https://doi.org/10.29321/MAJ.10.A03706

Development of New Male-Sterile Sorghums

by
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Introduction: In *Sorghum*, male-sterile Combine Kafir-60 served an immediate purpose in the hybrid project New male steriles will always be welcome, for a wider use of the available *Sorghum* germplasm, in the production of hybrids. Development of male-sterile lines in fertility restorers however requires special breeding techniques. The actual results of adopting such techniques and their efficacy are discussed in this paper.

Material and Methods: Male-sterile Combine Kafir-60, (m s.C.K. 60), with its maintainer, Combine Kafir-60 (C.K. 60), formed the cytoplasmic source, while *Sorghum roxburghii* (A.S. 3180 a promising lax panicled type and *S. subglabrescens* (Co. 18) a popular strain, were proposed to be developed into male-steriles. The method of simultaneous test crossing cum back crossing described by Appadurai (1963) was adopted. Instead of the testcross however, selfing was done in the development of the sterile line.

For the development of the non-restoring maintainer line an alternative method was adopted by which the cytoplasm from the recurrent parent itself could be used. If the cytoplasm of the recurrent parent proved to be of the 'normal' type, a few plants in the recurrent parent would be hand-pollinated by not less than seven plants chosen at random from the third backcross (m.s.C K. 60 recurrent parent, 4 times) whose corresponding selfed second backcross lines exhibited a proportion of completely male sterile plants. Thus the fourth backcross with cytoplasm from the recurrent parent was proposed to be obtained directly. The presence of recessive genes for male-sterility in this cross and its subsequent selfed generations could be verified by the use of test crosses with m.s.C.K. 60.

Results: *A. Development of male-sterile (A-line):* (a) *S. roxburghii (A.S. 3880):* All the plants in the first backcross (m.s.C K. × A.S. 3880 × A.S. 3880 had normally dehiscing anthers and good seed set when selfed. Of the six lines from the selfed seeds of 26 first backcross plants two lines (lines 6 and 7) had male-sterile plants. Plants from the corresponding two second backcross lines were simultaneously selfed and backcrossed a third time to A.S. 3880. Of the thirtytwo selfed second backcross lines 16 segregated for male-fertility, of which, 6 lines exhibited a few completely male-sterile plants (Table 1). The corresponding six third backcross lines were simultaneously selfed and backcrossed for the fourth time with A.S. 3880.

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Received on 31—1—68.

Eighteen out of 35 selfed third backcross lines, segregated for male fertility (Table 1). Plants from the corresponding fourth backcross line (Line 34) were selfed and simultaneously backcrossed to *S. roxburghii*. While three lines segregated for male-fertility one out of 12 selfed fourth backcross lines give a single completely male-sterile individual (Table 1).

TABLE 1. *Expression of male-fertility in selfed, backcross & test cross populations*

S. No.	Nature of population	No. of lines raised	Total No. of plants studied	No. of lines segregating for male-fertility	No. of lines having completely male-sterile plants
1	2	3	4	5	6
A.	Development of male-sterile A Line				
	1. *S. roxburghii* (A. S. 3880)				
	a. First backcross (m.s. C.K. 60×A.S. 3880)×A.S. 3830	1	26	Nil	—
	b. Selfed first backcross	6	120	2	2
	c. Second backcross (m s. C K. 60×A.S. 3880)×A.S.3880 twice	6	120	Nil	—
	d. Selfed second backcross	32	1362	16	6
	e. Third backcross (m.s. C.K. 60×A.S. 3880)×A.S.3880 thrice	30	776	Nil	—
	f. Selfep third backcross	35	1137	18	4
	g. Fourth backcross (m.s. C.K. 60×A.S. 3880)×A.S. 3880 four times	1	16	Nil	—
	h. Selfed fourth backcross	12	179	4	1
	2. *S. subglabrescens* (Co. 18)				
	a. Selfed first backcross [(m.s. C.K.60×Co. 18) F2×Co. 18] selfed	3	132	3	3
	b. Second backcross (m s. C.K. 60×Co. 18) F2×Co. 18 twice	3	59	Nil	—
	c. Selfed second backcross	13	270	13	2
B.	Development of maintainer B Line				
	1. *Through Cytoplasm from C.K. 60*				
	a. *S. roxburghii* (A.S. 3880) Test cross of first backcross m.s. C K. 60×[(C.K. 60×A.S. 3880)× A.S. 3880]	14	463	9	6
	b. *S. subglabrescens* (Co. 18) Test cross of first backcross m.s. C.K. 60×[(C.K. 60×Co. 18)×Co. 18]	16	596	10	10

TABLE 1. *Expression of male-fertility in selfed, backcross & test cross populations. (Contd)*

1	2	3	4	5	6
2.	*Through Cytoplasm from S. rexburghii* (A.S. 3880)				
a.	Selfed third backcross [(m.s. C K. 60×A.S. 3880)×A.S. 3880 thrice] selfed	12	280	4	2
b	Fourth backcross A.S. 3880×[(m.s. C.K. 60×A.S. 3880× A.S. 3880 Four times]	12	79	Nil	—
c.	Test cross of 4th backcross m s. C.K. 60×4th backcross	15	329	2	2
d.	Selfed 4th backcross	15	318	Nil	—
e.	Test cross of selfed 4th backcross m.s. C.K. 60×selfed 4th backcross	41	866	35	32
f.	Second generation Selfed 4th backcross	14	373	Nil	—
g.	Test cross of 2nd generation Selfed 4th backcross m.s. C.K. 60×2nd generation Selfed 4th backcross	9	169	All plants in all lines were almost male-sterile.	
h.	Third generation Selfed 4th backcross	9	176	—	—

(b) *S. subglabrescens (Co. 18):* Three sterile segregates from the F2 of the hybrid m.s.C.K. 60× Co. 18 were backcrossed to Co. 18 for the fist time. The first backcross plants were selfed and simultaneously backcrossed a second time with Co. 18. All the three selfed first backcross segregated for male-fertility. Six plants from line 2 and seven from line 3 of the corresponding 2nd backcross were simultaeously selfed and backcrossed a second time with Co. 18. Of the 13 selfed 2nd backcross lines two (lines 11 and 20) gave completely male sterile individuals.

B. *Development of the non-restoring, maintainer line*: (a) *Using cytoplasm from C.K. 60:* (i) *S. roxhurghii* (A.S. 3880): The F1, C.K.60× A.S. 3880 and its first backcross with A.S. 3880 were both giving full seed set when selfed. Fourteen plants from the first backcross were test crossed with m.s.C.K. 60 and simultaneously backcrossed to A.S. 3880 a second time. Nine out of fourteen testcross lines segregated for male-fertility. Two lines among the corresponding fourteen second backcross were utilised for further test crossing simultaneously effecting the third backcross.

(ii) *S. subglabrescens* (Co. 18): Hybrid combination C.K. 60×Co. 18 and its first backcross with Co. 18 had normally dehiscing anthers giving full seed set in covered panicles. Plants in the first backcross were simultaneously test crossed with m.s.C.K. 60 and back crossed for the second time with Co. 18.

Of the 16 testcross lines studied in 1963, ten segregated for partially male-fertile and completely male-sterile plants. The corresponding 2nd back crosses were utilised for effecting the 3rd backcross.

(b) *Using Cytoplasm from recurrent parent :* Line 29 of the third backcross m.s.C.K. 60×(A.S. 3880, 4 times) was inferred to carry recessive genes for male-sterility, as the corresponding selfed second backcross line segregated for male fertility. Individual plants from A.S. 3880 were pollinated by plants from line 29 to obtain the 4th backcross with A.S. 3880 cytoplasm. The same plants from line 29 were also simultaneously selfed.

The selfed third backcross exhibited 4 segregating lines. Plants from the corresponding fourth backcross (with A S. 3880 cytoplasm) were test crossed with m.s.C.K. 60 and aiso selfed. Of the 15 test crosses studied in 1964 summer, two lines segregated for male-sertility.

Fourtyone plants in the corresponding selfed fourth backcross with A.S. 3880 cytoplasm, were selfed (advanced to F3) and testcrossed simultaneously with m.s.C.K. 60.

The 41 test crosses, exhibited varied types of segregation for male-fertility. Line 12 for instance was segregating for partially fertile (almost male-sterile) and completely male-sterile plants. Individual plants from the corresponding F3 lines from the fourth backcross were selfed (advanced to F4) and simultaneously test crossed to m.s.C.K. 60. All the test cross lines consisted of only partially male-sterile plants with a very low degree of male-fertility. The fertility in these lines was so low that the major gene for complete fertility restoration can be taken to have been completely eliminated and substituted with its recessive allele for male-sterility in the selfed P4 lines from the fourth backcross. The plants in the F4 at this stage have also attained uniformity in respect of almost all characters and were almost identical in phenotype with *S. roxburghii* (A.S. 3880) the recurrent backcross parent.

Discussion: *Sorghum* types which possess the recessive non-restorer genes for male-sterility in m.s.C.K 60 can be easily developed into male-steriles through direct genome substitution by backcrossing. Craigmiles and Newton (1958), and Craigmiles (1961) transferred the cytoplasmic-genic male-sterility from the grain sorghum (Day Milo) to Rhodesian Sudan grass *(S.arundinaceum)* by the above method. A number of *Sorghum* lines were developed into male-steriles by Kalisnik (1963). Eckhardt (1954) developed a method for simultaneously developing a male-sterile as well as a restorer line in any non-restoring type of *Zea mays.* By this method Thomas (19C0) converted 14 pop corn inbreds into male-steriles and fertility restorers at the same time. The problem in the present study is however one of simultaneously producing the male-sterile and its non-restoring maintainer line, as all the types tested, proved to possess fertility restorers. Craigmiles and Newton (1958) reported that because of the presence of fertility restoring genes the male-sterility could not be transferred to Tift Sudan grass *(S. Sudanense).*

In the presence of fertility restorers, the recessive m. s. genes from the donor parent m. s. C. K. 60 will not be expressed in the backcross hybrids. A selfing or testcross with m. s. C. K. 60 at every backcross stage however will reveal their presence. Since it was not already known as to which of the plants in the backcross possessed the genes for male-sterility all the plants selfed or testcrossed were also simultaneously backcrossed with the recurrent parent. Following the estimates of Mather (1951) and the suggestions of Harrington (1952), a minimum number of seven plants was proposed to be chosen at each backcross, based on a report of menogenic inheritance for fertility restoration in m. s. C. K. 60 (Maunder and Pickett, 1959; Sreeramulu, 1961). To suit the actual inheritance, however (Appadurai 1967), the programme had to be taken up by stages, one gene at a time

The F1. m. s. C. K. 60 × *S. roxburghii* proved to consist of plants all of which were male-fertile. Some of the selfed progenies of the different backcrosses at various stages of the backcrossing, segregated for male-sterility as expected and a few of them gave one or more completely male-sterile segregates. The first, second and third backcross of the male-sterile segregates in the F2 of the hybrid m. s. C. K. 60 × *S. subglabrescens*, with S *Subglabrescens* had normally dehiscing anthers giving full seed set when selfed. Selfed progenies of the first and second backcross, in the case of hybrids involving *S. subglabrescens* have been obtained in this programme. Selfed generations of these hybrids yielded male-sterile segregates. These lines have already attained many of the charecteristics of the respective recurrent backcross parents and a high degree of uniformity. Selfed progenies from a few plants from these lines are expected to continuously yield completely male-sterile plants for future work.

The cytoplasm of *S. roxburghii* was inferred to be similar to that of C. K. 60 (Appadurai 1965). Hence *S. roxburghii* itself could be used as the seed parent for crossing with chosen plants in one of the third backcross lines with m. s. C. K. 60 cytoplasm, already inferred to possess the genes for male-sterility. This resulted in the direct production of the fourth backcross with normal cytoplasm. Through the use of testcrossess with m. s. C. K. 60, the fourth backcross with normal cytoplasm and its first, second and third selfed generations could be tested for the presence or absence of fertility restoring genes. By such a process, lines which mostly resembled *S. roxburghii* parent but which did not possess the major gene for complete fertility restoration could be developed. Selecton in such lines and testcrossing with m. s. C. K. 60 are expected to reveal plants completely devoid of genes for fertility restoration.

As the cytoplasm of *S. Subglabrescens* was inferred to be of the male-sterility inducing type (Appadurai 1965) the cytoplasm from C. K. 60 was already found to possess panicles resembling those of *S. Subglabrescens*. The second backcross with 'normal' cytoplasm from C. K. 60 was already found to possess panicles resembling those of *S. Subglabrescens*, the backcross parent. The testcross of the first backcross with m. s. C. K. 60 indicated that ten second backcross lines though by themselves fertile, possesed plants carrying the recessive genes for male-sterility.

Joglekar and Deshmukh (1961) developed male-sterile and maintainer lines in four fertility restorers of male-sterile Kafir. They applied selection in the F2 itself for choosing the desired phenotypes. In the present investigation a systematic backcross programme for reconstituting the recurrent parent characteristics after incorporation of the genes contributed by the donor parent (m. s. C. K. 60) was followed. Based on the present investigation, it is suggested that the simultaneous selfing (or testcross) cum backcross method for the development and maintenance of male-sterile lines will be of general application for any fertility restoring Sorghum type.

Summary: Attempts were made to develop new male-sterile lines in two Sorghum types *S. roxburghii* (A. S. 3880) and *S. subglabrescens* (Co. 18) through the use of m. s. C. K. 60. As genes for fertility restoration in m. s. C. K. 60 were present in the pollen parents, a procedure of simultaneous selfing (or test-crossing) cum backcrossing was adopted. The process attained the fourth backcross stage for *S. roxburghii* and the third backcross stage for *S. subglabrescens*. Backcross lines thus derived have attained a high degree of uniformity possessing recurrent parent characteristics in panicle shape and grain quality. For the development of a maintainer the major gene for full fertility restoration has been substituted with its recessive allele for male-sterility in *S. roxburghii*.

Acknowledgements: The author is highly indebted to the University of Madras for kindly giving permission to publish the doctoral thesis. The valuable guidance given by Dr. B. W. X. Ponnaiya, Professor of Genetics and Plant Breeding in the conduct of the studies and the keen interest evinced by Dr. V. Santhanam, Head of Regional Centre, Indian Council of Agricultural Research, Coimbatore, throughout the investigations are acknowledged with gratitude.

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