Some Observations on the Reproduction of 
*Tridax procumbens* L.

I. Pollen mother cells and pollen development

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**Abstract**

Chromosome data place *Tridax procumbens* at the tetraploid level. Regularity of bivalent formation suggests that little homology exists between the constituent genomes but meiotic irregularities nevertheless occur frequently and result in some 15% of the pollen being obviously non-viable. Premiotic aberrations in the walling of the sporogenous tissue give rise to 'normal' and 'giant' pollen mother cells. The latter are characterised by their abnormal size and often by their massive walls and elongated "tails" of either walled cytoplasm or wall material alone. The largest giant cells are binucleate.

Meiotic irregularities in the 'normal' pollen mother cells include the formation of a quadrivalent or a trivalent and univalent; one or more lagging bivalents; first and second division bridges and the failure of spindle formation at either the first or second divisions. In the 'giant' cells delay in the onset of meiosis occurs frequently, cells showing prophase or diakinesis being found among tetrads or differentiating pollen grains. Other giant cells show highly irregular divisions culminating in a variable number of nuclei. An attempt at cleavage into microspores occurs in a minority of these cells. The nuclei in binucleate giant cells do not necessarily behave synchronously and, as in the uninucleate giant cells, often show delayed or arrested division. In the minority, where both nuclei negotiate the division successfully a "tetrad" of eight apparently normal microspores may result. While the cause of the initial irregular cytoplasmic cleavage remains unknown, it is tentatively suggested that the subsequent meiotic retardation and irregular division in the giant cells may be related to their abnormally high cytoplasmic/nuclear volume.

**Introduction**

*Tridax procumbens* is an annual species native to S. America but widely distributed in Tropical America, Africa and Asia, where it is a highly successful weed of civilization. It is a member of the family Compositae and tribe Senecioneae with heterogamous capitula having 4–7 pale cream ray florets, which are effectively female, and a variable number of up to seventy hermaphrodite disc florets. Being therophytic, it is dependent on a high set of fertile seed, yet observations on wild populations consistently reveal a proportion of abnormally small or shrivelled and non-viable pollen grains and empty cypselas. In both cases these account for some 15% of the total. The investigation was undertaken to try to determine the causes of this sterility and to ascertain whether any apomictic mechanism operates in this species since agamospermy is widespread in the Compositae.
Materials and methods

Buds approximately 5 mm in diameter show a series of stages from fully differentiated pollen grains in the outermost disc florets to premeiotic sporogenous tissue in florets at the centre of the capitulum. Only a very small proportion of the anthers from any one capitulum shows diakinesis or metaphase I. Raghavan and Venkatasubban (1941) imply that there may be a diurnal periodicity in the meiotic cycle, alternatively these stages may be passed through very rapidly. Acetic and propionic orcein both give excellent results for squash preparations of pollen mother cells and root tips following fixation in acetic alcohol. The anther squash preparations were supplemented with serial sections stained in Feulgen or Meyer’s haemalum.

Observations

1. Chromosome counts

Both root tip and pollen mother cell preparations confirm, for West African material the diploid number of 36 reported by Raghavan and Venkatasubban (1941) for the species in India.

All the capitula examined show two types of pollen mother cell within the developing anthers:

1) Cells approximately 16μ in diameter at prophase increasing to 22μ by the end of the second meiotic division. These will be designated ‘normal’ pollen mother cells, since the majority go through a regular meiosis producing a tetrad of viable pollen grains.

2) A minority of the mother cells are recognised from their abnormally large size as ‘giant’ mother cells. They show varying degrees of meiotic irregularity and seldom give rise to functional pollen grains.

2. Meiotic behaviour in ‘Normal’ pollen mother cells

Chromosome pairing is generally regular, eighteen bivalents being observed at diakinesis. More than half of these are ring bivalents characterised by two strongly terminalised chiasmata. The remaining bivalents form a single chiasma, so that the average number of chiasmata per mother cell is 30, or 1.6 per bivalent. The chromosomes lack any strongly characteristic features and the single satellited chromosome reported by Raghavan and Venkatasubban (1941) was not observed in this material. One quadrivalent, or a trivalent and a univalent occur in a small proportion of the pollen mother cells. The univalents fail to get on to the spindle at metaphase I. In a number of cases one lagging bivalent is also observed. This may persist as such into the second division or may separate at that time into two univalents. In either case incorporation into micronuclei is likely to be the ultimate result.

Anaphase bridges occur with a low frequency in all capitula, though they may have a very high local frequency in individual pollen sacs (Figs.
As observed by Dodds and Simmonds (1946) in *Musa*, these bridges are not always accompanied by chromosome fragments. Breakage of the bridges prior to the second division may result in either nuclei with deficiencies and duplications, or two nuclei both with deficiencies and a broken "bridge fragment" that might ultimately become incorporated into a micronucleus. Persistence of bridges into the second division has been observed and results in configurations of cross bridges depending on how the chromatin stands involved attempt to segregate in the second division. Further bridges may occur between the separating daughter chromatids in the second division and these may also result in chromatin fragments ultimately incorporated into micronuclei. The "tetrads" that result from these aberrations are characterised by having between three and seven more or less normal sized though probably non viable microspores, together with one or more small supernumary nuclei formed from chromatin fragments and lagging chromosomes (Fig. 1g).
A different type of meiotic breakdown in 'normal' pollen mother cells appears to be associated with the failure of spindle formation in either the first or second divisions. In the former case separation of the chromosomes into irregular masses appears to follow directly upon diakinesis. In the latter the chromosomes segregate into irregular groups after anaphase I.

These irregularities would account for the proportion of non viable pollen grains and small grains containing micronuclei which occur in all pollen samples.

3. The origin of the 'Giant' pollen mother cells

Apart from their abnormal size, these cells are of the same general form as a normal pollen mother cell and are invested by the same type of rather massive mucilaginous wall. The cells lie in file with the normal pollen mother cells and although they may protrude into the tapetum and even cause distention of the anther, there is no evidence for their origin from relation to the tapetal cells and they must be regarded as aberrant pollen

Fig. 2. Giant cells. a, irregularities of walling in the sporogenous tissue resulting in two potential giant cells (g'g'') and an enucleate cell (e). b, uninucleate giant cell showing eighteen bivalents. c, attempted division resulting in numerous irregular groups of chromatin. d, meiosis resulting in eight nuclei but no attempt at cytoplasmic cleavage. The cell may have been originally binucleate. e, thick walled giant cell with irregular chromatin groups. f, giant cell showing irregular cleavage into microspores. g, binucleate giant cell, both nuclei at early prophase with two small enucleate cells. h, binucleate giant cell, both nuclei at diakinesis and showing eighteen bivalents. All ×468.
mother cells. They would seem to arise as a result of irregular walling in the sporogenous tissue and, while the reasons for such developmental irregularity remain obscure, observation of the subsequent sequence of events leaves little doubt that the subsequent development of any pollen mother cell into a 'normal' or a 'giant' is determined at this stage. The observed evidence may be summarised as follows:

1. Enlarged pollen mother cells are visible in premeiotic and early 1st division anthers (Fig. 2a, Figs. 3 1a and b).
2. The giant cells are very variable both in shape and size. In shape they range from more or less spherical to irregularly elongated, the latter not infrequently have an extended 'tail' of wall material. They ultimately attain a size ranging from 30-100µ in length compared with a diameter of approximately 22µ for a mature normal tetrad. They are generally, though not invariably, accompanied by small, thick walled enucleate cells filling any gaps in the files of mother cells resulting from the presence of a giant cell (Fig. 2g, Figs. 3 1a and f).
3. Irregular walling seldom occurs only once in a pollen sac—thus a pollen sac is either without giant cells or contains several (Fig. 1a).
4. The majority of giant cells are uninucleate and differ from normal cells only in their increased volume of cytoplasm. However, as a result of their mode of origin a minority are, from their inception, binucleate. The largest giant cells are, as would be anticipated, almost invariably binucleate, whilst the smaller are generally uninucleate (Figs. 2g and h). The two nuclei in a binucleate cell maintain their identity (i.e. there is no fusion) and do not necessarily behave synchronously so that it is possible for one to be at diakinesis whilst the other shows no signs of division.

4. Meiotic behaviour in giant pollen mother cells

Giant pollen mother cells seldom produce fully differentiated microspores, though in many stages of meiosis may be observed. The main features of nuclear behaviour are summarised below:

1. Giant cells frequently show delayed division. Thus giant cell nuclei at prophase, diakinesis and metaphase I may be found in tetrad stage pollen sacs (Fig. 3e). Eighteen bivalents are readily counted in these diakinesis nuclei (Fig. 2b), thus invalidating the theory advanced by Raghavan and Venkatasubban (1941) that the giant cells arise by fusion of pollen mother cells. In binucleate giant cells, each nucleus appears to have the normal diploid chromosome complement though since they seldom behave synchronously, it is not often possible to verify this (Fig. 2h). This delayed onset of meiosis may result in the giant cells having failed to complete the first meiotic division when their walls, together with those of the
Fig. 3. a, irregular walling of sporogenous tissue in the anther. ca.×783. b, large giant cell in prophase anther. ca.×533. c, giant cell showing attempted cytoplasmic cleavage among normal tetrads. ca.×783. d, giant cell without wall among pollen grains. ca.×783. e, two giant cells showing delayed meiosis. ca.×533. f, giant cell with many irregular chromatin groups and two small enucleate cells among tetrads. ca.×333. g, thick walled giant cell with eight nuclei. ca.×1050. h, two tetrads containing six and seven microspores respectively. The former also has a micronucleus rounding off to form a small pollen grain. ca.×533. e-h, are squash preparations.
normally differentiated tetrads disintegrate. Breakdown of the cytoplasm of the giant cells does not occur until the tapetum disintegrates so that they may be observed without walls but still undergoing meiosis, among the differentiating pollen grains (Fig. 3d).

(2) Divisions which take place more or less simultaneously with those of normal pollen mother cells vary in their degree of irregularity. Partially regular divisions result in mother cells containing between 3 and 8 nuclei (Fig. 3g and Fig. 2d) and such divisions are sometimes followed by an attempt at cytoplasmic cleavage and microspore formation, as in the rounding off of normal microspores (Fig. 2f and Fig. 3c). Partial or complete delimitation of the microspores, which are probably non viable, may occur and this stage has again been interpreted erroneously by Raghavan and Venkatasubban (1941) as resulting from the fusion of pollen mother cells. Synchronous and fairly regular division of a binucleate giant cell may occasionally produce a more or less regular looking tetrad of six to eight fully formed microspores (Fig. 3).

(3) Between those cells in which meiosis is delayed and the minority in which a division is more or less completed are the majority of giant cells in which meiotic breakdown occurs during an attempted division (Fig. 3f). Such cells ultimately show a varying number of highly irregular groups of chromatin (Figs. 2c and e). There is no attempt at cytoplasmic cleavage and disintegration follows upon the normal dissolution of the mother cell wall, although recognisable residues of such cells may be seen in anther sacs containing fully developed pollen grains.

Discussion

Compared with a diploid chromosome number of \(2n = 18\) for *Tridax trilobata* (Hjelmqvist 1951) which is the only other species for which a chromosome count has been recorded, \(2n = 36\) for *T. procumbens* places it at the tetraploid level. Until more information is available regarding other members of the genus, no surmises can be made regarding either the origin of the constituent genomes of *T. procumbens* or its probable evolutionary history. Although the infrequency of multivalent formation indicates that there is little homology between the constituent genomes, the frequent occurrence of other meiotic irregularities does suggest that there is some degree of genetic unbalance within the species. The aberrations observed are undoubtedly similar in nature to those reported earlier by Raghavan and Venkatasubban (1941) and they would seem, therefore, to be of general occurrence within the species and not confined to any one locality or part of the species area.

The only obvious difference between a ‘normal’ pollen mother cell and
uninucleate 'giant' would seem to be in the volume of cytoplasm. It is clearly impossible to describe a regular sequence of events in the highly aberrant form of development shown by these cells, but the observations made suggest that while relatively small increases in the proportion of cytoplasm to nucleus are associated with some delay in the onset of meiosis or failure of the second meiotic division and the subsequent cleavage into microscopes, large increases result in extreme retardation or arrest of meiosis and the presence of early meiotic configurations in anther sacs containing tetrads. In the binucleate giant cell the volume of cytoplasm/nucleus does not differ so widely from that in a normal uninucleate pollen mother cell. Observation of "tetrads" containing 7 or 8 normal sized nuclei or fully walled microspores indicates that a few of these binucleate giants negotiate meiosis in a nearly, if not entirely, normal manner. The largest binucleate cells show a similar extreme retardation of meiosis to that in the bigger uninucleate cells.

**Literature cited**

