Cytotaxonomic Studies on the Two Cultivated Species and the Wild Relatives in the Genus *Echinochloa*

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Introduction

The genus *Echinochloa* comprises about twenty species of annual or perennial grasses, and many of them are common to paddy fields.

The cultivated species, *E. frumentacea* (Roxb.) Link, is known as one of millets; and Sawa millet, Sanwa millet, poor man's millet, billion dollar grass, Japanese barnyard millet, barnyard millet and Japanese millet, etc. have been applied as the popular name to this cultivated species. This millet is grown for its grain as well as its forage in many parts of the Far East, being cultivated often in places where rice does not grow well. This crop is called Sawa or Sanwa (Mansfeld 1952), and locally known as Sama in India (Richharia 1957). Not much genetic work has been done on this crop.

In an earlier paper the author (Yabuno 1953) described that the basic number of *Echinochloa* is 9, and Japanese wild species, *E. crus-galli* (Linn.) Beauv., contains tetra- and hexaploid varieties, and Japanese cultivated strains are hexaploid. At the same time it was concluded that Japanese cultivated strains and Japanese hexaploid varieties of *E. crus-galli* have the same genome constitution in common, and it was further assumed that hexaploid varieties of *E. crus-galli* is an ancestral form of the cultivated species. Afterwards seeds of Malayan and Javanese strains of *E. colona* (L.) Link and Indian and Chinese cultivated strains could be obtained. Cross experiments were tried using these strains and hexaploid varieties of *E. crus-galli*. Their hybrids were easily obtained. In this paper the phylogenetic relationships of the cultivated species to the wild species of *Echinochloa* will be considered on the basis of the results of cytological investigations on their hybrids.

Materials and methods

In this study four strains of *E. crus-galli*, two strains of *E. colona* and five cultivated strains were used. The sources of these materials are shown in Table 1.

Of these materials W3 is the strain which was used in the controlled crosses with Japanese cultivated strains in a previous study (Yabuno 1953).

For cytological studies, spikelets were fixed in acetic alcohol (1:3) and

the chromosomes in PMC's were observed by the acetocarmine stain technique. The technique of hybridization is the same as that previously employed (Yabuno 1953).

Table 1. Source of materials

Species	Strain	Source
<i>Echinochloa crus-galli</i> (L.) Beauv.	563	Nepal
<i>E. crus-galli</i> var. <i>praticola</i> Ohwi	W3	Japan
<i>E. crus-galli</i> var. <i>caudata</i> (Roshev.) Kitagawa	In-9 U.S.A.-C	Coimbatore, India California, U.S.A.
<i>E. colona</i> (L.) Link	M-1 J-5	Malaya Java
The cultivated species		
Indian strain	In-1 In-3 In-6 In-11	Coimbatore, India do. do. do.
Chinese strain	5844	Hulan, China

In order to examine pollen fertility, anthers were collected at interval of a few days from florets just flowering, and pollen grains were stained by iodine-potassium iodide.

Observations

A. Morphology of the wild and the cultivated species

Two wild species, *E. crus-galli* and *E. colona*, can be distinguished in some characteristics. Principal characters distinguishing them are given in Table 2.

Table 2. Principal characters distinguishing the two wild species of *Echinochloa*

Characters	Species	
	<i>E. crus-galli</i> Beauv.	<i>E. colona</i> Link
Panicle	Usually nodding	Erect
Rachilla	Rather close on a rachis, more or less branched	Rather distant on a rachis, simple
Spikelet	Often not arranged in rows	Usually arranged in 4 rows
Awn	Variable in length	Awnless
Stigma color	White or red	Blackish-purple
Anther color	Brown or yellow	Purple

Panicles of some strains of these two wild species and the cultivated species are shown in Figs. 1a and 1b.

The essential difference between the cultivated and the wild species can be recognized in the deciduous character of spikelets, i.e. the cultivated species has non-deciduous character and both *E. colona* and *E. crus-galli* possess deciduous character of spikelets.

Leaf, stem and panicle of the cultivated species are more gigantic than that of the wild species. With respect to panicle characters Indian and Chino-Japanese cultivated strains tend to resemble *E. colona* and *E. crus-galli* respectively.

Fig. 1. Panicles of the two wild species and the cultivated species of *Echinochloa*. a, *E. colona* and Indian cultivated strains. b, *E. crus-galli* and Japanese cultivated strain. Panicles of the cultivated strains are indicated by arrows.

B. Cytology of the wild species, the cultivated species and the F_1 hybrids

Meiosis was observed in all strains of *E. colona* and *E. crus-galli* and in five cultivated strains. It was normal in all of them, and 27 bivalents were observed at metaphase I. *E. colona*, Indian and Chinese cultivated strains and varieties of *E. crus-galli* used in this study can be therefore said to be hexaploid because the basic number of *Echinochloa* is 9. All these strains showed the high pollen- and seed fertility.

Four strains of *E. crus-galli* used in this study hybridize easily between them, and it was confirmed that they have the same genome constitution. All five F_1 hybrids between two strains of *E. colona* and four Indian cultivated

strains were quite normal in the meiotic division of PMC's, and 27 bivalents were observed at metaphase I (Fig. 2d). Many characters of the F_1 hybrids are intermediate between those of the parent plants except for the deciduous character of spikelets (Fig. 2a-c).

It can be therefore concluded that Indian cultivated strains consist of genomes which are completely homologous with that of *E. colona*.

In spite of the normal meiotic division all the F_1 hybrids showed a marked high sterility in various degree (Table 3). Significant difference in pollen fertility was not found between anthers which were collected at intervals of a few days. Normal pollen percentage seems to depend upon the combination of parental strains.

The growth of four F_1 plants

between Indian cultivated strains and *E. crus-galli* and that of an F_1 plant of Indian strain $\times$ Chinese strain of the cultivated species were as vigorous as their parents (Fig. 3).

Fig. 2. *E. colona*, Indian cultivated strain and the F_1 hybrid. a, *E. colona* (J-5). b, Indian cultivated strain (In-3). c, the F_1 . d, metaphase I of the F_1 showing 27 bivalents.

Meiotic irregularities, i.e. quadri- trivalents, univalents, laggards, and micronuclei, were observed in all of these hybrids. The general features of meiotic division of these five hybrids were as follows :

Table 3. Pollen fertility of the F_1 s between *E. colona* and Indian cultivated strains

Hybrid	No. of pollen grains examined	No. of good pollen grains	Normal pollen percentage
J-5×In-1	6050	1918	31.70
J-5×In-3	29354	5555	18.92
M-1×In-6	9390	3628	38.63
M-1×In-3	6771	2022	29.86

Fig. 3. *E. crus-galli*, Indian cultivated strain and the F_1 . a, *E. crus-galli* (U.S.A.-C). b, the F_1 . c, Indian cultivated strain (In-6).

The chromosome conjugations at metaphase I of all these hybrids were very variable, and some bivalents loosely associated. Fig. 4a shows a meiotic metaphase I of the hybrid of Indian and Chinese cultivated strains. At anaphase I univalents could be observed in process of division at the equator of the spindle (Fig. 4b). At a somewhat later stage the half-univalents proceeded to the opposite poles. A few fragments remained in the cytoplasm were seen at telophase I. The single chromatids that derived from the division of univalents at anaphase I, were distributed to the poles at random at anaphase II. Laggardism was also observed at this stage.

A summary of the chromosome configurations of these five hybrids is given in Table 4.

The data for these five hybrids were combined in the column of total

of Table 4 since all hybrids behaved much the same in meiosis.

The average chromosome configurations of hybrids In-6 $\times$ U.S.A.-C,

Fig. 4. Meiotic division of the F_1 hybrid between Indian cultivated strain and Chinese cultivated strain. a, chromosome pairing at metaphase I, $2_{III}+11_{II}+26_I$. b, anaphase I showing division of univalents at the equator.

In-9 $\times$ In-1, W3 $\times$ In-1, 563 $\times$ In-1, and In-11 $\times$ 5844 are

$$0.15_{IV}+1.14_{III}+11.29_{II}+27.40_I,$$

$$0.11_{IV}+0.86_{III}+11.60_{II}+27.78_I,$$

$$0.31_{IV}+1.38_{III}+11.63_{II}+25.36_I,$$

$$0.08_{IV}+1.08_{III}+13.36_{II}+23.72_I,$$

and $0.70_{III}+11.27_{II}+29.36_I$, per cell respectively,

and the average chromosome configuration which can be obtained by combining the data for all these five hybrids is

$$0.13_{IV}+1.03_{III}+11.81_{II}+26.77_I.$$

The chromosome pairing observed in the hybrids may be auto- or allosyndetic. At the present there is no evidence for judging whether auto- or allosyndesis are taking place.

In the consequence of the irregular meiotic division of the F_1 hybrids described above, micronuclei were formed in the microspores of the quartets. The frequency of the microspores with various numbers of micronuclei is shown in Table 5.

The distribution of micronucleus numbers was ranged from one to five, and the deviation in percentage of microspores with micronuclei was considerably marked between the hybrids.

Pollen grains were empty in all the F_1 hybrids between Indian cultivated strains and *E. crus-galli* or Chinese cultivated strain, and anthers did not dehisce at flowering time. The hybrid plants set no seed in free pollination.

Table 4. Frequency distribution of the number of multivalents and bivalents in five F_1 hybrids

Hybrid	No. of bivalents																No. of trivalents				No. of quadri- valents		Total number of PMC's observed
	4	6	7	8	9	10	11	12	13	14	15	16	17	18	1	2	3	4	1	2			
In-6×U.S.A.-C	1	2	1	3	6	9	4	8	11	10	2				14	14	5	2	9		57		
In-9×In-1				2	5	7	10	4	4	8	2	1			15	8	2		3	1	43		
W3×In-1	1	2	4	5	2	3	5	13	3	5	1				15	9	8	1	12	1	44		
563×In-1				3	2	6	5	5	12	8	1	4	1		12	10	5	1	4		47		
In-11×5844				1	5	9	11	11	7	2	1				20	5	1				47		
Total	1	3	3	10	24	29	34	33	40	35	18	3	4	1	76	46	21	4	28	2	238		

Discussion

E. colona (L.) Link is an annual tufted grass, and is widely distributed in the tropics of Asia, America and Africa. When in the young state this grass closely resembles the young rice plant, and consequently is considered to be an obnoxious pest in rice fields (Bor 1960). This grass is called Shama in India (Mansfeld 1952), and was at one times cultivated in Egypt. The chromosome number of this species was reported to be $2n=48$ by Krishnaswamy and Raman (1949).

Both Javanese and Malayan strains of this species and Indian cultivated strains used in the present investigation possess $n=27$ chromosomes. The cultivated species, *E. frumentacea* (Roxb.) Link has been classified as a variety of *E. colona*, *E. colona* var. *frumentacea* Ridley, by some taxonomists. Krishnaswamy described in his paper (Krishnaswamy 1951) that *E. colona* hybridizes with the cultivated millet, *E. colona* var. *frumentacea*, giving rise to a large number of intermediates.

The hybrids of *E. colona* and Indian cultivated strains could be easily obtained in the present experiment. All these hybrids formed 27 bivalents at metaphase I, and the meiotic divisions proceed normally, although a marked high pollen sterility appeared in all of them. It can be thus concluded *E. colona* and Indian cultivated strains have the same genome constitution.

This relationship can be compared with that of hexaploid varieties of *E. crus-galli* (L.) Beauv. and Japanese cultivated strains, which was elucidated by a previous study (Yabuno 1953).

E. crus-galli is a polymorphic species in ecological and morphological characters, and can be found all round the world, rather more plentifully in warm temperate

regions than in the tropics. Four F_1 hybrids between Indian cultivated strains and hexaploid varieties of *E. crus-galli* and an F_1 hybrid of Indian- and Chinese cultivated strains were found to show meiotic irregularity, and they

Table 5. Frequency of microspores with micronuclei

No. of micronuclei/microspore	Frequency of microspores			
	In-9×In-1	W3×In-1	563×In-1	In-11×5844
0	16 (8.6%)	86 (39.4%)	1197 (64.3%)	68 (23.9%)
1	98 (52.7)	102 (46.8)	581 (31.2)	110 (38.7)
2	51 (27.4)	22 (10.1)	82 (4.4)	77 (27.1)
3	16 (8.6)	7 (3.2)	2 (0.1)	23 (8.1)
4	3 (1.6)	1 (0.5)	0 (0.0)	5 (1.8)
5	2 (1.1)	0 (0.0)	0 (0.0)	1 (0.4)
Total number of microspores observed	186	218	1862	284
Percentage of microspores with micronuclei	91.3%	60.5%	35.7%	76.0%

showed a wide distribution in the range of chromosome pairing. But all these hybrids behaved much the same in meiosis. From the data on chromosome associations for these five hybrids the average chromosome configuration,

$$0.13_{IV} + 1.03_{III} + 11.81_{II} + 26.77_I ,$$

could be summarized.

Although in this study the genomic formula can not be given to the two wild species, i.e. *E. colona* and *E. crus-galli*, and Indian and Chino-Japanese cultivated strains, it can be now safely said that *E. colona* and hexaploid varieties of *E. crus-galli* do not have the same genome constitution, and the same conclusion can be adopted to Indian and Chino-Japanese cultivated strains.

E. frumentacea (Roxb.) Link is the specific name originally given to the cultivated species of India (Roxburgh 1820), but it has been applied further to Chino-Japanese cultivated strains by many taxonomists and agronomists. It is therefore reasonable to separate Chino-Japanese cultivated species from *E. frumentacea* considering the cytological evidence mentioned above, and the new specific name, *E. utilis* Ohwi et Yabuno was given to Chino-Japanese cultivated strains. Accordingly, all four Indian cultivated strains and Chinese cultivated strain, which were used in this study, belong to *E. frumentacea* and *E. utilis* respectively, and the four Japanese cultivated strains which were used in the previous cross experiments reported in 1952 by the author are not *E. frumentacea* but *E. utilis*.

Ohwi described that "*E. frumentacea* of Japan has been said to be derived from *E. crus-galli* or *E. colona* but it is morphologically tends to

resemble the former rather than the latter species" (Ohwi 1942).

On the basis of morphology and cytology the present author assumes that *E. frumentacea*, which is the cultivated species of India, has been derived from *E. colona*, on the other hand, *E. utilis*, which is Chino-Japanese cultivated species, has been derived from hexaploid variety of *E. crus-galli*.

One of the popular names of the cultivated species of *Echinochloa*, Japanese millet must have originally been given to Japanese cultivated strains, therefore, this popular name is justifiable one to *E. utilis*. On the other hand *E. frumentacea* is called 'Sawa' in India (Mansfeld 1952) and Sawa millet seems to be a suitable popular name for *E. frumentacea*.

Summary

Echinochloa colona (L.) Link of Malaya and Java, and Indian strains of cultivated species, *E. frumentacea* (Roxb.) Link, were certained to be hexaploid, $n=27$, and to have the same genome constitution in common.

Although the F_1 hybrids of these two species showed a low degree of fertility, it can be assumed that *E. colona* is a wild form of *E. frumentacea*.

In earlier paper the author (Yabuno 1953) has confirmed that hexaploid varieties of *E. crus-galli* (L.) Beauv. and Japanese cultivated strains have the same genome constitution, and that the former is a wild form of the latter.

Four F_1 hybrids between Indian strains of *E. frumentacea* and hexaploid varieties of *E. crus-galli*, and an F_1 hybrid between Indian strain of *E. frumentacea* and Chinese cultivated strain were cytologically investigated. It is concluded that *E. colona* and *E. crus-galli* do not possess genomic constitution in common. The same conclusion can be adopted to Indian and Chino-Japanese cultivated strains. But any genomic formula was not assigned to these species in this paper.

Indian and Chino-Japanese cultivated strains have been maintained under the specific name, *E. frumentacea*. On the basis of the results of cytological investigations Chino-Japanese cultivated species was separated from *E. frumentacea*, and the new specific name, *E. utilis* Ohwi et Yabuno was given.

E. colona and *E. crus-galli* can be distinguished in some morphological characters, particularly with respect to panicles character, and these two wild species differ more or less in geographic distribution.

E. utilis and *E. frumentacea* resemble to *E. crus-galli* and *E. colona* respectively in the characteristics of panicle. From the evidences mentioned above, it is assumed that *E. utilis* has arisen from the hexaploid varieties of *E. crus-galli* probably in the Far East, and *E. frumentacea* from *E. colona* in the tropics.

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