Cytology of Normal and Seasonally Aberrant Microsporogenesis in *Asclepias* and *Dregea* (Asclepiadaceae)

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Temperature induced meiotic instability is known in several plants. In one of earlier findings, Sax (1937) recorded that high temperature conditions caused chromosomal aberrations in *Tradescantia*. Later, Jain (1962) observed the temperature sensitivity of the division cycle in *Lolium*. High temperature treatments of $37^{\circ}\pm 1^{\circ}\text{C}$ resulted in disturbances in microsporogenesis (Rana 1965) including formation of univalents (Utkhede and Jain 1974) in wheat. A reference to environmental effects—especially of high temperature—on sporogenesis and pollen viability has been made by Rana and Swaminathan (1964). Mostly, data for previous investigations were obtained from laboratory grown plants subjected to high temperatures. However, we detected microsporogenetic aberrations in natural populations of *Asclepias* and *Dregea* during summer months at a lower temperature range than that reported earlier. Both plants exhibited only transient sterility since they reverted to normal microsporogenesis with the advent of monsoon which brought about a general fall in temperature. The present paper discusses the cytological basis of this seasonal sterility.

Materials and methods

Four genera of Asclepiadaceae—*Asclepias*, *Dregea*, *Calotropis* and *Telosma*—which flower around the year were studied cytologically. All plants were collected from an area within two kilometers of the Indian Space Research Organisation Laboratories (ISRO) at Thumba, Trivandrum, from where the information on weather was obtained. Observations were also made on cultures of these four genera established at the Kerala University Botanical Gardens situated in the same area.

Conventional cytological methods were employed to prepare squashes of flower buds and root tips. The peak of cell division appeared to be between 9 and 10 A.M. in flower buds and around 2 P.M. in root tips and the respective materials were collected accordingly. Preparations for microsporogenesis presented some problems due to the limited number of pollen mother cells in each anther, the small size of the chromosomes and the presence of heavy inclusions of fat globules which interfered with staining. A drop of 1% haematoxylin, added to the acetocarmine squash preparations before sealing with the cover slip, improved contrast.

Variation in the shape and size of pollen mother cells and microspores was studied by taking microtome sections of appropriately developed flower buds. The usual methods of embedding, sectioning and staining were adopted.

Effects of seasonal variations on pollen viability were tested from June 1976 to May 1977. During this period, fresh pollinia from the four genera under study were cultured daily on a basic sugar media in a germination chamber. Within 90 min of transfer, pollen tubes emerged through the pollinial wall. Those which failed to produce even one pollen tube after two hours were taken as sterile. Such sterile pollinia were cultured with controls (fertile pollinia from other genera) in the same germination chamber.

Observations

Uniform climatic conditions prevailed over the area under study. Seasons are not well marked but the summer, characterized by high temperature and low precipitation, extends from February to May.

Meteorological data recorded during the last sixty years show that the daily maximum temperature crosses 32°C by the middle of February and remain, with few exceptions, in the range of 32°–35°C until the first or second week of May. Temperature values for 1976 and 1977 follow this general pattern. Fluctuations in humidity occur but they are not correlated with temperature variations.

Microsporogenesis in both garden and field grown plants of *Asclepias curassavica* and *Dregea volubilis* is normal except during summer when temperature induced abnormalities occur. A description of normal and aberrant cell divisions is given below.

Normal cell division

In both *Dregea* and *Asclepias*, root tip mitosis revealed 22 chromosomes and is normal throughout the year (Figs. 1, 3). In normal meiosis, 11 bivalents could be detected (Figs. 2, 4). Chromosomes of both genera are relatively small with a range in length from 1 to 3 μ .

As in other members of the Cynanchoideae sub-family of Asclepiadaceae, *Asclepias* and *Dregea* hold bag-like pollinia which enclose pollen grains. Anthers and pollinia have near oval shape and the PMCs are closely packed in them. Because of the space restrictions so imposed by the uneven shape of the pollinium, the PMCs take rectangular to wedge shaped forms and show a variation in length from 75 to 125 μ . Consequently, the meiotic products—the pollen grains—are also not of uniform shape or size. However, these differences in PMC morphology are not reflected in division processes.

Meiotic prophase in both genera is characterized by segmentation of the nucleolus. The PMC shows a single prominent nucleolus as it enters meiosis but by leptotene-zygotene, the nucleolus fragments into 3–11 pieces. These nuclear moieties take deeper stain than the embedding chromatin and can be clearly distinguished until they disappear by diakinesis.

Pollen grains also present atypical features. At the time of germination, the non-aperturate wall of the pollen grain breaks letting out the protoplast through the opening so formed. The free protoplast so formed is covered with only intine (Fig. 14), but exineless protoplasts also put forth tubes which grow normally.

The pollinia of four genera tested germinated easily and the process followed

the general pattern already described for *Calotropis* (Jalaja and Namboodiri 1975, Sreedevi and Namboodiri 1977). Normally, about 20–25 pollen tubes emerge out of the pollinium within 90 min of transfer to the medium.

Aberrant microsporogenesis and sterility.

The induction of aberrations in microsporogenesis is indicated by the failure

Figs. 1–8. 1,2, somatic anaphase ($2n=22$) and metaphase I ($n=11$) in *Dregea*. 3,4, somatic metaphase I ($2n=22$) and diakinesis ($n=11$) in *Asclepias*. 5–8. Aberrant microsporogenesis in *Asclepias*. 5, metaphase I, bivalents and association of chromosomes. 6, precocious separation of chromosomes at metaphase I. 7, univalents and unusual associations. 8, two late metaphase groups showing stickiness of chromosomes. Figs. 1, 3–8. $\times 1500$; Fig. 2. $\times 1100$.

Figs. 9-14. Microsporogenetic abnormalities in *Dregea*. 9, non synchronous chromosome condensation in metaphase II. 10, initiation of furrowing in a PMC with tripolar grouping of chromosomes. 11, an unreduced microspore with degeneration of cytoplasm. 12, two dyads with irregular nuclear division. Cytokinesis in left dyad results in nucleate and anucleate daughter cells; asynchronous division is seen in the two daughter cells of the dyad on the right side. 13, a large and a small microspore formed from irregular cytokinesis. 14, normal and exineless pollen grains of *Asclepias*.

of pollinia of *Asclepias* and *Dregea* to germinate from the middle of February to the second week of May 1977. During this period, the maximum daily temperature varied from 32° to 35°C. With the onset of monsoon in May-June, the maximum temperature dropped below 30°C and by this time normal germination of pollinia was restored. Germination of the pollinia of *Calotropis* and *Telosma*, however, was not affected by the seasonal variations in temperature.

Relative humidity showed wide fluctuations though unrelated to seasons or temperature variations. In 1976 and 1977, daily maximum humidity varied between 72 and 100% and the minimum between 34 and 75%. On individual days the variation in humidity may be anywhere from 1 to 60%. Because of these uncertain shifts no correlation could be established between relative humidity and pollen viability.

Association between high temperature and sterility was more definitive and loss of pollen viability during summer months was accompanied by a wide spectrum of microsporogenetic aberrations. No variation from the normal was observed in the early stages of meiosis—up to diakinesis. By the beginning of metaphase I, various kinds of chromosome associations reduced the normal haploid number; the most frequent number counted during this stage was 8 instead of the usual 11 bivalents (Fig. 5). Another aberration noticed was precocious separation of chromosomes (Fig. 6). Presence of univalents (Fig. 7), stickiness of chromosomes and irregular separation of chromosomes (Fig. 8) are also observed. Disturbances in anaphase separation sometimes result in tripolar separation and grouping of chromosomes. Non synchronised condensation of chromosomes is seen even in metaphase II (Fig. 9). All these aberrations result in pollen inviability and consequent failure of pollinial germination.

Pollen inviability in these genera may also be due to various cytokinetic aberrations which occurred in about 30% of the PMCs counted. In both genera, cytokinesis is normally successive, but in aberrant PMCs the origin and plane of segmentation show variations. Tripolar separation in anaphase I is followed by initiation of cytoplasmic furrowing from three points as is demonstrated in Fig. 10. In other PMCs, segmentation may not be completed by the time of synthesis of the wall resulting in the production of a macrospore (Fig. 11). Irregular cytokinesis may also be associated with cytoplasmic and chromatinic disintegration as seen in Fig. 11. Unequal segmentation also results in the formation of large and small inviable spores (Fig. 13). Instances have been observed where the first division is normal but one of the cells of the dyad fails to undergo any further division while the nucleus of its companion cell divides unequally leading to the formation of daughter nuclei of different sizes (Fig. 12). The spores formed at the end of aberrant microsporogenesis showed wide variations in size and chromosome contents and all of them were invariably inviable.

Discussion

The chromosome number of *Asclepias* and *Dregea* (as well as several other genera of Asclepiadaceae) has been already recorded (Fedorov 1969) and this has

been confirmed in this report. There are, however, certain interesting points of their microsporogenesis which deserve attention. Even in normal meiosis pollen grains are not uniform in size or shape although all of them are viable. The presence of exineless pollen protoplasts at the time of germination and their normal development are also atypical features.

Nucleolar fragmentation reported here has a parallel with the segmentation and embedding of the nucleolar pieces in the chromatin observed in the meiotic prophase of *Lathyrus* by Sciple and Jhonston (1962) but the apparent coalescing of the nucleolar fragments reported by them does not occur in *Dregea* and *Asclepias*.

Temperature induced meiotic aberrations such as condensation differences, occurrence of univalents, multiple association of chromosomes, non-synchronisation of anaphase separation and degeneration or disorganisation of chromosomes have been reported by various authors (Jain and Rana 1963, Rana 1965, Utkhede and Jain 1970, 1974). Such aberrations also occur in the microsporogenesis of *Asclepias* and *Dregea* during summer months. In addition, these plants show various cytokinetic abnormalities leading to the formation of pollen of various sizes and chromosome contents. Many of the cytokinetic aberrations noted here were of the patterns described by Tara and Namboodiri (1964, 1976) but they had observed these abnormalities in a genetically sterile mutant of *Impatiens sultani* and not in association with temperature effects.

In some of the earlier reports, temperature sensitivity of microsporogenesis was determined at a relative humidity of 70%. Wolgast and Stout (1977) found a direct relationship between relative humidity and fruit set in bear oaks grown under controlled conditions but not in field grown plants. The plants of the present study were growing under conditions where diurnal variations in humidity sometimes exceeded 50% and it is therefore not surprising that correlations could not be established between relative humidity and sterility.

Previous reports mostly were based on work with flowers exposed to high temperatures in the laboratory but the temperature influenced aberrations reported here occur in flowers of field or garden grown plants. Also the literature reports that aberrations are induced at a temperature of $37^{\circ}\pm 1^{\circ}\text{C}$, but the present investigation shows that in *Asclepias* and *Dregea* a range of 30° – 35°C is adequate to cause the types of chromosome aberrations previously reported, as well as several unlisted heat associated cytokinetic abnormalities. It may also be noted that these plants which produce aberrant microspores when exposed to summer conditions revert to normal microsporogenesis with the fall of temperature below 30°C .

It may be pointed out that although four genera of Asclepiadaceae, viz. *Calotropis*, *Telosma*, *Asclepias* and *Dregea* flower from early February only the last two show temperature sensitivity. Seven more genera of Asclepiadaceae frequently found in the same habitat flower only by the beginning of monsoon in May-June or by the time the normal microsporogenesis of *Asclepias* and *Dregea* is restored. No aberration was detected in any of them.

It is known that if temperate plants are artificially exposed to high temperatures or introduced into tropical regions microsporogenetic aberrations leading to sterility occur. However, *Asclepias curassavica* is a tropical American plant

naturalised in South U. S. (Bailey 1951) and in India (Bazbaruah 1963). *Dregea volubilis* is indigenous. Therefore, the seasonal sterility exhibited by these plants is not because of their sudden exposure to an unacclimatised environment. Rather, the transient microsporogenetic aberrations are due to the plants' inability yet to adapt to the range of high temperature prevalent during summer months.

Summary

The chromosome number of *Asclepias curassavica* and *Dregea volubilis* is confirmed as $n=11$ and $2n=22$. Normal microsporogenesis in both genera is characterized by the following atypical features: marked variations in the size and shape of the pollen mother cells and pollen grains, the segmentation of the nucleolus in the leptotene-zygotene stage and the presence of germinating exineless pollen protoplasts with normal tube growth.

Both plants exhibit seasonal sterility during summer. Microsporogenetic aberrations leading to pollen inviability occur at a temperature range of 30° – 35°C in contrast to the previously reported induction of aberrations at $37^{\circ}\pm 1^{\circ}\text{C}$. Various chromosomal aberrations associated with high temperatures such as presence of univalents, chromosome associations, unequal anaphase separation as well as unlisted heat associated cytokinetic abnormalities involving incomplete cytoplasmic cleavage and irregular segmentation result in the production of spores with wide variation of size and chromatinic contents. Consequently, pollen grains produced by these plants during summer are almost totally sterile. However, with the fall in temperature below 30°C , plants revert to normal microsporogenesis and pollen viability is restored.

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