

# THE INHERITANCE OF CYTOPLASMIC GENETIC MALE STERILITY IN SORGHUM<sup>(1)</sup>

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## Introduction

Since heterosis has been successfully used in corn breeding, many workers had focused their attention on the finding of male sterile lines in the many different crop species in the hope of discovering the possibilities for utilizing heterosis. Of all these workers, J. C. Stephens was the one who made substantial contribution in this field. He discovered the cytoplasmic genetic male sterility in sorghum resulted from the introduction of kafir chromosomes into milo cytoplasm and also provided a more satisfactory method for the commercial production of hybrid sorghum seeds (1954).

The investigations undertaken in this study are designed to explore the mode of inheritance of cytoplasmic genetic male sterility and the variations of viable pollen percentage among segregating populations in sorghum.

## Literature Review

The phenomenon of pollen sterility in sorghum was first reported independently by Ayyanger *et al.* (1937) in India and by J. C. Stephens (1937) in America. In each case anthers were devoid of pollen but stigmas were receptive, monogenic inheritance was observed with male sterility recessive, and the symbols  $ms_1$  and  $ms_2$  were used to represent the factors involved, in these two cases respectively (Stephens and Quinby, 1945). Kapper and his associates (1938) reported two additional male steriles and designated them  $ms_3$  and  $ms_4$ , but they give no data regarding inheritance. Stephens J. C. and J. R. Quinby (1945) reported that  $ms_2$  (male sterile) linked with a (awned) and  $V_{10}$  (virescent-yellow plant) with crossover percentages 10.9 for  $ms_2$ -a and 9.1 for a- $V_{10}$ . They also found that  $ms_1$  and  $ms_2$  were genetically independent.

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G. H. Kuykendall (1943) discovered a new kind of male sterility in the Day variety which differed from any previously reported cases. He found a single plant with partial male-sterility which segregated into normal, partial male-sterile and completely male-sterile in later generation. The most interesting phase of the Day male-sterile was that all plants in the  $F_1$  generation of Day with certain varieties were male-sterile or partially male-sterile; whereas, when the Day or  $F_1$  male-steriles were pollinated by certain other varieties, all plants in the next generation were completely fertile. Based on these facts, Stephens and his associates (1952) proposed a method for producing hybrid sorghum seed with three-way cross, but the mode of inheritance of this type of male-sterility was not clearly understood at that time.

In order to determine whether or not cytoplasmic factors might account for the  $F_1$ 's male-sterility in the combination of msms Day  $\times$  kafir, Stephens and Holland (1954) made all possible crosses between Msms Day, Double Dwarf Yellow Sooner milo, and Texas Blackhull kafir. The evidence indicated that the male-sterility in certain combinations was caused by interaction between milo cytoplasm and kafir nuclear factors. They also provided a more satisfactory method for producing hybrid sorghum seed than did the three-way cross which was dependent solely on nuclear genes.

Maunder A. B. and R. C. Pickett (1959) used male-sterile Combine Kafir 60 (with milo cytoplasm and kafir nucleus) as female parent to cross with 23 fertile varieties and hybrids to study the genetic inheritance of cytoplasmic-genetic male sterility in grain sorghum. Through observing and counting the segregation ratio of normal and sterile plants in  $F_2$  and backcross populations, they concluded that this type of male sterility was dependent on a single pair of recessive genes,  $ms_c ms_c$ , interaction with sterile cytoplasm.

The cytoplasmic male-sterile gene,  $ms_c$ , was found to be independent of the awned gene which was known to be linked to the genetic sterile gene,  $ms_2$ , as reported by Stephens and Quinby (1945).

#### Materials and Methods

Most of tested varieties and male-sterile Combine Kafir-60 plants were introduced from the United States in 1957. The seeds of male-sterile Combine Kafir-60 ( $ms$ ) which was obtained in a cross of 3197A male sterile and 3197B maintainer were used as female parents in all crosses and backcrosses. In order to prove the purity of the fertile varieties, selfing was carried out at the University Farm, Taipei in 1958. The selfing progenies were quite uniform and no phenotypic segregation appeared. By crossing the male-sterile line with all fertile varieties, hybrids were obtained and grown in 1959. All the materials,

parents,  $F_2$  and backcross progenies were planted in the Spring of 1960. Progenies, either  $F_2$  or backcross or both which showed complex inheritance, were again planted in the Fall of 1961 at the same location. The materials were randomly thinned at seedling stage to keep 30 plants for  $F_1$  and 100 plants for  $F_2$  and backcross progenies respectively. Part of the panicle was collected before pollen dehiscence from each plant and stored immediately in 70% alcohol. For making pollen counts, three florets were sampled from each panicle. All anthers from each floret were smeared in a drop of diluted aqueous iodine-potassium iodide solution and examined under a microscope. Pollen grains which were round and stained in dark-brown or black were considered as normal. Any pollen being shrunken and stained in light-yellow or colorless, it was classified as abortive. Those plants completely free from normal pollen grain were classified as sterile, otherwise they were fertile.

Individuals of  $F_2$  of cross combinations of Kafir-60 male sterile with Shallu and other varieties were tagged before heading to maintain their identity. Notes on glume tip color were taken before the plants reached maturity. Other characters, such as panicle type, glume type, condition of awn and seed color were observed and classified after maturity.

### Results and Discussion

#### *Interaction between milo cytoplasm and nuclear genes of varieties.*

All plants of the Combine Kafir-60 male sterile (with milo cytoplasm and kafir nucleus) were completely male sterile. All varieties used as male parent were completely fertile. The normal pollen ranged from 85 to 95 percent.

In  $F_1$  progenies, five plants of each cross were classified by microscopic examination of the pollen. The result was summarized in Table 1.

**Table 1.** *The pollen grain observations of Combine Kafir-60 male sterile crossed with fourteen grain sorghum varieties.*

Combinations	Normal pollen percentage	
	Range	Mean
ms × Westland	0	0
ms × Kafir 1	0	0
ms × Coastland	0	0
ms × Martin	0	0
ms × Taipei Farmer	0	0
ms × Caprock (Taichung)	60-95	79.0
ms × R7000	90-98	94.8
ms × Arizona	98	98.0
ms × Redbine	80-90	88.0
ms × Shallu	90-100	96.0
ms × Caprock 7000	50-90	65.0
ms × Combine 7078	98	98.0
ms × Hegari	95-100	97.6
ms × Shallu (Taichung)	80-90	82.0

Five of these crosses were completely male sterile. This would be expected if these male parental varieties used possessed the same male sterile nuclear gene as that of the Combine Kafir-60 male sterile. The remaining nine, on the other hand, had more or less normal pollen indicating that these varieties possessed restored gene which was dominant.

The percentage of normal pollen of  $F_1$  progenies varied with cross combinations. Crosses, ms  $\times$  R7000, ms  $\times$  Arizona, ms  $\times$  Shallu, ms  $\times$  Combine 7078 and ms  $\times$  Hegari had higher percentages. Crosses, ms  $\times$  Redbine, ms  $\times$  Shallu (Taichung) and ms  $\times$  Caprock (Taichung) had the percentage less than 90 percent.  $F_1$  of ms  $\times$  Caprock 7000 was the lowest, only 65 percent.

In  $F_2$  progenies, there was a continuous range of variation from completely sterile to completely fertile. No attempt was made to separate individual plants of each segregating progenies into several classes. The plants which did not have single normal pollen were classified as sterile plant. Plants with even less than ten percent of normal pollen were grouped as fertile. Since male sterile was controlled by a single factor (Maunder and Pickett, 1959), Ms factor might be modified by either modified gene or genes or by environment. Consequently, variation in pollen fertility might be a natural result (Hadley and Singh, 1961).

From the data in  $F_2$ , the segregation ratios of cross combinations of ms  $\times$  Caprock (Taichung), ms  $\times$  Combine 7078, ms  $\times$  Hegari, ms  $\times$  Caprock 7000 and ms  $\times$  Shallu were satisfactory for the explanation that a single recessive gene interacted with milo cytoplasm was responsible for the action of pollen sterility as Maunder and Pickett (1959) reported. The ratio of other combinations, ms  $\times$  Shallu (Taichung), ms  $\times$  Redbine, ms  $\times$  Arizona and ms  $\times$  R7000 deviated significantly from a 3:1 ratio. The result is summarized in Table 2.

**Table 2.** Pollen sterility of  $F_2$  progenies of male sterile Combine Kafir-60  $\times$  Varieties. The P values were the  $X^2$  test for monofactorial hypothesis.

Cross Combinations	No. of Total Plants	No. of Fertile Plants	No. of† Sterile Plants	P-Value	Percentage of normal pollen††	
					Range	Mean
ms $\times$ Caprock (Taichung)	54	39	15	0.50-0.75	10-95	67.77
ms $\times$ Combine 7078	27	20	7	0.90-0.95	30-95	65.45
ms $\times$ Hegari	93	70	23	0.05-0.10	30-95	81.50
ms $\times$ Shallu (Taichung)	216	146	70	0.01-0.05*	10-95	67.25
ms $\times$ Caprock 7000	96	71	25	0.90-0.95	10-95	64.22
ms $\times$ Redbine	83	49	34	<0.01**	20-95	61.49
ms $\times$ Arizona	46	41	5	0.01-0.05*	10-95	76.31
ms $\times$ Shallu	215	154	61	0.2 -0.3	10-95	80.71
ms $\times$ R7000	51	49	2	<0.01**	10-95	75.23

\* Significant P value

\*\* Highly significant P value

† No normal pollen formed

†† Individuals classified as fertile plants

In the backcross progenies there was also a wide range of variation from completely sterile to completely fertile as in the  $F_2$  progenies. The results are summarized in Table 3.

**Table 3.** *Pollen sterility of backcross progenies of ms × (ms × variety). The P values from the  $X^2$  test for monofactorial hypothesis are included.*

Cross Combinations	No. of Total Plants	No. of Fertile Plants	No. of Sterile Plants	P-value	Percentage of normal pollen††	
					Range	Mean
ms × (ms × Caprock Taichung)	99	45	54	0.25-0.5	10-70	36.20
ms × (ms × Combine 7078)	26	13	13	1.00	50-95	65.50
ms × (ms × Hegari)	68	41	27	0.05-0.10	10-95	66.27
ms × (ms × Shallu Taichung)	96	58	38	0.01-0.05*	10-95	68.50
ms × (ms × Caprock 7000)	96	41	55	0.10-0.25	10-95	66.27
ms × (ms × Redbine)	98	43	55	0.10-0.25	10-95	62.86
ms × (ms × Arizona)	71	52	19	<0.01**	10-95	67.77
ms × (ms × Shallu)	101	50	51	0.90-0.95	30-95	73.60
ms × (ms × R7000)	95	63	32	<0.01**	10-95	63.03

\* Significant P value

\*\* Highly significant P value

† No normal pollen formed

†† Individuals classified as fertile plants

The classification of sterile and fertile plants was the same as in  $F_2$  progenies. Six combinations, ms × (ms × Caprock Taichung), ms × (ms × Combine 7078), ms × (ms × Hegari), ms × (ms × Shallu), ms × (ms × Caprock 7000) and ms × (ms × Redbine) fitted well with the one factor hypothesis. This also indicated that a single dominant gene restored fertility. The other three progenies, ms × (ms × Shallu Taichung), ms × (ms × Arizona), and ms × (ms × R7000) were deviated significantly from a 1:1 ratio. The results are presented in Table 3.

From the data of  $F_2$  and backcross progenies, the ratio of ms × Arizona and ms × R7000 did not fit one factorial hypothesis but agreed very closely with an expected ratio of 15:1 in  $F_2$  and 3:1 in backcross progenies. This would mean that two independent recessive genes with duplicate effect were involved.

The ratio of the other two combinations, ms × Shallu (Taichung) and ms × Redbine in  $F_2$  deviated significantly from a 3:1 ratio. Both could not be explained by a simple factor hypothesis.

*Tests for association between sterility and other characters.*

The association between male sterile and awned characters were studied by using the Chi-square test for independence. The data on sterility and awn character were obtained from six  $F_2$  progenies. The result is shown in Table

4 with one degree of freedom. The P value was very high, so there seemed to have no correlation between these two characters in all of these crosses examined. It indicated therefore that neither the proposed recessive male-sterile gene of Caprock (Taichung) and Combine 7078 nor the two independent recessive male sterile genes of R7000 was identical to or involved with the genetic male sterile  $ms_2$  which was linked to the awned gene as Stephens and Quinby reported (1945).

The results of the studies of associations between male sterile and characters including panicle type, glume type, glume tip color, and seed color in the cross of Kafir-60 male sterile  $\times$  Shallu are summarized in Table 5. The probability values were all far above 0.05, indicating no association between male sterile and any of these characters.

**Table 4.** *Chi-square tests for independence of inheritance of the male sterility and awn character of six crosses of Combine Kafir-60 ms and varieties.*

Cross Combinations	N	D. F.	P Value
ms $\times$ Caprock (Taichung)	28	1	1.00
ms $\times$ Combine 7078	24	1	0.50-0.75
ms $\times$ Redbine	78	1	0.10-0.25
ms $\times$ Shallu	89	1	0.50-0.75
ms $\times$ R7000	34	1	0.25-0.50
ms $\times$ Shallu (Taichung)	87	1	0.25-0.50

**Table 5.** *Chi-square tests for independence of inheritance of the characters in the cross Kafir-60 male sterile  $\times$  Shallu.*

Characters compared	N	D. F.	P value
F <sub>3</sub> male sterility and F <sub>2</sub> panicle type	115	1	0.30-0.50
F <sub>2</sub> male sterility and F <sub>3</sub> glume type	115	1	0.90-0.95
F <sub>2</sub> male sterility and F <sub>2</sub> glume tip color	121	1	0.95-0.98
F <sub>3</sub> male sterility and F <sub>2</sub> seed color	115	1	0.50-0.70

### Summary

Using the male sterile Combine Kafir-60 as the female parent, fourteen cross combinations were made. In the F<sub>1</sub> plants of ms  $\times$  Westland, ms  $\times$  Kafir,

ms × Coastland, ms × Martin and ms × Taipei Farmer were completely male sterile. It was assumed that these varieties had a male sterile gene which was identical to the male sterile Combine Kafir-60 gene.

The data for both  $F_2$  and backcross in combinations of ms × Caprock (Taichung), ms × Combine 7078, ms × Hegari, ms × Caprock 7000 and ms × Shallu did fit very well the one gene hypothesis.

Cross combinations of ms × Arizona and ms × R7000 did not fit the one gene hypothesis but agreed very closely with an expected 15:1 ratio in  $F_2$  and 3:1 ratio in backcross progenies.

Cross combinations of ms × Shallu (Taichung) and ms × Redbine in  $F_2$  were deviated significantly from 3:1 ratio.

The percentage of normal pollen in fertile plants either the parents used or in  $F_1$  or again in segregating progenies showed a great variation. This variation might be explained by modifying genes or by environmental factors.

There was no correlation between the sterility and any of these characters including panicle type, glume type, glume tip color, seed color and presence of awn.

## 高粱 (蜀黍) 細胞質與遺傳因子間控制花粉不稔性 之遺傳研究

畢 中 本 武 光 東

本試驗在探求高粱不同品種間對於花粉不稔性之遺傳關係。所用母本為 ms Combine Kafir-60, 具有 Milo 花粉不稔細胞質與 Kafir 花粉不稔遺傳因子。以此品系與 14 個栽培品種雜交, 觀察  $F_1$  結果, Westland, Kafir, Coastland, Martin 與臺北農家種等五個品種具有與 ms Combine Kafir-60 相同的花粉不稔因子。根據  $F_2$  及  $BC_1$  之分離比, Caprock (臺中), Combine 7078, Hegari, Caprock 7000 及 Shallu 等五個品種與 ms Combine Kafir-60 品系間對於花粉不稔性狀之遺傳, 係一對因子關係, 花粉不稔為隱性性狀。Arizona 與 R-7000 二品種為二對重複因子之關係。Shallu (臺中) 與 Redbine 二品種與母本間對於花粉不稔性狀之遺傳則不能以簡單之遺傳現象說明之。

親本,  $F_1$  及分離系統內各株之正常花粉百分率常有變異, 特別在分離系統內, 變異極大。發生變異之原因, 可以修改因子或環境因子與花粉不稔因子間相互作用之關係說明之。

花粉不稔性狀與穗形, 護穎, 穎尖色, 種子色及芒之有無等性狀間均無連繫。(摘要)

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