Aneuploidy in Wild *Picris babylonica* (Asteraceae) in Kuwait

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Summary A cytological study of wild *Picris babylonica* in Kuwait revealed several aneuploids; monosomic ($2n=9$), trisomic ($2n=11$) and tetrasomic ($2n=12$) in addition to the prevailing euploidy condition ($2n=10$). Morphological features were not appreciably different among the aneuploid plants. Karyotype analyses showed that the first chromosome pair was probably involved either in trisomic or monosomic plants. Two univalents plus 4 bivalents were also detected in some analyzed PMC's. The possible origin of aneuploids and their probable relevance in speciation and evolution in plants are discussed.

Key words Aneuploidy, Kuwaiti flora, *Picris babylonica*.

Picris babylonica Hand.-Mazz., an annual herb that normally flowers and fruits between February and May, is widely distributed in Kuwait, Iraq and Saudi Arabia. A detailed description and comprehensive morphological study of *P. babylonica* was provided by Al-Rawi (1987). Malallah *et al.* (1997) and Malallah and Brown (1999) made the first cytological studies on this species, showing that the somatic number of this species is 10 and the gametic number is 5 with description of the 10 chromosomes.

Aneuploidy, a common phenomenon in plants, occurs when the chromosome number is not the exact multiple of basic number (x) of the true diploids. In the case of chromosomal excess many plant species, particularly the primitive or the wild plant forms, can generally tolerate a few additional chromosomes (Khush 1970, Rick 1971, Rick and Notani 1961). On the other hand, most of the diploid plants with chromosomal decrease (monosomics, $2n-1$) are weak and hardly viable, although in few cases as in tomato and maize they can be sufficiently vigorous to flower and even reproduce. Aneuploidy arises mainly through deviating meiosis (Khush and Rick 1966, Weber 1983) either spontaneously (Khush 1973) or in response to treatments with chemicals or radiation (Wang and Iwata 1996). It is an important phenomenon not only because of its application in plant cytogenetical studies but also due to its probable role as one of the evolutionary mechanisms in the plant. The present study shows the finding of aneuploidy condition, and other meiotic peculiarities in *P. babylonica*. The possible origin and significance of aneuploidy in speciation and evolution in plants are discussed.

Materials and methods

Seeds and flower buds of *Picris babylonica* Hand.-Mazz. were collected from groups of plants growing at various locations in Kuwait (the area of Kuwait is 17818 km² approx.) during the years 1993, 1998 and 1999. Dr. M. Mathew and M. Al-Doseri (Herbarium of Faculty of Science, Kuwait University, KTUH) made the identification. Voucher specimens were deposited at KTUH. No morphological differences were noticed among the collected plants, so all plants were treated the same during the preparation for chromosome analysis.

Young flower buds were fixed and preserved in Carnoy's solution (ethanol:glacial acetic acid, 3:1 v/v). The smears of anthers were stained with aceto-orcin. Seeds were germinated in a con-

trolled growth chamber, the root tips were collected and treated with 0.05% colchicine for 3 h at room temperature. The root tips were then fixed in Carnoy's solution and preserved in 70% ethanol in a refrigerator until use. For chromosome analysis, the conventional squash method was performed using the stain aceto-carmin. Karyotype analyses were based on at least 5 cells with well-spread chromosomes. Photo-ideograms were prepared by cutting out individual chromosomes and arranging them in a descending order on the basis of total length, centromere position, arm ratio and existence of a satellite. Standard error (S.E.) was estimated.

Results

In the majority of analyzed mitotic and meiotic cells, *Picris babylonica* exhibited a diploid chromosome number of 10 (Figs. 1a, 2, 7). This value was recorded from large number of plants during the present and previous work. The karyotype of $2n=10$ plants consisted of one pair of sub-terminal, 3 pairs of submetacentric and one pair of metacentric chromosomes (Fig. 1a, Table 1). In addition, aneuploid plants of *P. babylonica* with chromosome numbers of 11 and 9 were observed. In analyzed seedlings, the trisomic condition ($2n=11$) was detected in the cells of the root tips of several plants such as those shown in Figs. 3 and 4, while the monosomic condition ($2n=9$) was observed in all cells of only one root tip (Fig. 5). These aneuploid cells were found in samples collected from the same locality (Shuwaikh University Campus, 5 km north of Kuwait City in 1998–1999). The tetrasomic number of $2n=12$ (Fig. 6) was previously detected in root tip samples collected from another locality (Qurtoba, 8 km east of Kuwait City in 1993 unpublished data). Another plant collected from the same locality in 1998 showed 6 bivalents at metaphase I (photo is not shown) confirming the tetrasomic condition of $2n=12$. Karyotype analyses strongly suggested that the extra chromosome in cells with $2n=11$ and the missing chromosome in cells with $2n=9$ belong to the first pair (Table 1, Fig. 1b–e). This has been based on the total chromosome length and the presence of the satellite in the studied plants of both aneuploid cases. In the analyzable pollen mother cell with $2n=10$, although meiosis took place normally with complete pairing in most cases (Fig. 7), in few cells 2 univalents (about 15% of the analyzed pollen mother cells) were observed in diplotene or diakinesis of some plants indicating synaptic failure of chromosomes (Fig. 8).

It is worthy to mention that this species is commonly grown in many localities in Kuwait and no morphological differences were observed among the plants during every season collection.

Discussion

The basic chromosome number of the genus *Picris* was reported to be $x=5$ (Darlington and Wylie 1955). The gametic number of *P. saharae* from Kuwaiti flora was found to be $n=5$ (El-Bayoumi 1973). The name *P. saharae* was wrongly applied in earlier literature in Kuwait for *P. babylonica*. However, all the taxonomic studies conducted in Kuwait revealed that *P. babylonica* is the only species of this genus that was identified from this region (Al-Rawi 1987, Boulos and Al-Dosari 1994). Malallah *et al.* (1997), Malallah and Brown (1999) reported the somatic chromosome number of this species as $2n=10$ and the gametic number of $n=5$. Although the present study confirmed these previous findings, it demonstrated the existence of aneuploid conditions ($2n=9, 11, 12$) in *P. babylonica*. The monosomic condition was found in cells of only one plant among the *P. babylonica* populations studied. The seeds of the aneuploid plants (with 11 and 9 chromosomes) were collected from the same locality and treated the same as no morphological differences were observed among them. These aneuploid plants were detected among so many other euploid plants from the same locality. The most probable origin of these aneuploids seems to be from the spontaneous errors in meiotic process that lead to the failure of pairing and consequently to the unequal distribution of chromosomes (Weber 1983, Wiggers *et al.* 1990, Sybenga 1992, Kim 1994). It is not

Figs. 1–8. 1) Karyotypes of *Picris babylonica* with normal and aneuploid conditions. 2) Diploid cell of *Picris babylonica* ($2n=10$). 3 and 4) Trisomic state ($2n=11$). 5) Monosomic state ($2n=9$). 6) Tetrasomic state ($2n=12$). 7) PMC showing 5 bivalents. 8) PMC showing 4 bivalents and 2 univalents.

known whether the environmental conditions in the area could lead to such numerical chromosomal aberrations. These aberrations have not been seen in many other plants of the same species from other localities. However, the tetrasomic condition was seen in plant collected in 1993 after the Gulf War, which might be related to the polluted environment at that time because in another study, abnormalities were seen in samples collected from the same area during 1992 and 1993 (Malallah *et al.* 1997). The presence of univalents observed in the pollen mother cells in the present study demonstrates the failure of pairing which might lead to aneuploidy of *P. babylonica*. Because of the

Table 1. Karyotype analysis of *Picris babylonica*

Karyotype number (2 <i>n</i>)	Arm length ratio (μm)	Chromosome										
		Extra Chromosome	Pair 1		Pair 2		Pair 3		Pair 4		Pair 5	
			1	2	3	4	5	6	7	8	9	10
a. 10	Short arm		0.55	0.60	0.55	0.50	0.65	0.60	0.59	0.59	0.41	0.40
	Long arm		1.24	1.27	1.09	1.09	0.80	0.80	1.19	1.13	1.31	0.84
	Centromeric index		1.79	1.87	1.64	1.59	1.44	1.39	1.78	1.72	1.72	1.24
	L/S Ratio		2.28	2.13	2.00	2.20	1.23	1.33	2.02	1.92	3.19	2.12
	Type		sm	sm	sm	sm	m	m	sm	sm	st	sm
b. 11	Short arm	0.60	0.50	0.50	0.50	0.40	0.70	0.75	0.45	0.45	0.55	0.40
	Long arm	1.34	1.39	1.39	1.09	1.09	0.89	0.89	1.04	1.04	0.89	0.84
	Centromeric index	1.94	1.89	1.89	1.59	1.49	1.59	1.64	1.49	1.49	1.44	1.22
	L/S Ratio	2.25	2.81	2.81	2.20	2.76	1.29	1.20	2.34	2.34	1.64	2.12
	Type	sm	sm	sm	sm	sm	m	m	sm	sm	sm	sm
c. 11	Short arm	0.70	0.84	0.80	0.55	0.55	0.70	0.84	0.45	0.50	0.60	0.55
	Long arm	1.19	1.34	1.29	1.19	1.24	1.20	1.00	1.39	1.29	1.29	1.00
	Centromeric index	1.89	2.19	2.09	1.74	1.79	1.89	1.84	1.84	1.79	1.89	1.54
	L/S Ratio	1.72	1.59	1.62	2.18	2.28	1.72	1.18	3.12	2.60	2.16	1.82
	Type	sm	sm	sm	sm	sm	m	m	st	sm	sm	sm
d. 11	Short arm	0.55	0.65	0.60	0.60	0.55	0.70	0.70	0.45	0.40	0.40	0.35
	Long arm	1.34	1.44	1.33	1.33	1.39	1.00	1.00	1.28	1.05	1.00	0.83
	Centromeric index	1.89	2.09	1.93	1.93	1.93	1.69	1.69	1.72	1.45	1.40	1.18
	L/S Ratio	2.46	2.24	2.23	2.23	2.54	1.44	1.44	2.86	2.66	2.51	2.37
	Type	sm	sm	sm	sm	sm	m	m	sm	sm	sm	sm
e. 9	Short arm			0.60	0.55	0.50	0.70	0.65	0.45	0.40	0.35	0.40
	Long arm			1.20	1.34	1.20	0.80	0.84	0.84	0.94	0.94	0.89
	Centromeric index			1.79	1.89	1.69	1.49	1.49	1.29	1.34	1.29	1.29
	L/S Ratio			2.00	2.46	2.41	1.15	1.31	1.89	2.38	2.72	2.25
	Type			sm	sm	sm	m	m	sm	sm	sm	sm
S.E. for Total Length		0.01	0.05	0.05	0.07	0.08	0.08	0.08	0.1	0.08	0.11	0.06

m=median, sm=submedian, st=subterminal.

relative close lengths of all chromosomes of this species and depending only on the photographs, it is unknown whether the univalents are the members of the first pair or not. Nevertheless the involvement of the first pair is strongly indicated by karyotype analyses shown in Table 1 and Fig. 1. The failure of pairing was previously reported by El-Bayoumi (1973) who found in addition to univalents, a reduced chiasma frequency (1.12) per bivalent.

Oberprieler and Vogt (1993) reported that the root tips of *Picris saharae* showed $2n=10+1B$, indicating the presence of one B chromosome in addition to the diploid value of 10. The origin and development of B chromosome were discussed in several studies (Brown 1972, Brown and Bertke 1974, White 1973, Dyer 1979). Those studies concluded that the non-disjunction (resulting from failure of pairing) and the dysploid, (where the number of chromosomes in the genome is altered with no significant change in the genotype) create the progenitors B chromosome. Wild populations of many plant species contain B chromosomes that are mostly heterochromatic and with infrequent presence in somatic tissue.

Aneuploidy can generally play an important role in plant evolution and stepwise progression of chromosome number. Detailed studies of aneuploidy and changes associated with them have provided valuable chromosomal information to botanists and taxonomists of various flowering plant groups. Aneuploidy may lead to creation of new chromosome numbers other than the known basic

one; a change which may be associated with new characteristics that could be of some adaptive value. Such a process has been proposed to be effective in the evolution in the genus *Mikania* of the *Asteraceae* (Ruas Claudette and Aguiar-Perecin Margarida 1997). In Family *Asteraceae*, aneuploid plants were found in *Aster occidentalis* and were assessed for morphological variability and geographical distribution (Allen Geraldine 1984). Kim (1994) discussed aneuploidy, including their origin and possible role in the evolution of the 2 families *Asteraceae* and *Onagraceae*. An evolutionary trend involving chromosomal rearrangement in addition to polyploidy and aneuploidy has also been suggested for *Stevia* in southern Brazil (Frederico Andriana *et al.* 1996). Therefore, it would be interesting to illustrate the importance of aneuploidy in evolution of *P. babylonica*.

A better understanding for the causes and the consequences of incidences of aneuploidy, particularly in relation to evolutionary processes in natural populations of plants, is urgently needed. This goal may be realized through advanced analysis by modern methods of cytogenetics and biochemistry, and if achieved, it will certainly help to broaden our knowledge on the cytoevolutionary processes, that occur in flowering plants.

The observation of different chromosome numbers in the some plants of *P. babylonica* is documented here for the first time; this represents a case of aneusomaty, which refers to the presence of somatic cells with varying chromosome numbers. Aneusomaty has also been observed in sunflower (*Helianthus annuus* L.), mainly in root and shoot tips or during embryo development (Cremonini and Cavallini 1986, Cavallini and Cremonini 1985, 1987). In this case, aneusomaty was found to progressively decrease in the course of plant growth and disappear before meiosis commenced in the anthers. Whether a similar process of selection against aneuploid cells during plant development occur in the aneuploids of *P. babylonica* is currently unknown in the documented aneuploids in the present work as they were found accidentally.

In order to further assess the extent of aneuploidy in Kuwait populations of *P. babylonica* and its effects on meiosis, fertility and other selected characters, an intensive cytological examination of additional *Picris* populations is currently underway.

An interesting thing was found in 6 specimens of *P. babylonica* in that all the inflorescence composed exclusively of tubular flowers. The specimens were found in several occasions during 1997–1999 and now they are deposited in KTUH. Unfortunately, none of them contain buds or seeds to examine the cytological bases of this morphological difference. It is worthwhile to mention that this species shows flowers with 3 types of heads, homocarpus, heterocarpus and mixture of both as shown by Mandaville (1990) who stated that all of these types should be treated as forms of *P. babylonica*. However, the dissecting tubular flowers showed abnormal ovaries, which might lead to unviable seeds.

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