rate and vitality decrease. Darlington (1937) considered that the optimal number is conditioned by the size of the metaphase plate and the availability of extra space to permit an increase in the number of chromosomes. According to him, plants with a relatively small number of chromosomes and with small chromosomes are, generally speaking, more suitable to polyploid induction than plants with a large basic chromosome number in their genome or with large chromosomes. For this reason he considered the angiosperms more likely to react favourably to induction than the other classes of plants.

Pauley (1949) stated that triploidy seems to be the natural limit in the genus Populus and, therefore, the optimal number. Liellander (1950) believed that the optimal number of chromosomes in Norway spruce (Picea Abies (L) Karst) is $2n = 24$ and any increase of the number of chromosomes above this number would result in poor growth and lowering of vitality.

VARIABILITY OF POLYPLOIDS

Polyploidy, which is essentially the quantitative increase of the chromosome complement of plants, produces various results in different individuals of the same species. This is particularly true in cross-fertilized plants because in such cases there tends to be an effective recombination of the genetic material. One source of the increased variation in autopolyploids can be sought in the disturbances during meiosis which may lead to numerical aberrants and aneuploids. There is, however, the possibility that the effects of such disturbances may be eliminated to a great degree at the gametic stage. Another source of increased variation in polyploids may be the increased number of loci and consequently the greater possibility of recombination afforded. If the characteristic investigated is determined by several genes simultaneously with extreme factorial combinations, an increased variation is doubtless also to be expected in the polyploids (Johnsson,1950).

That the variability of polyploids is greater than that of the corresponding diploids in various types of plants is a fact stated by many workers. Genetic differences of diploid plants are accentuated in the polyploids. In order to produce a superior strain of polyploids, selection is necessary prior to induction as well as after it.

POLYPLOIDS AND ENVIRONMENT

It is widely accepted at present that the process of chromosome doubling in allopolyploids has played a great role in the evolution of plant species. There is however, still a great diversity of opinion among the various workers with regard to the evolutionary significance of chromosome doubling in autopolyploids. Muntzing (1933) considered autopolyploidy to be of importance in plant evolution because it:

a) affects both the morphology and the physiology of plants.
b) causes sexual isolation of polyploids.
c) may give rise to polyploid races and species in nature.

Stebbins (1947), on the other hand, considered that the evolutionary processes in nature are likely to limit the importance of autopolyploidy because of the reduced fertility and the inability to give rise to anything new among the derivatives of autopolyploids. According to him, the importance of chromosome duplication in the evolution of plants is, in the main, in fixing and spreading hybrid combinations, either intervariate or interspecific.

The fate of a spontaneous autopolyploid within a population of a diploid prototype from which it arose has not been investigated for forest trees but two studies conducted on barley species throw light on the problem. Sakai and Suzuki (1955a,b) have found that autotetraploid

strains of barley were almost always poor competitors against the diploids. On the other hand, allopolyploids were found to be superior to both parental strains. These findings might explain the survival of Sequoia sempervirens, an autoallopolyploid with 2n = 44 (Stebbins,1948), and Aesculus carnea, an allopolyploid with 2n = 80 (Upcott, 1936).

If the above findings are universally true for plants, it is apparent that there are good prospects for future allopolyploids which could be developed artificially or naturally. It is also likely that autopolyploids would have to be propagated vegetatively for each generation in cases where they do not produce fertile seeds.

TRIPLOIDY IN WOODY PLANTS

Triploids of several woody plants exhibit an increased growth rate in comparison with diploids of the same species. In the genus Populus both spontaneous and induced triploids are much faster growing and bigger than the corresponding diploids (Muntzing, 1936, and others). Triploidy also produced good results in Alnus glutinosa (L) Gaertn; young seedlings of this species were studied by Johnsson (1950) and found to be 50% taller on the average than the diploids. The real difference in height attributable to triploidy was probably higher since the triploid seedlings included many aneuploids with retarded growth. In Betula verucosa triploidy produced robust seedlings with a larger average diameter than that of the diploids but with a somewhat reduced height (Johnsson, 1956).

In the conifers, examples of spontaneous autotriploidy are unknown. In these the potentialities of induced autotriploidy are at present unknown. In a private communication to the author, Kiellander (1957) expressed the opinion that: "artificial triploids of such hardwoods as Betula, Populus, Alnus, and Ulmus possess good growing capacities. Autotriploids among the conifers have not, however, been produced as yet; consequently we know nothing about autotriploid conifers".

Allotriploid larch (Larix decidua/Larix occidentalis) was obtained by Larsen and Westergaard (1938). This is

the only mention found in this literature survey of triploidy of any kind in the conifers. This allotriploid was the only progeny obtained from a cross between the two species of larch. Soegard (1957) reported on the development of the above allotriploid: "it has an excellent rate of growth and in January, 1957 was 14.5 metres tall which is almost the height of a hybrid larch Larix decidua/Larix leptolepis of the same age."

The main difficulty in the production of triploids of both hardwoods and conifers is that they cannot be induced directly from diploids. The case of the allotriploid larch cited above is uncommon and probably took place because the two parent species were mutually incompatible and could not produce a diploid progeny of the cross. Normally a triploid has to be produced through a cross between a tetraploid and a diploid. The fact that tetraploids bear fruit at an older age than diploids makes the problem more serious in woody perennials. One way of overcoming this difficulty is to graft tetraploids on diploid stock. Larson, (1956) and others, with the development of new techniques in connection with seed orchard work, found in recent years that grafting is conducive to fruit bearing at a younger age.

Triploids, owing to their degree of sterility, present another difficulty in the fact that they are incapable of bearing a progeny true to type. At least two types of gametes, univalents and bivalent, and in many cases unreduced trivalent gametes, have been reported in trip-

loids. The high frequency of occurrence of aneuploids, in addition to the variety of euploids with different chromosome count, which have been reported as the progeny of triploids (Johnsson,1942) makes it clear that vegetative propagation would be the only practical means of reproducing triploids. The extra cost involved in such propagation would be a limiting factor in undertaking cultivation of triploids. In the case of triploid clones of poplar such a means of propagation was found worthwhile and has been employed in Sweden for some time. Whether such a method also would be worthwhile in other species will have to be decided individually for each species after the added value of the triploid crop has been compared with the extra cost involved in vegetative propagation.

ALLOPOLYPLOIDY IN WOODY PLANTS

Allopolyploidy is directly effective in producing new species since such polyploids result from species hybridization followed by a chromosomal increase. The duplication of each of the parental genomes restores fertility and the hybrid breeds true to type. Thus the allopolyploid is a constant species hybrid which has characteristics of a true species, and in the case of generic crosses may merit a generic rank (Sax,1936).

Allopolyploids are known to occur in nature and under cultivation, and have been produced experimentally in several different families. Examples of natural allopolyploid woody perennials are Sequoia sempervirens (2n = 44) among the conifers (Stebbins,1948) and Aesculus carnea (2n = 80) among the hardwoods (Upcott,1936).

Allopolyploids with superior growth qualities have been produced artificially and in numerous cases rendered infertile crosses fertile. It is significant that many of the most important cultivated plants today are of known or supposed allopolyploid origin. Very little use has been made in forestry thus far of allopolyploidy in the propagation of hybrid varieties of trees.

POLYPLOIDY AMONG THE CONIFERS

Changes in the chromosome number have been of little importance in the differentiation between families and genera of most coniferous trees. Sax and Sax (1933) believe that the stability of the conifers with respect to polyploidy indicates that evolution in this group has passed its climax and that the existing forms are survivors of long natural selection. In another study, Sax (1932) stated that the comparatively high number of chiasmata with the prevalence of interstitial chiasmata account for the great uniformity of chromosome number and the general stability of that group. He attributes the rarity of polyploids to the large number of chiasmata in the diploid stage. There is an average of 2.4 interstitial chiasmata per bivalent and this number seems to be remarkably constant in all the conifers covered in his study. Any autopolyploids produced would be likely to form closely paired tetravalents and the segregation of homologous chromosomes would be too irregular to produce a high degree of fertility. Muntzing (1933) explained the rarity of polyploids among the conifers by the absence of double fertilization in this group and the fact that diploid and polyploid races can cross readily and, consequently, the polyploid forms are not isolated and developed independently. This hypothesis might also explain the differentiation of relatively few species and genera

among the conifers. The mechanism of evolution in the conifers has involved a gain or loss of one chromosome, structural rearrangement, and gene mutations, but polyploidy has played a small role. The loss of a chromosome has been responsible for the evolution of Taxodiaceae and and Cupressaceae. This involved a loss of a centromere which follows translocations of all essential genes to the rest of the centromeres. Instances of a gain of a chromosome are few and occur only in the genus Pseudotsuga and in the family Araucariaceae. This always involves duplication of a centromere and could be achieved by a system of translocations as proposed by Darlington (1937). The majority of the conifers are thus found to have a haploid chromosome number of 12 (n = 12). The only known genera with deviating chromosome numbers are Taxodium, Thuja, and Juniperus, each of which has a haploid complement of 11 (n = 11) and Pseudotsuga and Araucaria with a haploid number of 13 (n = 13).

Spontaneous stabilized polyploids among the conifers are very rare. The only cases known are that of Juniperus chinesis var. Pfitzeiania, an autotetraploid with a 2n = 44 number of chromosomes, and Sequoia sempervirens, an autoallopolyploid also with a chromosome complement of 2n = 44. Occasional occurrence of spontaneous polyploids among the conifers is reported from time to time but none of these has proved to be stabilized. The oldest reported such polyploid is that found by Christiansen (1952) in Denmark.

This autotetraploid, Larix decidua Miller, which was between 56 and 58 years old at the time of discovery, was 15.2 metres tall and 97.5 cms. at breast height. The tetravalent chromosome complement was found to act very irregularly during meiosis and most of the seeds produced were found to be hollow.

SPONTANEOUS CONIFEROUS POLYPLOIDS IN NURSERY STOCK

The occurrence of spontaneous polyploids among coniferous nursery stock has been reported for several species. Kiellander (1950) reported such an occurrence in two-year-old Picea Abies. The seed material for these seedlings was gathered from a large number of trees and it was found that the polyploid aberrants were a rare occurrence among the progeny of many different trees. The calculated frequency of occurrence for Picea Abies was 8 per 100,000 seedlings. The polyploid aberrants resembled the polyploids of the same species which were produced by means of colchicine treatment. They exhibited the same slowness of growth and thick needles. The ratio of the total height of these two-year-old seedlings to the total height of normal seedlings of the same age was found to be 0.3.

Zinnai (1955) made a study of spontaneous polyploidy in nursery stock of Cryptomeria japonica and found an occurrence of 5.15 per 10,000 in unthinned seedbeds and only 1.64 in thinned seedbeds. Zinnai did not give any figures on length ratio of polyploid and normal seedlings, but stated that among seedlings with heights from 6.5 to 7.5 cm. there were 2.23 polyploids compared with 1.65 per 10,000 seedlings with a height of 7.5 to 9.0 cm. and only 0.56 per 10,000 in the 9.0 to 15.0 cm. height group. He also found that there was a high occurrence of polyploids

among the seedlings removed by thinning. This agrees with Kiellander's explanation for the rarity of polyploid spruce and the reason that polyploids of this and other conifers have hitherto escaped observation. Apparently autotetraploids in the conifers are poor competitors of the diploids and in addition, they do not reproduce true to type.

Chiba and Watanabe (1952) have investigated the occurrence of autotetraploids in nursery stock of Larix kaempferi and found that they lacked the giant form of the autotetraploid Cryptomeria japonica seedlings, reported earlier by Chiba (1950).

No account has been found in the literature of the occurrence of spontaneous polyploidy in Douglas fir. Only one instance, (Meyer,1951) is known of induction of polyploidy in that species. Meyer immersed seeds for four days in a 0.2% solution of colchicine in water and obtained seedlings which proved to be tetraploids. Dean George S. Allen, Faculty of Forestry, University of British Columbia, and Mr. R. R. Silene, U. S. Forest Service, Pacific N. W. Forest Experiment Station, Corvallis, Oregon, tried to induce polyploidy in Douglas fir by treating seed with colchicine and have obtained abnormal seedlings similar to those described for other coniferous species.

POTENTIALITIES OF POLYPLOIDY IN FORESTRY

In concluding this review of literature, it can be said that, despite the many futile attempts to find or induce superior varieties of polyploids in forest trees, all the possibilities have by no means been exhausted. It is believed that polyploidy can provide a good means by which existing varieties can be improved. Allopolyploidy, in particular, can be useful in developing fertile hybrids. The chemical and physical changes that take place in polyploids of various species should be investigated in more detail. It is possible that these modified characteristics might be more desirable than those of the corresponding diploid varieties. On the whole, much more investigation of polyploidy is necessary before this field is abandoned. Improved methods of induction would have to be found in order to produce true polyploids in which the increased number of chromosomes tends to produce better varieties. The optimal number of chromosomes would have to be determined for each species. In any event, selection is as essential in a polyploid population as in the variants of diploid forms. The added value resulting from the production of superior polyploid varieties would have to be determined and weighed against the extra cost involved in their production and cultivation.

Improved methods of inducing polyploidy in all tissues of the plant would entail different applications of colchicine or a combination of this and other chemicals to

ensure penetration. In the past, colchicine treatment was confined to germinating seeds and shoots. One attempt to induce polyploidy in excised embryos of pine was carried out without success, by Hyun (1954). In excised embryos the meristematic tissue is more exposed than in seeds and treatment of excised emryos in culture should give better results. Haddock (1954) described a method of growing excised embryos of sugar pine in culture, and such a method, if it proves to be successful in other conifers, could be used in polyploidal induction work.

The fact that there is no double fertilization in the conifers could be used in attempts to induce doubling of the chromosomes in the gametic stage and might prove to be useful in the production of coniferous autotriploids. In the angiosperms, on the other hand, doubling of the chromosomes in the gametic stage is less likely to succeed since double fertilization and the different chromosomal count in the embryo and the endosperm tissues might cause difficulties in the development of the seed.

PART B

A PRELIMINARY STUDY OF ABERRANT SEEDLINGS IN DOUGLAS FIR

INTRODUCTION

The occurrence of aberrants in nursery stock at the B.C. Forest Service nursery at Duncan has been noticed for several years. Mr. Jack Long, superintendent of the nursery, has separated some of these aberrants and planted them in two rows on the nursery grounds. The oldest of these aberrants is now ten years old, but none has produced any cones thus far.

The aberrants are characterized by thicker, darker needles than those in normal seedlings and by a retarded growth. The buds of the aberrants are more round and smaller than the normal buds (Figs. 1,2,3,4).

The frequency of occurrence of the aberrants has been estimated by Mr. Long as from 6 to 10 per million seedlings. It is possible that some were unnoticed and the frequency of occurrence may actually be higher. The different phenotypes that are found among the aberrants and their presence each year suggest that they are of rare occurrence among the progeny of various trees. The external features of these aberrants and their spontaneous and rare occurrence every year strongly suggest autopolyploidy. They resemble autopolyploids which have been described for other coniferous species (Kiellander, 1950; Chiba, 1952; and others). The presence of normal twigs on some of the aberrants (Fig. 4) resemble sectorial chimeras in autopolyploids.

The investigation to determine whether these were

really polyploids was undertaken in the summer 1957, following a suggestion by Dr. P. G. Haddock and Dr. A. Orr-Ewing. The study consisted of:

a) The measurement of the length of aberrant seedlings and comparing them to normal seedlings.

b) The embedding of cross-sections of aberrant needles and detecting any abnormalities in them.

c) The comparison of stomata and guard cell lengths in the aberrants and in normal seedlings.

d) The breaking of dormancy in aberrant and normal seedlings to facilitate a cytological study.

e) The finding of a suitable squash technique for examining vegetative buds of Douglas fir.

f) The preparation of slides of chromosomes in the dividing cells of buds in aberrant and normal Douglas fir seedlings.

MATERIALS AND METHODS

The study was confined mostly to 22 aberrant seedlings (5 One-year-old and 17 two-year-old) and an equal number of normal seedlings of the same age groups which were shipped to Vancouver, thanks to the courtesy of Mr. J. Long. The normal seedlings were picked at random from among nursery stock. A measurement of the length of the aberrants and normal seedlings was carried out prior to planting them in pots in the University of British Columbia green-house on November 25, 1957.

Needles of aberrant and normal seedlings were embedded in paraffin, cut into cross-sections, mounted, and stained with safranin and fast green.

Mounts of stomata from aberrant and normal needles were prepared by peeling off the lower epidermis of needles after boiling in water for a few minutes, then inserting in cold water (Johanssen, 1940). This method of peeling off the epidermis was found to be more satisfactory than the method employing collodion films (Long and clements, 1934). The stomata were stained with aceto-carmine. The length of the guard-cells in the aberrant and in the normal seedlings was measured and compared.

In order to break the dormancy of the seedlings the following treatments were tried:

a) Chilling the seedlings for 24 hours prior to planting in the green-house by placing the seed-

lings in a refrigerator with their roots covered with wet soil contained in a plastic bag. (6 aberrants and 6 normal seedlings).

b) Coating some of the buds of seedlings grown in the green house with giberellic acid in lanolin paste (Marth et al, 1956). (4 aberrants and 4 normal seedlings).

c) Increasing the photoperiod in the green house by 2 hours daily. (5 aberrants and 7 normal seedlings).

d) Increasing the photoperiod in the green house by 4 hours daily. (5 aberrants and 7 normal seedlings).

e) Treating plants grown indoors, in a heated laboratory, with artificial light from ordinary table lamps for alternate periods of 24 hours, in addition to fertilizing with small amounts of ammonium sulfate. (4 aberrant seedlings).

During the course of the study it was found that there was no completely satisfactory squash technique for Douglas fir. Neither the conservative method with aceto-carmine (Johanssen,1940) nor the method suggested by Mergen and Novotny (1956), using crystal violet stain, gave good results. By a process of trial and error, various other schedules were used and it was found that an adaptation of the aceto-lacmoid squash method suggested by Mikaelson (1952) for staining spruce and birch was the most suitable for Douglas fir. The fixative used by him, however, was found to result sometimes in plasmolysis of Douglas fir cells and did not give as good results as acetic alcohol. Only temporary slide preparations, sealed with nail polish, were used. Mikaelson suggested that these slides could be made permanent

by removing the seal after four days and mounting in well aged (acid) euparal.

The following schedule has been tried in chromosome staining of vegetative buds of Douglas fir and found to be most suitable:

Step 1. Actively dividing buds are cut off the seedling or tree and dissected longitudinally.

Step 2. (This step is carried out only when an inhibition of the spindle formation and shortening of the chromosomes are desired; otherwise it may be omitted). Dissected buds are treated as follows:

a) Placed in a solution of 1% colchicine in water for one hour under artificial light from an ordinary table lamp. (Mergen and Novotny, 1957)

b) Placed in a 0.002M solution of 8 hydro-oxyquinoline in water for 24 hours. (Illies, 1952)

c) Placed in a saturated solution of paradichloro-benzene in water for 24 hours. (Hyun, 1954).

Step 3. Dissected buds are killed and fixed in acetic alcohol 3:1 (Glacial acetic acid to 96% ethanol in the ratio of 3:1) (Darlington,1950).

Step 4. Single needles are excised and hydrolized in a mixture of 96% ethanol and concentrated hydrochloric acid in the ratio of 1:1 for 5 to 15 minutes, depending on hardness of the material. (Mikaelson,1954).

Step 5. Hydrolizing fluid is washed off with water. (Mikaelson,1954).

Step 6. Single needles are placed on a slide and stained

with standard acetic lacmoid stain (1% resorcin blue in 45% acetic acid). (Mikaelson,1954).

Step 7. Needles are squashed with a cover glass. The slide is placed in a bibulous book and pressure is applied to it. Tapping of the slide may help to obtain polar views and scatter the chromosomes. (Darlington and La Cour, 1950).

Step 8. Slide is examined under a microscope and, if under-stained more stain is applied. (Darlington and La Cour,1950).

RESULTS

The measurements of average length of aberrants and normal seedlings are presented in Table 1.

TABLE 1. Comparison of stem and root lengths of one-and two-year-old aberrants with those of an equal number of normal seedlings picked at random.

No. of seedlings	Type	Length, inches Stem	Root	Total	Range of Total Length, inches	Ratio of Length Aberrant/ normal
One-year-old						
5	Normal	4.9	4.7	9.6	8.0-10.1	
5	Aberrant	2.9	3.0	5.9	4.5-6.0	0.6
Two-year-old						
18	Normal	12.5	9.0	21.5	13.5-25.4	
17	Aberrant	6.6	6.0	12.6	8.5-21.5	0.6

From the table it can be seen that the ratio of the total length of aberrants to that of normal seedlings remained constant on the average, for the one-and the two-year-old seedlings.

The ranges of the one-year-old seedlings did not overlap and, in the two-year-old seedlings, only three aberrants overlapped the range of the normal seedlings.

A "t" test conducted on the two-year-old seedlings indicated that the normal and aberrant seedlings were from different populations with a confidence level of much less than 1 per cent.

No irregularities in the epidermal layer and the size of the mesophyll cells were noted in the cross-sections of the aberrant needles. Such irregularities were described in autopolyploids of pine (Hyun, 1954). This was the first indication that the aberrants might not be polyploids.

A subsequent study of the size of stomata and guard cells failed to reveal any significant difference between the aberrants and the normal seedlings. The length of the average guard cell in both the aberrants and the normal seedling was around 57 microns.

Of the five various treatments to break the dormancy of the seedlings all the four aberrant seedlings grown indoors and fertilized with ammonium sulfate (treatment e) resumed their growth ten days after the start of the treatment. Of the 5 aberrants and 7 normal seedlings grown under increased photoperiod of 4 hours (treatment d) only two aberrants and one normal seedling resumed their growth three weeks after the start of the treatment. The other three treatments failed to break dormancy in any of the seedlings treated.

Cytological study of six aberrants and four normal seedlings failed to show any increase above the normal complement of 26 chromosomes (Zenke, 1953 and others). This finding (Figs. 5,7,9,11,13,15,17,19,21) was consistent for all plants studied and in various tissues of the bud.

DISCUSSION

In spite of the fact that the external features of the aberrant seedlings strongly suggest polyploidy it is obvious that the cause for the aberrant shape must be other than polyploidy. Illies (1952) described a similar phenomenon in dwarf spruce with all the external features of a polyploid but with a regular diploid number of chromosomes in the somatic tissue. The cause of the aberrant form in such cases must be due to some sort of a mutation and this would require a further study. The fact that some of the aberrants have normal branches below the aberrant ones suggests that at least in some of the aberrants the appearance of the mutation took place in the somatic tissues. Some of the older aberrants in the Duncan nursery have normal branch whorls above aberrant whorls which suggests that the mutation can be normalized as well as formed in the somatic tissues of the plant.

It is of interest to note that the aberrants studied strongly resemble the foliage of trees expressing the phenomenon of brooming which was described in older Douglas fir trees (Buckland and Kuijt, 1957). This brooming appears at a late stage in the development of the tree. The possibility that the same causes underlie the formation of brooming as well as the aberrant form under study cannot be excluded.

During the course of the foregoing study it has been

noted that at anaphase only the haploid number of chromosomes is discernible in all Douglas fir bud cells studied and this peculiarity would require further study.

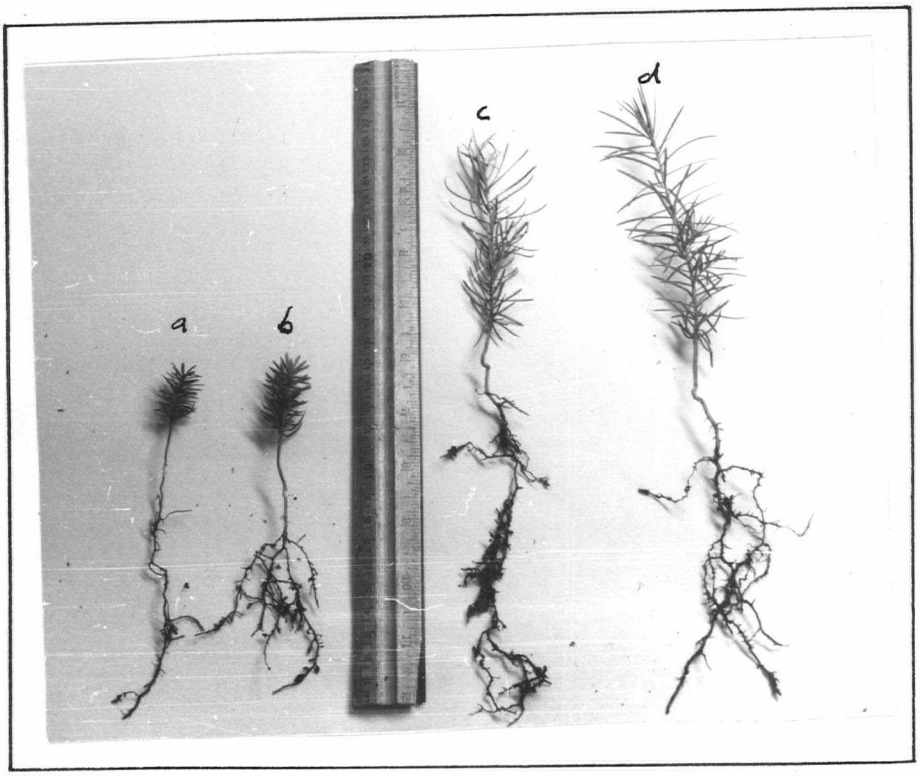

Fig. 1. Normal and aberrant one-year-old seedlings: a, b, aberrant seedlings; c, d, normal seedlings

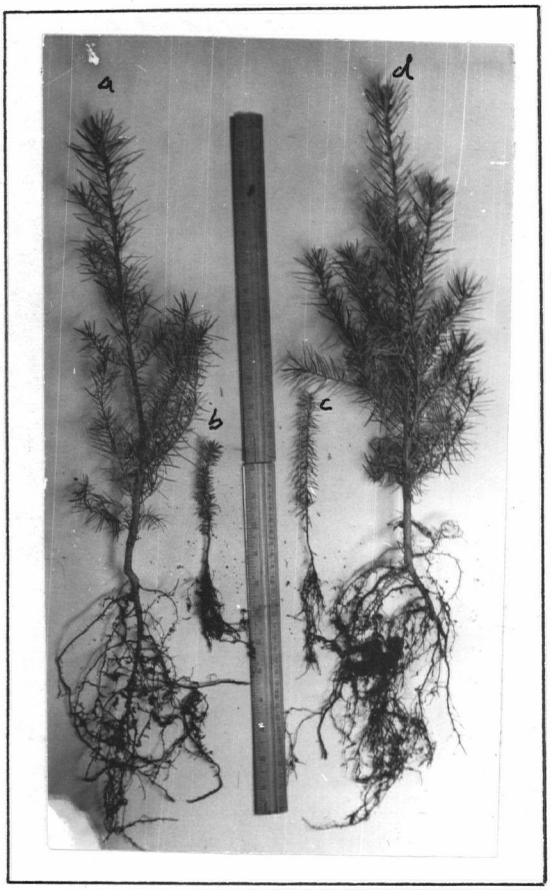

Fig. 2. Normal and aberrant two-years-old seedlings: a, d, normal seedlings; b, c, aberrants

Fig. 3 Stem differences in aberrant and normal seedlings: a, normal seedling; b, aberrant seedling. (Note difference in green hue of the needles and in the shape of buds)

Fig. 4 Aberrant seedling with a normal branch: a, normal branch; b, c, aberrant branches

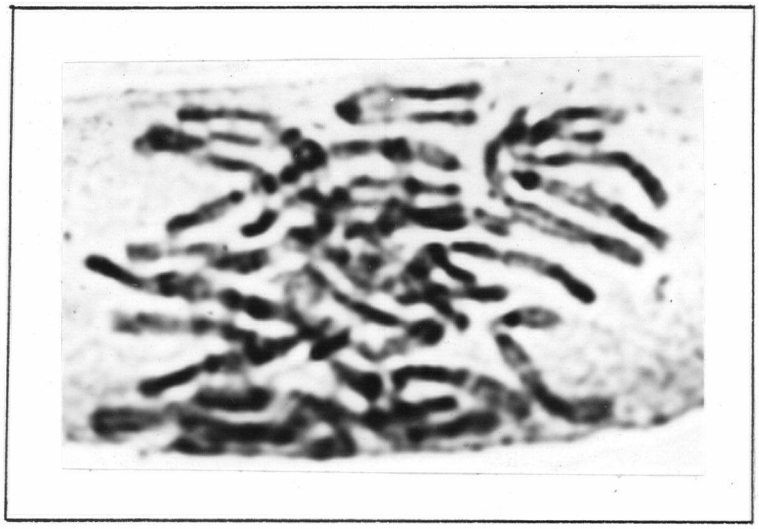

Fig. 5. Pro-metaphase in an aberrant seedling (X2400)

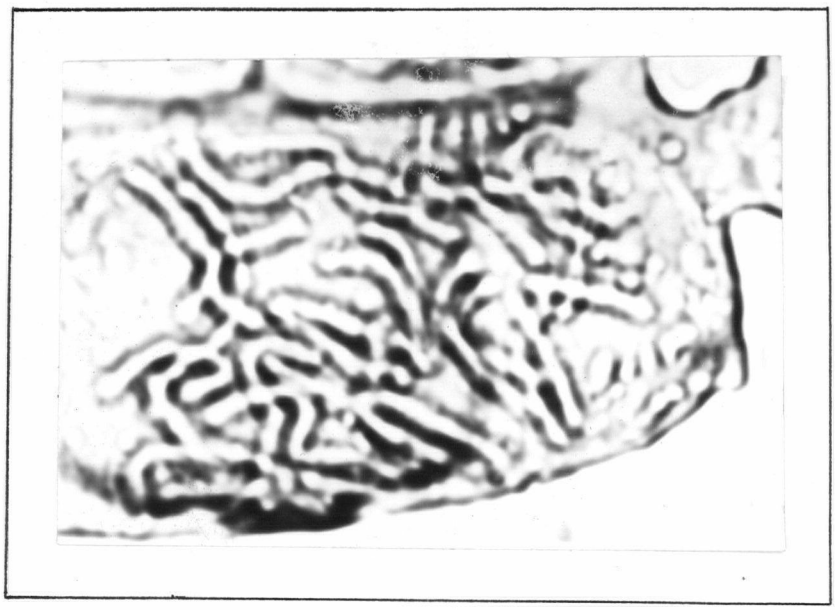

Fig. 6. Prometaphase in a normal seedling. (X2300)

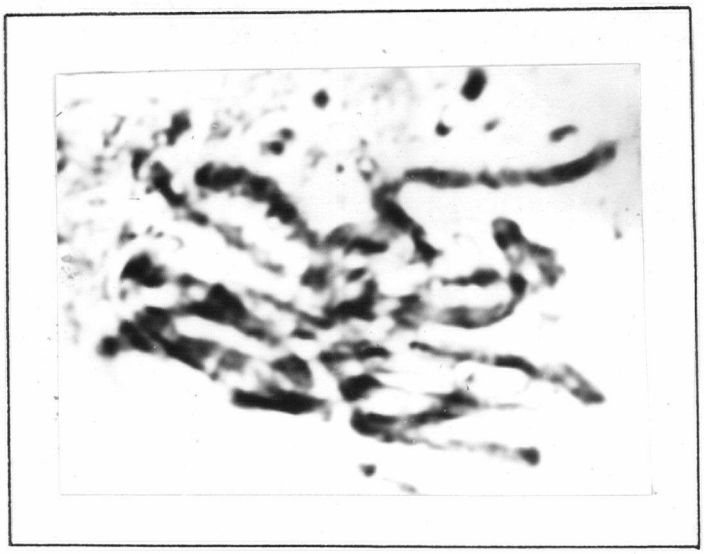

Fig. 7. Metaphase in an aberrant seedling. (X3000)

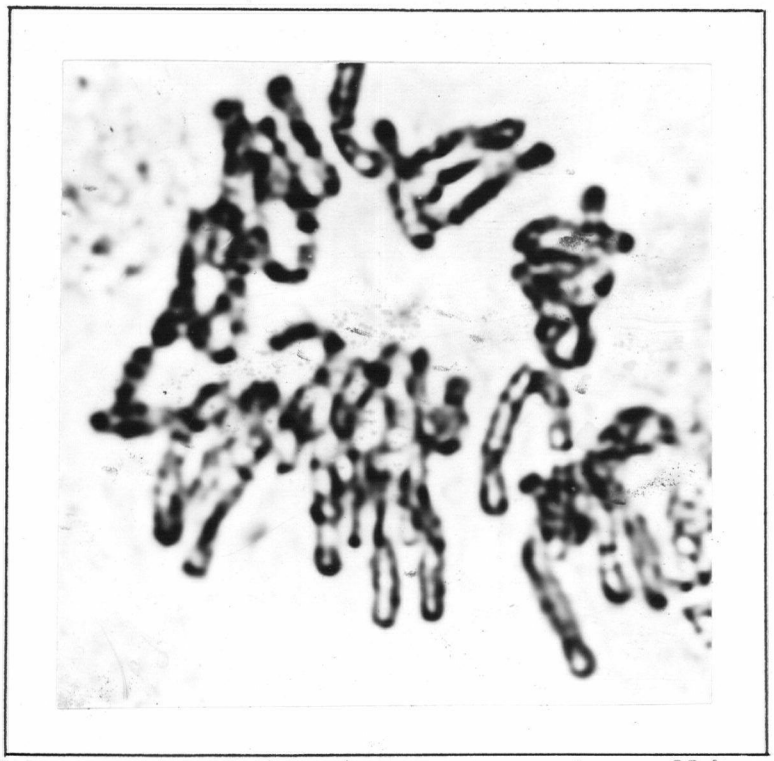

Fig. 8. Metaphase (polar view) in a normal seedling. (X2500)

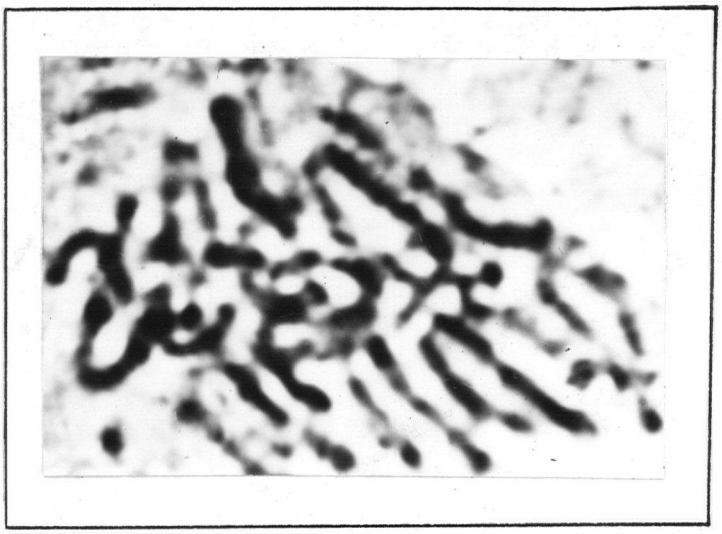

Fig. 9. Early anaphase in an aberrant seedling. (X3000)

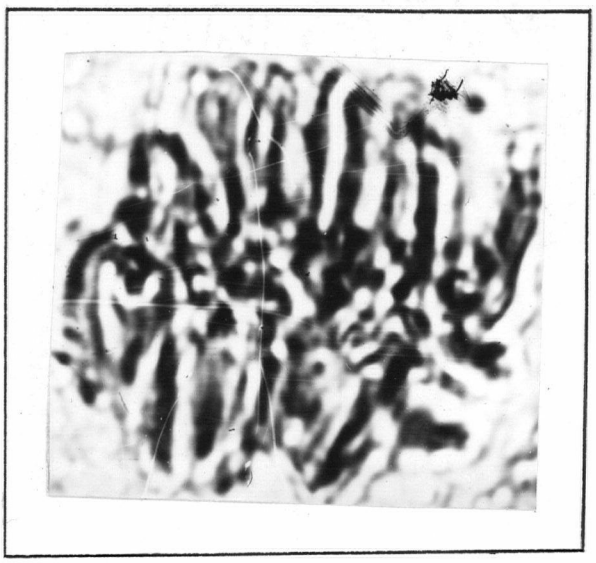

Fig. 10. Early anaphase in a normal seedling. (X2900)

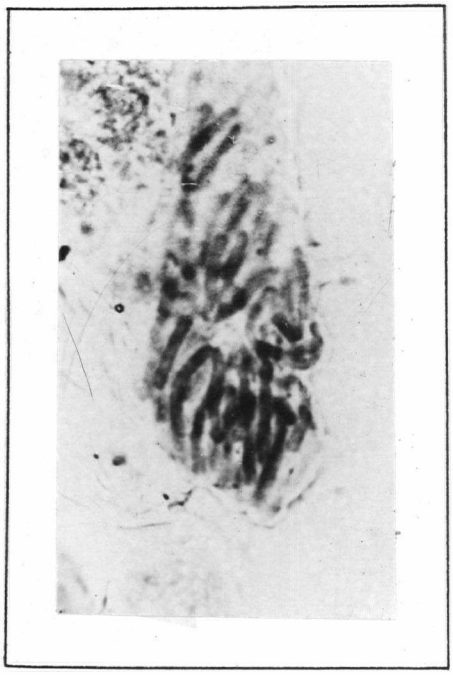

Fig. 11. Pro-metaphase in a normal twig on an aberrant seedling. (X1350)

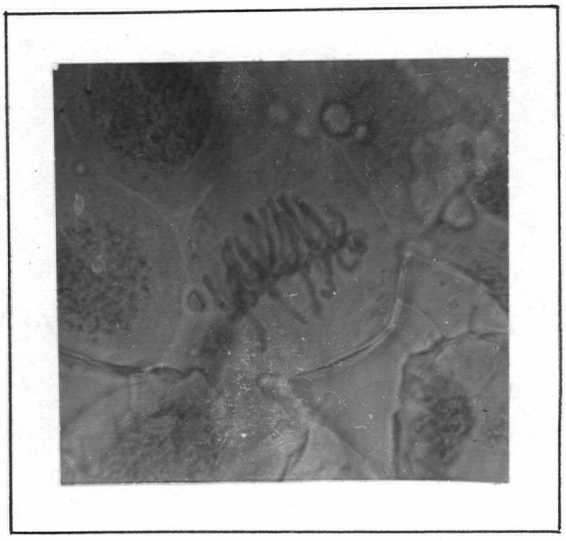

Fig. 12. Metaphase in a normal twig on an aberrant seedling. (X1350)

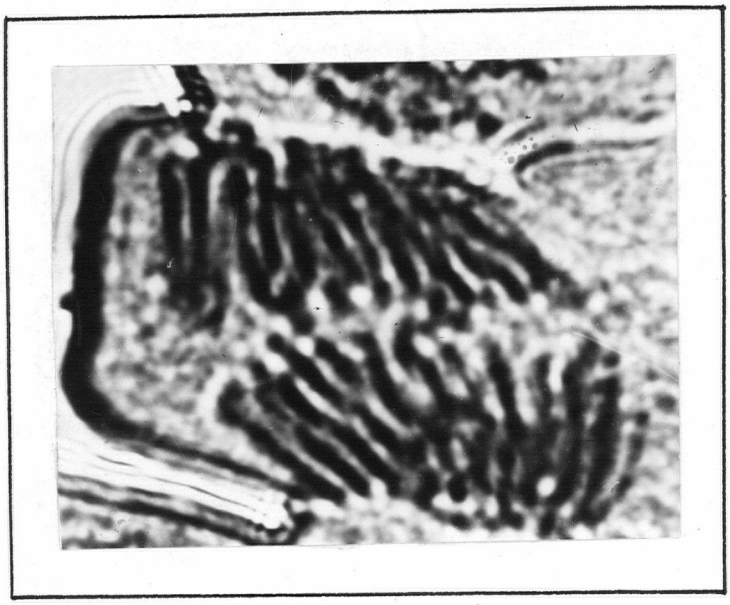

Fig. 13. Anaphase in an aberrant seedling. (X2400)

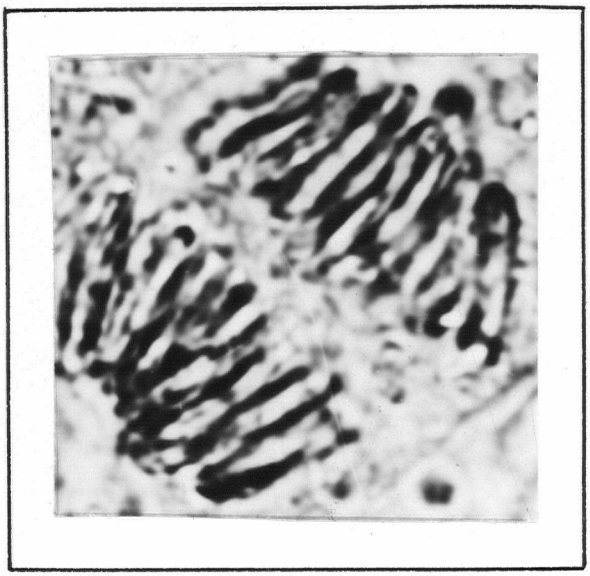

Fig. 14. Anaphase in a normal seedling. (X2900)

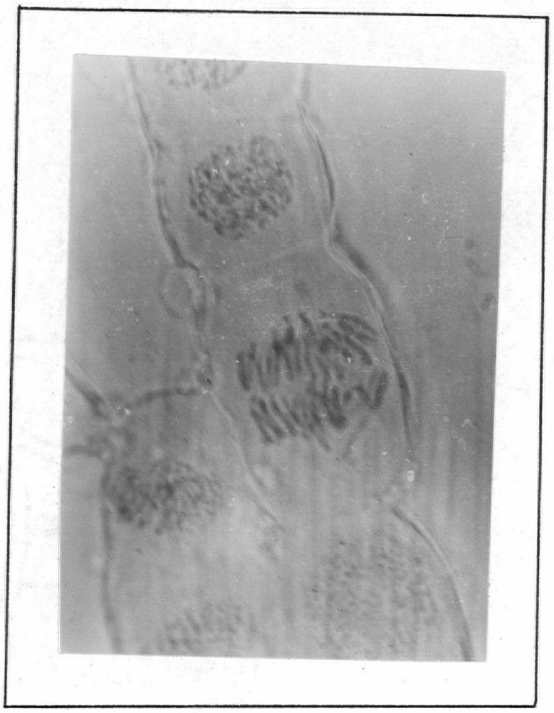

Fig. 15. Anaphase in an aberrant seedling. (X1350)

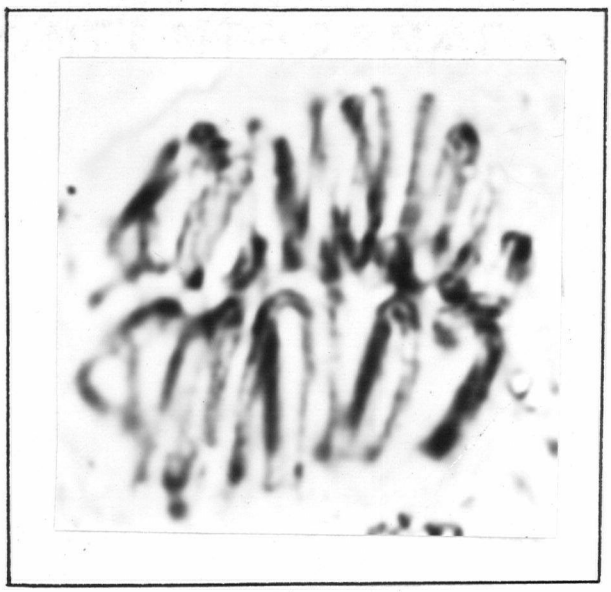

Fig. 16. Pro-metaphase in a normal seedling. (X2900)

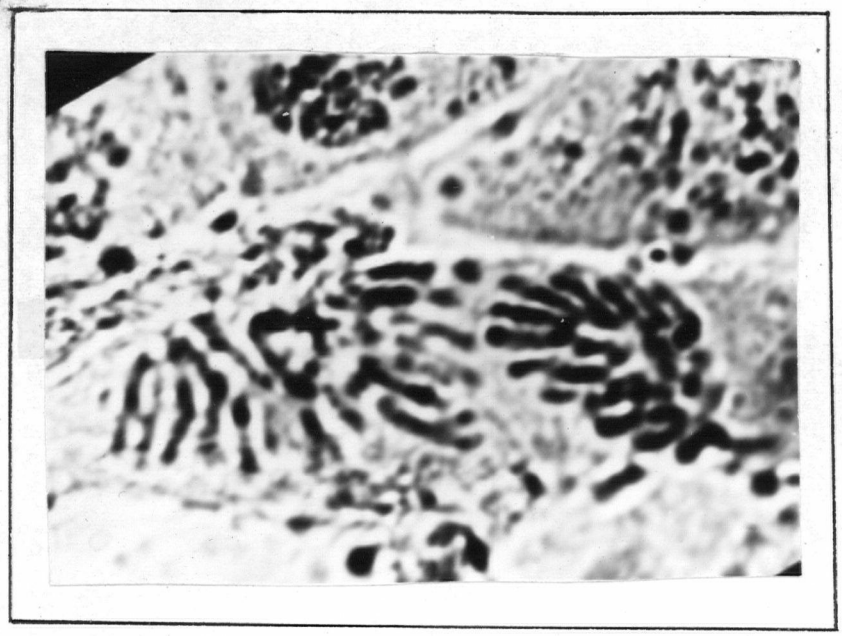

Fig. 17. Telophase (tilted polar view) in an aberrant seedling. (X2020)

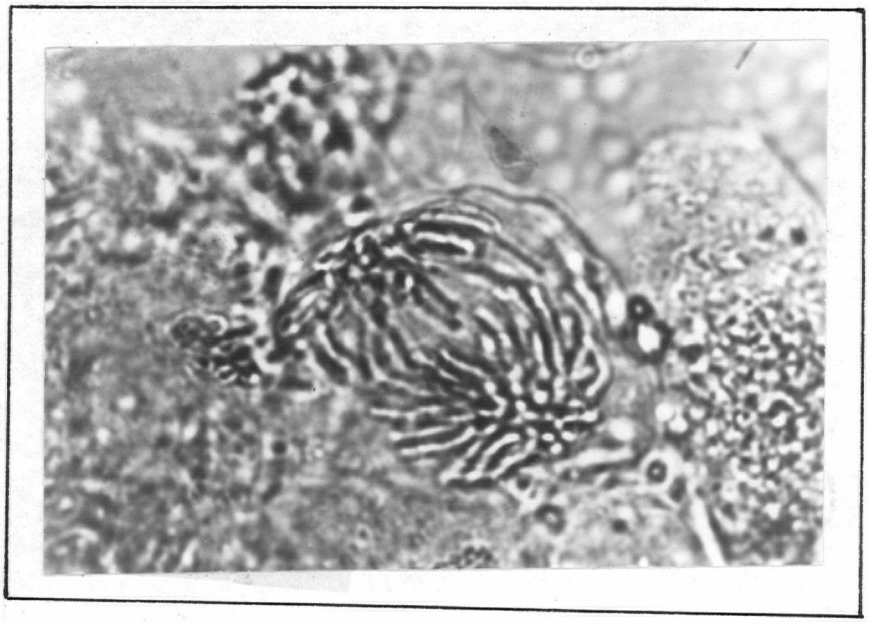

Fig. 18. Telophase (tilted polar view) in a normal seedling. (X1950)

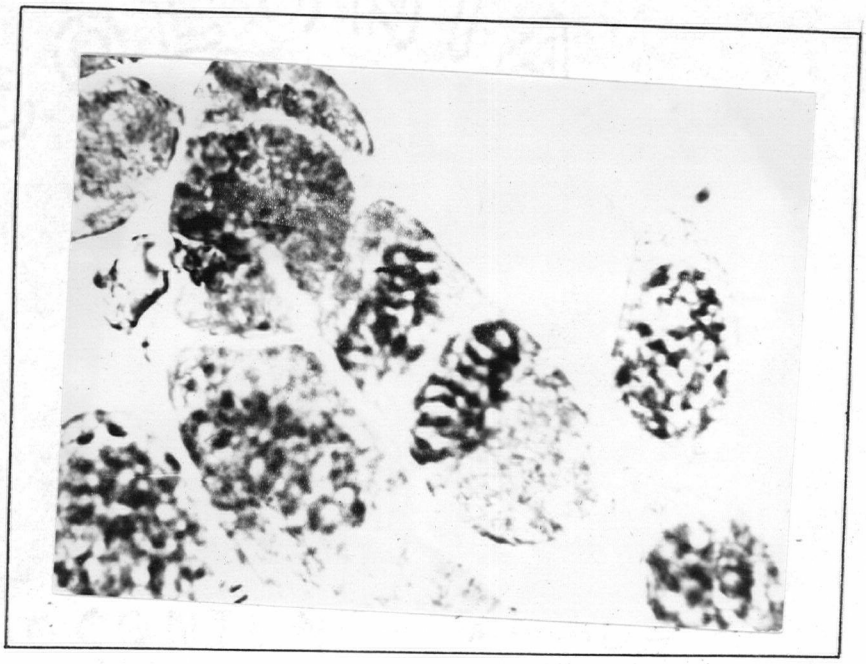

Fig. 19. Telophase in an aberrant seedling. (X1350)

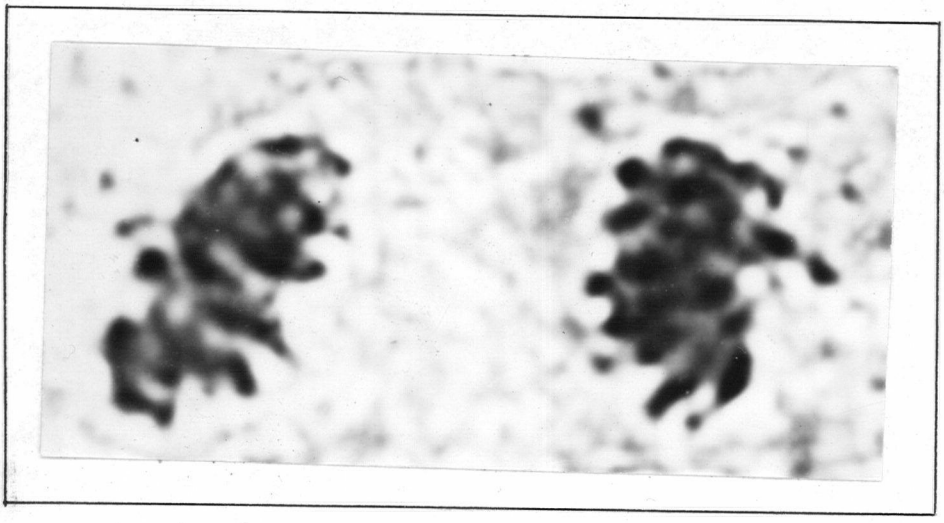

Fig. 20. Telophase in a normal seedling. (X2730)

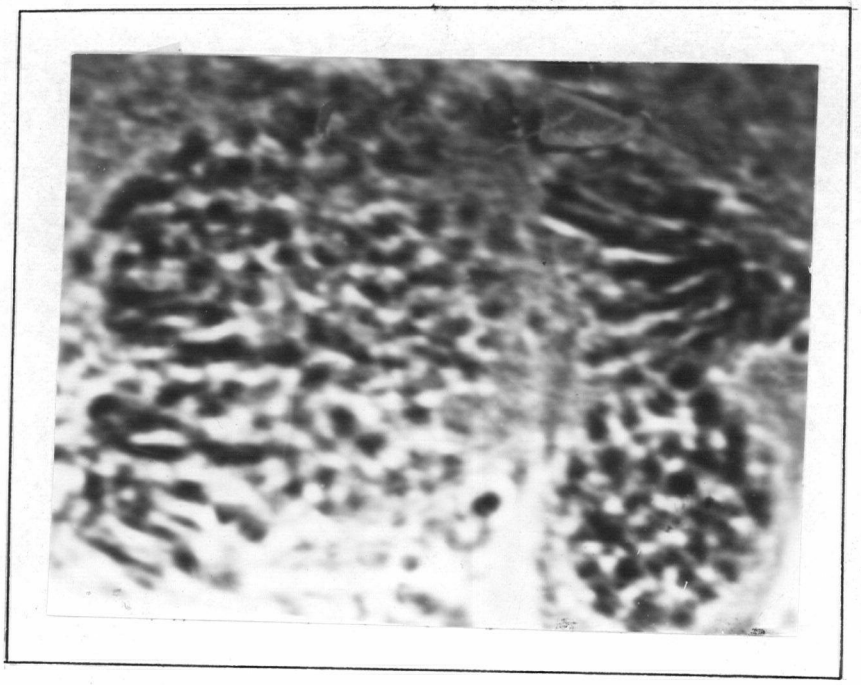

Fig. 21. Telophase in an aberrant seedling. (X1900)

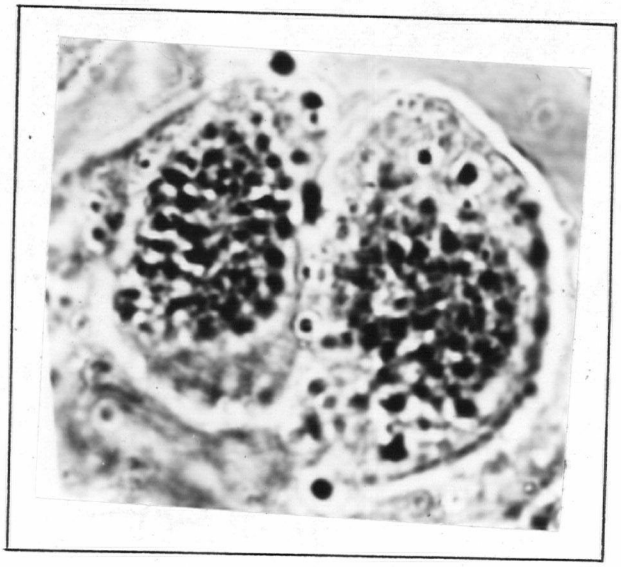

Fig. 22. Telophase in a normal seedling. (X1900)

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