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Karyotype of apomictic Dandelion (*Taraxacum officinale*), a wild plant with high medicinal value

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Abstract

Taraxacum officinale (Dandelion) includes sexually diploid as well as apomictic polyploid, mostly triploid plants. Apomixis holds great promise in agriculture and biotechnology as it fixes traits regardless of their complexity in genetic control. *Taraxacum officinale* serves as a model system to investigate the genetics, ecology and evolution of apomixis. The *Taraxacum* seeds were allowed to germinate, The colchicine treated metaphasic cells of the dividing root tips were fixed, and processed for chromosome analysis. It is found that *Taraxacum officinale* of Kashmir is a triploid (2N=3X=24), based on X=8. The karyotyping reveals symmetrical karyotype of the genus where in centromere is metacentric in six triplets and sub-metacentric in two triplets.

Keywords: Dandelion, *Taraxacum officinale*, apomixis, chromosome, karyotype

INTRODUCTION

The Dandelion (*Taraxacum officinale*) is found in all parts of the North Temperate Zone, in pastures, meadows and on waste ground, and is so plentiful that farmers everywhere find it a troublesome weed. Although its flowers are more conspicuous in the earlier months of summer, it is found in bloom, and consequently also prolifically dispersing its seeds, almost throughout the year. It is used as a medicinal plant especially by tribal people for liver, kidney and joint ailments and in inflammatory diseases [1]

Genus *Taraxacum* serves as a model system to investigate the genetics, ecology, and evolution of apomixis. The genus includes sexually diploid as well as apomictic polyploidy, mostly triploid, plants. Apomictic *Taraxacum* is diplosporous, parthenogenetic and has autonomous endosperm formation. It has been suggested that these three apomictic elements are controlled by more than one locus in *Taraxacum* and that diplospory inherits as a dominant, monogenic trait. [2]

MATERIALS AND METHODS

Seed collection

The seeds were collected from fully matured parachute bearing *Taraxacum officinale* plants located in the vast beautiful lawns of Kashmir University located at *Hazratbal,* Srinagar during March to May, 2005. The seeds were air dried and preserved in blotting paper for further use.

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Seed germination

The petri-plates containing wetted blotting papers were sterilized by dry heat in an oven at 60ºC for two hours. The seeds were soaked in 0.6% acetic acid for two hours. After sterilization, 20 seeds were kept in each of the four Petri-plates. Gibberlin (GA3) at a concentration of 0, 25, 50 and 100 ppm was added into these plates respectively. The number of seeds germinated in each Petri-plate and the time required for germination was recorded.

Pretreatment

The germinating root tips were excised early in the morning. Excised root tips were thoroughly washed with tap water and pretreated with 0.1% aqueous colchicine or a saturated solution of Para-dichlorobenzene (PDB) for about 3-4 hours at room temperature.

Fixation and preservation

After four hours, the pretreated root tips were thoroughly washed with tap water to remove traces of colchicine, which otherwise could impair penetration of the fixative into the root tip cells and also affect proper staining of the chromosomes. The root tips were immediately fixed in Carnoy's fixative I (1:3 glacial acetic acid : ethanol) for 24 hours and then washed thoroughly to remove the traces of acetic acid. These were then preserved in 70% ethanol under refrigeration.

Staining

Staining of mitotic chromosomes was done by conventional Feulgen staining method using 0.5 % Feulgen solution [3]. The preserved root tips were washed thoroughly with water and hydrolyzed in 1N HCL at 60^{0}C for 8 minutes. The hydrolyzed root tips were put in Feulgen Stain for 35-45 minutes in dark till the meristematic tissue got prominently stained. The Schiff`s reagent

was drained off and the root tips were placed in 1% acetocarmine for 1 to 3 minutes. A drop of the acetocarmine in 45% acetic acid was added to the material on a clean glass slide and a cover slip was carefully laid on it. The material was then squashed in between the two layers of filter paper. The prepared slide was gently heated to destain the cytoplasm for better contrast.

The temporary slides were thoroughly scanned for well spread, non-overlapped & properly stained metaphase chromosome preparations. 5-7 such cells were analyzed using 15X x 100X (oil immersion) lens combination.

The outline drawings of the chromosomes were drawn with the help of Camera Lucida under a uniform magnification of 15X x 100X. Further, each chromosome was observed very keenly to study the number, structure & position of centromere.

Chromosome measurements absolute length & arm ratio) were made from the Camera Lucida drawings. Homologues were identified on the basis of absolute length and arm ratio (long arm / short arm) according to Bothmer, 1977 [4]. For identification, recommendations by Levan et al, 1965 [5] were followed using measurements from three well spread metaphase preparations, and an average ideogram was constructed.

F% & TF% were calculated using the following formulae.

$$F\% = \frac{\text{length of short arm of all chromosomes}}{\text{Total length of all chromosome}} \times 100$$

$$TF\% = \frac{\text{Total length of short arm of all chromosomes}}{\text{Total length of all chromosomes}} \times 100$$

The degree of karyotype asymmetry was estimated following Stebbin's two-way system [6,7]

Microphotography

Photographs of well spread mitotic preparations were taken from temporary slides with the help of Olympus PM-35 mm camera fitted on Meopta (Germany) binocular microscope at 10X x 100X magnification using a black and white Kodalith Ortho film 6556 type 3 sp 653 (made in USA). For developing the photomicrography film Sterling Universal developer was used. The films were printed on a black and white RC glossy enlarging paper. The temporary cytological preparations were later made permanent by placing the slide; inversely i.e. cover slip facing downwards, in 1: 1 acetic acid and butanol solution till the cover slip got detached from the slide .The slides and the cover slips were then transferred to a solution of 100% butanol for 1 minute. Then the dried slide and cover slips were reannealed by a drop of DPX (A mixture of distyrene (a polystyrene), a plasticizer (tricresyl phosphate), and xylene).

RESULTS AND DISCUSSION

Seed germination

Taraxacum seeds were collected from fully mature parachute bearing plants and left to germinate in presence of different concentrations of gibberline. The germinating seeds were continuously observed and the results are given in Table 1.

Table 1. Germination record of *Taraxacum* seeds at different GA3 concentrations.

Plate Number	Concentration of GA3 (ppm)	Total No. of germinated seeds		
		5th day	7th day	10th day
1	25	4	16	20
2	50	9	18	20
3	100	5	9	19
4 (control)	0	0	4	18

Gibberline serves as a stimulus to expedite the process of germination .The optimum concentration for stimulation was 50 ppm. The *Taraxicum* seeds however, germinated, equally in the control plate which did not contain any gibberline, thus showing that the seeds do not undergo any dormancy.

Chromosome morphology

The metaphase slides were thoroughly scanned for well spread, non overlapped chromosomes using 15 X x 100 X (oil immersion) lens combinations. Three such cells are shown in Fig 1. Several other cells with similar metaphase chromosomal preparations were observed and analyzed to study the number and structure of their chromosomes. *Taraxacm officinale* is a triploid plant (3x) with base number (x) equal to 8 (Fig.1).

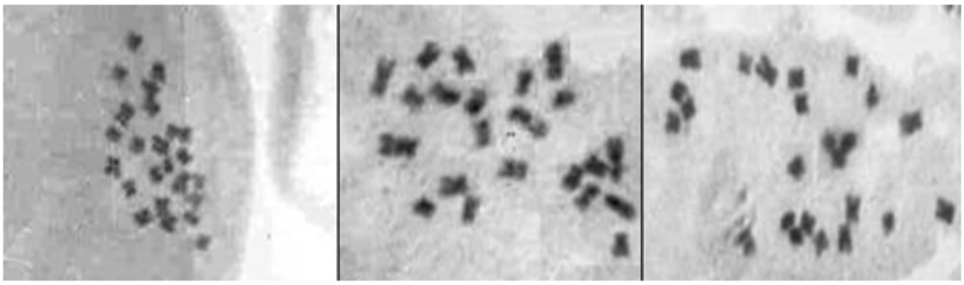

Fig 1. Chromosomes in the metaphase stage observed in three different cells

The karyotype (Figs. 2) exhibits Stebbins IA class of asymmetry, which is the most symmetrical class and considered as primitive. [6,7)

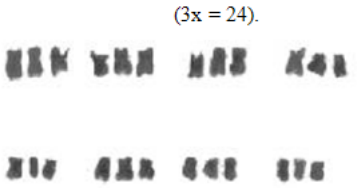

Fig 2. A karyotype for *Taraxacum officinale*

For identification, recommendations by Levan and his colleagues were followed [5] using measurements from three well spread metaphase preparations, and an average ideogram was constructed (Fig.3)

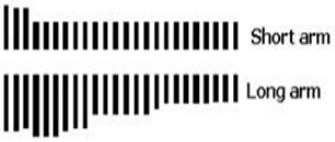

Fig 3. Average karyoidiogram of *Taraxacum officinale*

Chromosome measurements (absolute length & arm ratio) were made from the Camera Lucida drawings. The data showing short arm and long arm measurements, total length of short and long arm, short to long arm ratio and F% are presented in Table 2.

Table 2 : Analysis of chromosomes in terms of length , number and arm ratio.

Triplet No.	Chromosome Number	Length of short arm "S"(μ)	Length of long arm "L"(μ)	(L+S)	(L/S)	(F%)	Position of centromere
1st	1	1	1.31	2.31	1.3	42.1	M
	2	0.93	1.31	2.24	1.4	41.6	M
	3	0.93	1.25	2.18	1.3	42.8	M
2nd	4	0.62	1.43	2.05	2.3	30.3	Sm
	5	0.62	1.43	2.05	2.3	30.3	Sm
	6	0.62	1.43	2.05	2.3	30.3	Sm
3rd	7	0.62	1.31	1.93	2.1	32.2	Sm
	8	0.62	1.25	1.87	2.0	33.3	Sm
	9	0.62	1.25	1.87	2.0	33.3	Sm
4th	10	0.62	0.93	1.55	1.5	40.0	M
	11	0.62	0.93	1.55	1.5	40.0	M
	12	0.62	0.93	1.55	1.5	40.0	M
5th	13	0.62	0.93	1.55	1.5	40.0	M
	14	0.62	0.93	1.55	1.5	40.0	M
	15	0.62	0.93	1.55	1.5	40.0	M
6th	16	0.62	0.81	1.43	1.3	43.3	M
	17	0.62	0.68	1.30	1.1	43.3	M
	18	0.62	0.68	1.30	1.1	47.6	M
7th	19	0.62	0.68	1.30	1.1	47.6	M
	20	0.62	0.68	1.30	1.1	47.6	M
	21	0.62	0.68	1.30	1.1	47.6	M
8th	22	0.62	0.62	1.24	1.0	50.0	M
	23	0.62	0.62	1.24	1.0	50.0	M
	24	0.62	0.62	1.24	1.0	50.0	M

m, metacentric ., sm , sub metacentric . , S , short arm ., L , long arm L/S , Ratio of long arm to short arm., PDB , Para-dichlorobenzene

Homologues were identified on the basis of absolute length and arm ratio (long arm / short arm) according to Bothmer [4]. The chromosomes are similar in shape, Very difficult to be differentiated under light microscope at low magnification. They are also very small in size ranging from 1.24 to 2.31μ. With respect to the position of centromere, the chromosomes are metacentric in six triplet groups, and sub-metacentric in the remaining two triplet groups.

Taraxacum officinale (local name Handh) is a perennial herb, which grows widely throughout the valley of Kashmir. This plant has been in vogue as cooking vegetable as well as medicinal herb in the valley since ancient time.

Apomixis is the process that confers the advantage of clonal uniformity with the cost effectiveness in the propagation of the plant [8]. *Taraxacum officinale* is an apomict that does not even require the pollen grains to trigger the development of the seed .Thus this herb can be cultivated for commercial purposes on large scale without any impediments [9, 10] However, to ensure that *Taraxacum officinale* growing in Kashmir is an obligate apomict, working out of somatic chromosome number essential. The present investigation has established that *Taraxacum officinale* of Kashmir is a triploid (2N=3X=24), based on X=8

The karyotyping of *Taraxacum officinale* reveals symmetrical karyotype of the genus in which centromere is metacentric in six triplets and sub-metacentric in two triplets. The present investigation gives the first report for the karyotype of *Taraxicum* and establishes that *Taraxacum officinale* growing in Kashmir is a triploid (2N=3X=24), based on X=8 and an obligate apomict.

Conclusion

It is found that *Taraxacum officinale* of Kashmir is a triploid (2N=3X=24), based on X=8. The karyotyping reveals symmetrical karyotype of the genus where in centromere is metacentric in six triplets and sub-metacentric in two triplets, and is an obligate

apomicts.

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