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Occurrence of a mosaic-aneuploid in polyembryonic Norway spruce seed

Mosaik-aneuploid embryo i polyembryonalt granfrö (Picea abies (L.) Karst.)

by

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Introduction

Experiments were started some years ago at the Royal College of Forestry in Sweden to produce haploid individuals in forest tree species, a goal that, if it could be reached, would provide new theoretical and practical aspects for forest tree breeders (Simak, 1965; Stettler and Howe, 1965; Schreiner, 1967). These studies were made simultaneously with other projects in studying polyembryonic Scots pine and Norway spruce seed because in similar materials, such as those found in angiosperms, a relatively high frequency of haploids arose (Kimber and Riley, 1963; Magoon and Khanna, 1963). These investigations on haploidy are still going on, and this paper presents only one interesting example of a mosaic-aneuploid embryo that was found in polyembryonic seeds of Norway spruce (*Picea abies* (L.) Karst.).

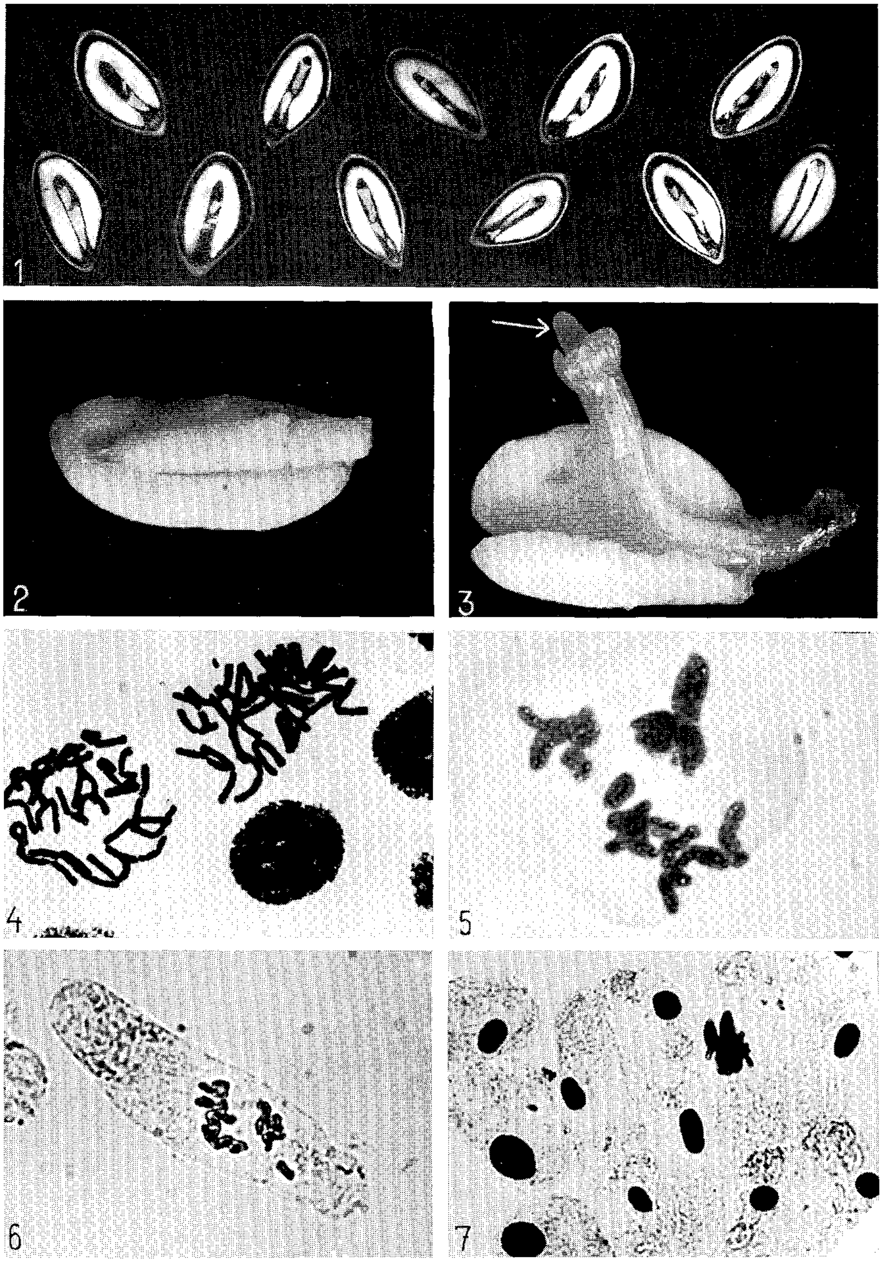
Material and methods

An abnormal embryo was found in a seed with twin embryos that originated from a northern Swedish spruce population (location: Njeltik; latitude: 65° 40'; altitude: 430 m; crop year, 1967). Polyembryonic seeds, which occur very frequently in the northern latitudes in Sweden, can be considered in Scots pine and Norway spruce embryogenesis as a modified stage caused mostly by the harsh climates (Simak and Gustafsson, 1954; Sarvas, 1962; Dogra, 1967). These polyembryonic seeds were easily recognized and separated from the monoembryonic seeds by the x-ray radiographic method (Fig. 1). After 48—72 hours in the germinators, embryos from the yet-ungerminated polyembryonic seeds were dissected and investigated cytologically by squash techniques.

Results

In this experiment, 220 mono- and polyembryonic seeds were analysed cytologically. Embryos in these seeds, observed on the x-ray radiographs, totalled 415. By dissection after 24—72 hours, however, only 348 embryos were obtained.

The mosaic-aneuploid embryo was smaller than its twin partner; both embryos, however, were morphologically differentiated, and had the yellowish-green color and the hard consistency that are characteristic of viable embryos. If competition did not occur between the two embryos, probably



Figs 1 — 7:

1. Radiographs of polyembryonic seeds of *Pinus silvestris* and *Picea abies*. The seed on the extreme right has a fully developed embryo (monoembryonic seed).
   2 — 7. *Picea abies* material.
2. A dissected seed with large (left) and small (right) embryos.
3. A dissected seed with a large embryo absorbing a small one (marked with arrow).
4. Cells from a normal embryo (2n = 24). Note the size of the nuclei and that of the chromosomes.
5. A cell with 14—16 chromosomes in the mosaic-aneuploid embryo.
6. A cell with 12—14 rather strongly contracted chromosomes in the mosaic-aneuploid embryo.
7. The nuclei in the mosaic-aneuploid embryo are very small and pycnotic, with the chromosomes showing clumping.

both would germinate and grow in the germinator. The larger embryo had the normal number of chromosomes, 2n = 24. The smaller, abnormal embryo showed 34 cells in different stages of cell divisions (excluding interphase), and the chromosome numbers in this deviant varied from 12 to 24, with a tendency toward the lower number (Fig. 8). In addition to the deviations of chromosome numbers from the normal, other abnormalities, such as strong chromosomal contractions (Fig. 5 and 6), clumpings (Fig. 7), pycnotic nuclei (Fig. 7) and micronuclei were also observed. We do not know whether or not the morphological aberrations in the chromosomes mentioned above are artifacts. The occurrence of micronuclei is, however, a common process in the first mitotic divisions in germinating embryos (Simančik *et al.*, 1966) and may not, therefore, necessarily be associated with the mosaic-aneuploidy.

These anomalies made difficult the determination of the exact number of chromosomes in each cell. For this reason, a chromosome number was estimated for each of the 28 investigated cells within the range in which the real chromosome number surely lay (Fig. 8). The exact chromosome numbers could not be determined in six other cells because of a heavy clumping effect. Rough estimates, however, indicated that their chromosome numbers were lower than 24 (cf. Fig. 7).

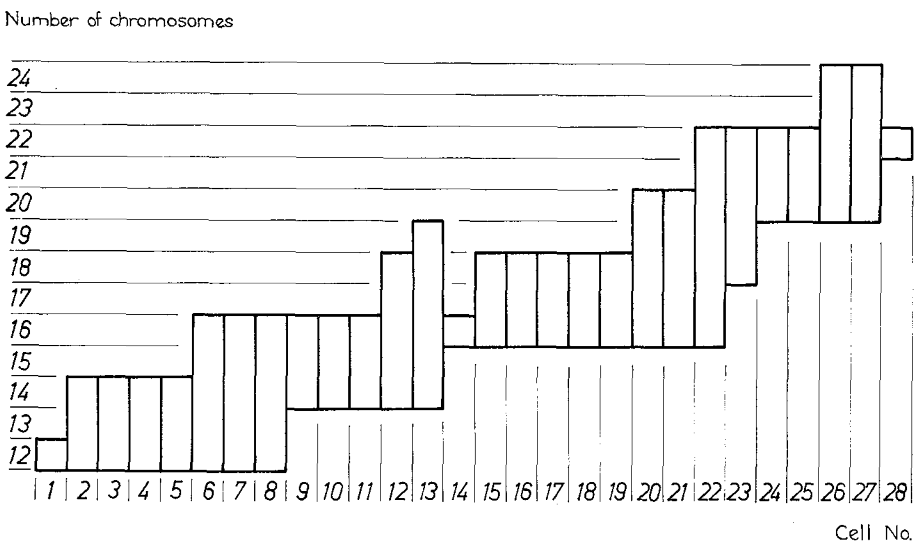


Fig. 8. The number of chromosomes was determined for each of the 28 investigated cells within the range in which the real number of chromosomes certainly lies. Example: In cell No. 13 the number of chromosomes is between 14 and 19.

Discussion

Most observers have found that spontaneous aberrations in chromosome number of somatic tissues of spruce belong to mixoploid, polyploid, or aneuploid individuals with chromosome numbers higher than 2n = 24 (Kiellander, 1950; Illies, 1958; Eifler, 1966). The individuals with chromosome numbers lower than the diploid level are of unusual interest, because they could originate from a haploid.

The first report of haploidy in chromosome number lower than 2n in Norway spruce was made by Illies in 1964. She obtained ten haploid seedlings from a greenhouse sowing of spruce seeds. Among the ten individuals, five arose from polyembryonic seeds and five came from seeds with reverse embryos. Illies's investigation showed for the first time that spontaneous haploids can occur even in gymnosperms. This important discovery would have been more conclusive if other information, such as the number of cells per plant investigated and the occurrence of aneuploid cells or haploid counts in all cells, had been included. From Illies's work no living haploid plants for further developmental studies were obtained, because the materials were processed for cytological examination.

Another report of haploidy in conifers was made recently by Pohlheim (1968), who found that *Thuja gigantea gracilis* is haploid (n = 11), the only known incidence of living spontaneous haploid individuals in gymnosperms. Demonstration by *Thuja plicata* and other species of the same genus, as well as *Picea omorica*, of a very high degree of self fertility indicates the occurrence of few recessive lethal factors in these species. Here is a possible explanation for the viability of *Thuja gigantea gracilis* in spite of its haploidy. It was also the reason why *Thuja plicata* and *Picea omorica* were recommended by Simak (1965) as suitable materials for investigation of haploidy.

These investigations show that spontaneous haploid plants with chromosome number lower than 2n do occur in coniferous species. Mehra and Dogra (cit. in Dogra, 1966) observed in archegonia of *Pinus spp.* the early stages of initiation of haploid proembryos. One may speculate that haploids may arise, under certain conditions, in unfertilized archegonia because of some stimulant from other, already fertilized archegonia.

Investigating the haploids in polyembryonic seeds is associated with the problem of how to keep alive the maximum numbers of embryos in these seeds. X-radiography can be used to select polyembryonic seeds; the microscopically small embryos cannot, however, be easily detected from the photographs (Dogra, 1967). These extremely small embryos are usually not viable (Simak, 1966). Moreover, we were not able to find, in the germinator or during the dissecting process, all of the well-developed embryos that were shown on the x-ray radiographs. This finding has been made repeatedly in Scots pine and Norway spruce during the early stages of germination when competition among embryos resulted in elimination of weak embryos (Simak, 1966; and Kim Ching and Simak, unpublished). Studied materials, therefore, must be kept in the germinator only until the first mitosis has started (from 48—72 hours), and sometimes even this period may be too long. Then, embryos should be dissected earlier and cultivated in artificial media to keep them growing. Suitable methods for collecting, storing, and extracting cones and seeds should be devised so that weak embryos will not be damaged or killed by these processes. Great care must be exercised to preserve these embryos if we expect to increase the recovery of haploids; their chromosome numbers could well make the individual much weaker than those with the normal complement. The possibility of obtaining living plants from these haploids is enhanced if cultivation and treatments are performed carefully. The ten haploid plants described by Illies (1964), cultivated in the greenhouse, lasted through the germination period without any special tender care.

Finally, the results of such experiments depend greatly on the genotypic constitution of the studied materials. Not only species such as *Thuja plicata* and *Picea omorica* have shown a high degree of self-fertility, but individuals within other species that have the same tendency (Ch. Plym Forshell, 1953, in Scots pine), may be suitable for haploid investigations.

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Summary

In this investigation, the chromosome number in the embryos of polyembryonal *Picea abies* seeds was determined. Polyembryony in seeds of Norway spruce is of rather common occurrence in the northern regions of Scandinavia. The selection of such seeds for experimental purposes can be easily made with the help of x-ray radiography.

Among 220 polyembryonal seeds investigated there was one with two embryos. One of the embryos was normal ($2n = 24$), the other was a mosaic aneuploid with chromosome number ranging from 12 to 24 (Fig. 8). Some other cytological abnormalities were also observed in this embryo. The possibility of getting plants from haploid embryos in polyembryonal seeds is discussed.

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Sammanfattning

Mosaik-aneuploid embryo i polyembryonalt granfrö (*Picea abies* (L.) Karst.)

I denna undersökning har studerats kromosomantalet i embryo, vilka härstammade från polyembryonalt granfrö. Förekomst av sådant frö är ganska vanlig i de nordligaste regionerna i Skandinavien. Urval av för dessa studier lämpligt polyembryonalt frö utfördes med hjälp av röntgenografisk metod. Bland de 220 analyserade fröna har vi hittat ett med två embryoner, av vilka ett var normalt (2n = 24) och det andra var mosaik-aneuploid med kromosomantal som varierade mellan 12—24. Dessutom har i detta embryo även fastställts förekomst av andra cytologiska anomalier. Möjligheten hur man till livet kan rädda ett svagt haploid embryo, vilket eventuellt kan isoleras ur polyembryonalt frömaterial, har också diskuterats.