Cytogenetical Studies on the Hybrids of *Echinochloa oryzicola* Vasing. and the Thai Tetraploid Strain of *E. stagnina* (Retz.) Beauv. with the West African Species *E. obtusiflora* Stapf

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Received November 2, 1981

Species of the grass genus *Echinochloa* form a polyploid series with the basic chromosome number of $x=9$ (Yabuno 1953, 1966). *E. crus-galli* (L.) Beauv. is an annual hexaploid species ($2n=6x=54$) distributed throughout the world, rather more plentifully in warm temperate regions than in the tropics. This species is the presumable wild form of the Asian cultivated species *E. utilis* Ohwi et Yabuno (Yabuno 1953, 1962). The other annual species *E. oryzicola* Vasing. is distributed in Japan, Korea, China, U. S. S. R., Northern India, Sri Lanka, Italy, France and U. S. A. as a weed in lowland rice fields. This species is tetraploid with $2n=4x=36$ (Yabuno 1953). It was cytogenetically concluded that *E. oryzicola* is the donor of the two genomes to *E. crus-galli* (Yabuno 1966). Therefore the cytogenetical identification of diploid carrier of the third genome of *E. crus-galli* is the next main concern in the author's work. On the other hand a corm bearing, partially self-incompatible strain referred to as *E. pyramidalis* (Lamk.) Hitchc. et Chase was collected by Dr. A. V. Bogdan in Kitale, Kenya in 1964. This strain is diploid with $2n=2x=18$ (Yabuno 1968). Cytogenetical evidence indicated that the genome of the Kenyan diploid strain may be related to one of the two genomes of *E. oryzicola* (Yabuno 1976).

The self-pollinated, stoloniferous perennial species *E. stagnina* (Retz.) Beauv. occurs in swampy places or in floating rice fields as a weed in tropical Asia and Africa. The present author obtained the 4x, 6x, 12x and 14x strains which have been included under *E. stagnina* by taxonomists (Yabuno 1968, 1981 a). Cytological relationship between the 12x and 14x strains was described in a previous paper (Yabuno 1970). Since hybrid between the 4x and 6x strains has not yet been obtained, there is no evidence on their chromosomal relationship.

Recently the present author collected the annual diploid species *E. obtusiflora* Stapf in Nigeria.

Hybrids of *E. oryzicola* and the Thai 4x strain of *E. stagnina* with *E. obtusiflora* were artificially produced in 1980. In this paper their cytological relationships are described.

Materials and methods

Echinochloa obtusiflora collected in an upland rice field in Fufore, Nigeria, a Japanese strain of *E. oryzicola* and a Thai 4x strain referred to as *E. stagnina* were

Fig. 1. Panicles of three *Echinochloa* species and their F_1 hybrids. a, *E. oryzicola*. b, The Thai tetraploid strain of *E. stagnina*. c, *E. obtusiflora*. d, the F_1 of *E. oryzicola* $\times$ *E. obtusiflora*. e, the F_1 of *E. stagnina* (Thai strain) $\times$ *E. obtusiflora*.

used in this study. Panicles of these three species are shown in Figs. 1-a, b, c. The technique of hybridization was same as that previously employed (Yabuno 1953). Young panicles of the resulting F_1 hybrids were fixed in Farmer's solution and processed as usual to make acetocarmine squash preparations of PMCs. A potassium iodide solution (I_2 -KI) was used to determine pollen stainability.

Results

General characteristics of the strains of *E. oryzicola*, *E. stagnina* and *E. obtusiflora* used in this study

The Japanese strain of *E. oryzicola* had the characteristics of the C-form, the surface of lemma of the lower floret being coriaceous, convex and lustrous, and was awnless. The lower floret sterile. The lower empty glume $1/2-3/5$ the length of the spikelet. The spikelets 4.5 mm long, 2.2 mm wide. The plant type erect.

The Thai 4x strain of *E. stagnina* was stoloniferous, and was of shorter stature as compared with the 6x, 12x and 14x strains which have been referred to as *E. stagnina* by taxonomists. The ligule transformed into hairs. The lower floret male. The spikelets 4.0 mm long, 1.5 mm wide, and awn 5 mm long.

Table 1. Seed fertility of two plants of *E. obtusiflora* following self- and cross-pollination

Plant no.	Seed fertility when selfed			Seed fertility when bagged together with a panicle of another plant			Partner bagged together
	No. of florets	No. of seeds	Seed fertility (%)	No. of florets	No. of seeds	Seed fertility (%)	
4	2092	138	6.5	1007	419	41.6	12
12	4363	193	4.4	4009	949	23.6	4

E. obtusiflora was slightly tufted erect to 1 m high. Leaf 20-40 cm long, up to 9 mm broad. The ligule represented by a fringe of hairs. Panicles 10-30 cm long. The branches more or less erect. The lower floret male. The spikelets markedly blunt at the tip, 3 mm long, 1.5 mm wide, awnless, often in four rows. The lower empty glume obtuse, about a third as long as the spikelet. A chromosome number of $n=9$ was reported for this species for the first time by the present author (Yabuno 1981 a) (Figs. 2 a and b). When panicles of this species were self-pollinated by bagging, they showed a very low seed fertility in spite of their high pollen fertility. But when two panicles of different plants were bagged together, the seed fertility was remarkably improved, indicating the nature of partial self-incompatibility in this species (Table 1).

Morphology and cytology of the F_1 hybrids

The F_1 hybrid, *E. oryzicola* ($n=18$) $\times$ *E. obtusiflora* ($n=9$):

A panicle of this hybrid is shown in Fig. 1 d.

The surface of lemma of the lower floret was coriaceous, convex and shiny as that of the C-form of *E. oryzicola*. The lower floret male, and the ligule trans-

formed into hairs as that of *E. obtusiflora*. The spikelets 3.4–4.0 mm long, 1.8 mm wide. Although both parents were annual, the F_1 hybrid was unexpectedly perennial.

The data on chromosome configurations in the F_1 hybrid are presented in Table 2. Tri- and bivalents were formed, ranging from 0 to 1, and 0 to 4, respectively. All the bivalents were rods with a single terminalized chiasma. The univalents were irregularly scattered on the spindle, and some of them divided longitudinally at AI. About 30% of the PMCs had 27 univalents (Fig. 2 c). The mean chromosome configuration was $0.04_{III} + 1.54_{II} + 23.80_I$. This fact indicates that both genomes of *E. oryzicola* are different from the genome of *E. obtusiflora*. Restitution nuclei were occasionally observed in the first meiotic division (Fig. 2 d). The F_1 plant had 2.2% stainable pollen. The darkly stained pollen grains were usually larger than the empty ones (Fig. 2 e). The former might have an unreduced nucleus. Some pollen grains were dumb-bell shaped (Fig. 2 e).

Table 2. Chromosome configurations in the F_1 hybrids,
E. oryzicola ($n=18$) $\times$ *E. obtusiflora* ($n=9$)

Configurations	No. of PMCs
27_I	32
$1_{II} + 25_I$	20
$2_{II} + 23_I$	19
$3_{II} + 21_I$	15
$1_{III} + 2_{II} + 20_I$	3
$4_{II} + 19_I$	11
$1_{III} + 3_{II} + 18_I$	1
	18
	12
Mean chromosome configuration	Total number of PMCs
$0.04_{III} + 1.54_{II} + 23.80_I$	101

The F_1 hybrid, *E. stagnina* (Thai strain, $n=18$) $\times$ *E. obtusiflora* ($n=9$):

A panicle of this hybrid is shown in Fig. 1 e.

The F_1 hybrid was stoloniferous perennial and morphologically resembled the Thai strain, but was awnless. The spikelets 3–4 mm long, 1.4 mm wide. The ligule represented by a fringe of hairs, and the lower floret male as those of the parents.

The data on chromosome configurations in the F_1 hybrid are presented in Table 3. Chromosome configuration in the F_1 hybrid was variable. There were 0 to 2 trivalents and 1 to 10 bivalents (Fig. 3). Some of the bivalents were ring type. The univalents usually splitted equationally at AI. The average chromosome configuration was $0.3_{III} + 6.1_{II} + 13.9_I$. The pollen stainability was 0.09%, and the selfed seed fertility was 0%.

Fig. 2. a and b, diakinesis and MI in *E. obtusiflora*, respectively, showing 9_{II} . c, chromosome configuration at MI in the F_1 hybrid of *E. oryzicola* $\times$ *E. obtusiflora*, showing 27_I . d, restitution nucleus in the first meiotic division in the F_1 hybrid. e, pollen grains of the F_1 hybrid.

Discussion

The habitat of *E. oryzicola* ($n=18$) is confined to rice fields and considered as a companion weed of common rice *Oryza sativa*. *E. phyllopogon* Stapf subsp. *oryzicola* (Vasing.) Koss. is a synonym of this species (Yabuno 1981 b). It is assumed that *E. oryzicola* was introduced to U. S. S. R., Italy, France and U. S. A. from the Orient. According to Barrett and Seaman (1980), *E. oryzicola* was probably introduced as rice seed contaminant at the beginning of rice culture in California, the 1920's, and rice agronomists in California have referred to this species as "Japanese, Orient or White Water Grass".

Table 3. Chromosome configurations in the F_1 hybrid, *E. stagnina* (Thai strain, $n=18$) $\times$ *E. obtusiflora* ($n=9$)

Configurations	No. of PMC
$1_{II}+25_I$	1
$2_{II}+23_I$	1
$1_{III}+1_{II}+22_I$	2
$3_{II}+21_I$	4
$4_{II}+19_I$	5
$1_{III}+3_{II}+18_I$	1
$2_{III}+2_{II}+17_I$	1
$5_{II}+17_I$	10
$1_{III}+4_{II}+16_I$	3
$6_{II}+15_I$	11
$1_{III}+5_{II}+14_I$	3
$7_{II}+13_I$	17
$1_{III}+6_{II}+12_I$	9
$2_{III}+5_{II}+11_I$	1
$8_{II}+11_I$	8
$1_{III}+7_{II}+10_I$	6
$2_{III}+6_{II}+9_I$	1
$9_{II}+9_I$	9
$1_{III}+8_{II}+8_I$	1
$10_{II}+7_I$	1
$1_{III}+9_{II}+6_I$	1
Mean chromosome configuration $0.3_{III}+6.1_{II}+13.9_I$	Total number of PMCs 96

Fig. 3. Chromosome configuration at MI in the F_1 hybrid of *E. stagnina* (Thai strain) $\times$ *E. obtusiflora*, showing $5_{II}+17_I$.

E. crus-galli ($n=27$) is a phenotypically and ecologically extremely polymorphic species. This species is distributed rather more plentifully in warm temperate regions than in the tropics, and usually grows in swampy places. In the meiosis in the F_1 hybrid, *E. crus-galli* ($n=27$) $\times$ *E. oryzicola* ($n=18$), chromosome configurations $18_{II}+9_I$ and $1_{III}+17_{II}+8_I$ were formed with high frequencies, total up to 98% of the PMCs (Yabuno 1966). Therefore the present author assumed that two of the three genomes of *E. crus-galli* are homologous to those of *E. oryzicola*. On the basis of evidence from morphology, cytology and geographical distribution, it was concluded that *E. crus-galli* is an allohexaploid produced by the natural hybridi-

zation of *E. oryzicola* with a not-yet-discovered diploid species of *Echinochloa* or its related genus and by the subsequent chromosome doubling (Yabuno 1966).

The F_1 hybrid of *E. oryzicola* with the Kenyan diploid strain referred to as *E. pyramidalis* had a mean chromosome configuration of $0.2_{III} + 6.0_{II} + 14.4_I$ (Yabuno 1976). This indicates that the genome of the Kenyan diploid strain is related to one of the genomes of *E. oryzicola*. While on the other hand the mean chromosome configuration in the F_1 hybrid of *E. oryzicola* ($n=18$) $\times$ *E. obtusiflora* ($n=9$) was $0.04_{III} + 1.54_{II} + 23.80_I$. The most characteristic feature of chromosome configuration in this hybrid was the high frequency of univalents. Moreover this hybrid was rarely capable of producing unreduced nuclei in the first division in PMCs. Therefore it may be said that the West African diploid species *E. obtusiflora* has more or less the cytological characteristics which are requisite for a donor of the third genome present in the hexaploid *E. crus-galli*.

At present the diploid species of *Echinochloa* are known only from Africa. But when the distributional patterns of *E. oryzicola* and *E. crus-galli* are considered, the possibility of diploid carrier of the third genome being Oriental species cannot be ruled out. Further collection of diploid species of *Echinochloa* and studies on species hybrids are required to elucidate the origin of the third genome of *E. crus-galli*.

E. stagnina is distributed in tropical Asia and Africa. The 4x, 6x, 12x and 14x strains which have been treated as *E. stagnina* by taxonomists are different from each other not only in chromosome number but also in some morphological characters (Yabuno 1968). F_1 hybrid between 4x and 6x strains has not yet been obtained. Therefore at present their genomic constitutions are not distinct. But in this study it was elucidated that one of the two genomes of the Thai 4x strain of *E. stagnina* may be partially homologous to the genome of *E. obtusiflora*.

E. obtusiflora has been collected in Gurin, Fufure, Yola, Jalingo, Fika, Gombe, Gaya, Jos, Katsina, Zaria, Kaduna, Wurno and Tegna, Nigeria and in Maroua, Cameroun. These collection sites in Nigeria are localized in the area where African rice *Oryza glaberrima* is predominantly cultivated. The present author confirmed in Nigeria that *E. obtusiflora* occurs in shallow pools or wet places often mixed with *E. pyramidalis* and also in upland rice fields in the northern area. This fact suggests that the distribution of *E. obtusiflora* has been connected with African rice cultivation. *E. obtusiflora* has been much less known but the present author regards this species as a companion weed of African rice *O. glaberrima*.

Summary

The West African annual diploid *E. obtusiflora* ($2n=2x=18$) is characterized by the morphology of its spikelets and is partially self-incompatible. The result of the author's field observation in Nigeria indicated that this species is a companion weed of African rice *Oryza glaberrima*. The annual tetraploid *E. oryzicola* ($=E. phyllopogon$ Stapf subsp. *oryzicola* (Vasing.) Koss., $2n=4x=36$) is a companion weed of common rice *Oryza sativa*, and also is the carrier of two of the three genomes of the annual hexaploid *E. crus-galli* ($2n=6x=54$), the wild form of the Asian cultivated species *E. utilis*. The self-pollinated, stoloniferous perennial species *E. stagnina* is

distributed in tropical Asia and Africa, and includes some cytootypes. The F_1 hybrids of *E. oryzicola* and the Thai 4x strain ($2n=4x=36$) of *E. stagnina* with *E. obtusiflora* were artificially produced. The results of the cytogenetical studies on these F_1 hybrids indicated that the genome of *E. obtusiflora* may be related to one of the genomes of the Thai 4x strain and that *E. obtusiflora* has more or less the cytological characteristics which are requisite for a presumable diploid carrier of the third genome of *E. crus-galli*.

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