Theor Appl Genet (2000) 100:315–318

G.A. Akpan

Cytogenetic characteristics and the breeding system in six *Hibiscus* species

Received: 19 February 1999 / Accepted: 17 June 1999

Abstract Cytogenetic characteristics confirm that *Hibiscus acetosella* and *Hibiscus cannabinus* are outbreeders, while *Hibiscus asper, Hibiscus physaloides, Hibiscus sabdariffa* and *Hibiscus surattensis* have evolved into inbreeders. The inbreeding species appear to have co-evolved a floral structure in which some anthers abut on the stigma prior to anthesis.

Key words Cytogenetic characteristics · Floral structure · *Hibiscus* · Inbreeders · Outbreeders

Introduction

Hibiscus, the type-genus of th tribe Hibisceae, has over 300 species. Many of these yield bast fibres and are used for cordage throughout tropical and sub-tropical regions (Cobley 1976). Some of the species are economically important as sources of food and medicines (Wilson and Menzel 1964; Ugborogho and Shofoyeke 1983). Other species are useful as ornamentals (Sharma and Sharma 1962; Singh and Koshoo 1989). The morphology and cytology of some of the species, especially in the section Furcaria, have been much investigated (Skovsted 1935, 1941; Ford 1938; Tjio 1948; Sanyal and Kundu 1959; Sharma and Sharma 1962; Menzel and Wilson 1966, 1969; Menzel and Martin 1970, 1971; Wise and Menzel 1971). No attempt has however been made to explore the relationship between these characters and the breeding system of these species.

Ugborogho and Shofoyeke (1983) called for the breeding of some of the *Hibiscus* species in order to improve on their useful characteristics. Since it is important to understand the breeding system in order to devise a successful breeding programme, an attempt has been made to elucidate the cytogenetics of the breeding system in six *Hibiscus* species that grow in Nigeria.

Communicated by H.F. Linskens

G.A. Akpan
Department of Botany,
Horticulture and Microbiology University of Uyo, P.O. Box 2735, Uyo, Nigeria

Materials and methods

Seeds of *Hibiscus acetosella* Welw., *Hibiscus asper* Hook., *Hibiscus cannabinus* L., *Hibiscus physaloides* Guil and Perr., *Hibiscus sabdariffa* L. and *Hibiscus surattensis* L. were collected from dif-

Table 1 Collection sites of the six *Hibiscus* species studied

Plant	Accession number and site
Hibiscus acetosella Welw.	CL-841: Botany experimental garden, University of Calabar, Calabar UY-843: Flower bed at No 105J/1 Nwaniba Road, Uyo
H. asper Hook.	IF-863: Grassland area, 5 km along Ife-Ede road, Ile-Ife JL-856: Abandoned farm at the College of Education, Jalingo NS-862: Grassland, 3 km along Nsukka-Ibagua road, Nsukka
H. cannabinus L.	BA-851: Abandoned farm near the University Campus, Bauchi JS-852: Roadside, 5 km along the Jos-Zaria road, Jos VK-851: Roadside, opposite Government Secondary School, Vandeikya
H. physaloides Guill & Perr.	AB-852: Roadside, 8 km along the Abak-Ikot Okoro road, Abak IT-861: Roadside at Mbak Atai, Itu UY-841: Roadside, 6 km along the Uyo-Itu highway, Uyo
H. sabdariffa L.	JL-851: Farm near the College of Education, Jalingo JL-854: Farm near the Government Reservation Area, Jalingo NS-843: Farm, 19 km along the Nsukka-Ibagua road, Nsukka
H. surattensis L.	CK-842: Roadside at Okoyong-Creek Town road JS-851: Roadside, 2 km along the Jos-Mangu road, Jos UY-842: Field at No. 21 Brooks street, Uyo

Table 2 Summary of the morphological characteristics of the accessions of the six species of *Hibiscus*

Species	Vegetative characteristics	Floral characteristics
Hibiscus acetosella CL-841	Red, erect annual up to 2.5 m tall. Stems smooth; 8–10 branches. Leaves alternate; shallowly 3–5-lobed; gland at base or nerves. Soft to touch	Actinomorphic; bisexual; calyx, corolla, carpel pentamerous. Stamens monadelphous. Corolla large, brownish-pink with dark centre. Stigma lobes and stalked anthers do not abut on the stigma in bud
UY-843	As CL-841 above	As CL-841 above
H. asper IF-863	Erect annual; up to 1.0 m tall. Stem densely covered with broad-based setae; 6–8 branches. Leaves alternate; simple; palmately lobed; gland at base of nerves. Leathery to touch	Actinomorphic; bisexual; calyx, corolla, carpel pentamerous. Stamens mondelphous. Corolla large yellow with dark centre. Stigma lobes not stalked; anthers abut on the stigma in bud
JL-856	As IF-863 above	As IF-863 above
NS 862	As IF-863 above	As IF-863 above
H. cannabinus BA-851	Erect annual; up to 3.0 m tall. Stems smooth; not branched. Leaves alternate; simple; not lobed; gland at base of nerves. Soft to touch	Actinomorphic; bisexual; calyx, corolla, carpel pentamerous. Stamens mondelphous. Corolla large, yellow with dark centre. Stigma lobes stalked; anthers do not abut on the stigma in bud
JS-852	As BA-851 above	As BA-851 above
VK-851	Leaves lobed. All other characteristics as BA-851 above	As BA-851 above
H. physaloides AB-852	Erect annual; up to 1.2 m tall. Stem, petioles, leaves, pedicels; epicalxy covered with stiff pungent hairs. 12–17 branches. Leaves alternate; shallowly 3 to 5-lobed; white incrustations at base of nerves; no gland on nerves. Prickly to touch	Actinomorphic; bisexual; calyx, corolla, carpel pentamerous. Stamens monadelphous, corolla yellow with dark centre. Stigma lobes not stalked; anthers abut on the stigma in bud
IT-861	As AB-852 above	As AB-852 above
UY-841	As AB-852 above	As AB-852 above
H. sabdariffa JL-851	Erect annual; up to 1.5 m tall. Stems petioles pedicels smooth; 10–13 branches. Leaves alternate; deeply 5–7-lobed; gland at the base of nerves. Soft to touch	Actinomorphic; bisexual; calyx, corolla, carpel pentamerous. Stamens monadelphous, corolla large, yellow with dark centre. Stigma lobes not stalked anthers abut on the stigma in bud
JL-852	As JL-851 above	As JL-851 above
NS-843	Leaves broadly 5-lobed. All other characteristics as JL-851 above	Corolla pink with dark centre. All other characteristics as JL-851 above
H. surattensis CK-842	A scrambling annual, stem, petioles, pedicels densely covered with retrorse prickles. Leaves deeply palmately lobed; gland at the base of nerves. Prickly to touch	Actinomorphic; bisexual; calyx, corolla, carpel pentamerous. Stamens monadelphous, corolla large, yellow with dark centre. Stigma lobes not stalked; anthers abut on the stigma in bud
JS-851	As CK-842 above	As CK-842 above
UY-842	As CK-842 above	As CK-842 above

ferent sites in Nigeria and given the accession numbers shown in Table 1. The identities of the plants were confirmed by comparing them with voucher specimens at Ile-Ife herbarium, of the Obafemi Awolowo University. Voucher specimens of the plants have been deposited in the Department of Biological Sciences, University of Calabar, Calabar. The seeds were raised in a nursery and seedlings were transplanted to permanent beds in the garden when they had at least four foliage leaves. Morphological observation of the plants began at first following.

Young flower buds were fixed in acetic alcohol (1:3 v/v) for 24 h with iron as a mordant. A few anthers were smeared in a drop of aceto-orcein. The chiasma frequency per bivalent was determined at the diplotene-diakinesis stages of meiosis by dividing the total chiasmata per pollen mother cell by the number of bivalents in that cell. Fifteen pollen mother cells were analysed for each accession and the mean chiasma frequency per bivalent was determined for each species. The distribution of chiasmata on the bivalents was also observed, as well as the general trend of meiosis.

Older flower buds were used for the determination of pollen fertility. The pollen grains were stained by the method of Alexander (1969). The percentage of fertile pollen grain was determined by scoring five slides per accession and the mean per cent pollen fertility was determined for each species. The mean per cent pollen fertility was arc-sin transformed using the formula

$$\text{Sin}^{-1}\sqrt{\frac{\%}{100}}$$ (Zar 1974).

Flower buds at various stages of development prior to anthesis were dissected and the disposition of the anthers towards the stigma as well as the structure of the stigma were observed.

Results

The morphological characteristics of the six species of *Hibiscus* studied are given in Table 2.

H. asper, H. cannabinus and *H. surattensis* had 2n = 36 chromosomes and regularly formed 18 bivalents at meiosis. *H. physaloides* had 2n = 40 chromosommes and formed 20 bivalents at meiosis. *H. acetosella* and *H. sabdariffa* had 2n = 72 chromosomes and regularly formed 36 bivalents at meiosis. The bivalents were small and displayed one or two chiasmata at the diplotene-diakinesis stages of meiosis. Anaphase disjunctions were normal. Regular tetrads were formed and pollen fertility was high in all six species.

The mean chiasma frequency per bivalent and the mean pollen fertility of the six species are given in Table 3. The mean chiasma frequency per bivalent ranged from 1.43 in *H. cannabinus* to 1.94 in *H. surattensis*. The mean pollen fertility ranged from 64.91 in *H. surattensis* to 82.21 in *H. acetosella*.

Table 3 Mean chiasma frequency per bivalent and mean pollen fertility of the *Hibiscus* species studied (± 95% C.L.)

Species	Mean chiasma frequency per bivalent	Mean pollen fertility
H. acetosella	1.56 ± 0.01	82.21 ± 1.89
H. asper	1.71 ± 0.02	72.87 ± 1.30
H. cannabinus	1.43 ± 0.05	68.08 ± 2.77
H. physaloides	1.64 ± 0.04	81.71 ± 1.07
H. sabdariffa	1.66 ± 0.02	82.19 ± 2.38
H. surattensis	1.94 ± 0.02	82.19 ± 2.38

Discussion

The large, brightly coloured flowers of the species studied give the impression that the species are cross-pollinated. However, the low mean chiasma frequency per bivalent of 1.43 and 1.56 in *H. cannabinus* and *H. acetosella*, respectively, shows that they are outbreeders. Kumar and Dutt (1989, 1990) found a similar low chiasma frequency per bivalent in the outbreeders *Gmelina, Verbena* and *Vitex*. *H. asper, H. physaloides, H. sabdariffa* and *H. surattensis*, with a high mean chiasma frequency per bivalent, are inbreeders. Kumar and Dutt (1989) associated such a high chiasma frequency per bivalent with the inbreeder *Lippia*.

It was observed that in the inbreeding species of this study, the anthers abutted on the stigma in the flower bud before anthesis. This arrangement gives self-pollen a head-start advantage in the race for the fertilization of the ovules. Sanyal (1959) found that in *Hibiscus* self-pollen germinated profusely on the stigma and grew vigorously down the styles. The immediate fitness of the progeny is thus assured (Kumar and Dutt 1990) despite the high level of recombination indicated by the high chiasma frequency per bivalent. Inbreeding therefore appears to have supplanted outbreeding in these species despite the showy flowers: inbreeders constantly arise from outbreeders (Allard 1960). Judged by the mean chiasma frequency per bivalent (Table 3) the progress towards inbreeding has been somewhat variable among the species, with *H. surrattensis* being the most advanced while *H. physaloides* is the least advanced.

The regular meiosis and high pollen fertility (Table 3) indicate that self-fertilization is not deleterious in these species. The high recombination is, however, useful in that it provides the species with the genetic flexibility to exist outside its adapted environment. Despite this high recombination, the total zygotic genetic endowment of the species remains unchanged because of self-fertilization. This preservation of the total zygotic genetic endowment is further enhanced by the population structure of the *Hibiscus* species studied, which consists of widely separated individual stands or small, widely separated, populations (Martin and Menzel 1972).

References

Alexander MP (1969) Differential staining of aborted and non-aborted pollen. Stain Technol 44:117–122

Allard RW (1960) Principles of plant breeding. John Wiley and Sons, New York

Cobley LS (1976) An introduction to the botany of tropical crops (2nd ed, revised by W.M. Steele) Longmans, London

Ford CE (1938) A contribution to the cytogenetical survey of the Malvaceae. Genetica 20:431–452

Kumar P, Dutt B (1989) Cytogenetic basis of breeding system in some verbenaceous species. Cytologia 54:347–353

Kumar P, Dutt B (1990) Cytogenetic basis of breeding systems in two verbenaceous species. Nucleus 33:121–123

Martin DW, Menzel MY (1972) Identity of the genome shared by African and New World species of *Hibiscus* sect. *Furcaria*. J Hered 63:235–240

Menzel MY, Martin DW (1970) Genome affinities of four African diploid species of *Hibiscus* sect. *Furcaria*. J Hered 61: 178–184

Menzel MY, Martin DW (1971) Chromosome homology of some intercontenental hybrids in *Hibiscus* sect. *Furcaria*. Am J Bot 58:191–202

Menzel MY, Wilson FD (1966) Hybrid and genome relations of *Hibiscus sabdariffa, H. meeusei, H. radiatus* and *H. acetosella*. Am J Bot 53:270–275

Menzel MY, Wilson FD (1969) Genetic relationship in *Hibiscus* sect. *Furcaria*. Brittonia 21:91–125

Sanyal P (1959) Studies on pollen tube growth in six species of *Hibiscus* and their crosses in-vivo. Cytologia 23:460–467

Sanyal P, Kundu BS (1959) Cytological study of the *Hibiscus radiatus – Hibiscus cannabinus* hybrid and genomic relationship of the two species. Nucleus 11:99–108

Sharma AK, Sharma A (1962) Polyploidy and chromosome evolution in *Hibiscus.* Cellule 62:281–300

Singh F, Khoshoo TN (1989) Cytogenetic basis of evolution in garden *Hibiscus*. Nucleus 32:62–67

Skovsted A (1935) Chromosome numbers in the Malvaceae 1. J Genet 31:263–293

Skovsted A (1941) Chromosome numbers in the Malvaceae II. C Rend Trav Lab Carlsberg, Ser Physiol 23:195–242

Tjio JH (1948) The somatic chromosomes of some tropical plants. Hereditas 34:135–146

Ugborogho RE, Shofoyeke BA (1983) Observations on the morphology, reproductive biology and eonomic importance of *Hibiscus esculentus* L. and *H. sabdariffa* L. (Malvaceae) in Nigeria. Nigrian Field 48:79–80

Wilson FD, Menzel MY (1964) Kenaf (*Hibiscus cannabinus*), roselle (*Hibiscus sabdariffa*). Econ Bot 18:80–91

Wise DA, Menzel MY (1971) Genetic affinities of the North American species of *Hibiscus* sect. *Trionum.Brittonia* 23: 425–437

Zar JH (1974) Biostatistical analysis. Prentice Hall, Englewood Cliffs, New Jersey, USA