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Potential advantages of male-sterile F_1 hybrids for use as seed parents of three-way hybrids in pearl millet

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Abstract

Nine male-sterile F_1 hybrids, developed from crosses between A-lines and distantly related maintainer lines (B-lines) and intended for use as seed parents of three-way hybrids, were compared with their 10 inbred parental lines across 11 year-location environments in India. The F_1 hybrids from crosses between A-lines and more distantly related B-lines yielded 64–107% more than their respective higher-yielding inbred seed parents. Such high grain yields were achieved even in those F_1 seed parents that were as early in maturity as their earlier-maturing inbred seed parents. Thus, flowering of high-yielding but the otherwise late maturing inbred seed parents can be modified in F_1 seed parents such that the latter can be used to produce seed of early maturing three-way hybrids without any need for staggering with the early maturing pollen parents. The dominant nature of downy mildew resistance observed in this study indicated that F_1 seed parents can help salvage and extend the commercial viability of promising inbred seed parents that might have become susceptible during long-term and large scale deployment. F_1 seed-parent technology provides more cost-effective and more predictable seed production than is possible with inbred seed parents. It also provides for more effective manipulation of flowering of the later-maturing inbred seed parents towards earliness and better downy mildew disease management.

Keywords: Pearl millet; *Pennisetum glaucum*; Male sterility; F_1 seed parent; Heterosis; Three-way hybrid; Seed production

1. Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a highly cross-pollinated crop (Burton, 1974). A high degree of heterosis reported in the literature, with seed yield of F_1 hybrids exceeding that of the higher-yielding parental lines by up to 400% (Virk, 1988), and the discovery of a stable cytoplasmic-nuclear male sterility (Burton, 1965) opened up the possibility of commercial exploitation of heterosis through hybrid cultivars. Currently, single-cross grain hybrids, produced on inbred seed parents, occupy more than 5.5 M ha of the total 10 M ha planted to pearl millet in India. Since pearl millet displays high degrees of inbreeding depression for grain yield (Khadr and El-Rouby, 1978; Rai et al., 1985), seed production of single-cross hybrids becomes relatively less cost-effective due to low seed yields obtained from male-sterile inbred lines (A-lines). This was the main reason for the corn (*Zea mays* L.) seed industry to resort to double-cross hybrids (Hallauer and Miranda, 1981) and for sorghum (*Sorghum bicolor* (L.) Moench) research programs to assess the potential of male-sterile F_1s to breed three-way hybrids (Walsh and

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Atkins, 1973; Gorz et al., 1984). There was also perception that seed yields of inbred parents would be less dependable as homozygosity has been suggested to be associated with reduced developmental buffering (Allard and Bradshaw, 1964). Thus, higher and more dependable seed yield of male-sterile F_1 hybrids used as seed parents might provide an opportunity for a more cost-effective seed production of three-way hybrids.

The narrow genetic base of inbred seed parents and genetic uniformity of their single-cross grain hybrids have led to downy mildew [*Sclerospora graminicola* (Sacc.) J. Schröt] epidemics during the 1970s and 1980s in India (Dave, 1987). F_1 seed parents provide an option to achieve intra-locus genetic diversity and inter-locus combination of alleles for downy mildew resistance at the hybrid seed production stage to a level that is comparable to those of single-cross hybrid cultivars for grain production. Some of this intra-locus diversity for downy mildew resistance in F_1 seed parents will be lost in three-way hybrids, but it would not be of practical consequence as long as the restorer line is resistant. Three-way hybrids, made possible with the use of these F_1 seed parents, would make further contribution to diversifying the genetic base of hybrids that could have more stable downy mildew resistance. The objective of this research was to assess the potential advantageous features of male-sterile F_1s as seed parents compared to inbred seed parents, in terms of seed yield potential and its stability, and opportunities for manipulation of maturity and downy mildew disease resistance.

2. Materials and methods

2.1. Experimental materials

The materials in this study consisted of 10 male-sterile inbred lines (A-lines or inbred seed parents) and nine male-sterile F_1 hybrids (F_1 seed parents) produced from crosses between some of these A-lines and unrelated maintainer lines (B-lines) (Table 1). In terms of parentage and phenotype, there was considerable diversity among seed parents within both groups. However, some of these inbreds, and consequently

Table 1
Parentage/origin of inbred seed parents and F_1 seed parents in pearl millet

Seed parent	Height group	Parentage/origin of B-line
Inbred seed parent (A-line)[a]		
AKM 1163	d_2 dwarf	Selection from Kansas State University (KSU) line 81-1163B
ICMA 89111	d_2 dwarf	[843B×(GNS×SS-48-40-4)-1-9-8)]30-B-B-1
843A	d_2 dwarf	Selection from Kansas State University BKM 2068
81A	d_2 dwarf	Gamma ray-induced downy mildew resistant version of Tift $23d_2A$
ICMA 88004	Non-d_2	Selection from an *iniadi* S_2 progeny Togo-11-5-2
ICMA 88006	d_2 dwarf	[(81B×SRL 53-1)×843B]-30-2-B
863A	Non-d_2	Selection from an *iniadi* S_2 progeny Togo-13-4-1
5054A	Non-d_2	Developed by Indian Agricultural Research Institute, New Delhi
852A	Non-d_2	(MC-103×Serere 17B-12-2)-1
862A	d_2 dwarf	(B 816×3/4EB-105-6-1)-4
F_1 seed parent		
F_1SP_1	d_2 dwarf	AKM 1163×ICMB 89111
F_1SP_2	d_2 dwarf	843A×ICMB 89111
F_1SP_3	d_2 dwarf	81A×ICMB 89111
F_1SP_4	Non-d_2	ICMA 88004×ICMB 89111
F_1SP_5	Non-d_2	ICMA 88004×863B
F_1SP_6	Non-d_2	ICMA 88006×863B
F_1SP_7	Non-d_2	5054A×852B
F_1SP_8	Non-d_2	81A×852B
F_1SP_9	d_2 dwarf	843A×862B

[a] Nuclear genotype of A-line is identical to its counterpart B-line. A-line with its sterility-inducing cytoplasm is male-sterile and a B-line with its fertility-inducing cytoplasm is male-fertile.

their F_1s, were related. For instance, 843A (=AKM 2068) is derived from a population of a cross involving Tift $23d_2B$ and an early maturing landrace from Ghana (Stegmeier et al., 1998). BKM 1163 is also derived from the same cross. Line 843B is involved in the parentage of ICMB 89111, and along with 81B, it is also involved in the parentage of ICMB 88006. ICMB 88004 is derived from a half-sib progeny of an *iniadi* landrace, originating from northern Togo (Rai et al., 1995). Line 863B is also derived from the same population as ICMB 88004, but from a different half-sib progeny. All the B-lines used in this study are similar to their counterpart A-lines with regard to nuclear genome, differing only for cytoplasm.

2.2. Yield trials

The 19 entries in the trial, along with an inbred male-sterile line (841A) as check, were tested in the rainy season of 1989 and 1990 (including a summer season crop at Patancheru), covering a latitudinal range from 11°N at Bhavanisagar in southern India to 28°N at Hisar in northern India. In each environment, a trial was planted in a randomized complete block design with three replicates in 4-row plots of 4 m length, at row-to-row spacing of 0.75 m at Patancheru and Hisar, 0.6 m at Gwalior, and 0.5 m at Bhavanisagar and Jalna. The trials received 80–90 kg N and 40–45 kg P ha^{-1} at Patancheru (high fertility) and Bhavanisagar (also 45 kg K at Bhavanisagar), 40 kg N and 20 kg P at Gwalior, Patancheru low fertility and Jalna (also 20 kg K at Jalna), and 20 kg each N and P at Hisar. In each environment, the trials were planted in large experimental fields that included breeding lines and hybrids of a wide range of maturity. This ensured abundant pollen supply throughout the flowering period of test entries in the trials.

Time to 50% flowering was recorded when main panicle of 50% of the plants had fully exserted stigmas. Three plants chosen at random from the central two rows of each plot were used to determine plant height. Grain yield was determined by harvesting panicles from the entire 4 m length of two central rows (3 m length at Patancheru and Hisar in 1990). Panicles were sun dried for more than 10 days before threshing.

2.3. Downy mildew evaluation

Two field trials, consisting of the previously mentioned 19 seed parents and a highly susceptible hybrid (HB-3) were conducted in a downy mildew disease nursery at Patancheru. The 1989 trial consisted of 1-row plots of 4 m (20–30 plants) while that in 1991 consisted of 2-row plots of 4 m (50–90 plants). Both trials were sown in a randomized complete block design with three replications. Field disease-nursery management and downy mildew evaluation were done following standard procedures (Williams et al., 1981).

A glasshouse seedling inoculation test of the above 20 entries was also conducted using Patancheru isolate from the diseased plot. The trial was sown in a randomized complete block design with two replications. A single 4 in. pot (30–40 seedlings) constituted a plot in each replication. Seedling inoculation and downy mildew evaluation were done following the procedure developed by Singh and Gopinath (1985).

2.4. Statistical analysis

The yield trials were analyzed following a mixed model analysis of variance, where genotype (G) effects were considered fixed and environment (E) effects as random to test statistical significance of differences among genotypes and G × E interactions (McIntosh, 1983). The difference between the F_1 and its higher-yielding inbred parental line was tested using least significant difference (LSD) calculated from the standard error of difference (SE_d) obtained from the analysis of variance. In cases where this difference was significant ($P < 0.05$), better-parent heterosis was calculated to express this difference as percent of the higher-yielding parental line.The stability of F_1 seed parents and their inbred seed parents to varying environmental conditions over the 11 environments was assessed using an extended version of the linear regression approach of Eberhart and Russell (1966). In that approach, a stable genotype is one that has unit linear regression coefficient ($b = 1$) and small deviation from regression ($s_d^2 = 0$). The stability parameter s_d^2 for an individual genotype is computed as $s_d^2 = s_{res}^2 - s_e^2$, where s_{res}^2 is the regression residual mean square (RRMS) for the genotype in question and s_e^2 is the pooled error MS. The latter is computed from a combined analysis over all environments. Since it is the same value of s_e^2 that is subtracted from s_{res}^2 for any individual genotype, the term s_d^2 essentially is a reflection of s_{res}^2 for the genotype. The subtraction of s_e^2 from s_{res}^2 for any individual genotype, therefore, does not seem to play any role in reflecting the deviations from regression, except to correct for the overall background noise in the trial as a whole. A low s_{res}^2 will imply a low s_d^2 whether or not s_e^2 is subtracted from s_{res}^2.The responsiveness of a genotype was assessed by testing the null hypothesis H_0: $\beta = 1$ against the alternative hypothesis H_A: $\beta \neq 1$ using a *t*-test. In this study the stability of a genotype was assessed using the new parameter "adjusted R^2", denoted as R_{adj}^2 and defined as follows:

$$R_{adj}^2 = 1 - \frac{s_{res}^2}{s_T^2} \tag{1}$$

where s_T^2 is the total mean square (TMS) for the genotype in question. The fact that a low s_{res}^2 for any genotype implies a low s_d^2 may not necessarily guarantee, unless accompanied by a high TMS (s_T^2) for that genotype, that its deviations from regression, in terms of R_{adj}^2, will be necessarily smaller than for other genotypes with higher s_d^2. In our view R_{adj}^2 is a more appropriate measure of the degree of deviation from regression for a genotype than s_d^2 as it takes its s_{res}^2 into account relative to its TMS ($=s_T^2$), the latter acting as a scaling factor to permit comparison of different genotypes for their relative stability. The mathematical relationship between R_{adj}^2 and s_d^2, based on Eberhart and Russell's definition $s_d^2 = s_{res}^2 - s_e^2$, can be expressed as

$$s_d^2 = (1 - R_{adj}^2)s_T^2 - s_e^2 \tag{2}$$

which leads to the equation

$$R_{adj}^2 = 1 - \left[\frac{s_d^2 + s_e^2}{s_T^2}\right] \tag{3}$$

In Eq. (3), s_e^2, is being a constant for all genotypes, does not play any role in determining the relative values of R_{adj}^2, although its magnitude affects the absolute value of R_{adj}^2. A high R_{adj}^2 necessarily implies low deviation from regression and, therefore, seems more reliable than s_d^2 to assess the stability of a genotype. Also, in contrast to s_d^2 being an absolute measure, R_{adj}^2 is a relative measure properly adjusting for the differences in total MS for different genotypes. Consequently, R_{adj}^2 can be expected to reliably rank the genotypes according to their degree of stability. In this way stable genotypes have high R_{adj}^2. In case of heterogeneous error variances across environments, a weighted regression analysis for each genotype may be used to compute its *b* and R_{adj}^2 values. Based on our practical experience in regression analysis, a genotype may be called stable if it has $R_{adj}^2 \geq 0.7$. However, such a cut-off point may not be necessary if the objective of the research, as in the present case, is to rank the genotypes for relative stability. In this paper, *b* is considered a measure of responsiveness and R_{adj}^2 a measure of stability of the responsiveness of a genotype, and both of these are considered together with the mean grain yield for the characterization of genotypes.

Analysis of variance of downy mildew incidence using a fixed model on arcsine scale led to similar conclusions as that on the original scale with respect to overall statistical significance, and made little difference with respect to comparison of F_1 seed parents with their inbred parental lines. Therefore, downy mildew data were analyzed on the original scale. All computations of the yield and downy mildew data were made with the statistical package GENSTAT5 Release 4.1 (GENSTAT 5 Committee, 1993).

3. Results

3.1. Grain yield and agronomic traits

Grain yield of all nine F_1 seed parents, averaged over 11 environments, was significantly greater than their respective higher-yielding (i.e., better) inbred seed parents (Table 2). The average heterosis of F_1 seed parents over better inbred parental lines (i.e. better-parent heterosis) varied from 27% for F_1SP_2 to 107% for F_1SP_8. There was clear indication of greater heterosis in those F_1s that involved more distantly related inbred lines. For instance, the three F_1 seed parents (F_1SP_7, F_1SP_8 and F_1SP_9) in which both parents were most distantly related had 104–107% heterosis. In case of F_1SP_2 and F_1SP_5, better-parent heterosis was smallest (27 and 36%) because ICMB 89111 involves 843B in its parentage, and 863B and ICMB 88004 are derived from two different half-sibs of the same *iniadi* landrace population. The other

Table 2
Grain yield, time to 50% flower and plant height of F_1 seed parents and their inbred seed parents (P_1 and P_2) in pearl millet[a]

F_1 seed parent ($P_1 \times P_2$)	Designation	Grain yield (g m^{-2})			Time to 50% flowering (days)			Plant height (m)		
		F_1	P_1	P_2	F_1	P_1	P_2	F_1	P_1	P_2
AKM 1163×ICMB 89111	F_1SP_1	274 (41)[b,**]	176	194	46	47	54	1.2	1.0	1.1
843A×ICMB 89111	F_1SP_2	247 (27)[*]	147	194	46[**]	43	54	1.1	0.9	1.1
81A×ICMB 89111	F_1SP_3	286 (47)[**]	165	194	50[**]	54	54	1.3[**]	1.1	1.1
ICMA 88004×ICMB 89111	F_1SP_4	319 (64)[**]	191	194	48	49	54	1.7[**]	1.4	1.1
ICMA 88004×863B	F_1SP_5	260 (36)[**]	191	112	47	49	51	1.7[**]	1.4	1.3
ICMA 88006×863B	F_1SP_6	288 (78)[**]	162	112	48	50	51	1.8[**]	1.1	1.3
5054A×852B	F_1SP_7	293 (105)[**]	143	116	48[**]	51	57	1.7	1.3	1.7
81A×852B	F_1SP_8	342 (107)[**]	165	116	52	54	57	1.9[**]	1.1	1.7
843A×862B	F_1SP_9	319 (104)[**]	147	156	49[**]	43	62	1.5	0.9	1.4
SE_d		±20.8			±1.1			±0.06		

[a] In case of grain yield, the F_1s were tested against the corresponding higher-yielding inbred parental lines, in case of time to 50% flowering against the earlier flowering inbred parental lines, and in case of plant height against the taller inbred parental lines.
[b] Values inside the parentheses for grain yield indicate better-parent heterosis (%).
[*] $P < 0.05$.
[**] $P < 0.01$.

two F_1 seed parents with low heterosis were F_1SP_1 (41%) and F_1SP_3 (47%). In these two cases also, inbred parental lines were related. For instance, 843B and BKM 1163 are derived from the same cross, and 843B is involved in the parentage of ICMB 89111. Similarly, 81B was derived as gamma-ray-induced downy mildew resistant version of Tift $23d_2B$ (Anand Kumar et al., 1984) and Tift $23d_2B$ is involved in the parentage of 843B which, in turn, is involved in the parentage of ICMB 89111. Thus, male-sterile F_1s developed from distantly related inbred parental lines had ≥64% better-parent heterosis.

The higher-yielding inbred lines did not necessarily produce high-yielding F_1s. For instance, ICMB 89111 was the highest-yielding inbred line, but its hybrid with ICMA 88004 was in the high-yielding group, whereas that with 843A was in the low-yielding group. This lower seed yield of the hybrid made on 843A could, however, be partly due to its shorter plant height and earlier maturity. On the other hand, some of the low-yielding parental lines produced high-yielding F_1s (e.g., F_1SP_8 and F_1SP_9).

Early maturity and short plant height, besides high seed yield potential, are the most important considerations in the development and seed production of hybrid cultivars. Thus, it is significant that in several instances, a yield advantage in F_1s was achieved in spite of flowering as early as the earlier maturing inbred parent. In cases where F_1s differed significantly from their early flowering inbred parental lines, the difference between the F_1 and early parental line was less than the difference between the F_1 and its late-flowering parental line. In two instances (F_1SP_3 and F_1SP_7), the F_1s flowered even earlier than the parental lines. Also, the height of F_1s based on d_2 dwarf parental lines generally did not differ significantly from their shorter parental lines. In a case where this difference in the dwarf height group was significant (i.e., F_1SP_3), the increased height of the F_1 still remained within the acceptable limit (<1.5 m) for efficient field management under high-input conditions. In most cases, where the height of F_1s transgressed into an unacceptable range (above 1.5 m) and exceeded the height of their taller parents, at the most one parent was d_2 dwarf.

3.2. Yield stability

Combined analysis of variance for grain yield revealed highly significant differences among genotypes as well as highly significant genotype×environment interactions ($P < 0.01$). These two sources and the error term accounted for 43, 17 and 11% of the total variability, respectively. We assumed, following

Eberhart and Russell, that the genotype×environment interactions are linearly related to environmental indices. The linear regressions accounted for 15% of genotype×environment interaction sum of square. Though this does not account for a sizable portion of genotype×environment interaction, it was statistically significant ($P < 0.05$) when tested against pooled deviation and pooled error. Hill et al. (1998) have suggested that under such circumstances, a linear model still retains considerable predictive value, even though it does not account for a significantly large amount of the genotype×environment interaction variance.

Large differences in responsiveness (b) among seed parents were observed in both inbred and F_1 seed parent groups (Table 3). F_1 seed parents, in general, appeared slightly more responsive to changes in productivity, although the differences were not statistically significant. There were seven F_1s out of nine that had $b > 1$, but only five out of 10 A-lines. The least responsive inbred seed parent (863A with $b = 0.44$) was involved in the two least responsive F_1s (F_1SP_5 with $b = 0.29$ and F_1SP_6 with $b = 0.78$). ICMA 89111 and 862A were the two most responsive inbred seed parents. These were also involved in some of the most responsive F_1s (F_1SP_2 and F_1SP_9).

Table 3
Stability parameters for grain yield (g m^{-2}) of inbred and hybrid seed parents in pearl millet (the null hypothesis was that $b = 1$ and $s_d^2 = 0$)

Seed parent	b	s_d^2	RRMS[a]	TMS[b]	R^2_{adj}
Inbred seed parent					
AKM 1163	0.64	562	1261	2711	0.54
ICMA 89111	1.33	2424***	3124	9517	0.67
843A	0.68	137	837	2497	0.67
81A	1.06	233	932	5067	0.82
ICMA 88004	0.81	865*	1564	3896	0.60
ICMA 88006	1.01	675*	1375	5166	0.73
863A	0.44**	1587***	2286	2788	0.18
5054A	1.04	1231**	1930	5873	0.67
852A	0.92	1809***	2508	5490	0.54
862A	1.27	3747***	4446	10161	0.56
F_1 seed parent					
F_1SP_1	1.14	1106***	1805	6625	0.73
F_1SP_2	1.27	387	1086	7160	0.85
F_1SP_3	1.15	1276**	1976	6786	0.71
F_1SP_4	1.20	5857***	6556	11384	0.42
F_1SP_5	0.29**	1442**	2141	2253	0.05
F_1SP_6	0.78	766*	1465	3630	0.60
F_1SP_7	1.28	2321***	3020	8974	0.66
F_1SP_8	1.22	1196**	1895	7402	0.74
F_1SP_9	1.42*	1661***	2360	9840	0.76

[a] Regression residual mean square.
[b] Total mean square.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Based on conventional s_d^2 as a measure of stability, three inbred seed parents (AKM 1163, 843A and 81A) were found to be stable, followed by other two inbreds (ICMA 88004 and ICMA 88006) that were relatively less stable (Table 3). In comparison, only one F_1 seed parent (F_1SP_2) was found to be stable and another one (F_1SP_6) relatively less stable. All the remaining five inbreds and seven F_1s were unstable. This pattern of relative stability of A-lines and F_1 seed parents is unexpected as F_1s being more heterozygous are expected to be developmentally more buffered and hence more stable than homozygous inbred lines.

It is clear from Eq. (3) that a low s_d^2, unless accompanied by a high s_T^2, may not lead to a high value of R^2_{adj}, even when the null hypothesis H_0: $s_d^2 = 0$ is not rejected by Eberhart and Russell's approximate F-test. Therefore, s_d^2 would not be expected to provide a reliable measure of the relative stability of genotypes. To illustrate this, consider the two genotypes AKM 1163 and ICMA 88006. AKM 1163 had $s_d^2 = 562$ ($P > 0.05$), and $R^2_{adj} = 0.54$, while ICMA 88006 had $s_d^2 = 675$ ($P < 0.01$), and $R^2_{adj} = 0.73$. Though AKM 1163 had statistically non-significant and lower s_d^2 than ICMA 88006, it deviated more from the regression as reflected in the lower value of R^2_{adj} (Table 3). This occurred due to the difference in their total mean squares. Consequently, ICMA 88006 with higher R^2_{adj} would be considered more stable. Using this extended model based on R^2_{adj}, five F_1 seed parents (F_1SP_1, F_1SP_2, F_1SP_3, F_1SP_8 and F_1SP_9) were found to be stable compared with only two inbred seed parents (81A and ICMA 88006). The two latter are related: ICMB 88006 involves 81B in its parentage. Of the five stable F_1 seed parents, three (F_1SP_1, F_1SP_8 and F_1SP_9) involved only unstable inbred parents, while in the other two F_1 seed parents one inbred parent was stable and the other unstable. Of the four unstable F_1 seed parents, three (F_1SP_4, F_1SP_6 and F_1SP_7) had only unstable inbreds in their parentage, while the most unstable F_1 seed parent (F_1SP_5) involved one stable and one unstable inbred seed parent.

Table 4
Downy mildew incidence in F_1 seed parents and their inbred parental lines (P_1 and P_2) in downy mildew disease nurseries during the rainy season of 1989, dry season of 1991, and glasshouse (GH) inoculation during 1991 rainy season, Patancheru

F_1 seed parent ($P_1 \times P_2$)	Designation	1989 rainy season			1991 dry season			GH 1991 rainy season		
		F_1	P_1	P_2	F_1	P_1	P_2	F_1	P_1	P_2
AKM 1163×ICMB 89111	F_1SP_1	4.1	9.1	2.8	0.4	1.4	3.4	0.0	32.0*	5.2
843A×ICMB 89111	F_1SP_2	5.8	33.9*	2.8	2.4	11.7*	3.4	0.0	48.5*	5.2
81A×ICMB 89111	F_1SP_3	1.9	39.6*	2.8	0.4	10.1*	3.4	4.6	41.8*	5.2
ICMA 88004×ICMB 89111	F_1SP_4	0.0	2.6	2.8	0.5	0.0	3.4	0.0	0.0	5.2
ICMA 88004×863B	F_1SP_5	6.2	2.6	5.3	0.0	0.0	0.0	0.0	0.0	0.0
ICMA 88006×863B	F_1SP_6	2.7	20.1	5.3	1.0	1.8	0.0	0.0	11.3	0.0
5054A×852B	F_1SP_7	1.1	0.9	3.7	0.4	1.2	5.6	0.0	6.1	4.0
81A×852B	F_1SP_8	10.7	39.6*	3.7	3.8	10.1*	5.6	9.8	41.8*	4.0
843A×862B	F_1SP_9	18.0	33.9	23.6	3.7	11.7*	1.4	21.9	48.5*	23.9
HB-3 (susceptible control)			73.3			66.1			93.1	
SE_d			±12.2			±2.8			±8.7	

* $P < 0.05$ for the difference between P_1 and P_2 of a hybrid.

3.3. Downy mildew incidence

High downy mildew pressure developed in all the three trials, with disease incidence in the susceptible check (HB-3), ranging from 66% in 1991 dry season disease nursery to 93% in 1991 rainy season glasshouse inoculation test (Table 4). Parental lines of three F_1 seed parent hybrids (F_1SP_2, F_1SP_3 and F_1SP_8) had significant differences for downy mildew incidence in all the three trials. Parental lines of F_1SP_1 had significant difference for downy mildew incidence in the glasshouse test, while those of F_1SP_9 had significant differences in 1991 dry season field test and 1991 rainy season glasshouse test. In all these 12 instances, there was no significant difference between the downy mildew incidence of F_1 seed parents and their more resistant inbred seed parents.

4. Discussion

In a limited number of nine F_1 seed parents evaluated in this study, the mean better-parent heterosis for grain yield varied from 64 to 107% in those instances, where F_1s involved distantly related inbred lines in their parentage. Grain yield advantage of this order makes male-sterile F_1s of significant economic value in three-way hybrid seed production. Evaluation of a larger number of hybrid combinations is likely to identify male-sterile F_1s with even higher grain yield advantage as compared to their higher-yielding A-lines. There was no indication in this study that high-yielding male-sterile F_1s necessarily involved higher-yielding inbred lines as one or both of their parents. Comparison of F_1s and A-lines in sorghum has also shown that high-yielding A-lines do not necessarily produce high-yielding male-sterile F_1s (Hookstra and Ross, 1982; Gorz et al., 1984).

Based on the regression coefficient of grain yield of individual genotypes on mean grain yield of environments, F_1 seed parents were generally observed to be slightly more responsive than A-lines to changes in the productivity levels of environments. The least responsive F_1s involved at least one least responsive inbred line (863B) in its parentage, and two of the most responsive inbreds (ICMB 89111 and 862B) were involved in some of the most responsive F_1s. This suggests that probability of producing more responsive F_1s can be considerably increased by including in their parentage at least one highly responsive inbred line, or by excluding inbred lines(s) of poor responsiveness. Based on R^2_{adj}, grain yield of F_1s was found, in general, to be more stable than that of A-lines, and there was no indication of more stable F_1s having necessarily any stable inbred in its parentage. However, a most unstable inbred line (863B) was again involved as a parent in a most unstable F_1. These results showed that with all the three yield parameters ($\overline{X}$, b, R^2_{adj}) considered together, it would be possible to combine high stability and responsiveness with high grain-yield potential in F_1s but not in A-lines.

Simultaneous flowering of A-line and R-line is desirable for economic seed production of hybrids. A majority of the productive and downy mildew resistant pearl millet A-lines developed at ICRISAT are of medium (50–55 days to 50% flowering) and late (>55 days to 50% flowering) maturity. The dominant nature of earliness observed in this study would enable the development of early maturing F_1 seed parents from these productive but late-maturing A-lines, when crossed with early maturing B-lines, and vice versa. Such F_1 seed parents can be crossed with early maturing R-lines without a staggering in planting, leading to a cost-effective production of early maturing three-way hybrids. Production of foundation seed of F_1 seed parents from an A-line and an unrelated maintainer line (B-line) of differing maturity would, of course, require staggered plantings, making the foundation seed production of F_1s more costly than that of A-lines. However, foundation seed production of seed parents requires a much smaller operation than does the certified seed production of hybrids. Furthermore, the seed yield advantage of F_1s over A-lines would more than offset the additional cost of foundation seed production of F_1s seed parents resulting from staggered planting of A-lines and unrelated B-line of differing maturity.

Tall stature of A-lines creates management problems during seed production, by reducing the efficiency of roguing of off-type plants and pollen shedders. Also, since most of the hybrid seed production is done under high management conditions (well-fertilized and irrigated fields), the risk of lodging is more in A-lines of tall stature than in dwarfs. This study showed that in d_2 dwarf genetic background (i.e., $d_2 \times d_2$) crosses, F_1 seed parents either did not differ significantly from their dwarf inbred parents, or where the difference was significant, it was manageable (<1.5 m). In non-d_2 material of medium height (i.e., non-d_2×non-d_2 crosses, or d_2×non-d_2 crosses and vice versa), there was a significant increase in the height of F_1 seed parents over the taller inbred parental lines. Thus, the advantage of F_1 seed parents with respect to height can be better exploited in dwarf than in non-dwarf genetic background.

Large-scale deployment of inbred parents in single-cross hybrids increases their susceptibility to downy mildew, leading ultimately to withdrawal from cultivation. Such epidemics have occurred during the 1970s and 1980s in India, leading to large grain yield losses (Dave, 1987). Recently, downy mildew incidence of epidemic proportions has occurred on three hybrids, each based on one of the three A-lines 81A, 843A and ICMA 88006 (R.P. Thakur, ICRISAT, personal communication). Results of this study showed that promising inbred seed parents that have become susceptible to downy mildew over time, forcing their withdrawal from seed production, can be salvaged by crossing them with a resistant maintainer line and using the resulting F_1 seed parents in breeding three-way hybrids. These hybrids will be more resistant than the single-cross hybrids made on the susceptible inbred seed parent. Thus, the application of F_1 seed-parent approach in hybrid breeding from the beginning of the program has the advantage of prolonging the commercial viability of inbred seed parents. Witcombe and Hash (2000) showed that F_1 seed-parent technology allows strategic deployment of resistance (*R*) genes in which pyramids of smaller number of complementary *R*-genes are produced by marker-assisted selection in the two B-lines and pollen parents. These are then used to produce three-way hybrids which are heterozygous and heterogeneous at the target resistant loci. Downy mildew resistance of heterogeneous hybrids is expected to last longer than of uniform single-cross hybrids.

There is no published information on grain yield potential of three-way vis-à-vis single-cross hybrids in pearl millet. Sorghum studies show that three-way hybrids yield as well as single-cross hybrids (Walsh and Atkins, 1973; Patanothai and Atkins, 1974; Kide et al., 1985). Due to genetic heterogeneity, three-way hybrids are expected to be more stable for grain yield than genetically homogeneous single-cross hybrids. At the same time, greater phenotypic variability within three-way hybrids may be an impediment to their acceptance by farmers. Phenotypic variability in three-way hybrids will generally be between what is observed in single-cross hybrids and open-pollinated cultivars. Phenotypic variability in open-pollinated varieties, once defined, has not been an obstacle to their certification and acceptance by farmers in agricultural systems in India. By implication, phenotypic variability of three-way hybrids, once defined, is unlikely to be an obstacle, especially in the marginal environments of India and many African countries. The advantage of F_1 seed parents in terms of seed yield and stability, and their positive attributes in relation to maturity manipulation of seed parents and downy mildew disease management for seed and grain production, would far outweigh the within-cultivar variability. The key to success of the F_1 seed-parent approach and three-way hybrids will lie in their yield potential and effectiveness in downy mildew disease control.

5. Conclusions

The high degree of better-parent heterosis for grain yield observed in this study indicated that the use of F_1 seed parents offers a considerable economic advantage over inbred seed parents in hybrid seed production of pearl millet. The magnitude of yield advantage of F_1 seed parents will be even higher under high productivity conditions, where most of the seed production is undertaken and the seed yield of F_1 seed parents across the environments will be slightly more stable than that of inbred seed parents. Other positive attributes of F_1 seed-parent technology include its effectiveness as a bridge for the exploitation of a late maturing but the otherwise high-yielding seed parents in producing early maturing three-way hybrids, and strategic deployment of resistance genes for the management of downy mildew and other diseases. Application of this technology in breeding and seed production of forage hybrids should find immediate wide acceptance. The success of this technology for grain hybrid development would depend, however, on grain yield advantage of three-way hybrids, the extent of within-cultivar phenotypic variability, and its bearing on farmers' acceptance, and its effectiveness in downy mildew disease control.

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