Nucleus and Breeder Seed Production in Sorghum

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Introduction

Of all the inputs, seed is the most basic and vital input for increased productivity of any crop variety. The actual impact of a variety on agricultural production depends on the extent of coverage and the level of performance on farmers' fields. Like any other industrial product, the originator of plant varieties, the plant breeder, has to make his variety available in required quantity and original quality for its wide adoption, popularity and real benefit to the farmer. Even an outstanding variety can fail to catch up because farmers do not get genuine seed with the stated purity and/or seed capable of reproducing the claimed performance of the variety. For example, one of the main reasons for the non-adoption of highly popular sorghum (*Sorghum bicolor*) cultivars in Africa, such as ICSV 111 and ICSV 400 in some regions of Nigeria (Ogungbile et al. 1999), and S 35 in some regions of Chad (Yapi et al. 1999) and Cameroon and Mali (Ndjomaha et al. 1998), have been attributed to non-availability of seed of these cultivars.

Classes of Seed

The breeder usually has a small quantity of seed of very high genetic purity, which only after successive multiplication in two or three stages provides required and good quality seed to farmers and producers. It is in this context that different classes of seed are recognized, whose production is planned and monitored under the process of seed certification by a governmental agency to make available required quantities of seed of targeted variety to farmers. In most countries, including India, three to four classes of seed, nucleus, breeder, foundation and certified seed, are recognized. While nucleus and breeder seed production do not require certification, foundation and certified seed production require a rigorous process of seed certification. Official/formal release of a variety is a prerequisite for its entrance into the seed production chain. However, private seed companies do not follow such rigorous release procedures and are allowed to market their products (mostly hybrids) as truthfully labeled seed in India. At the time of submitting a proposal for the release of any new variety, a breeder is required to have some quantity of seed for further multiplication and must supply sufficient quantities to the designated government agency to multiply for commercial cultivation. Each country has its own established well-defined seed production standards/systems governed by seed legislation. As nucleus and breeder seed are sources for further multiplication before reaching the farmers, utmost care has to be exercised during maintenance and production of these classes of seed. While procedures and techniques of foundation and certified seed production of pure-line varieties and hybrids are documented in several textbooks and/or seed production manuals, the same is not true in case of nucleus and breeder seed production. Such information is useful for all those (breeders and seed production specialists) who are engaged in maintenance and production of these classes of seed in sorghum to ensure adequate and timely supply of high quality seed of varieties and hybrids to the farmers. In this context, an attempt has been made in this article to describe a step-wise procedure for maintenance and production of nucleus and breeder seed in sorghum.

Nucleus seed. It is the initial handful of seed originated through selection/breeding by the breeder. It is the only class of seed, which is regenerated from itself and is produced in very small quantities under the supervision of the originating breeder or a designated qualified breeder. The varietal purity of subsequently multiplied breeder seed, foundation seed and certified seed depends on the purity of nucleus seed and therefore, it must be produced with utmost care. Sorghum is predominantly a self-pollinated crop, which suffers little inbreeding depression. However, outcrossing to an extent of 5 to 25% (House 1985) does occur depending on the climatic conditions and the genotype/panicle type (compact or loose). The availability of stable cytoplasmic-nuclear male-sterility (CMS) in sorghum (Stephens and Holland 1954) also makes it possible to develop hybrids for commercial cultivation. Three lines, seed parent/male-sterile line/A-line, maintainer/B-line (to maintain A-line) and restorer/R-line to restore fertility in the hybrids when the A-line is crossed with the R-line, are required. Both varieties and hybrid parental lines are theoretically pure lines and should be easy to maintain; however, sometimes due to factors beyond the breeder's control such as chance out-crossings, rare mutations and mechanical admixtures, a variety or parental line deteriorates and therefore, requires needed maintenance to conform to its designated characteristic features at least once every four to five years. Nucleus seed production procedures for pure-line variety and hybrid parents are described below.

Pure-line variety: For the maintenance and production of nucleus seed of a sorghum variety, head-to-row procedure is suggested. The seed from selected individual self-pollinated panicles (that are true to type of the designated

variety) of the plants raised from the original/previous nucleus stock is grown in head-to-row progenies in subsequent season. The rows are carefully scrutinized for their conformity to the defined characteristics of the designated variety. Any progeny row(s) that show variation and deviation from the descriptors of the variety are discarded. Seed from only those selfed progeny rows that are uniform and conform to the characteristic features of the designated variety are bulked to constitute nucleus seed, which becomes a source for breeder seed production.

Hybrid parents: In case of hybrids, nucleus seed production involves the production of A-, B- and R-lines. The nucleus seed of A- or B-line is produced from paired plant-to-plant crossing of A- and B-lines. The A- and B-lines are grown in alternate rows and plants are carefully examined for their uniformity. Since A- and B-lines are isogenic lines except for male-sterility in A-lines, both A- and B-lines should conform to the same defined descriptors of the designated hybrid parents. Any off-types and those not conforming to the descriptors are discarded before anthesis. Apart from off-types, pollen shedders can be a problem in A-lines (a pollen shedder is a male-fertile plant in an A-line that results either from a breakdown of male-sterility or due to mechanical mixture during the previous harvest) and therefore, such plants should be rouged out and all the panicles before anthesis in A- and B-lines are bagged. The breakdown of male-sterility in A-lines may occur under high temperatures (>38°C) during flowering. Hence, it is recommended to avoid nucleus seed production of A-lines during summer season in the locations where temperature exceeds 38°C during flowering period. After rouging off-types and pollen shedders, only those plants in B-lines that conform to the defined characteristic features are used for pollinating similarly selected plants in A-lines in a paired plant-to-plant crossing system. Plants used as pollinators in B-lines as well as the plants that are pollinated in A-lines are bagged. Seeds harvested from bagged panicles from A- and B-lines are grown separately as head-to-row progenies in subsequent season. The progeny rows are examined for defined characteristics and seeds from only those progeny rows, which are uniform, are separately harvested in A- and B-lines and bulked to constitute nucleus seed of A- and B-lines. The nucleus seed production of R-lines is similar to that of a pure-line variety described earlier.

Breeder seed. Breeder seed is produced from nucleus seed in small quantities on experiment stations by the sponsoring breeder under his direct supervision. The organization sponsoring cultivar release has the responsibility for the supply and safe storage of breeder seed. Sponsored breeders can also produce breeder seed. In such cases, the originating breeder supplies the breeder seed to different institutions such as agricultural universities, central and state research institutions, and other recognized/sponsored organizations. The breeder seed required for national varieties in India is arranged through the Department of Agriculture and the National Seeds Corporation Limited, Government of India. The breeder seed for state varieties is produced by breeders of the states concerned. Breeder seed plots are monitored by a team consisting of breeders, representatives of the State Seed Certification Agency (SSCA) and the seed producing agency. Breeder seed production of hybrid sorghum involves the production of A-, B- and R-lines. Seed production of B- and R-lines is similar to that of any pure-line variety.

The production of breeder seed is expensive with an associated risk of contamination by repeated multiplication and of loss due to adverse production conditions. Therefore, the quantity of breeder seed required for about 3 to 4 years is produced by the breeder and deposited in cold storage. Highest standards of genetic purity must be maintained in the production of breeder seed since it is the base material for all further multiplication. Breeder seed might be produced under such controlled conditions as selfing by bagging if the requirement is small. Otherwise, it should be carried out under isolation. The minimum isolation distance to be maintained from any other sorghum crop is provided in Table 1. The breeder provides small quantities of breeder seed of the varieties and A-, B- and R-lines to foundation seed producers. The breeder also provides complete and accurate description of all distinguishing morphological and seed characters of the varieties, and A-, B- and R-lines, in case of hybrids, because the certification process depends upon these descriptions. The experiment station sponsoring the release of the hybrids trains the technical staff involved in the production and certification of hybrid seed and familiarizes them in the identification of distinguishing characters of the parents and the hybrid. The procedures for breeder seed production of hybrid parents and varieties are described below.

Male-sterile lines: Breeder seed production of A-lines is carried out by growing the A-line and its corresponding B-line together in an isolated plot. The isolation distance required for A-line × B-line production fields is >300 m. A row ratio of 4A:2B or 6A:2B is maintained and the borders of the field are sown with the B-line. The A-line and its B-line flower at about the same time and thus there are typically no problems of asynchronous flowering. Pollen produced by the B-line fertilizes the A-line plants thus maintaining the A-line.

Rouging in A-line seed production plots should be more stringent for pollen shedders because A-line and B-line

plants cannot be distinguished after flowering. The pollen shedders in A-lines must be identified and uprooted each morning during the flowering period. Utmost caution must be exercised in labeling and harvesting A-line and B-line rows. The B-line rows are harvested first, followed by the A-line rows. Purity of the A-line is very important and any lapses can lead to huge losses of time and resources spent in rouging during hybrid seed (A × R) production plots in the next generation. Because of the reasons stated earlier while discussing nucleus seed production, it is recommended to avoid breeder seed production of A-lines during summer season in locations where temperature exceeds 38°C during flowering. Since the A- and B-lines exhibit synchronous flowering, seed yields of the A-line in the A/B seed production plots are relatively better than in the A-line × R-line (hybrid) production plots. Seed of the B-line harvested from the A-line and B-line production plots can be reused for the next generation, depending on the seed laws of the country.

Maintainer and restorer lines and varieties: Since varieties, B-lines and R-lines are pure-lines, their seed increase are somewhat similar. Seed multiplication plots of these types, particularly B- and R-lines are sown in an area isolated by a radius of >300 m distance (>200 m in case of varieties) from other sorghum cultivars (Table 1). If Johnson grass (*Sorghum halepense*) or any other forage or grassy sorghum types are growing in the vicinity, an isolation distance of 400 m is recommended (Reddy 1997). Any plant in these plots appearing different from the true to type (as described by the breeder) for any character (major or minor) should be uprooted, or rouged, before anthesis. Although the process of rouging, or removal of off-types, starts soon after the seedling stage, the boot leaf and the panicle emergence stages are most critical because detection of off-types is easier during these stages. If the off-types are allowed to flower, their pollen will cross with genuine type plants and contaminate seed in the next generation. Off-types that escape detection during the flowering stage should still be removed before harvest to minimize contamination. It is recommended that plants of doubtful status should also be removed. Purity of the hybrid parents (A/B- and R-lines) is very important because it affects the quality of hybrid seed that is generated.

Planning Nucleus and Breeder Seed Production

Nucleus and breeder seed should be produced under optimum production conditions. Plots endemic to serious diseases such as downy mildew (*Peronosclerospora sorghi*) and ergot (*Claviceps* spp), obnoxious weeds like *S. halepense* and *Striga,* and areas prone to natural disasters such as floods, excessive bird damage or hailstorms should be avoided. Excessive rains or high humidity during the grain-filling stages of sorghum could cause grain molds, discoloration, weathering and pre-harvest sprouting, all of which affect germinability. Productivity *vs* cost, and climatic conditions, particularly during grain-filling stages, should be important considerations when selecting plots for seed production. If seed production is planned for the off-season, access to irrigation facilities is important.

The quantity of breeder seed required should be roughly estimated on an annual basis in advance, depending upon the projected demand for the commercial hybrid under cultivation (Table 2). It is desirable to maintain sufficient quantities of carry-over seed as an insurance against unforeseen seed crop losses. The progress of seed production and status of seed stocks should be reviewed annually in joint meetings among representatives of seed growers, foundation seed agencies and national seed agencies. The various activities of the multiplication chain of breeder seed, foundation seed and certified seed should be coordinated (Murty et al. 1994).

Table 1. Minimum isolation distance requirements for breeder seed production in sorghum.

Type of genetic material	Isolation distance (m)	Isolated from the fields of
Hybrid parents	300	Parents of other grain or dual-purpose sorghum hybrids or varieties
	300	Same hybrid parents not conforming to purity requirements
	400	Johnson grass and forage sorghum
	400	Dual-purpose (both grain and fodder) sorghum but mainly meant for fodder
Variety (pure-line)	200	Other varieties of grain or dual-purpose sorghum or hybrid parents
	200	Same variety not conforming to varietal purity requirements
	400	Johnson grass and forage sorghum
	400	Dual-purpose (both grain and fodder) sorghum but mainly meant for fodder

Table 2. Annual estimates of land and seed requirements for various classes of hybrid parents and hybrid seed production in sorghum.

Projected area of commercial hybrid (ha)	Certified seed		Foundation seed[1]		Breeder seed[1]	
	Seed requirement (t)	Area to be sown (ha)	Seed required for certified seed production (kg)	Area to be sown (ha)	Seed required for foundation seed production (kg)	Area to be sown (ha)
100,000	1000	1000	6000 (A-line)[2]	6	60	0.01
			×			
			4000 (B-line)	4	40	0.07
			4000 (R-line)[3]	4	40	0.07
200,000	2000	2000	12000 (A-line)[2]	12	120	0.20
			×			
			8000 (B-line)	8	80	0.14
			8000 (R-line)[3]	8	80	0.14

1. A seed rate of 10 kg ha^{-1} and seed yield of 1000 kg ha^{-1} were assumed for certified and foundation seed production plots. Breeder seed production plot yields were estimated on the basis of 600 kg ha^{-1}.
2. A-line seed production.
3. R-line seed production.

Purity Test of Hybrids, Parental Lines and Pure-line Varieties

Purity of seed is a very important criterion for the acceptance of a variety. This is ensured through various technical methods involved in seed production. In spite of the best efforts in maintaining all the recommended standards from seed production to packaging, possibilities always exist for contamination of the original seed lots by unwanted seeds of other cultivars or other types. Contamination can occur due to several factors in the seed production plots such as natural crossing with another cultivar, mutation and unclean harvesting equipment, and during postharvest processing such as carelessness at the processing plant, and mistakes in bagging and tagging. There are chances that some impurities or mixture like seed of different color and size, immature seeds, weed seeds, etc may still be present in the harvested seed lot. Therefore, in addition to assessing genetic purity and inert material in the harvested seed, it needs to be assessed for its quality in terms of germination, moisture content, etc so that it is capable to raise a normal healthy crop, befitting the standard of the stated variety. Hence, the harvested seed is sent to the Seed Testing Laboratory notified by the State for purity, seed germination and moisture content tests. The minimum acceptable standards for genetic purity, germination and moisture content in nucleus and breeder seed in sorghum are furnished in Table 3.

Packaging and Labeling

Breeder seed of sorghum should be packaged in small quantities of 1–10 kg. This seed can be packaged in advance in standardized units, or prepared from bulk upon receipt of the request from a seed multiplication agency. It is important that the packaging should be clean, moisture

Table 3. Purity standards of nucleus and breeder seed of varieties and hybrid parents in sorghum.

Standards	Nucleus and breeder seed
Genetic purity (%)	100
Crop standards (%)	
Off-types	0
Pollen shedders	0
Diseased heads	0
Seed standards (%)	
Pure seed	100
Inert matter	0
Other crop seed	0
Weed seeds	0
Germination	>80
Moisture (in ordinary container)	<12
Moisture (in vapor proof container)	<8

proof, well labeled and not easily damaged in transit. The seed packet should have a breeder seed tag attached (preferably) or enclosed indicating the crop, label number, variety, quantity, seed lot number, seed class and actual data indicating inert matter, germination and genetic purity, with a date for the germination test. In addition, the producing institution should be indicated, along with the signature of the breeder responsible for multiplication of the seed lot. Different colors of tags are used for different classes of seeds. For nucleus seed and breeder seed, golden yellow tag is used.

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Dominant Nuclear Male Sterility in Sorghum Induced by Hybridization of Two Fertile Lines

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Introduction

It is clearly established that in hybrids of genetically distant sorghum (*Sorghum bicolor*) accessions interaction of nuclear and cytoplasmic genes may cause cytoplasmic-genic male sterility. At the same time little is known on hybrid male sterility that results from interaction of different nuclear genes between themselves. This type of male sterility induced by specific crossing combinations has been revealed in some dicotyledonous species of higher plants (Kaul 1988).

Previously, by substitution backcrosses of male-sterile somaclones from the haploid and autodiploid plants of sorghum cv Milo-145 with the line SK-723, which is a fertility-restorer of *milo* cytoplasm, the analogues of SK-723 that contained a dominant nuclear mutation of male sterility were obtained (Elkonin et al. 1994, Elkonin 2000, Tsvetova and Elkonin 2003). Assuming that initial male-sterile plants have been isolated among somaclones (R_0-R_2 generations) we supposed that this mutation has been induced in tissue culture. However, further investigations revealed a more complicated nature of this sterility. In this article we report on isolation of nuclear dominant mutation of male sterility, *Ms-h* (male sterile from hybridization), in the progeny of the Milo-145 × SK-723 hybrid obtained by common pollination of emasculated panicles of Milo-145 with the SK-723 pollen, without tissue culture stage.

Material and Methods

The autodiploid line used in this investigation was obtained by VS Tyrnov, Saratov State University, Saratov, Russia. It was derived from a spontaneously diploidized sorghum haploid, which was isolated among the seedlings of Milo-145. The line SK-723 is a selection from a hybrid population derived from an open-pollinated panicle of *Feterita.* Direct and reciprocal F_1 hybrids, F_2 and BC generations as well as the progenies

from sib-crosses, selfed fertile siblings and the testcross hybrids were grown in an experimental field in 4–5 m rows. Fertility was determined by percentage seed set on panicles bagged before anthesis. The plants were classified as sterile (0% or 1–2 single seeds), partially sterile (1–50%, usually 5–15%) or fertile (>50%). The partially sterile plants were characterized by sectors of fertile flowers on one or few panicle branches usually located at the basal part of sterile panicle. The χ^2-test was used to determine the fit of observed segregation ratios of sterile and fertile plants to the expected segregation ratios.

Results and Discussion

In the F_1 obtained by hybridization of the lines Milo-145 and SK-723, plants with partial or complete male sterility were observed. Remarkably, sterility was observed only on the panicle of the first tiller while the second and the third tillers had fertile panicles. Such plants with complete or partial male sterility appeared both in direct (Milo-145 × SK-723) and reciprocal (SK-723 × Milo-145) crossing combinations. This seems to indicate that this type of male sterility is controlled by interaction of nuclear genes rather than by interaction of nuclear and cytoplasmic genes.

In BC_1 obtained by pollination of sterile panicles of the Milo-145 × SK-723 hybrids with the pollen of SK-723, segregation for male-sterile and male-fertile plants corresponding to a 1:1 ratio was observed (Table 1). Male-fertile plants proved to be homozygous and did not segregate male-sterile plants in their self-pollinated progenies. At the same time, in the progenies obtained by crossing male-sterile to male-fertile siblings segregation of sterile and fertile plants was observed, thus suggesting heterozygosity of sterile plants for the nuclear gene-inductor of male sterility. In some progenies segregation corresponded to the ratio 1 fertile : 1 sterile. In BC_2 obtained by repeated crossing of sterile BC_1 plants to the line SK-723, there were families which segregated for sterile and fertile plants in the ratio 1:1, and families almost completely made up of male-sterile plants only. At the same time, after crossing sterile plants from BC_1 to the line KVV-181, another fertility restorer of the *milo* cytoplasm, complete restoration of male fertility was observed (Table 1).

These data could be explained by two different hypotheses. One of them assumes that isolated type of male sterility is conditioned by the action of one dominant gene. To search for this supposed dominant gene-inductor of male sterility, both lines SK-723 and Milo-145 have been testcrossed to a number of fertile and male-sterile sorghum lines (Table 2). No plants with male sterility were observed in the F_1 of these crosses with SK-723. Additionally, these testcrosses showed the ability to restore male fertility in the *milo* cytoplasm, ie, it contained dominant genes in the nuclear loci controlling expression of this type of cytoplasmic male sterility (CMS). This indicates that this type of male sterility does not result from interaction of nuclear gene of SK-723 in the locus controlling CMS, and sterile cytoplasm of the maternal line (Milo-145).

Table 1. Inheritance of male sterility arising after crossing of two fertile sorghum lines, Milo-145 and SK-723.

Hybrid combination	Generation	Number of plants[1]			Ratio of (f+ps) to s	χ^2	*P*
		f	ps	s			
Milo-145 × SK-723	F_1	5	2[2]	3[2]			
SK-723 × Milo-145	F_1	11	7	3			
(Milo-145 × SK-723) × SK-723 (cross N1)	BC_1	8	2	14	1:1	0.667	0.25–0.50
(Milo-145 × SK-723) × SK-723 (cross N2)	BC_1	11	6	10	1:1	1.815	0.10–0.25
BC_1 sterile × fertile (sib-cross N1)	F_1	1	8	10	1:1	0.053	0.75–0.90
Fertile (from sib-cross N1) selfed	F_2BC_1	21	–	–	–		
BC_1 sterile × fertile (sib-cross N2)	F_1	11	3	6	1:1	2.000	0.10–0.25
Fertile (from sib-cross N2) selfed	F_2BC_1	18	–	–	–		
BC_1 sterile × fertile (sib-cross N3)	F_1	4	2	7	1:1	0.077	0.75–0.90
Fertile (from sib-cross N3) selfed	F_2BC_1	18	–	–	–		
BC_1 plant N1 × SK-723	BC_2	11	5	9	1:1	1.960	0.10–0.25
BC_1 plant N2 × SK-723	BC_2	7	4	10	1:1	0.048	0.75–0.90
BC_1 plant N3 × SK-723	BC_2	17	7	6	1:1	10.800	<0.05
BC_1 plant N4 × SK-723	BC_2	1	–	23	–		
BC_1 sterile × KVV-181	F_1	17	2	1	–		

1. f = Fertile; PS = Partially sterile; s = Sterile.
2. Panicles on the second and third tillers of these plants were fertile.