Cytoembryological Studies on Indian *Taraxacum* Weber

I. Microsporangium, microsporogenesis and microgametogenesis

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Received February 19, 1980

The European *Taraxacum* spp. have been the subject of several cytological investigations in recent years. Battaglia (1948) described meiosis in three apomictic species of *Taraxacum*. She observed several meiotic irregularities resulting in 1 (restitutional) 2, 3 or 4 nuclei at the time of heterotypic division. These at the end of meiosis produced dyads, tetrads and polysporads. Malecka (1961, 1964, 1965) investigated the karyotypes and meiosis of 20 species of *Taraxacum*. In *T. serotinum* (Malecka 1964) about 44 percent PMC's showed meiotic irregularities and the formation of multinucleate pollen grains as a result of defective cytokinesis. Richards (1973) came to the conclusion that the genus arose in the West Himalayas during the cretaceous era. The cytoembryological studies on the Indian representatives of the genus *Taraxacum* has not been worked out adequately and therefore, the present investigation is taken up. The present paper deals with the ten species of the genus.

Materials and methods

The material for the present investigation was collected from various localities in Kashmir, India (see Table 1) and voucher specimens are preserved in Botany Department, Rajasthan University, Jaipur, India. It was fixed in acetic alcohol (1:3) and stored in 70% ethyl alcohol. Usual method of dehydration through T. B. A. and embedding in paraffin wax were followed. Microtome sections about 8-10 μ were cut and stained in Heidenhain's hematoxylin iron alum.

Palynological preparations were made following the Erdtman's (1952) modified acetolysis method of Nair (1960). For studying the range of size variability of pollen grains of different *Taraxacum* spp. measurements of 100 pollen grains from each of the five different heads was taken up. Their mean was taken out and it was plotted on graph. Pollen viability was determined by 1% lactic acid-iodine.

Observations

Microsporangium: Each anther contains four microsporangia and receives

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a single vascular strand. The young anther consists of homogeneous parenchymatous cells. A single series of archesporial cells differentiates in the hypodermal position in each corner. Each archesporial cell divides periclinally and gives rise to an outer parietal and inner sporogenous cell. During further growth the cells in the parietal layer divide anticlinally and periclinally. The latter results in the formation of two secondary parietal layers (Figs. 1A, R, VV), of which the inner functions as tapetum, whereas another periclinal division in the outer layer results in the formation of the middle layer and the endothecium. Thus, the development of the anther wall is of 'Dicotyledonous' type and it comprises epidermis, endothecium, single middle layer and a glandular tapetum (Figs. 1B, X, HH, II, NN, EEE).

Table 1. List of plants investigated

S. no.	Name	Locality	Collected or sent
1.	<i>Taraxacum flavum</i> v. Soest	Between Tangmarg and Gulmarg (Kashmir)	Vijay Koul
2.	<i>T. gulumargense</i> v. Soest.	Tangmarg Dak Bungalow	—
3.	<i>T. harbhajan-singhii</i> ssp. <i>pahalgamense</i> v. Soest.	Pahalgam	—
4.	<i>T. tenebristylum</i> v. Soest.	Gulmarg	—
5.	<i>T. vulpinum</i> f. <i>pallidocarpum</i> v. Soest.	Yarikhah Tangmarg	—
6.	<i>T. himalaicum</i> ssp. <i>himalaicum</i> v. Soest.	Gulmarg	—
7.	<i>T. banhyhalense</i> v. Soest.	Verinag	—
8.	<i>T. polyodon</i> Dahlstedt.	Nedou's Hotel Srinagar	—
9.	<i>T. duplidense</i> Lindberg f.	Kashmir Univ. Campus	—
10.	<i>T. wallichii</i> De Candolle	Udampur Jammu.	Mr. Bhardwaj, K. L.

The tapetum is of dual origin and its cells on the connective side differentiates from the ordinary somatic cells. At first each cell of the tapetum is small uninucleate with small amount of cytoplasm. As the anther grows further, the tapetal cells stretch tangentially and their nuclei undergo a series of mitotic divisions prior to meiosis in the microsporocytes (Figs. 1, II, PP). Simultaneously active synthesis in the cytoplasmic contents take place and the tapetal cells are full of homogeneous contents in which distinct vacuoles are not formed. Although the tapetal cells become multinucleate in all the species, the number of nuclei shows a slight range of variation (Fig. 1PP). In *Taraxacum flavum* there are upto 6 nuclei whereas it varies from 8–16 in the remaining species (see Table 2). The nuclei in the tapetal cells do not show any fusion until the microspore tetrads have been formed.

Fig. 1 A–JJJ. Microsporangium, microsporogenesis and microgametogenesis in *Taraxacum* spp. A–K, *T. polyodon*. A–D, stages in the development of anther sacs. Note the fibrous thickenings in endothecium in 'D'. E–K, dyads, tetrads, polysporads of microspores and the development of male gametophyte. Note multinucleate giant pollen grains in 'K'. L–O, *T. duplidense*, stages

in the development of anther sacs. Note the radial enlargement of tapetal cells and digestion of the meiocytes in 'Q'. R-W, *T. wallichii*, stages in the development of anther sacs, tetrads of microspores and male gametophyte. X-CC, as above in *T. banhyhalense*. DD-GG, as above in *T. himalaicum* ssp. *himalaicum*. HH-MM, same in *T. vulpinum* f. *pallidocarpum*. NN-UU, same in *T. tenebristylum*. Note the multinucleate condition in tapetal cells in 'PP' and variations in sporad types 'QQ-SS'. VV-DDD, same in *T. harbhajan-singhii* ssp. *pahalgamense*. Note the formation of restitutional nucleus during meiosis I and irregularities in homotypic division. EEE-HHH, same in *T. flavum*. III, JJJ, uninucleate pollen grains of *T. gulmargense*. (ispl, inner secondary parietal layer, ospl, outer secondary parietal layer, tp, tapetum).

During microgametogenesis they fuse forming first several polyploid masses and simultaneously the cell contents are depleted. The tapetal cells disappear by the time 2-celled pollen grains result.

In *T. duplidens* which show total male sterility, the behaviour of the tapetal cells after the differentiation of the microsporocytes differ considerably from that of the other investigated species. The tapetal cells usually remain uninucleate throughout their existence (Figs. 1L–O) and only rarely some cells become 2-nucleate. They either degenerate as such or more often they show pronounced radial enlargement and press upon the microspore mother cells. The tapetal cells as well as the microsporocytes are exhausted and finally collapse rendering the anthers totally male sterile (Figs. 1P, Q).

Table 2. Data on tapetum, sporad types and pollen viability in *Taraxacum* spp.

Name	Tapetum	Frequency in % of sporad types					
	No. of nuclei	Tetrad	Triad	Dyad + micro nuclei	Dyad	Poly sporad	Pollen viability
<i>T. harbhajansinghii</i> ssp. <i>pahalgamense</i>	9–14	2	3	20	75	—	30
<i>T. tenebristylum</i>	14	38	12	—	6	44	20.5
<i>T. vulpinum</i> f. <i>pallidocarpum</i>	10	39	6	10	—	45	27
<i>T. wallichii</i>	10–15	91	—	9	—	—	86
<i>T. bankhyhalense</i>	12	26	10	28	20	16	26
<i>T. himalaicum</i> ssp. <i>himalaicum</i>	12	29	12	32	—	27	25
<i>T. polyodon</i>	10	50	17	16	—	17	37

The middle layer is ephemeral and shows signs of degeneration as the microspores prepare to enter meiosis (Figs. 1C, DD, EEE). However, its remnants can be identified even after the formation of tetrads of microspores.

The endothelial cells stretch soon after their formation and during microgametogenesis show radial enlargement. They acquire fibrous thickenings at maturity in all the species except *T. duplidens* (Figs. 1D, S, OO). The endothecium persists in anthers which dehisce introrsely by two longitudinal slits.

Microsporogenesis: The primary sporogenous cells directly function as microspore mother cells. They round up prior to entering meiosis. In *T. duplidens* the sporogenous tissue degenerate at microspore mother cell stage and never enters meiosis (Fig. 1C). In *Taraxacum wallichii*, the only diploid and sexually reproducing species, meiosis is fairly regular forming more than 90 percent normal tetrads of microspores (Figs. 1T, U). In the remaining species *T. harbhajansinghii* spp. *pahalgamense*, *T. tenebristylum*, *T. vulpinum* f. *pallidocarpum* and *T. polyodon* considerable degree of meiotic disturbances have been recorded.

Due to small quantity of material meiosis was studied from paraffin sections cut at 8–10 μ thick and stained with Heidenhain's iron-alum haematoxylin. Structure of tetrads of microspores and pollen viability is determined in all the species investigated except *T. duplidens* in which the sporogenous tissue degenerate at

microspore mother cell stage and the taxon shows total male sterility. However, details of meiosis are observed in *T. harbhajan-singhii* ssp. *pahalgamense* triploid with $n=12$ and *T. tenebristylum* tetraploid with $n=16$ only.

The observation reveal that meiocytes follow a regular as well as an irregular course. During regular meiosis the chromosomes pass through normal stages (Figs. 2A–E). They arrange at equatorial plate in metaphase and undergo equal and synchronous distribution of chromosomes to the poles, 12 chromosomes at each pole in *T. harbhajan-singhii* ssp. *pahalgamense* and 16 chromosomes at each pole in *T. banhyhalense*. The interkinesis period as dyad is short and second division figures are observed in the same head and often in the same floret. As a result of normal course of meiosis and cytokinesis by furrowing tetrahedral or isobilateral types of tetrads of microspores are formed (Figs. 2 D, E).

In almost all heads, the florets showed serious meiotic disturbances both in triploid *T. harbhajan-singhii* ssp. *pahalgamense* and tetraploid *T. tenebristylum* (Figs. 1WW, AAA). The most common of these is the precocious separation of 2 or more chromosomes in one or more groups during 1st meiotic division (Figs. 2F, G). The lagging of univalents during anaphase I is observed quite frequently (Fig. 2H). The lagging univalents which are 2–4 in number result in the formation of micronuclei in dyads which after division forms polysporads of different chromosome numbers (10:10:2:2 chromosomes). Besides this, unequal distribution of chromosomes at anaphase I (Figs. 2I, J) and II forming nuclei of unequal sizes is also observed (Fig. 2J). In *T. harbhajan-singhii* ssp. *pahalgamense* the most common distribution of chromosomes observed is 26 and 8 and also 20 and 4 chromosomes. But in *T. tenebristylum* it was not possible to ascertain the exact number of chromosomes at each pole, but in many cells it was more than 16 chromosomes at one pole. These result in the nuclei of unequal size and after completing the equational division (2K, L) result in the formation of 4 nuclei of different sizes (Figs. 2M, N).

In 50 to 70 percent PMC's the chromosome pairing was poor or lacking and the univalents are scattered along the spindle (Figs. 2P, Q). The chromosomes fail to separate forming a restitution nucleus (Fig. 2R). This nucleus divides usually forming two nuclei (Fig. 2S). Occasionally during this division unequal distribution of chromosomes and formation of one or two micronuclei as a result of precocious separation of chromosomes and lagging chromosomes during division is also observed. In these cells, cytokinesis follows immediately resulting in the formation of unreduced dyads and dyads with one or two microcytes (Figs. 2T, U). The dyad cells round up and secrete a wall. An interesting behaviour observed in large numbers of these dyads is that they become subsequently two nucleate (Fig. 2V) and finally their nuclei fuse to form a single nucleus (Fig. 2W). Although the chromosome number could not be known for the second division, it seems that it is mitotic and the resulting nucleus is of hyper unreduced type i.e. $4r$ of Battaglia (1955). Formation of monads is also quite frequently observed in these species (Fig. 2X).

These meiotic irregularities result in the production of sporads with varied number of cells. Except the sexual *T. wallichii* which produces a very high per-

Fig. 2 A-X. Stages in the meiosis of *Taraxacum harbhajan-singhii* ssp. *pahalgamense* (A-R, U-W) and *T. tenebristylum* (S, T, X). A-E, normal meiosis forming tetrads of microspores. F, G, metaphase showing precocious separation of chromosomes. H, leaggards at anaphase I. I, J, unequal distribution of chromosomes and formation of micronuclei. K-M, equational division in dyad with unequal nuclei and formation of tetrads. N, microspore tetrad, note a large and 3 small microspores. O, polysporads. P-T, stages in the formation of restitution nucleus and unreduced dyads. U, as above, note the unequal size of microspores. V, W, 2-nucleate dyad and fusion of nuclei in each cell. X, monads.

centage of normal tetrads of microspores, a wide range of variability in spore types is formed in the remaining species (see Table 2). This leads to the formation of pollen grains of various sizes.

Microgametogenesis: The young microspores on separation vary in size. They enlarge, become spherical and their wall differentiates into exine and intine. A vacuole appears in the cytoplasm and the nucleus is pushed close to the wall. It divides mitotically forming a large vegetative cell and a small lenticular cell. The latter separates and the pollen grains are shed at 2-celled stage (Figs. 1 I-K, V, W, BB, CC, FF, GG, KK-MM, TT, VV, CCC, DDD, FFF-JJJ).

Mature pollen grains which represent various degree of ploidy show considerable variation in size. In the diploid *T. wallichii* the pollen grains range between $18\ \mu$ to $22.5\ \mu$ whereas in the remaining species the diameter of pollen grains vary from $9\ \mu$ to $36\ \mu$ (see Table 3). The acetocarmine smears of pollen grains revealed that the percentage of pollen viability is high in *T. wallichii* while in the remaining species it ranged from 18–37 percent only.

Table 3. Range of pollen grain size in *Taraxacum* spp.

Species	$9\ \mu$	$13.5\ \mu$	$18.0\ \mu$	$22.5\ \mu$	$27.0\ \mu$	$31.5\ \mu$	$36.0\ \mu$	Total
<i>T. flavum</i>	35	85	45	65	125	125	20	500
<i>T. gulmarganse</i>	15	80	110	60	125	80	30	500
<i>T. harbhajan singhii</i> ssp. <i>pahalgamense</i>	10	65	75	130	150	55	15	500
<i>T. tenebristylum</i>	10	70	85	100	120	95	20	500
<i>T. vulpinum</i> f. <i>pallidocarpum</i>	90	100	50	20	115	125	—	500
<i>T. wallichii</i>	—	—	90	410	—	—	—	500
<i>T. banhyhalense</i>	40	45	70	70	150	115	10	500
<i>T. himalaicum</i> ssp. <i>himalaicum</i>	10	70	70	50	175	95	30	500
<i>T. polyodon</i>	20	80	45	75	220	35	25	500

The pollen grains are spheroidal 3-zono-aperturate, covered by a thick exine which form reticulate ridges on the surface. The exine is covered uniformly by spine. Multinucleate, particularly 2-nucleate pollen grains are observed. In *T. polyodon* a few giant grains with 3 nuclei have also been observed (Fig. 1K). In *T. tenebristylum* in few heads pollen grains were formed inside the microspore mother cell wall (Fig. 1SS). An operculum is observed in the mature pollen grains of *T. wallichii*.

Discussion

The microsporogenesis in *Taraxacum* spp. is both of normal and abnormal types resulting in the formation of regular tetrads, dyads, triads, pentads and hexads etc. The resulting spores are reduced as well as of unreduced and hyper-unreduced types. The method of formation of the sporad type is described in detail

Table 4.

A. Reduced (r)

in *T. harbhajan-singhii* ssp. *pahalgamense* and *T. tenebristylum* in the present study. Malecka (1961, 1964, 1965, 1967) has shown the same for *T. pieninicum*, *T. serotinum*, *T. palustre*, *T. comitans*, *T. eriopodum*, *T. sinicum* whereas Battaglia (1955) and Poddubnaja-Arnoldi *et al.* (1935) have reported meiotic disturbances in *Rudbeckia sulvantii* and *Scorzonera tau-saghyz* respectively. Malecka (1962) and Battaglia (1955) have also figured schemes showing various courses of abnormal meiosis in *T. pieninicum* and *R. sulvantii* respectively. The authors have attempted to prepare a scheme (Table 4) to cover majority of the most frequent abnormalities during microsporogenesis in *Taraxacum* spp.

The present study of meiosis in *T. harbhajan-singhii* ssp. *pahalgamense* and *T. tenebristylum* shows that dyads are usually formed following the formation of

Table 4. (cont.)

B. Unreduced (2r) C. Hyper unreduced (4r)

Schematic representation of meiotic steps forming different sporad types

heterotypic restitution nucleus at the end of meiosis I (see also Battaglia 1947, 1948, 1955) but Malecka (1964) considers their formation even from the reduced nuclei in *T. pienicum*. This however needs checking. Davis (1964) reported in *Brachycome ciliaris* the formation of some unreduced dyad cells but Kapil and Sethi (1962) observed dyads with reduced nuclei in *Ainsliaea aptera*. Murty (1964) has given a long list of plants forming dyads and triads. Though personally we doubt the formation of dyads and triads having reduced nuclei in *Taraxacum* spp. such courses have been included in the scheme represented by dotted lines, on account of their report at present in quite a few cases.

The above meiotic disturbances are attributed to thermal shocks in *Scorzonera* (Proddubnaja-Arnoldi *et al.* 1935), since these were observed in buds collected after strong frost. Malecka (1961) who fixed the buds of *T. pienicum* both during a long fine weather and after strong frost, observed the meiotic disturbances in the apical parts of the anther irrespective of the weather. She regards them to be due to some physiological factor namely the weak supply of the nutrients. The meiotic

disturbances in the *Taraxacum* spp. investigated presently is neither confined to any particular region nor is affected by weather conditions. The meiotic behaviour of the presently investigated plants was more or less similar in the native areas in Kashmir valley and the hostile Jaipur (Rajasthan) climate. The authors are of the opinion that it is inherent of the species and controlled by genetic factors. However, the effect of environment in microsporogenesis is not unfamiliar.

An interesting feature of *Taraxacum* spp. is the formation of pollen grains of different size on account of highly irregular meiosis. Tschermak-Woess (1949) and Holmen (1952) drew graphs of variability of pollen grains size in species investigated by them and concluded that the diploid species show a limited scale of variability and the graphs are unimodal, whereas, in polyploid forms an enormous range of variation is found to occur. Similar graphs are drawn for all the investigated species during the present study and *T. wallichii*, a diploid species, showed a limited scale of variability, whereas, a wide range is recorded for the triploid species. Malecka (1965) observed a narrow range of variability in diploid *T. comitans* but considerable variations in *T. serotinum*.

Another feature observed in apomictic *Taraxacum* species is the formation of giant pollen grains having 2, 3 or 4 nuclei. They have been reported in *T. serotinum* (Malecka 1964) such pollen grains are the result of inhibition of cytokinesis, a rather rare phenomenon.

Acknowledgement

We are grateful to Prof. J. L. van Soest of Netherland for the identification of the *Taraxacum* species.

Summary

Microsporogenesis in 10 species of *Taraxacum* was studied. Meiosis is both of regular and irregular type resulting in the formation of regular tetrads, dyads, triads, pentads and hexads etc. The resulting spores are reduced as well as of unreduced types. A scheme is prepared to cover the majority of the most frequent abnormalities during microsporogenesis in *Taraxacum* ssp. The reason for the meiotic disturbances is also discussed.

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