Cytology of *Thelepogon elegans* Roth ex Roem et Schult¹

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Introduction

Thelepogon Roth ex Roem et Schult, a monotypic genus, has only one species namely *elegans*. It is a member of the tribe Andropogoneae of Gramineae. The grass is a coarse annual herb. Some information has been gathered about the distribution of the grass after consulting the following works, Hooker 1897, Cooke 1906, Gamble 1922, Rhind 1945, Raizada *et al.* 1957, Santapau 1957 and Bor 1960 etc. The grass is distributed in India and tropical Africa. In India it grows in Madhya Pradesh at Indore, Central India, Bombay, Poona, Nasik, Purandhar, Hyderabad State at Ellora, Madras, the Concan, Malabar, Belgaum and W. Peninsula. The grass is fairly common on hill sides upto about 3,000 feet in Burma. It is eaten by cattle but never sufficiently abundant to count much in the fodder supply (Rhind 1945). This is reported to be a bitter grass but it is eaten by horses (Raizada *et al.* 1957), possibly in default of anything better. It is a gregarious species and according to Blatter is very abundant on the "bunds" between the rice-fields in the Carnatic (Bor 1960). It is a fair fodder grass (Whyte 1963). No cytological studies have so far been conducted on this monotypic genus. In the present paper cytogenetical studies of this grass are described.

Materials and methods

The grass seeds used in the present study were obtained through the courtesy of Dr. Bhatt, Agricultural College, Indore (M.P.). The seeds were sown in the month of July, 1946 in the garden of the Botany Department of Allahabad University. During flowering season (October to November) the flower buds were fixed in Carnoy's fluid (absolute alcohol, glacial acetic acid and chloroform in the proportion of 3:1:1). A trace of ferric acetate added to the fixative was found to yield better results. The anthers were then squashed in acetocarmine. Photomicrographs were taken from permanent slides which were made by using n-butyl alcohol-acetic acid series and mounted in Canada balsam.

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Figs. A₁-A₂. Somatic metaphases of *Thelephogon elegans*. Figs. 1-22. 1, diakinesis showing 5 bivalents. 2, metaphase I in equatorial view showing 5 bivalents. 3, metaphase I in

Observations

Mitosis: The chromosome number in this grass is determined from the study of root-tip squashes. The $2n$ chromosome number is found to be 10 (Figs. A₁ and A₂). This is first record of chromosome number in this species. The sizes of the chromosomes vary from $3.00\ \mu$ to $4.8\ \mu$. On the basis of sizes the chromosomes could be classified into the following types:

Type A—A pair of long chromosomes with sub-median primary constrictions,

Type B—Two pairs of long chromosome with sub-median primary constrictions,

Type C—A pair of median-sized chromosomes with sub-median primary constrictions,

Type D—A pair of short chromosomes with median primary constrictions and each having a satellite at the shorter arm.

Meiosis in normal plant: Early stages of prophase of meiosis could not be undertaken. At diakinesis five bivalents are clearly seen, one of which is attached to the nucleolus (Figs. 1, 23). The mean number of chiasmata per cell is 16 and the mean number of chiasma frequency per bivalent is 3.20. At metaphase I five bivalents always assembled at the equational plate (Figs. 2, 24). In few cases four bivalents are seen arranged on the equator and one bivalent is lying away from the equator (Figs. 3, 25). At anaphase I generally there is normal separation of the chromosomes. In few instances chromatin bridge is seen (Figs. 4, 26). The number of such bridge is one. The chromatin bridge in most cases is unaccompanied by any fragment. The bridge is seen to be simply stretched out chromatin material. Rarely four chromosomes reach each of the two poles and 2 are seen lagging at the equator at anaphase I. From this stage it appears that this bivalent which could not orient at the

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equatorial view showing non-orientation of a bivalent. 4, anaphase I showing a chromatin bridge. 5, anaphase I showing two laggards. 6, anaphase I showing normal separation. 7, metaphase II with 5 chromosomes in each plate. 8, anaphase II showing normal separation. 9, four microspores. 10, diakinesis showing 10 bivalents in a syndiploid cell. 11, metaphase I showing 10 bivalents in a syndiploid cell. 12, diakinesis showing a chain of four chromosomes and three bivalents. 13, diakinesis showing a chain of six chromosomes in which one bivalent is nucleolar, and two bivalents. 14, diakinesis showing an open ring of eight chromosomes in which one bivalent is nucleolar, and one bivalent. 15, prometaphase showing a chain of six chromosomes and two bivalents. 16, metaphase I in equatorial view showing an open ring of eight chromosomes and two chromosomes resulted from a precocious separation of a bivalent. 17, metaphase I in polar view showing a chain of six chromosomes in which one bivalent is interlocked, and two bivalents. 18, metaphase I in equatorial view showing a chain of four chromosomes, two bivalents and 2 chromosomes due to precocious separation of a bivalent. 19, anaphase I showing delayed disjunction of a bivalent. 20, anaphase I showing 5 chromosomes at each pole. 21, metaphase II showing 5 chromosomes in each plate. 22, anaphase II showing normal separation.

Magnification: Figs. 1-22. $\times 775$, except Figs. A₁-A₂. $\times 600$. Figs. 10-11. $\times 335$.

Figs. 23-29. 23, *Thelepogon elegans*. Diakinesis showing 5 bivalents. 24, metaphase I showing 5 bivalents in equatorial view. 25, metaphase I showing non-orientation of a

equator has now reached the equator and disjoin into two chromosomes (Figs. 5, 27). Though the anaphasic separation is delayed in the different P.M.Cs, yet the chromosomes reach each pole in equal number. Fig. 6 shows five chromosomes at each pole. At telophase I the two daughter nuclei are separated through a wall formation which is of the successive type.

Prophase II is very short and the chromosomes immediately acquire metaphase appearance. Five chromosomes are observed at each equator at metaphase II (Fig. 7). The division in both cells of a dyad is synchronous. Anaphase II is quite regular (Fig. 8). At telophase II a wall is layed at right angles to the previous one and this results in the formation of isobilateral tetrads of microspores. They soon separate (Fig. 9) and develop into healthy pollen grains.

Pollen viability is about 98%

In few P.M.Cs ten bivalents are seen at diakinesis. The size of the P.M.C. is just double than the normal P.M.C. One chromosome is seen attached at the each of the two nucleoli (Figs. 10, 28). At this stage chromosomes originating from different nuclei became intermingled and at metaphase I ten bivalents are congressed on a single metaphase plate (Figs. 11, 20). This is due to the fact that the nuclear fusion occurred after synapsis, no greater association than bivalents would be expected. This is a case of syndiploidy.

Meiosis in a plant showing chain and open ring formation: While the meiotic chromosomes of *Thelepogon elegans* show the normal structure as described above, in the case of one plant certain spontaneously occurring deviations comprising formation of chains and open rings involving more than two chromosomes are met with. The plant under observation shows no morphological anomaly.

Table 1. Association at diakinesis

	1 _{VI} +1 _{II}	1 _{VI} +2 _{II}	1 _{IV} +3 _{II}	5 _{II}
No. of P.M.Cs observed	14	62	75	84
Percentage	5.91	26.38	31.91	35.75

The chromosome configurations are studied in 235 cells at diakinesis. 84 of those cells possess a normal number of five bivalents. The remaining 151 cells exhibit associations of eight, six and four chromosomes. The various types of chromosome associations observed in the plant are shown below in Table 1 along with the number of cells bearing the particular configurations:

Out of 235 cells studied at diakinesis 31.9% shows a chain of four bivalent on the equator. 26, anaphase I showing a single chromatin bridge. 27, anaphase I showing two laggards. Figs. 23-27. $\times 2325$. 28, diakinesis showing 10 bivalents in syndiploid cell. $\times 1000$. 29, metaphase I showing 10 bivalents in syndiploid cell. $\times 1000$.

chromosomes and three bivalents (Fig. 12). 26.38% shows a chain of six chromosomes and 2 bivalents (Fig. 13) and 5.91% shows an open ring of eight chromosomes and one bivalent (Fig. 14). A chain of six or an open ring of eight chromosomes is always seen associated with one of the chromosomes of a nucleolus organizing bivalent. At pro-metaphase a chain of six chromosomes and two bivalents are observed (Figs. 13, 30).

At metaphase I, the various types of associations observed are presented in Table 2.

Table 2. Association at metaphase I

	1vIII+1II	1vI+2II	1vI+3II	5II
No. of P.M.Cs observed	10	51	63	75
Percentage	5.02	25.62	31.68	37.68

Figs. 30-34. 30, prometaphase showing a chain of six chromosomes and two bivalents. 31, metaphase I showing an open ring of eight chromosomes and two chromosomes. 32, metaphase I showing a chain of six chromosomes and two bivalents. The hexavalent is interlocked with a bivalent. $\times 1000$. 33, metaphase I showing a chain of four chromosome, two bivalents and two chromosomes. 34, anaphase I showing delayed disjunction of a bivalent. Magnification: Figs. 30-34. $\times 2325$.

Out of 199 cells studied at metaphase I 37.68% shows normal five bivalents. 5.02% shows an open ring of eight chromosomes and one bivalent. Figs. 16, 31 show an open ring of eight chromosomes and two chromosomes. An interlocking of bivalents is observed in some cells. 25.62% shows a chain of six chromosomes in which one bivalent is interlocked (Figs. 17, 32). 31.68% shows a chain of four chromosomes and three bivalents (Figs. 13, 33).

The maximum association observed is that of an open

ring of eight chromosomes. This indicates that four different bivalents involved in translocations. One bivalent is always found away from the open ring or chain. Not more than one chain or an open ring is observed in one P.M.C. The various configurations are observed in different P.M.Cs.

Sometimes one bivalent shows delayed disjunction at anaphase I (Figs. 19, 34). Anaphase I (Fig. 20) and telophase I are normal.

Five chromosomes are seen at each equator of the metaphase II (Fig. 21). Anaphase II (Fig. 22) and telophase II are found to be regular. Pollen viability is about 97%.

Discussion

Syndiploidy: In few P.M.Cs of *Thelepogon elegans* ten bivalents instead of five are seen at diakinesis and metaphase I. No multivalents have been seen. This appears to be a case of syndiploidy. Here the two daughter nuclei in the germinal cells at the last division before meiosis failed to separate and the individual nuclei develop synchronously and passed through leptotene, zygotene, pachytene and diplotene stage. The chromosomes originating from two nuclei became intermingled and at metaphase I ten bivalents congressed on a single metaphase plate.

The term syndiploidy was originally used by Strasburger (1907), who explains this condition as due to the fusion of nuclei to give a double chromosome number especially in the division immediately preceding meiosis. Darlington (1937) ascribed this phenomenon as due to reunion, or failure of separation of daughter nuclei in the germinal cells prior to meiosis. Levan (1941) uses the term syncyte in a wider sense wherein he does not only include the fusion of nuclei but also fusion of P.M.Cs. He finds the fusion from two to thirty P.M.C. in *Phleum pratense*. He observed that at metaphase I these syncytes behave as large cells, developing one bipolar spindle on which all the bivalents present are arranged into one regular equatorial plate. Price (1965) uses the term syncyte in which fusion of P.M.Cs has been observed in certain clones of *Saccharum* and *Erianthus*. He states that syncytes may be formed due to the breakdown of cell walls. He observed that two or only a few P.M.Cs unite through the dissolution of adjacent cell walls, the process had usually been called fusion.

In the case of *Thelepogon elegans* no fusion of P.M.Cs has been seen. Not only this, even fusion of not more than two nuclei has been examined. Therefore the original term syndiploidy is preferred here.

The evolutionary significance of syndiploidy lie in the production of diploid gametes, which ultimately produce polyploid forms. This is one of the major source of polyploidy in plants. Karpechenko (1927) found the progeny in which the chromosome number suggested the functioning of pollen derived from syndiploid cells.

Interchange heterozygosity: During the meiotic investigation of *T. elegans*,

an annual monotypic genus, having $n=5$ chromosomes, in the case of one plant spontaneous occurrence of chromosomal interchange has been observed between non-homologous chromosomes.

Out of 235 cells examined at diakinesis (Table 1), 5.91% shows an open ring of eight chromosomes and one bivalent, and 26.38% shows a chain of six and 2 bivalents and 31.91% shows a chain of four and 3 bivalents. At metaphase I, 199 cells studied (Table 2), in which 5.02% shows an open ring of eight chromosomes and one bivalent, 35.62% shows a chain of 6 chromosomes in which one bivalent is interlocked and 31.68% shows a chain of four and three bivalents. The maximum association was that of 8 chromosomes. One bivalent is always found away from the open ring or chain. Not more than one chain or open ring has been observed in one P.M.C. The various configurations have been observed in different P.M.Cs. The spontaneously occurring chain and open ring configurations in *Thelepogon elegans* indicate a failure of chiasma formation in one pairing segment in an association of four, six and eight. Such failure is generally brought about when the translocating segments are not very long as in the case of maize T3-6 (Clarke and Anderson 1935) and Barley (Burnham *et al.* 1954).

Venkateswarlu (1958) has reported a spontaneously occurring translocation heterozygote in *Coix aquatica*. Koul (1964) observed the association of 4 or 6 chromosomes in *Coix aquatica*. He explained the formation of four and six chromosomes as due to association between non-homologous heterochromatic segments, which also sometimes results in the interlocking of bivalents.

The first cytological demonstration of the location of an interchange between two non-homologous chromosomes was given by McClintock (1930) in a semi-sterile line of maize. Since then extensive cytological work has been carried out in maize as well as in other plants and animals. Burnham (1936) cited 40 genera in Dicotyledoneae and 47 genera in Monocotyledoneae in his review referring to such translocations. The spontaneously occurring interchange has been reported in different plants. Among others, Naithani and Raghuvanshi (1958 a, b) and Raghuvanshi (1962 a, b) have reported such translocation interchange in *Citrus assamensis*, for the first time in the genus. They observed the formation of multivalents, such as tri-, quadri- and hexavalent at metaphase I in P.M.Cs of *C. assamensis*, which is a diploid form. Rana and Jain (1965) observed a ring or chain of four chromosomes, their number varies from one to two in different populations of diploid annual *Chrysanthemum carinatum*. Khoshoo and Mukherjee (1966) reported a ring or a chain of 4 plus 7 bivalents in a diploid garden *Canna*.

Burnham (1956) presents seven possible ways in which reciprocal translocations may arise spontaneously. They are

- 1) Crossing over between duplicated segments which are present in non-homologous chromosomes,
- 2) The spontaneous association between heterochromatic regions,

- 3) The interlocking of bivalents at meiosis,
- 4) Accidental entanglement of chromosomes,
- 5) Chromosomal breakage followed by reunion of broken ends,
- 6) In progenies of plants, homozygous for the 'sticky chromosome' character (in maize) and
- 7) When plants are grown from aged seeds.

Out of these no evidence of chromosome duplication, spontaneous association between heterochromatic regions, spontaneous chromosome breakage or of sticky nature of the chromosomes so far observed in the present investigation. The plants were not raised from aged seeds.

If these configurations also are explained on the basis of reciprocal translocations it would mean that four of the five bivalents have undergone interchanges. This appears rather unlikely because pollen fertility of the plant is as high as 97%. As pointed out in the text these associations in the present case involved an interlocking of bivalents. This, associated with high pollen fertility of the plant amply justifies the conclusion that the associations of four, six and eight chromosomes is due to association by the interlocking of bivalents at meiosis or an accidental entanglement of chromosomes or both the ways indicated by Burnham.

Summary

1. Chromosome number of *Thelepogon elegans*, a monotypic genus, has been found to be $2n=10$. This is the first record of chromosome number in this monotypic genus. It forms five bivalents at metaphase I. The grass is diploid. Pollen viability is 98%.
2. In few P.M.Cs ten bivalents instead of five have been observed at diakinesis and metaphase I. This is a case of syndiploidy.
3. In one plant of this grass, spontaneously occurring associations of chain of four, six or an open ring of eight chromosomes have been observed showing interchange heterozygosity. Pollen viability is 97%.

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