https://doi.org/10.29321/MAJ.10.A03768

A Study of the Cyto-Nuclear Relationships in *Eu-Sorghum*

by

R. APPADURAI[2] and B. W. X. PONNAIYA[3]

Introduction: In an attempt to develop new male-sterile lines in chosen Indian Sorghum types *viz.* A. S. 3880 (*S. roxburghii* Stapf) and Co. 18 (*S. subglabrescens* Schweinf et Aschers) through the use of m. s. C. K. 60 (*S. caffrorum* Beauv, the male sterile combine Kafir-60), it was felt desirable to get a knowledge of the cyto-nuclear relationships among the types involved. The relationships were sought to be studied from the expression of male-fertility in various cross combinations among these varieties including C. K. 60 (*S. caffrorum*-Combine Kafir-60), the male-fertile, maintaining counter part of m. s. C. K. 60. The results obtained and their significance are discussed hereunder.

Materials and Methods: The Sorghum types utilized in the study were *S. caffrorum* (C. K. 60 and m. s. C. K. 60), *S. roxburghii* (A. S. 3880) and *S. subglabrescens* (Co. 18). The crosses were designed to bring different types of genomes in different kinds of cytoplasm. Male-fertility was assessed both by stainability of pollen and by estimating the selfed seed set. Three classes of male-fertility were recognised by pollen staining *viz.*, Fertile (F), Partially Fertile (P. F.) and Sterile (S) as also by seed set *viz.*, Full, Part and Nil. The conventional notation of representing the seed parent initially, in the expressions denoting the various types of crosses has been adopted throughout.

Results: *(a) Reaction of Cytoplasm from m. s. C. K. 60 to genes from S. roxburghii (A. S. 3880) and S. subglabrescens (Co. 18).* In the F1 of the cross m. s. C. K. 60 X A. S. 3880 all plants were pollen fertile while in that of cross, m. s. C. K. 60 X Co. 18, all F1 plants were partially pollen-fertile. In both the crosses all the plants recorded a good selfed seed set (Table. 1). The pattern of segregation for male-fertility in the F2 and B1 could not be easily fitted into standard *mendelian* ratios. Nevertheless, from the relative proportions of the different fertility classes in the F2 and test crosses the probable number of genes determining fertility restoration in m. s. C. K. 60 by *S. roxburghii* (A. S. 3880) and *S. subglabrescens* (Co. 18) has been arrived at elsewhere (Appadurai 1965).

---

[1] The paper forms part of the thesis submitted for the Degree of Ph. D. by the first author.

[2] Superintendent, Agricultural Research Station, Bhavanisagar.

[3] Dean and Professor of Genetics and Plant Breeding, Agricultural College and Research Institute, Coimbatore-3.

(b) The reaction of genes from S. roxburghii (A. S. 3880) and S. subglabrescens (Co. 18) in the cytoplasm of C. K. 60. All the plants in the cross C. K. 60 x A. S. 3880 had normally dehiscing anthers giving full seed set when selfed. All the 32 first back cross plants (C. K. 60 x A. S. 3880) x A. S. 3880 also had normally dehiscing anthers giving full seed set when selfed. The F1 of C. K. 60 x Co. 18 as well as the BC1 to Co. 18 showed normaly dehiscing anthers giving full seed set when selfed.

(c) The reaction of genes from C. K. 60 in the cytoplasm of S. roxburghii A. S. 3880 and S. subglabrescens (Co. 18). (i) All the plants in the F1, A. S. 3880 x C. K. 60, the BC of (A. S. 3880 x C. K. 60) to C. K. 60, and F2 of A. S. 3880 x C. K. 60, consisted of plants all of which were fully male-fertile giving full seed set when selfed. (Table 1.)

(ii) The F1 of Co. 18 x C. K. 60, the backcross of (Co. 18 x C. K. 60) to C. K. 60 and the F2 of Co. 18 x C. K. 60 were studied for male-fertility. (Table 1. C). The F1 was male fertile and there was segregation for male-fertility both in the F2 and backcross plants. In the F2 eleven fully fertile and 38 partially fertile plants were observed. While one completely male-sterile plant was observed in addition to six partially fertile and one fully fertile plant in the back cross.

(d) The expression of fertility in the hybrids between S. roxburyhii (A. S. 3880) and S. subglabrescens (Co. 18): The reciprocal hybrids of A. S. 3880 x Co. 18 were partially pollen fertile but there was full seed set on selfing. (Table 2.) Cytological examination of P. M. Cs. revealed no gross meiotic irregularities in these hybrids, The F2 of A. S. 3880 x Co. 18 and BC1 of (A. S. 3880 x Co. 18) to (Co. 18) were also studied for pollen stainability and selfed seed set. The backcross showed almost equal numbers of fertile and partially pollen fertile plants. On selfing 27 plants gave full seed set while three plants had partial seed set. In the F2, two plants were completely male-sterile, 34 were partially fertile and 25 were fully fertile.

The occurrence of partial fertility in F1 and completely male-sterile segregates in the F2 was indicative of a break down in the genetic balance established in the parents. The proportion of completely male-sterile individuals to the total number of plants in the F2 was suggestive of a difactorial inheritance. It appeared that two different genetic units might be determining male-fertility in either of the parents and that lack of both the units due to recombination in certain of the F2 individuals, might lead to complete male-sterility. The F1 which would be heterozygous for both

TABLE 1. *Segregation for male-fertility*

Cross	Pollen stainability				Selfed seed set			
	No. of plants				No. of plants			
	F	P. F.	S.	Total	Full	Part	Nil	Total
(a) *Reaction of genes from S. roxburghii (A. S. 3880) or S. subglabrescens (Co. 18) to Cytoplasm from m. s. C. K. 60*								
1. m. s. C. K. 60 x A. S. 3880 F1 ...	24	—	—	24	24	—	—	24
2. m. s. C. K. 60 x Co. 18 F1 ...	—	32	—	32	32	—	...	32
3. m. s. C. K. 50 x A. S. 3880 F2 ...	80	11	15	106	76	5	20	101
4. m. s. C. K. 60 x (m. s. C. K. 60 x A. S. 3880) B1 ...	24	4	14	42	26	4	11	41
5. m. s. C. K. 60 x Co. 18 F2 ...	22	59	11	92	46	20	15	81
6. m. s. C. K. 60 x (m. s. C. K. 60 x Co. 18), B1 ...	—	35	15	50	15	16	17	48
(b) *Reaction of genes from C. K. 60 to the Cytoplasm from S. roxburghii (A. S. 3,880)*								
1. A. S. 3880 x C. K. 60; F1 ...	6	—	—	6	11	—	—	11
2. A. S. 3880 x C. K. 60; B1 ...	50	—	—	50	50	—	—	50
3. (A. S. 3880 x C. K. 60) x C. K. 60; B1 ...	29	—	—	29	29	—	—	29
(c) *Reaction of genes from C. K. 60 to the cytoplasm from S. subglabrescens (Co. 18)*								
1. Co. 18 x C. K. 60; F1	Normal anthers				5	—	—	5
2. Co. 18 x C. K. 60; F2 ...	11	38	—	49	40	3	6	49
3. (Co. 18 x C. K. 60) x C. K. 60; B1 ...	1	6	1	8	4	—	3	7

the units for male-fertility and those which would be heterozygous for one unit but homozygous for the absence of the other unit, might be expected to be partially fertile. Such genotypes would form 8/16 of the F2 population if the inheritance is difactorial. The rest of the genotypes forming 7/16 of the F2 would be fully fertile. On the basis of these expectations the F2 segregation for pollen stainability was tested for a good fit with a 7 fertile, 8 partially fertile, 1 malesterile ratio. The difference between the observed and expected ratio was not significant (Table 2).

TABLE 2. *Expression of male-fertility in hybrids between S. roxburghii (A. S. 3,880) and S. subglabrescens (Co. 18)*

The hybrid population	Pollen stainability				Selfed seed set			
	No. of plants				No. of plants			
	F	P. F.	S	Total	Full	Part	Nil	Total
1. A. S. 3880 x Co. 18 F1 ...	—	16	—	16	16	—	—	16
2. Co. 18 x A. S. 3880 F1 ...	—	9	—	9	9	—	—	9
3. A. S. 3880 x Co. 18 F2 ...	25	34	2	61	44	11	2	57
Expected ratio ...	7	: 8	: 1		11	: 4	: 1	
X2 ...	1·365				2·022			
P between ...	0·50–0·70				0·30–0·50			
4. (A. S. 3880 x Co. 18) x Co. 18 B1 ...	14	16	—	30	27	3	—	30
Expected ratio ...	1	: 1			3	: 1		
X2 ...	0·133				3·600			
P between ...	0·70–0·80				0·05–0·10			

Forty four plants in the F2 had full seed set, eleven had partial seed set while two had no seed set, when selfed. It was recognised that as the F1 which was partially fertile had full seed set when selfed, the double heteroxygous class in the F2 might also be expected to give full seed set. This would result in the modification of the 7 : 8 : 1 ratio for pollen fertility into a 11 : 4 : 1, Full: Partial: Nil seed set ratio, since the double heterozygous class would form 4/16 of the F2 population. The observed ratio was also found to fit with the expected 11 : 4 : 1 ratio for seed set in the F2 (Table 2)

The backcross of the F1 with one of the parents according to the above scheme of inheritance would amount to a cross of the double heterozygous dominant for one gene and homozygous recessive for the other. There would result four genotypes in the backcross, two of which would be homozygous for one unit conferring male-fertility and be fertile. The other two genotypes would not possess either of the units for male-fertility in a

homozygous condition and be partially fertile. Therefore it might be expected that the backcross would segregate in equal proportions of fertile and partially fertile plants. The observed segregation for pollen fertility agreed with this requirement (Table 2). One of the four possible genotypes in the backcross would be heterozygous for one unit determining male-fertility but homozygous for the absence of the other unit. Thus ¼ of the backcross might be expected to be giving partial seed set according to the expectation made, for the F2 seed set. The observed segregation, 27 full seed set and 3 partial seed set fitted with a 3 : 1 ratio though the probability was low (Table 2).

Two portions in the panicle of one of the male-sterile plants in the F2 were separately dusted with pollen from A. S. 3880 and Co. 18. All the nine plants studied in the backcross with A. S. 3880 and eleven out of 12 plants in the backcross with Co. 18 produced partially stainable pollen. One plant in the backcross with *S. subglabrescens* however, was completely sterile. There was no seed set both under selfed and open pollinated conditions. The plant proved to be a haploid.

Discussion: Stephens and Holland (1954) observed that increasing doses of Kafir genome in 'Milo' cytoplasm progressively increased the expression of male-sterility finally resulting in a completely male-sterile line. The situation may be considered similar to the phenomenon observed in inter-specific and inter-racial hybrids in *Epilobium* (Michaelis, 1954), where increasing doses of *E. hirsutum* genome in *E. luteum* cytoplasm progressively increased sterility. The disturbance in the genic-cytoplasmic harmony established through several generations of close breeding has presumbly resulted in a loss or reduction in fertility in these cases.

From the occurrance of fully male-fertile individuals in the F2 of m. s. C. K. 60 x *S. roxburyhii* (A. S. 3880) and m. s. C. K. 60 x *S. subglabrescens* (Co. 18) (Table 1) it could be inferred that the 'Milo' cytoplasm of m. s. C. K. 60 while causing male-sterility by in teraction with the genes from *S. caffrorum* (C. K. 60) reacted normally with the genes from *S. roxburghii* or *S. subglabrescens* in homozygous condition. It is known that the cytoplasm from *S. caffrorum* (C. K. 60) is 'normal' for the genes causing male-sterility in the cytoplasm of male-sterile m. s. C. K. 60 derived from 'Milo'. The same cytoplasm in the present investigation was found to be 'normal' for the genes from *S. roxburghii*, (A. S. 3880) and *S. subglabrescens* (Co. 18). The absence of male-sterile segregates in the F1 (*S. roxburghii* A. S. 3880 x C. K. 60), the F2 and the backcross with C. K. 60 (Table 1), indicated that the cytoplasm of *S. roxburghii* was 'normal' to

the genes from *S. caffrorum* (C. K. 60). If otherwise some of the recombinant types in the segregating generations, homozygous for certain of the genes from C. K. 60 should have been male-sterile. But the occurrence of completely male-sterile and partially male-sterile segregates in the segregating generations of the hybrid *S. subglabrescens* x C. K. 60 (Table 1) revealed that unlike that of *S. roxburghii* (A. S. 3880) the cytoplasm of *S. subglabrescens* (Co. 18) might be disharmonious with the genes from *S. caffrorum* (C. K. 60). *S. subglabrescens* corresponds with the 'Milo' of American varietal classification. When cytoplasmic-genic male-sterility was originally discovered in *Sorghum*, it was the 'Milo' cytoplasm that brought about male-sterility by interaction of genes from 'Kafir' *(S. caffrorum)*. It is therefore reasonable to expect that the cytoplasm from *S. subglabrescens* (Co. 18) tested in the present investigation is of the sterility inducing type for the genes from *S. caffrorum* (C. K. 60).

Reduced fertility in the reciprocal first generation hybrids between *S. roxburghii* (A. S. *S.* 3880) and *S. subglabrescens* (Co. 18) and the segregation pattern for male-fertility in the F2 and test cross plants led to the conclusion that two different genetic units determined male-fertility in the parents and that by hybridization, a break down in the genetic balance occurred resulting in completely male-sterile individuals in the segregating progency (Table 2). The occurrence of reproductively defective individuals (male-sterile plants) in the hybrid progenies can be taken to indicate that a barrier for free interbreeding between these two species of *Sorghum* (Snowden, 1936) exists. Stebbins (1958) while discussing cytoplasmic-genic sterility, observed "that such a system of genic and cytoplasmic determiners of sterility could, by segregation of appropriate combination in a single population, cause such population to become partially isolated reproductively from other populations of its species and so initiate speciation".

The mechanism for differentiation as propounded by Stebbins seems to be in operation in the *Sorghum* types under study in the present investigation.

Summary: The cyto-nuclear relationship among *S. caffrorum* (m. s. C. K. 60), *S. roxburghii* (A. S. 3880) and *S. subglabrescens* (Co. 18) was studied by observing the expression of male-fertility in relevant crosses.

From the occurrance of fully male-fertile individuals in the F2 of the corsses, m. s. C. K. 60 x *S. roxburghii* (A. S. 3880) and m. s. C. K. 60 x *S. subglabrescens* (Co. 18), the cytoplasm of m. s. C. K. 60 was inferred to interact normally with the genes from *S. roxburghii* (A. S. 3880) and *S. subglabrescens* (Co. 18).

The first generation hybrids of C. K. 60 x *S. roxburghii* (A. S. 3880) and C. K. 60 x *S. subglabrescens* (Co. 18) and their backcrosses with the respective male parents possessed normal anthers thus indicating that the cytoplasm from C. K. 60 was 'normal' in relation to genes from *S. roxburghii* (A. S. 3880) and *S. subglabrescens* (Co. 18). The absence of male-sterile segregates in the F1 and F2 of *S. roxburghii* (A. S. 3880) x C. K. 60 and in the backcross with C. K. 60, indicated that the cytoplasm from *S. roxburghii* (A. S. 3880) reacted normally with genes from C. K. 60.

The occurrance of segregants with defective pollen in the F2 of the cross *S. subglabrescens* (Co. 18) x C. K. 60 and in the test cross with C. K. 60 revealed that the cytoplasm of *S. subglabrescens* (Co. 18) might be of the sterility inducing type for genes from C. K. 60. Reduced pollen fertility in the reciprocal hybrids of *S. roxburghii* (A. S. 3880) x *S. subglabrescens* (Co. 18) and the occurrence of completely male-sterile individuals in the F2 indicated a break down in the genetic balance for male-fertility in these hybrids.

It is sugested that the *Eu-Sorghum* types involved in the present study are genetically differentiated.

Acknowledgements: The first author is highly indebted to Dr. V. Santhanam, Head of Regional Centre, I. C. A. R. (PIRRCOM), Coimbatore, for his invaluable guidance and keen interest throughout the investigation and in the compilation of the thesis.

REFERENCES

Appadurai, R. 1965 Studies in Genus *Sorghum* (Moench.) Thesis for Ph. D.

Michaelis, P. 1954 Cytoplasmic inheritance in *Epilopium* and its theoretical significance. *Adv. Genet.*, 6: 388-401.

Snowden, J. D. 1936 *Cultivated races* of *Sorghum* Allard and Son Ltd., London.

Stebbins, G. L. 1958 The inviability, weakness and sterility of inter-specific hibrids *Adv. Genet.*, 9: 147-215.

Stephens, J. C. and E. F. Hollaad. 1954 Cytoplasmic male-sterility for hybrid *Sorghum* seed Prodduction *Agron. J.*, 46: 20-3.