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Hideyuki MATOBA and Hiroshi UCHIYAMA: **Chromosome Counts of Aquatic and Wetland Plants Growing in Acid Sulfate Soil from the Central Plain, Thailand**

タイ国中央平原の酸性硫酸塩土壌に生育する水湿地生植物の染色体数（的場英行，内山　寛）

Summary: Chromosome numbers were counted in root tip cells of 13 aquatic and wetland plants growing in acid sulfate soil in central Thailand. Results were as follows: *Cyperus haspan*, 2n = 26; *Eleocharis dulcis*, 2n = ca. 212; *Fimbristylis alboviridis*, 2n = 20; *Pycreus polystachyos*, 2n = 106 (*Cyperaceae*); *Blyxa aubertii*, 2n = 40 (*Hydrocharitaceae*); *Digitaria ciliaris*, 2n = 54; *Imperata cylindrica*, 2n = 20; *Panicum repens*, 2n = 45; *Paspalum orbiculare*, 2n = 40 (*Poaceae*); *Cleome rutidosperma*, 2n = 32 (*Capparaceae*); *Ludwigia adscendens*, 2n = 32; *Ludwigia hyssopifolia*, 2n = 16 (*Onagraceae*); *Melochia corchorifolia*, 2n = 36 (*Sterculiaceae*). Most chromosome numbers were confirmed in the previous reports, but they are reported here for the first time using plant materials from Thailand. The result of *Fimbristylis alboviridis* (2n = 20) is first report for the species.

Potential acid sulfate soil is widely distributed in coastal lowland areas of Southeast Asia. Once acid sulfate soil (ASS) is actualized, soil pH can drop to below 3.0 and most plant species cannot grow in actualized ASS fields. The recovery of vegetation then becomes complicated and the productivity of crop plants is vastly reduced. A limited number of plant species, however, can grow vigorously in actualized ASS fields. We previously performed research activities aimed at understanding the characteristic features of such plants under the research title, "Development of new bioremediation systems for the destroyed environment", launched as a 21st Century Center of Excellence (COE) Program (Sasaki 2008). We are also interested in increasing the available chromosomal information for plants in Southeast Asia, since such reports in the area are scarce. In this paper, we report the chromosome numbers of 13 aquatic and wetland plants collected from an ASS field near Bangkok, Thailand.

Materials for the present study are listed in Table 1. They were collected in the non-liming field of the Experiment Station for Royal Acid Sulfate Soil Project (14º14.6' N, 100º59.1' E) in Ban Na, Nakhon Nayok Province, Thailand, then cultivated in a greenhouse of the College of Bioresource Sciences, Nihon University, Japan. Voucher specimens were deposited in the herbarium of Nihon University, the College Museum of Bioresource Sciences, Fujisawa, Japan. Taxonomic treatment followed Simpson and Koyama (1998) for cyperaceaous species, Cook and Lüönd (1983) for *Blyxa* species and Raven (1977) for *Ludwigia* species.

Root tips were cut into 2–3 mm in length then pretreated with 2 mM 8-hydroxyquinoline at 20ºC for 3 h before being fixed in ethanol-acetic acid (3:1) at 4ºC for 24 h. Fixed root tips were washed three times in distilled water, then 10 to 30 samples were placed in a microcentrifuge tube containing 500 μL of enzyme solution (4% Cellulase Onozuka-RS (Yakult Honsha) and 2% Pectolyase Y-23 (Kikkoman, pH 4.2) and incubated at 37ºC for 1 h. After washing three times with distilled water, some of the washed root tips were placed on a glass-slide onto which 10–20 μL of fixative was dropped; the root tips were immediately spread apart. Air-dried slides were then mounted in Vectashield Mounting Medium (Vector Lab.)

Table 1. Chromosome numbers of the plant species investigated in this study

Species (Voucher)	Present count (2n)	Previous report		
		n	2n	Reference
Cyperaceae				
Cyperus haspan L. (Uchiyama 07068)	26	8		India (Sarkar et al. 1978)
			16	India (Sanyal 1972)
		13		India (Rath and Patnaik 1978)
			26	India (Nijalingappa et al. 1978, Tejavathi and Nijalingappa 1990)
			30	Nigeria (Baquar 1978)
		18		India (Mehra and Sachdeva 1975)
Eleocharis dulcis (Burm. f.) Trin. ex Hensch. (Uchiyama 07016, 07017)	ca.212	38		India (Rath and Patnaik 1978)
			c.196	Japan (Yano et al. 2004)
		>100		India (Rath and Patnaik 1974)
		c.108		India (Patnaik and Guru1968; cited in Rath and Patnaik 1974)
Fimbristylis alboviridis C. B. Clarke (Uchiyama 07036, 07038, 07039)	20			n. d.
Pycreus polystachyos (Rottb.) P. Beauv. (Uchiyama 07033–07035)	106		96	India (Sanyal 1972)
		53		India (Rath and Patnaik 1978)
		54		India (Sarkar et al. 1976)*
Hydrocharitaceae				
Blyxa aubertii Rich. var. *aubertii* (Uchiyama 07004, 07006)	40	8		India (Sarkar et al. 1977)
		12	24	India (Ghosh and Bhattacharya 1981)
			32	India (Fotedar and Roy 1974)**
			40	Japan (Uchiyama 1989)
Poaceae				
Digitaria ciliaris (Retz.) Koel. (Uchiyama 07002, 07037)	54	9		India (Mehra and Sharma 1973)
				India (Mehra and Sharma 1975)
		18		Argentina (Hunziker et al. 1998)
			36	Sudan (Trouin 1972)
				India (Sarkar et al. 1976)
				Kurile Islands (Probatova et al. 2000)
			36, 54	unknown (Gupta and Yashvir 1977)
		27		Costa Rica (Davidse and Pohl 1978)
				India (Mehra 1982)
		27	54	Japan (Hirayoshi and Yasue 1955)
			54	Vladivostok (Sokolovskaya and Probatova 1977; cited in Probatova et al. 2000)
			54, 72	Pakistan (Ahsan et al. 1994)
		36		India (Mehra and Remanandan 1973)
			72	Sri Lanka (Gould and Soderstrom 1974)
Imperata cylindrica (L.) Raeusch. (Uchiyama 07069)	20	10		Taiwan (Chen and Hsu 1962)
				India (Mehra 1982)
				Pakistan (Faruqi et al. 1979)
				Tanzania (Rao and Mwasumbi 1981)
		10	20	India (Singh 1964)
			20	Thailand (Larsen 1963)
				Sri Lanka (Gould and Soderstrom 1974)
				India (Christopher 1978)
		20		Libya (Faruqi et al. 1987)
			60	Portugal (Queirós 1973)

Table 1. Continued

Species (Voucher)	Present count (2n)	Previous report		
		n	2n	Reference
Panicum repens L.	45	9		Pakistan (Ahsan et al. 1994)
(Uchiyama 07003–07015)		18		Zaire (Dujardin 1979)
			36	South Africa (de Wet and Anderson 1956)
				India (Jauhar and Joshi 1966)
				Sri Lanka (Gould and Soderstrom 1974)
				Egypt (Haroun 1995)
		18, 30		India (Mehra and Chaudhary 1981a)
			45	Japan (Tateoka 1956)
				Thailand (Larsen 1963)
				India (Christopher and Abraham 1976)
		27		USA (Gould 1960)
				Spain (Devesa et al. 1991)
			54	Portugal (Queirós 1973)
		10		India (Sarkar et al. 1975, 1976)
			40	India? (Krishnaswamy 1941)
Paspalum orbiculare G. Forst.	40	10		Zaire (Dujardin 1979)
(Uchiyama 07010–07011)		20		India (Khosla and Mehra 1973)
				Nigeria (Olorode 1974)
		20	40	India (Christopher et al. 1987)
			40	Cote d'Ivoire (Kammacher et al. 1973)
				India (Dandin and Chennaveeraiah 1983)
		20	60	India (Mehra and Chaudhary 1981)
			60	Japan (Tateoka 1956)
			54	India (Nath et al. 1970)
Capparaceae				
Cleome rutidosperma DC.	32	10		India (Sarker et al. 1980)
(Uchiyama 07022–07024)		15		Nigeria (Pilz 1980)***
				India (Sarker et al. 1982)
Onagraceae				
Ludwigia adscendens (L.) H. Hara	32	16		India (Sarkar et al. 1972)
(Uchiyama 07047)				Hong Kong, India, Thailand (Raven and Tai 1979)
				Taiwan (Peng 1983)
			32	China (Gu et al. 1991)
				India, Taiwan (Zardini et al. 1991)
Ludwigia hyssopifolia (G. Don) Exell	16	8		India (Shetty and Subramanyam 1971)
(Uchiyama 07055)				Borneo, India, Nepal, Philippines, Taiwan, etc. (Raven and Tai 1979)
			16	Suriname (Gadella and Kliphuis 1968)
Sterculiaceae				
Melochia corchorifolia L.	36		36	China (Huang et al. 1989)
(Uchiyama 07040–07042)			46	India (Bir and Sidu 1975)

* as *Cyperus polystachyus* var. *ferruginosus*.

** as *Blyxa oryzetorum*.

*** as *Cleome ciliata*.

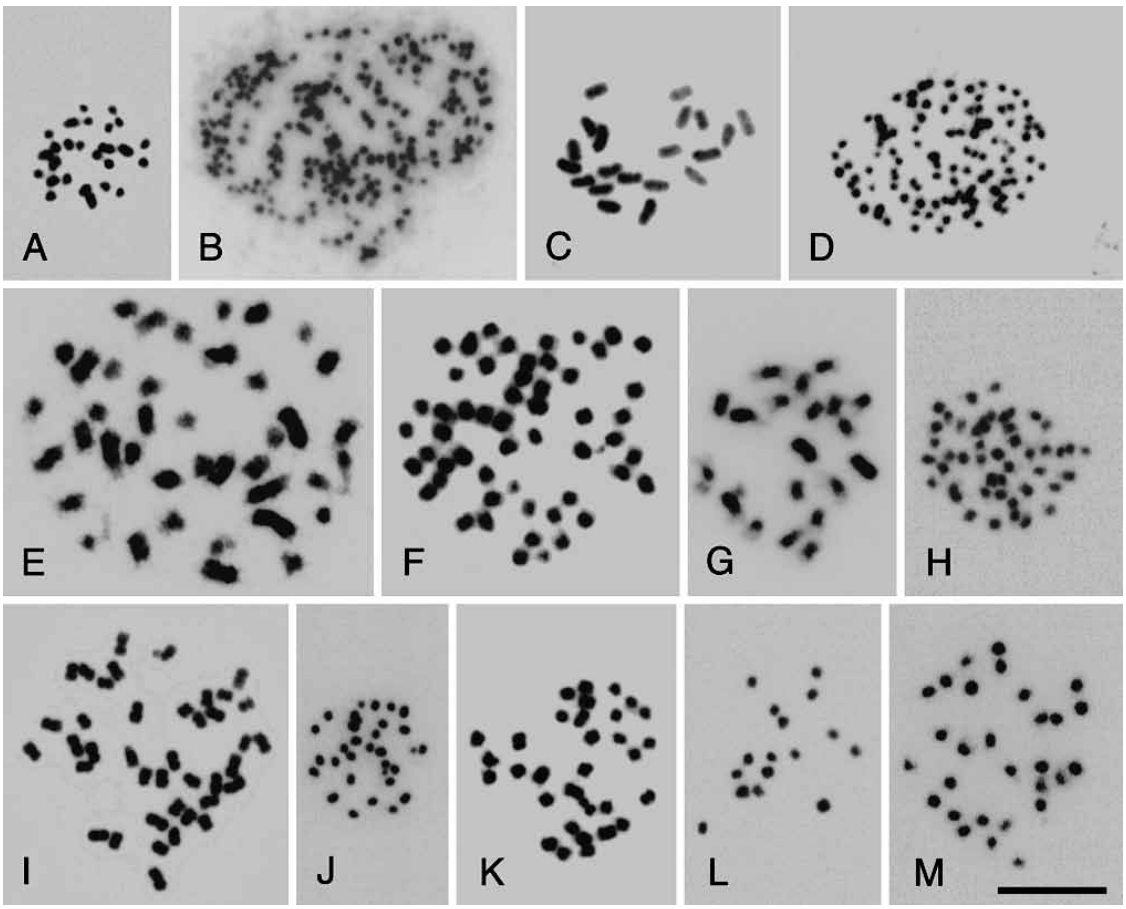

Fig. 1. Fluorescent photomicrographs of somatic chromosomes investigated in this study. A. *Cyperus haspan* (2n = 26). B. *Eleocharis dulcis* (2n = ca. 212). C. *Fimbristylis alboviridis* (2n = 20). D. *Pycreus polystachyos* (2n = 106). E. *Blyxa aubertii* (2n = 40). F. *Digitaria ciliaris* (2n = 54). G. *Imperata cylindrica* (2n = 20). H. *Panicum repens* (2n = 45). I. *Paspalum orbiculare* (2n = 40). J. *Cleome rutidosperma* (2n = 32). K. *Ludwigia adscendens* (2n = 32). L. *Ludwigia hyssopifolia* (2n = 16). M. *Melochia corchorifolia* (2n = 36). Scale bar = 10 μm.

containing 500 ng/mL of 4',6-diamidino-2-phenylinodole (DAPI). At least 10 metaphase plates in cytoplasm were observed and fluorescent photographs were taken with an Olympus microscope BX60 (Olympus Optical) using an Olympus DP50 CCD camera.

Chromosome numbers counted in this study and those reported previously are listed in Table 1. Localities of the materials used for previous chromosomal observations are also shown. Photomicrographs of somatic chromosomes investigated in this study are shown in Fig. 1A–M.

Cyperus haspan L. (*Cyperaceae*; Fig. 1A) Chromosome number was counted as 2n = 26, which agrees with reports from India (Rath and Patnaik 1978, Nijalingappa et al. 1978, Tejavathi and Nijalingappa 1990). Other chromosome numbers have also been reported for this species as follows: n = 8 (Sarker et al. 1978), 2n = 16 (Sanyal 1972) and n = 18 (Mehra and Sachdeva 1975) from India and 2n = 30 (Baquar 1978) from Nigeria. The plant used in this study did not have creeping rhizomes.

Eleocharis dulcis (Burm. f.) Trin. ex

Hensch. (*Cyperaceae*; Fig. 1B) Around 200 chromosomes have been observed in this species from India (Patnaik and Guru 1968, Rath and Patnaik 1974) and Japan (Yano et al. 2004), and n = 38 has also been reported from India (Rath and Patnaik 1978). Although we could not fix the chromosome number due to the small size of the chromosomes, approximately 212 were counted in many cell plates observed.

Fimbristylis alboviridis C. B. Clarke (*Cyperaceae*; Fig. 1C) The somatic chromosome number was counted as 2n = 20, which is a tetraploid of x = 5 in this genus. This is the first report for this species.

Pycreus polystachyos (Rottb.) P. Beauv. (*Cyperaceae*; Fig. 1D) Chromosome number was counted as 2n = 106. The chromosome number of this species has previously been reported from India only. Our count agreed with that of Rath and Patnaik (1978; n = 53), but not with those reports of Sanyal (1972; 2n = 96) and Sarkar et al. (1976; n = 54, as *Cyperus polystachyus* var. *ferruginosus*).

Blyxa aubertii Rich. var. *aubertii* (*Hydrocharitaceae*; Fig. 1E) The chromosome number of this taxon has been reported to be n = 8 (Sarkar et al. 1977), n = 12 and 2n = 24 (Ghosh and Bhattcharya 1981), and 2n = 32 (Fotedar and Roy 1974; as *B. oryzetorum*,) from India, and 2n = 40 (Uchiyama 1989) from Japan. Our observation agreed with the report from Japan. Several large chromosomes were observed in the chromosome complement, indicating a common feature with the Japanese materials.

Digitaria ciliaris (Retz.) Koeler (*Poaceae*; Fig. 1F) Intraspecific polyploidy, di-, tetra-, hexa- and octa-ploids of x = 9 have been observed in this species (Table 1). The plant used in this study was a hexaploid with 2n = 54. Hexaploids have been reported from Asia and Central America.

Imperata cylindrica (L.) Raeusch. (*Poaceae*; Fig. 1G) Chromosome number was counted as 2n = 20, which is a diploid of x = 10 in this genus. A diploid chromosome number has previously been reported from Thailand (Larsen 1963). In this species, diploid (var. *major*) is widely distributed in southeastern Africa, southern and southeastern Asia, and Australia, while tetra- (var. *europaea*) and hexaploids (var. *africana*) are mainly distributed in the Mediterranean and southern Africa, respectively (Roux and Adjanohoun 1958).

Panicum repens L. (Poaceae; Fig. 1H) Intraspecific polyploidy, di-(rare), tetra-, penta- and hexaploids of x = 9 have been reported in this species (Table 1). The plant used in this study was a pentaploid with 2n = 45. Pentaploids have only been reported from Asia, including Thailand. Reports of n = 10 (Sarker et al. 1975, 1976) and 2n = 40 (Krishnaswamy 1941) are considered a misidentification of this species or a miscount.

Paspalum orbiculare G. Forst. (*Poaceae*; Fig. 1I) Intraspecific polyploidy, di-, tetra- and pentaploids of x = 10 have been investigated in this species (Table 1). The plant used in this study was a tetraploid with 2n = 40, and was distinguished from *P. scrobiculatum* in accordance with De Koning and Sosef (1985). The report of 2n = 54 (Nath et al. 1970) is considered a misidentification of this species or a miscount.

Cleome rutidosperma DC. (*Capparaceae*; Fig. 1J) A somatic chromosome number was counted as 2n = 32, which is a new count for this species. This chromosome number, however, did not agree with that in previous reports of n = 10 (Sarker et al. 1980) and n = 15 (Pilz 1980, Sarker et al. 1982; both as *C. ciliata*). To clarify the ploidy status of this species further investigation is therefore required, since intra- and interspecific variation in chromosome number has been observed in the genus *Cleome*.

Ludwigia adscendens (L.) H. Hara (*Onagraceae*; Fig. 1K) Chromosome number was counted as 2n = 32, which is a tetraploid of x = 8 in this genus. This confirmed previous reports from Asia including Thailand (Sarkar et

al. 1972, Peng 1983, Raven and Tai 1979, Gu et al. 1991, Zardini et al. 1991).

Ludwigia hyssopifolia (G. Don) Exell (*Onagraceae*; Fig. 1L) Chromosome number was counted as 2n = 16, which is a diploid of x = 8 in this genus. This agrees with previous reports (Shetty and Subramanyam 1971, Raven and Tai 1979, Gadella and Kliphuis 1968).

Melochia corchorifolia L. (*Sterculiaceae*; Fig. 1M) Chromosome number was counted as 2n = 36, which agrees with the report of Huang et al. (1989) from China, but not with that of Bir and Sidhu (1975; 2n = 46) from India.

Most chromosome numbers examined in this study confirmed those in previous reports; however, they are reported here for the first time using the plant materials from Thailand. Interestingly, most species investigated possessed small chromosomes (< 2 μm) and were shown to be polyploid.

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タイ国中央平原の酸性硫酸塩土壌に生育する水湿地生植物13種の染色体数を根端細胞を用いて次の通りに算定した：*Cyperus haspan*, 2n = 26; *Eleocharis dulcis*, 2n = ca. 212; *Fimbristylis alboviridis*, 2n = 20; *Pycreus polystachyos*, 2n = 106（カヤツリグサ科）; *Blyxa aubertii*, 2n = 40（トチカガミ科）; *Digitaria ciliaris*, 2n = 54; *Imperata cylindrica*, 2n = 20; *Panicum repens*, 2n = 45; *Paspalum orbiculare*, 2n = 40（イネ科）; *Cleome rutidosperma*, 2n = 32（フウチョウソウ科）; *Ludwigia adscendens*, 2n = 32; *Ludwigia hyssopifolia*, 2n = 16（アカバナ科）; *Melochia corchorifolia*, 2n =36（アオギリ科）．算定した染色体数の大部分は過去の報告と一致したが，タイ国産の材料では多くが初めての報告である．*Fimbristylis alboviridis* の染色体数 (2n = 20) は今回初めて報告されるものである．

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