Expl Agric. (2005), volume 41, pp. 335–356 © *2005 Cambridge University Press*
doi:10.1017/S001447970500267X *Printed in the United Kingdom*

TOPCROSS HYBRIDS AS AN ENTRY INTO COMMERCIAL SEED PRODUCTION OF PEARL MILLET IN EASTERN AFRICA

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(*Accepted 18 November 2004*)

SUMMARY

Pearl millet topcross hybrids (inbred male-sterile seed parent × open-pollinated variety restorer) based on locally adapted varieties and publicly available seed parents provide an ideal entry point into the commercial hybrid seed business, which can stimulate commercial investment by prospective seed producers. To demonstrate this potential, fifteen topcross hybrids made with the widely adapted variety ICMV 221 were evaluated in Eritrea, Sudan and Kenya for overall field performance, and in India for mechanisms of expression of heterosis and for terminal drought tolerance. Across all evaluation environments, the mean yield heterosis was 8%, with a range of −1% to +19%; six hybrids had a statistically significant, positive across-environment yield heterosis (ranging from +11% to +19%). Significant grain yield heterosis in rainy season environments was a consequence of heterosis in both biomass and harvest index, but not necessarily in any specific yield component. Positive grain yield heterosis under terminal drought stress, a common occurrence in millet-growing environments, was related to positive heterosis for grain size. These results are discussed in terms of their support for topcross hybrids as an entry point for prospective millet seed producers, and a scheme presented for the rapid creation, evaluation and marketing of locally adapted topcross hybrids.

INTRODUCTION

Pearl millet (*Pennisetum glaucum*) is the third most important staple cereal in sub-Saharan Africa, after sorghum and maize, grown on approximately 14 million ha. It provides the major source of calories for subsistence farming families living in the drier semi-arid tropics of the Sahel and northern Sudan agroclimatic zones south of the Sahara, stretching from Senegal in the west to Eritrea in the east. It is also important locally in various parts of the semi-arid zone bordering the Kalahari desert in southern Africa and in drier areas in eastern Africa (Harinarayana, *et al.*, 1999). As such, it should be the focus of major agricultural research and development efforts in sub-Saharan Africa. However, the strong identification of the crop with environments which are by definition marginal for arable agriculture, and thus present limited opportunity for

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large-scale increases in productivity, has meant that the crop has generally not been a favoured target for investment of research resources. This is despite the fact that the crop is widely grown in marginal areas specifically because it provides farmers with the best available opportunity for a reliable harvest in environments with uncertain rainfall and low soil fertility levels (Bidinger and Hash, 2003). The importance of the crop is not likely to decline in the future, even with the development of better infrastructure, markets and economic incentives to invest in agriculture, as neither maize nor sorghum have the level of adaptation of pearl millet to the uncertain rainfall and high temperature environments of the Sahelian ecological zone.

Pearl millet occupies an analogous (or even drier) ecological zone in south Asia, which grows about 70% of the area of sub-Saharan Africa (approximately 10 million ha). But the crop in south Asia, while still largely a subsistence crop for marginal zones, has benefited from large-scale investments in rural development and rural infrastructure, and particularly from the development of a dynamic commercial pearl millet hybrid seed industry. An estimated 50% of the Indian pearl millet area is currently sown to hybrid seed (K. N. Rai, ICRISAT, personal communication), most of which is purchased and sown by resource-poor farmers who have land holdings of less than 5 ha. This seemingly incongruous use of hybrids by some of the world's poorest farmers is possible because the amount of hybrid millet seed required is small (3–5 kg ha^{-1}), allowing farmers to recover their seed costs with a modest grain yield increase (approximately 70 kg ha^{-1}, equivalent to a 10% increase at average national on-farm yield levels). This increase is well below the yield advantage of well-adapted hybrids over crops produced from farmer-saved seed. Seed multiplication rates are high (300–1000 fold), and demand is large, making pearl millet hybrid seed multiplication, distribution and marketing profitable for the private sector. Competition is strong among seed companies, which in turn assures a reasonable cost of seed and a ready seed supply in all market towns.

Pearl millet hybrids have had a dual role in the increase in productivity of the crop over the past three decades in India (from an estimated 339 kg ha^{-1} in 1960–1965 to 636 kg ha^{-1} in 1990–1995, Harinarayana, *et al.*, 1999). The change to hybrids from open-pollinated cultivars (for which farmers do not purchase seed annually, and which seed companies cannot protect from competitors) has brought private industry and competition into the market, assuring farmers of a ready supply of seed of a choice of improved cultivars. The greater yield potential of hybrids, compared to open-pollinated cultivars (AICPMIP, 1988), has encouraged farmers to increase the level of input use on the crop (fertilizers and, in some areas, supplemental irrigation), allowing them to exploit the synergism inherent in the combination of a higher yield potential and greater inputs. There is no effective price support programme for pearl millet in India (in contrast to the situation for the major cereals rice and wheat), so many farmers have used the increased productivity of millet hybrids to produce family grain needs on a smaller fraction of their land, and divert additional areas to crops such as oilseeds or pulses which have greater market opportunities than does pearl millet (Bidinger and Parthasarathy Rao, 1990).

The Indian pearl millet scenario has lessons for similar ecological zones in Africa, as the population pressures on both land and food supply which have driven much of the development of agriculture in India, are on the horizon in sub-Saharan Africa. In fact, in terms of the productive capacity of the land and climate, certain African countries, including many of those dependent on pearl millet, are already more densely populated than are analogous parts of India (Binswanger and Prabhu Pingali, 1988). Realistic technological options for increasing the productivity of pearl millet do exist in sub-Saharan Africa, particularly through the improvement of soil fertility in the Sahelian and northern Savanna Zones (e.g. Yamoah *et al.*, 2002), and new open-pollinated cultivars with superior yield potential and disease resistance (Andrews and Anand Kumar, 1996; Rai *et al.*, 1997), but the input markets to support the use of these technologies are almost non-existent in most areas, as are remunerative output markets for increased production. The development of supporting infrastructure (e.g. output markets, price policies, rural access to new technology) for changing agricultural productivity needs to be based on realistic technologies that can both increase productivity and improve quality – especially important for commercialized processing. One such technology has always been the reliable supply of good quality seed that provides opportunities for both increasing productivity and accessing product markets that demand a more standardized product quality. The option of hybrid seed, rather than open-pollinated variety seed, will encourage commercial investment in seed production, thus ensuring sustainability of seed supplies. The low seed rates required and modest cost of seed production of hybrid pearl millet should make this technology readily accessible to farmers and open new opportunities in this crop in sub-Saharan Africa.

Hybrid seed, rather than open-pollinated variety seed is, for reasons cited above, likely to be the option of greatest interest to private sector entrepreneurs. Topcross hybrids (open-pollinated variety pollinator × inbred male-sterile seed parent) rather than single cross hybrids (inbred pollinator × inbred male-sterile seed parent) offer the easiest entry point into the hybrid seed business. Topcross hybrids exploit heterosis to increase productivity (Mahalakshmi *et al.*, 1992; Yadav *et al.*, 2000), as do single cross hybrids, but breeding topcross hybrids is far simpler and quicker, and seed production is easier and more profitable. Specifically, topcross hybrids offer the following advantages (Talukdar *et al.*, 1999), which cumulatively mean that a new seed company can make and evaluate experimental topcross hybrids, and identify and produce seed of a first hybrid for large-scale on-farm testing, in as little as four years (see the Appendix):

- Topcross hybrids can be based on well-adapted and well-accepted local varieties as pollinators, which greatly increases the likelihood that the resulting hybrids will be adapted to the requirements of farmers' physical environments, management systems and cultural preferences, and will not adversely affect local cultivars through cross-pollination.
- A broad range of male-sterile lines are publicly and freely available as international public goods from the millet breeding programs in both India and Africa of the

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). These form the basis of the Indian hybrid millet industry.

- This route virtually eliminates the need to initiate a costly hybrid parent breeding programme with a typical product delivery time of 10 years. The 'breeding' of initial topcross hybrids involves little more than testcross evaluation of good local open-pollinated varieties on a range of available seed parents, to select the best combinations for on-farm evaluation with/by farmers in the target area.
- If desired, selected pollinator varieties can be readily re-selected to strengthen phenotypic characters of the hybrids (e.g. time to flowering, disease resistance, fertility restoration) by conventional population improvement means (Witcombe, 1999).
- Seed production of topcross hybrids is considerably easier than production of conventional single cross hybrids, as variety pollinators are more vigorous, shed pollen over a longer period of time than inbred pollinators and produce higher/more reliable seed yield on the seed parent (Talukdar *et al.*, 1999).

The objective of this paper is to assess the potential for topcross hybrids as a possible entry point for a hybrid seed initiative for eastern Africa (including both East Africa and the Horn of Africa), using as a test case a set of experimental topcross hybrids made with the widely-adapted pearl millet variety ICMV 221 (which has been released for cultivation in Eritrea and Kenya). These hybrids were evaluated in field trials conducted in Eritrea, Sudan and Kenya for general topcross hybrid performance, and in more detailed physiological evaluations in India, for the expression of heterosis and for drought tolerance in the experimental hybrids. Results are discussed in terms of the likely commercial viability of the best experimental hybrids as a basis for beginning a hybrid seed industry.

MATERIALS AND METHODS

Topcross hybrids

Seed of the experimental topcross hybrids was produced under isolation at ICRISAT, Patancheru, India, in the dry season (January–April) of 2001, by inter-planting open-pollinated variety ICMV 221 (as pollinator) with a selection of fifteen publicly available ICRISAT male-sterile lines (A-lines) with similar flowering times to ICMV 221 (Table 1). ICMV 221, also known as ICMV 88904, was bred at ICRISAT, India (Witcombe *et al.*, 1997) from largely early-flowering *Iniadi* germplasm (Andrews and Anand Kumar, 1996) and has been officially released in both Kenya (as KAT/PM3) and Eritrea (as Kona), as well as in India. It is well adapted to short season, more marginal areas of eastern and southern Africa, but retains the ability to yield well in improved environments. The A-lines used had varied genetic backgrounds, often including parents of both Indian and West African origin; a number also had *Iniadi* parentage in their pedigrees (notably ICMA 863, ICMA 98222, 98333, 99111 and 99222), as this is a major source of early maturity in the ICRISAT male-sterile breeding programme. The A-lines varied widely for phenotype (apart from maturity) and yield component expression, which was reflected in the variation in yield component expression and heterosis among the topcross hybrids. The planting arrangement for the hybrid seed production was four rows of ICMV 221 (two rows of which were planted one week before the A-lines and two rows of which were planted at the same time) to four rows of A-line (one row per individual A-line). This pattern was repeated four times to accommodate all fifteen A-lines.

Field trials in eastern Africa

The fifteen ICMV 221 topcross hybrids, plus ICMV 221 and a local variety as controls, were tested on research stations in Eritrea and Sudan in the Horn of Africa in the 2001 rainy season (June–September) and in Kenya in East Africa in the 2002 long rainy season (March–June). The Eritrean trial was grown at the Department of Agricultural Research and Human Resource Development research site at Hagaz, near Keren (16°N,38°E, 884 m asl), the Sudan trial at the Agricultural Research Council station at Wad Medani (14°N,33°E, 405 m asl) and the Kenya trial at the Kenya Agricultural Research Institute testing site at the Kiboko sub-centre (2°S,38°E, 915 m asl). Seasonal rainfall was 300 mm at Hagaz, 223 mm at Wad Medani and 174 mm (including supplemental irrigation) at Kiboko.

All trials were hand sown in a randomized complete block design with three replications. Plot size was four rows × 4 m × 0.75 m at Hagaz; four rows ×4 m × 0.90 m at Wad Medani and four rows × 3 m × 0.75 m at Kiboko. Trials received 18–20 kg N ha^{-1} and 19–22 kg P ha^{-1} as basal before planting and between 20 (Kiboko) and 45 (Hagaz) kg N ha^{-1} as side dressing three weeks after planting. Thinning was done approximately two weeks after planting and weeds were managed by a combination of cultivation and hand weeding. Observations in all trials were recorded on the central two rows of each plot. These included days to 75% flowering, numbers of plants and panicles per plot, plant height and panicle and grain yields. Panicle length (on five plants visually judged to be average) was recorded at Hagaz and Kiboko only. Grain yield and panicle number were converted to a m^{-2} basis for comparison across trials. The data for ICMA 96222 × ICMV 221 at Hagaz were discarded due to seeds being mixed up, so this hybrid was not included in the means for the eastern Africa trials (Tables 1 and 2). It was possible to include this hybrid in the across location analysis of heterosis (Table 5), however, as the Genstat META analysis model we used for this analysis (see below) permitted the estimation of values across all environments, despite the missing values for one location (Hagaz) within the eastern African environment.

Field trials in India

The full set of topcross hybrids, plus ICMV 221, was evaluated at the ICRISAT headquarters, Patancheru (18°N, 77°E, 545 m asl) in the rainy seasons of 2001 and 2002 (Patancheru trials) to assess the mode of expression of grain yield heterosis, in terms of growth rate, partitioning and yield component heterosis. They were also grown under two managed terminal drought stress environments (drought nursery trials) during the dry season of 2002, to specifically assess the expression of heterosis under drought stress, as drought stress is common in pearl millet growing environments. Both sets of trials were grown in well-fertilized conditions (18 kg N ha^{-1} and 20 kg P ha^{-1} banded into the ridges before sowing and 45 kg N ha^{-1} side dressed at 15 days after planting). Plots were four rows × 4 m long × 0.75 m wide in the rainy season and four rows × 4 m long × 0.6 m wide in the dry season. Plots were mechanically sown on ridges in a randomized complete block design with three replications (rainy season) and five replications (dry season). The two centre rows of the plots were harvested at maturity, and data recorded as described below. Seasonal rainfall in the rainy season trials was 342 mm in 2001 and 385 mm in 2002. The drought nursery trials were maintained by sprinkler irrigation until the onset of the drought treatments, which were controlled by the timing of the final irrigation, given by holding water in the furrows for four hours to completely fill the soil profile. The soil in the drought nursery field is a shallow (0.6–0.7 m) sandy Alfisol, containing about 50 mm of available water, sufficient to support potential transpiration of a full crop cover for about 7–8 days during the dry season, allowing a relatively precise control on the timing of stress occurrence. The stress in the early-onset stress treatment began just after the beginning of flowering (designed to affect both grain number and grain filling) and one week later in the late-onset treatment (designed to affect primarily grain filling). The drought nursery trial also included a non-stressed control, which received regular weekly furrow irrigation until physiological maturity.

For all the India trials, data were collected on days to flowering (stigmas visible on 50% of the main shoot panicles), productive panicle number, panicle and grain dry weights, total stover fresh weight, and the fresh and dry weights of a subsample of stover from the centre two rows of the plots (4 m × 1.5 m). These data were used to calculate dry stover yield (total stover fresh weight × subsample moisture percentage), above ground biomass (panicle + stover weights) and harvest index (grain yield/biomass). For the rainy season trials, crop duration (flowering + 25 days), vegetative growth rate (stover weight/days to flowering + 10 days; Bramel-Cox *et al.*1984), total growth rate (biomass/crop duration) and grain yield per panicle were also calculated. Yields, as appropriate, were expressed as $g\,m^{-2}$, and growth rates as $g\,m^{-2}\,d^{-1}$. Individual grain weight in the drought stress environments was determined from duplicate, weighed samples of 100 grains per plot and these were used to calculate grain number m^{-2} by dividing individual plot grain yields by individual plot grain mass.

Data analysis

Yield and yield component data were analysed by SAS PROC GLM for both individual trials and for the three different environmental sets of trials (eastern Africa, Patancheru and the drought nursery, according to the experimental design. The across-environment analysis (Table 5) was done initially with PROC GLM to estimate the variances for environment group, trial within environment group, genotype and genotype interactions with both aspects of environment. The META (multi environment trial analysis) procedure in Genstat was used to estimate across-location genotype means and associated *s.e.d.*, as it will estimate across-location values for entries (e.g. ICMA 96222 × ICMV 221) which are not included in all locations. For the purposes of this paper, heterosis was considered only in relationship to the measured performance of ICMV 221, as it is this comparison that will determine the viability of topcross hybrids as a new cultivar form for eastern Africa. This equates to best-parent heterosis for biomass and grain yield, but may not for individual yield components (e.g. tillering ability), for which individual male-sterile lines may exceed ICMV 221. For single locations, heterosis for each topcross hybrid was estimated on an individual replication basis, as the difference between the individual topcross hybrid value and the ICMV 221 parent value in that replication, and expressed as a percentage of the ICMV 221 parent value. This procedure permitted conventional statistical analysis of heterosis as an independent variable, and the calculation of an *s.e.d*. for heterosis. The *s.e.d*. provided a basis for distinguishing hybrids which exhibit heterosis (negative or positive) from those which do not, based on whether or not the difference between the hybrid mean and that of ICMV 221 is within the expected range of variation between two similar genotypes or not. A topcross hybrid whose% heterosis is within ± 1 *s.e.d*. of 0 (0 being the expected value for % heterosis if there were no differences between ICMV 221 and one of its testcrosses) was defined as non-heterotic. In comparison, a hybrid whose % heterosis is greater then $+1$ *s.e.d*. or less than -1 *s.e.d*., was considered as exhibiting 'real', i.e. non-zero, heterosis. Topcross hybrids meeting this latter criterion were thus considered to have either positive (% heterosis $>+1$ *s.e.d*.) or negative (% heterosis <-1 *s.e.d*.) heterosis. For the across environment analysis (Table 5), a conventional least significant difference (*l.s.d*. = appropriate student t value for a one tailed test × *s.e.d*., derived from the residual *m.s.*) was also calculated at $p < 0.05$, to identify hybrids with a significant positive heterosis. A hybrid whose mean heterosis exceeded the *l.s.d*., was considered to have a statistically significant, positive heterosis across all three groups of evaluation environments (eastern Africa, Patancheru and the drought nursery).

RESULTS

Eastern African trials

Evaluation environments in all three of the ICMV 221 topcross hybrids in eastern Africa were favourable, with mean trial grain yields ranging from 2.9 t ha^{-1} (Wad Medani) to 3.5 t ha^{-1} (Hagaz). Variation among the topcross hybrids (mean of all three locations) was small for time to flowering (45 to 48 days, as seed parents used were chosen for a similar time to flowering as ICMV 221), and for height (160–176 cm) and panicle length (20–26 cm) (Table 1). However, the choice of seed parent did affect topcross hybrid yield component expression to a much larger degree. Panicle number among the topcross hybrids ranged from 15 to 25 m^{-2}, a significant increase over ICMV 221 which had only 16 panicles m^{-2} (Table 1). However, this increase in panicle number was generally accompanied by a decrease in grain weight panicle^{-1}, with a range of 13–24 g panicle^{-1} among the topcross hybrids, compared to ICMV 221 with 20 g panicle^{-1} (Table 1). Differences in mean grain yield among hybrids were also highly significant, with a range of 292–395 g m^{-2}, compared to a yield of

Table 1. Mean time to flowering, grain yield and major yield components, plant height and panicle length of ICMV 221 topcross hybrids. Data are from replicated field trials in Hagaz, Eritrea; Wad Medani, Sudan and Kiboko, Kenya, 2001/2002.

Topcross hybrid	Time to flowering (days)	Grain yield ($g\,m^{-2}$)	Panicle number (m^{-2})	Grain weight (g $panicle^{-1}$)	Plant height (cm)	Panicle length (cm)†
ICMA 863 × ICMV 221	48.2	352	15.2	23.6	171	19.9
ICMA 00888 × ICMV 221	45.7	298	17.5	17.6	167	21.7
ICMA 89111 × ICMV 221	46.4	312	24.9	13.0	162	21.2
ICMA 91222 × ICMV 221	47.4	327	17.9	18.4	173	22.7
ICMA 91777 × ICMV 221	45.4	326	20.7	16.0	168	20.7
ICMA 92444 × ICMV 221	45.6	349	23.4	15.1	160	22.3
ICMA 95333 × ICMV 221	47.2	357	17.6	20.1	176	25.6
ICMA 96222 × ICMV 221‡						
ICMA 97111 × ICMV 221	46.8	317	19.1	16.9	166	19.7
ICMA 97333 × ICMV 221	45.7	395	24.3	16.8	171	20.7
ICMA 98222 × ICMV 221	45.8	262	17.1	15.8	162	20.7
ICMA 98333 × ICMV 221	46.1	301	19.3	15.7	161	20.8
ICMA 98777 × ICMV 221	47.0	314	21.6	14.3	159	20.0
ICMA 99111 × ICMV 221	47.1	350	17.5	20.7	170	21.8
ICMA 99222 × ICMV 221	47.3	292	14.8	20.4	163	20.8
ICMV 221	45.7	325	16.3	20.1	162	20.5
Local control variety	50.2	218	17.7	12.4	172	24.0
m.s. genotype (17) §	17.22	14 919	70.72	77.67	231.2	10.95
m.s. genotype × location (33)	9.08	2941	11.80	9.48	111.9	5.12
m.s. error (100)	2.85	2251	7.43	8.51	103.4	1.89
s.e.d. genotype means	0.80	22.4	1.29	1.38	4.8	0.65
Trial mean	47.0	320	18.9	17.6	165	21.3
s.e.m.	0.69	19.4	1.11	1.19	3.4	0.56
CV (%)	3.6	14.8	14.4	16.5	6.1	6.5

† Based on Hagaz and Kiboko only, data were not available from Wad Medani.
‡ Excluded from the Hagaz and across location analysis due to a mixed seed lot at Hagaz.
§ Numbers in parentheses following the *m.s.* are the degrees of freedom associated with each source of variation.

325 $g\,m^{-2}$ for ICMV 221 (Table 1). The choice of A-line for making topcross hybrids with ICMV 221, is therefore an effective mechanism for increasing productive tiller number and grain yield m^{-2} over that of ICMV 221, but with a likely cost in individual panicle productivity.

As improved grain yield potential is likely to be the major factor in decisions of both prospective seed companies and progressive farmers to invest in pearl millet hybrids, we examined grain yield heterosis (% increase/decrease compared to the yield of ICMV 221 itself) by hybrid and location (Table 2). Mean yield heterosis was positive and differences in heterosis among hybrids significant in Hagaz (mean heterosis of +6% and differences significant at $p < 0.014$) and Wad Medani (+10% and $p < 0.001$), but negative and non-significant in Kiboko (−6% and $p = 0.20$). By the criterion used for the existence of heterosis (absolute value of percentage heterosis exceeding the *s.e.d.* for heterosis), there was positive grain yield heterosis in six topcross hybrids at Hagaz, seven at Wad Medani and none at Kiboko, and negative heterosis in five hybrids at

Table 2. Grain yield heterosis in ICMV 221 topcross hybrids in replicated field trials in Hagaz, Eritrea; Wad Medani, Sudan and Kiboko, Kenya; in 2001/2002, and averaged across all three environments.

	Grain yield heterosis (%) by location			
Topcross hybrid	Hagaz	Wad Medani	Kibiko	Across
ICMA 863 × ICMV 221	18.9†	3.5	8.8	10.4†
ICMA 00888 × ICMV 221	−6.1	11.8†	−16.4†	−3.4
ICMA 89111 × ICMV 221	−0.4	10.1	−14.2†	−1.5
ICMA 91222 × ICMV 221	−7.8	14.0†	1.0	2.3
ICMA 91777 × ICMV 221	−0.4	6.0	0.2	2.0
ICMA 92444 × ICMV 221	12.8†	21.8†	−4.3	10.0†
ICMA 95333 × ICMV 221	31.0	−4.7	1.5	9.2†
ICMA 96222 × ICMV 221	NA‡	49.7†	11.8	NA‡
ICMA 97111 × ICMV 221	−1.8	12.4†	−11.6	−0.4
ICMA 97333 × ICMV 221	37.9†	33.7†	7.0	26.3†
ICMA 98222 × ICMV 221	−12.0	−21.5†	−16.4†	−16.8†
ICMA 98333 × ICMV 221	9.4	−15.6†	−13.8†	−6.7
ICMA 98777 × ICMV 221	20.3†	−0.2	−22.1†	−0.8
ICMA 99111 × ICMV 221	18.6†	19.7†	−7.3	10.4†
ICMA 99222 × ICMV 221	−11.2	−3.0	−12.3	−8.8†
Mean heterosis	6.1	9.5	−5.9%	2.8%
s.e.d. (heterosis ≠ 0)	12.67	10.94	12.41	6.87
m.s. genotype (14)§				1126.2
m.s. genotype × location (27)				292.1
m.s. error (68)				212.4
ICMV 221 (yield gm^{-2})	347	273	355	320

† Heterosis percentages that are different from 0 (< −1 *s.e.d.* or > +1 *s.e.d.*).
‡ Excluded from the Hagaz and across location analysis due to a mixed seed lot at Hagaz.
§ Numbers in parentheses following the *m.s.* are the degrees of freedom associated with each source of variation.

Kiboko (differences were non-significant), two at Wad Medani and none at Hagaz (Table 2). Across locations there were highly significant differences among topcross hybrids in yield heterosis ($p < 0.001$); five hybrids had an overall positive yield heterosis and two had an overall negative heterosis. The best combination was ICMA 97333 × ICMV 221, with an overall yield heterosis of +26% and a positive yield heterosis at Hagaz and Wad Medani. The other four combinations had an overall positive yield heterosis of approximately 10%, with positive heterosis at one or two locations and zero heterosis at the other(s) (Table 2). Positive heterosis for grain yield was not strongly associated with positive heterosis for either panicle number or grain yield per panicle, however (data not shown). These data, while including too few test locations to draw definitive conclusions, do indicate that it is likely that it will be possible to identify topcross hybrids with ICMV 221 as a pollinator with useful levels of positive grain yield heterosis.

Patancheru trials

The evaluation of the ICMV 221 topcross hybrid set at Patancheru was intended to determine the basis of the observed grain yield heterosis. To do this we estimated

Table 3. Mean percentage heterosis in grain yield, vegetative growth rate,crop duration, biomass and harvest index and yield components in ICMV 221topcross hybrids evaluated in Patancheru, India 2001 and 2002.

	Yield and yield component heterosis (%)						
Topcross hybrid	Grain yield	Growth rate	Growth duration	Biomass yield	Harvest index	Panicle number m^{-2}	Grain yield $panicle^{-1}$
ICMA 863 × ICMV 221	14.8†	3.7	2.2†	8.5†	5.7†	−6.8	23.5†
ICMA 00888 × ICMV 221	6.0	−3.6	−2.4†	−2.3	8.5†	6.1	−0.2
ICMA 89111 × ICMV 221	18.4†	7.2†	1.0†	13.7†	3.1	24.2†	−4.3
ICMA 91222 × ICMV 221	17.0†	11.7†	2.2†	14.1†	2.3	13.3†	5.7
ICMA 91777 × ICMV 221	27.9†	12.4†	7.2†	21.0†	−0.5	1.8	25.2†
ICMA 92444 × ICMV 221	6.7	2.7	−1.7†	3.8	2.8	18.4†	−9.5†
ICMA 95333 × ICMV 221	9.8†	2.8	1.9†	7.6†	1.5	−0.2	10.6†
ICMA 96222 × ICMV 221	12.5†	−1.5	2.7†	6.4†	5.4†	−0.9	14.3†
ICMA 97111 × ICMV 221	33.0†	10.0†	1.0†	21.0†	9.1†	7.3	24.3†
ICMA 97333 × ICMV 221	23.9†	9.6†	2.2†	16.9†	5.3†	7.1	16.4†
ICMA 98222 × ICMV 221	2.2	−5.4	−0.5	−0.7	6.3†	2.8	0.3
ICMA 98333 × ICMV 221	23.0†	2.8	−1.9†	9.9†	11.2†	16.2†	4.9
ICMA 98777 × ICMV 221	4.7	−5.5	1.0†	−1.9	6.5†	20.1†	−12.9†
ICMA 99111 × ICMV 221	5.6	−0.4	2.9†	3.4	1.4	−6.5	12.7†
ICMA 99222 × ICMV 221	10.8†	−14.7†	1.9†	−2.7	13.1†	−5.3	17.9†
Mean heterosis (%)	14.9	6.5	1.3	7.7	5.6	6.5	8.9
s.e.d. (heterosis ≠ 0)	8.19	6.25	0.89	6.00	3.55	8.23	6.68
m.s. genotype (14) ‡	501.5	307.2	33.45	381.7	84.86	602.1	880.6
m.s. genotype × year (14)	234.8	101.4	1.30	104.2	51.72	94.0	175.5
m.s. error (56)	201.3	117.0	2.40	107.9	37.80	203.4	133.7
ICMV 221 (actual value)	230 ($g\ m^{-2}$)	5.48 ($g\ d^{-1}$)	69.7 (d)	585 ($g\ m^{-2}$)	39.4 (%)	10.5 (m^{-2})	21.9 (g)

† Heterosis percentages that are different from 0 (< −1 *s.e.d.* or > +1 *s.e.d.*).
‡ Numbers in parentheses following the *m.s.* are the degrees of freedom associated with each source of variation.

heterosis for the growth rate and duration, biomass and harvest index, and panicle number and yield per panicle, in addition to heterosis for grain and stover yields. Differences in heterosis among individual entries were significant for all measured variables; mean location heterosis ranged from 14.9% for grain yield to 1.3% for growth duration and was positive for all variables (Table 3). Individual hybrid heterosis was positive in the majority of the hybrids for the following variables: growth duration (eleven hybrids), grain yield (ten hybrids), biomass and harvest index (nine hybrids) and grain yield per panicle (eight hybrids). In contrast, heterosis for vegetative growth rate and for panicle number was positive in only five hybrids (Table 3). Negative heterosis was uncommon, occurring in only growth duration (three hybrids), yield per panicle (two hybrids), and growth rate (one hybrid).

All but one of the ten topcross hybrids (that on ICMA 99222) that had positive heterosis for grain yield also had positive heterosis for biomass (Table 3). In comparison, only six of the ten hybrids with a positive heterosis for grain yield also had a positive heterosis for harvest index (Table 3). Of the nine hybrids with a positive heterosis for both grain yield and biomass, half had a positive heterosis for both growth rate and duration (five hybrids), and the other half for duration alone (four hybrids). Thus positive heterosis for all three factors (growth rate, growth duration and partitioning) was related to heterosis for grain yield in some of the hybrids, but there was no exclusive way in which grain yield heterosis was achieved. However, the hybrids with the greatest grain yield heterosis tended to have a positive heterosis for more than one of the three component factors, e.g. hybrids on ICMA 97111, ICMA 91777 and ICMA 97333 (Table 3). Similarly, a positive grain yield heterosis was not uniquely related to either a positive heterosis for panicle number or grain yield per panicle, although there were more associations of grain yield heterosis with grain yield per panicle heterosis (7) than with panicle number heterosis (3) (Table 3).

Drought nursery trials

The analysis of this data set focused on those yield components which are relevant to crop response to an unrelieved stress beginning at flowering: (1) grain number per unit area, which assesses the ability of a genotype to maintain grain numbers despite moisture stress affecting fertilization and embryo cell division; (2) individual grain mass, which assesses the ability of a genotype to fill grain despite the reduction in crop photosynthesis; and (3) harvest index, which measures the overall ability to translate biomass into grain, despite stress affecting normal reproductive processes. A stress adapted topcross hybrid, relative to ICMV 221 should maintain a positive heterosis for either or both grain number and grain mass under stress conditions, resulting in a positive heterosis for both grain yield and harvest index.

The late-onset stress treatment limited both the total numbers of grains (grain number m^{-2} was 31 400 in the early stress v. 37 500 in the control [data not shown]) and the ability to fill individual grains (mean individual grain mass was reduced from 9.6 mg in the control to 7.7 mg in the late stress [data not shown]). The grain yield of ICMV 221 was reduced to 218 $g\,m^{-2}$ from 340 $g\,m^{-2}$ in the control treatment (data not shown), and the trial mean grain yield heterosis was 11.7%. Ten of the hybrids had a positive heterosis for grain yield but differences among genotypes in grain yield heterosis among topcross hybrids (−1.8% to 28.3%) were not significant ($p < 0.17$; Table 4). In contrast, differences in heterosis among topcross hybrids were significant for both grain number ($p < 0.0007$) and individual grain mass ($p < 0.003$, Table 4). However the positive heterosis in grain number (treatment mean of 16.3%), was partly offset by a negative heterosis in individual grain mass (treatment mean of −2.2%, with only two hybrids having a positive heterosis for the latter variable, Table 4). Despite the differences in soil moisture availability in the irrigated control and late stress treatments, the pattern of yield component heterosis was similar in both – positive for grain number and negative for individual grain mass. Further, most of the hybrids which had a positive heterosis for grain number in the late-onset stress generally also had a positive heterosis for the same trait in the control (data not shown). Therefore it does not appear that the late-onset stress materially affected overall expression of yield component heterosis, and a number of hybrids still maintained a positive heterosis of grain yield in the late-onset stress, as was the case in the non-stress control treatment,

Table 4. Percentage heterosis in grain yield, harvest index, grain number per unit area and individual grain mass in ICMV 221 topcross hybrids in the early- and late-onset terminal stress treatments in the dry season drought nursery in Patancheru, India, 2002.

Topcross hybrid	Grain yield Early stress	Grain yield Late stress	Harvest index Early stress	Harvest index Late stress	Grain number Early stress	Grain number Late stress	Grain mass Early stress	Grain mass Late stress
ICMA 863 × ICMV 221	19.6†	15.2†	1.7	1.9	−1.9	8.4	24.9†	8.1†
ICMA 00888 × ICMV 221	−6.7	−1.8	−0.8	−1.8	−7.3	−2.2	2.6	1.9
ICMA 89111 × ICMV 221	−2.0	2.6	−9.6†	−10.4†	9.1	14.4†	−9.7†	−8.5†
ICMA 91222 × ICMV 221	6.5	11.9†	−1.3	−6.8†	−3.5	9.9†	10.3†	3.1
ICMA 91777 × ICMV 221	−8.9	17.9†	−14.5†	−9.4†	−18.8†	25.9†	12.1†	−4.3
ICMA 92444 × ICMV 221	−3.4	8.8	−5.8	−2.2	11.7†	29.3†	−16.1†	−12.7†
ICMA 95333 × ICMV 221	−16.7†	2.6	−21.1†	−14.3†	−3.7	13.8†	−12.4†	−9.3†
ICMA 96222 × ICMV 221	−19.8†	9.3†	−5.5	−5.5†	−12.3†	11.2†	−10.0†	−6.4
ICMA 97111 × ICMV 221	13.9†	12.1†	10.6†	−0.2	−10.0	0.0	17.6†	12.3†
ICMA 97333 × ICMV 221	−12.2†	10.8†	−12.6†	−5.6†	−7.6	14.0†	−4.7	0.0
ICMA 98222 × ICMV 221	20.3†	11.9†	10.3†	2.8	9.5	12.0†	10.1†	2.2
ICMA 98333 × ICMV 221	15.4†	28.3†	13.3†	8.6†	10.2	20.9†	5.7	6.5
ICMA 98777 × ICMV 221	−4.0	21.8†	−4.7	−0.7	9.0	46.8†	−12.7†	−15.3†
ICMA 99111 × ICMV 221	5.6	7.0	−2.3	−7.1†	−6.9	14.7†	12.6†	−3.1
ICMA 99222 × ICMV 221	−5.8	14.8†	−7.2†	1.2	−7.1	26.0†	1.9	−8.0†
Mean heterosis	0.3	11.7	−3.6	−3.2	−1.8	16.3	0.2	−2.2
Probability of m.s. for genotype	0.014	0.17	.002	.0001	0.23	.0007	.0001	.003
s.e.d (heterosis ≠ 0)	11.50	9.04	6.92	3.58	11.14	9.25	8.01	6.69
ICMV 221 (actual value)	145 ($g\,m^{-2}$)	218 ($g\,m^{-2}$)	35.2 (%)	44.9 (%)	24 820 (m^{-2})	27 820 (m^{-2})	0.587 (mg)	0.788 (mg)

† Heterosis percentages that are different from 0 (< −1 *s.e.d.* or > +1 *s.e.d.*).

even if differences among hybrids were not significant in the late-onset stress (Table 4). Where the effects of the stress on heterosis were slightly more apparent was in harvest index, which was reduced from a mean of −0.9% in the control to a mean of −3.2% in the late-onset stress.

The pattern of heterosis in more severe, early on-set stress treatment, in which the grain yield of ICMV 221 was reduced to 145 $g\,m^{-2}$, differed from that of the control and late-onset stress treatments in terms of both grain yield and yield component heterosis. There were significant differences in heterosis for grain yield ($p < 0.014$), harvest index and individual grain mass ($p < 0.0001$) among the topcross hybrids in the early-onset treatment; but no significant differences for grain number ($p < 0.23$, Table 4), In the early-onset stress, only one hybrid had a positive heterosis for grain number, compared to twelve in the late-onset treatment (Table 4). Evidently the earlier onset of the stress, which reduced mean grain number to a greater degree than in the late-onset treatment (24 000 m^{-2} v. 31 400 m^{-2}), suppressed the opportunity for expression of heterosis for grain number. However with fewer grains to fill, six of the hybrids had a positive heterosis for grain mass in early-onset treatment, compared to only two in the late-onset treatment (Table 4). Positive heterosis for grain yield in the early-onset stress treatment appeared to be related to positive heterosis for individual grain mass, as three of the four topcross hybrids that had a positive heterosis for grain yield – those made on ICMA 863, 97111A and 98222A – also had a positive heterosis for individual grain mass (range 10.1% to 24.9%) (Table 4). These were the only three topcross hybrids to have a positive heterosis for this trait in the early stress treatment. (The fourth topcross hybrid with a positive grain yield heterosis – that made on 98333A – also had a positive heterosis for harvest index, but neither for grain number nor grain mass). The early-onset stress treatment was therefore a more effective assessment of actual drought tolerance in the topcross hybrids than was the late-onset treatment.

The four topcross hybrids which had a positive grain yield heterosis in the early-onset stress conditions (listed above) also exhibited positive heterosis in the late-onset stress treatment (range 11.9–28.3%), even if the differences among hybrids in the late-onset treatment were not significant by the variance ratio test (Table 4). None of the four showed a positive heterosis for either grain yield or individual grain mass in the control treatment (data not shown), so that their heterosis under the early-onset stress environment is clearly an expression of a better adaptation to terminal drought stress than their pollinator parent, rather than simply a reflection of a general level of positive heterosis in the absence of stress. Three of the four – those on ICMA 863, 97111A and 98333A – did have a positive heterosis for harvest index in the control treatment, however, indicating a superior ability (compared to ICMV 221) to partition dry matter to the grain even in the absence of stress (Table 4).

Identification of promising experimental topcross hybrids

In an actual exercise to select the best experimental topcross hybrids for wider evaluation, a number of criteria would be considered: farmer preference, disease resistance, grain quality (especially where marketing to processors is an option), specific adaptation (e.g. to drought stress environments) and across-environment yield heterosis (i.e. broad adaptation). As an example of such an exercise, the across-environment heterosis was evaluated in this data set by grouping test environments by eastern Africa, Patancheru rainy season and the drought nursery terminal stress trials, and conducting an across-environment-group analysis for heterosis for time to flowering, grain yield and yield components. The effect of environment group was significant for heterosis for all variables except for time to flowering (data not shown). The contrast between the rainy season (eastern Africa and Patancheru) and drought nursery environment groups accounted for the majority of the environmental effects for the yield and yield component variables. This underlined the major differences in the drought stress and rainy season (non-stress) environments, and the need to select topcross hybrids with a positive yield heterosis in both types of environments.

Genotype and genotype × environment group effects were significant for all four heterosis variables (Table 5). Trial mean (across environment) heterosis was positive for all variables except grain weight per panicle (Table 5). Average grain yield heterosis was 7.8% with a range of 0 to +17%; seven topcross hybrids had a significant ($p < 0.05$) positive grain yield heterosis (Table 5). Of these, ICMA 97333 × ICMV 221 stood out

Table 5. Across-location mean heterosis for time to flowering, grain yield and yield components of ICMV 221 topcross hybrids. Analysis was based on the 15 ICMV 221 topcross hybrids, three environmental groups (three eastern African locations, the two years at Patancheru, India, and the early and late-onset terminal stress treatments in the drought nursery in Patancheru, India) and individual trials within location group.

	Percentage heterosis for			
Topcross hybrid	Time to flowering	Grain yield	Panicle number m^{-2}	Grain weight $panicle^{-1}$
ICMA 863 × ICMV 221	2.2	14.0*	−2.9	19.4*
ICMA 00888 × ICMV 221	−1.0	0.1	7.2	−6.1
ICMA 89111 × ICMV 221	1.4	4.8	37.4*	−21.9
ICMA 91222 × ICMV 221	5.0*	9.6*	15.3*	−3.5
ICMA 91777 × ICMV 221	5.9*	11.5*	7.7	6.1
ICMA 92444 × ICMV 221	1.1	7.4	26.8	−15.8
ICMA 95333 × ICMV 221	3.8*	2.4	6.9	−1.8
ICMA 96222 × ICMV 221	6.5*	12.3*	3.6	9.1*
ICMA 97111 × ICMV 221	0.6	13.9*	14.1*	1.8
ICMA 97333 × ICMV 221	1.0	17.2*	26.5*	−6.0
ICMA 98222 × ICMV 221	−0.7	0.1	6.7	−3.5
ICMA 98333 × ICMV 221	−1.6	11.3*	20.9*	−7.2
ICMA 98777 × ICMV 221	0.7	3.7	35.7*	−23.7
ICMA 99111 × ICMV 221	3.4*	7.6	1.9	5.7
ICMA 99222 × ICMV 221	3.2*	1.5	−2.3	8.3
Mean heterosis	2.1	7.8	3.7	−2.6
s.e.d. (heterosis ≠ 0)	1.40	6.36	5.67	5.49
l.s.d. (significant difference)	2.30	9.33	10.48	9.03
m.s. genotype (14) †	133.2	650.8	3989.9	2948.5
m.s. genotype × envir. group (28)	53.0	893.7	673.6	547.9
m.s. geno × location (envir.) (55)	15.4	294.3	347.1	264.3
m.s. error (250)	7.3	239.1	257.6	244.5

* Heterosis percentages that are significantly ($p < 0.05$) greater than 0 (> +1 *l.s.d.*).
† Numbers in parentheses following the *m.s.* are the degrees of freedom associated with each source of variation.

with a 17% greater grain yield than ICMV 221 across environments, followed by the hybrids on ICMA 863 and ICMA 97111A with a 14% advantage, those on ICMA 91222, 96222, 91777 and 98333 with an 10–11% advantage (Table 5). Four of these hybrids achieved their significant yield heterosis by a significant heterosis for panicle number (which might have been expected as ICMV 221 produces only a limited number of tillers). In only one case (ICMA 863) was this achieved by a significant heterosis for grain weight per panicle (Table 5).

Mean yield heterosis figures masked a significant amount of hybrid × environment group interaction, particularly for grain yield, however, where the *m.s.* for the genotype × environment group interaction exceeded the *m.s.* for genotype (Table 5). For example, ICMA 97333 × ICMV 221, the hybrid with the highest overall grain yield heterosis, had positive grain yield heterosis in both the eastern African (+26%) and the Patancheru trials (+24%), but not in the terminal stress trials (Tables 2, 3 and 4). ICMA 96222 × ICMV 221 exhibited a similar pattern of interaction, suggesting that both of these combinations are primarily adapted to favourable environments. In contrast, hybrids on ICMA 97111 and ICMA 98333 had positive yield heterosis at both Patancheru (+23% and +33%, respectively) and in the drought nursery (+14% and +12% in the early-onset treatment, and +15% and +28% in the late-onset treatment, respectively), but had no yield heterosis in the eastern African environments (Tables 2, 3 and 4). The most promising of the topcross hybrids appeared to be ICMA 863 × ICMV 221, which maintained a positive 10–20% grain yield heterosis across all environmental groups, was among the best hybrids in both the late and early stress treatments, and had the second highest grain yield heterosis overall; Tables 2, 3, 4 and 5). The expression of significant heterosis in this hybrid was unexpected, however, as ICMA 863, like ICMV 221, originates primarily from *Iniadi* germplasm. Most importantly, the data clearly demonstrate that it is possible to identify topcross hybrids, made with a well-adapted (even high yielding) local variety as a pollinator, that are statistically superior to their pollinator across a range of environments.

DISCUSSION

Magnitude of grain yield heterosis

Grain yield heterosis estimates for pearl millet in the literature vary widely, depending upon the type of parental materials used and the type of crosses from which heterosis was estimated (Virk, 1988). A review of the literature suggests that percentage grain yield heterosis estimates are partly a function of the yield level of the pollinators themselves, on which heterosis estimates are based, rather than the type of pollinator *per se* (e.g. Mahalakshmi *et al.*, 1992). Highest heterosis estimates (> 100%) are based on single cross hybrids made with highly inbred parents (Virk, 1988) which are often very low yielding due to inbreeding depression. Estimates of grain yield heterosis (hybrid v. pollinator) of topcross hybrids made with unimproved Indian landrace population parents are in the order of 20–40% (Mahalakshmi *et al.*, 1992; Bidinger *et al.*, 1995; Yadav *et al.*, 2000). These are comparable to mean grain yield heterosis estimates (hybrid v. mid-parent values) derived from population crosses among unimproved West African landraces (40%; Ouendeba *et al.*, 1993) or improved West African landrace-derived populations (22%; Lambert, 1983). At the extreme, grain yield heterosis (hybrid v. pollinator) for topcross hybrids based on high yielding Indian released varieties was only 15% (Mahalakshmi *et al.*, 1992).

In a fashion parallel to the effects of pollinator parent yield on hybrid heterosis cited above, mean grain yield heterosis among the various rainy season evaluation environments in this study also varied inversely with the yield level of ICMV 221. Heterosis ranged from −6% at Kiboko, where ICMV yielded 3.6 t ha^{-1} to 21% at Patancheru 2001, where ICMV 221 yielded only 2.1 t ha^{-1}. Over all environments, the variation in the yield of ICVM 221 itself explained nearly 80% of the variation in mean trial grain yield heterosis (data not shown). Given this, the mean grain yield heterosis of 8%, and the 15% yield heterosis in the best of topcross hybrids (Table 5) based on a widely adapted, high yielding open-pollinated variety (with mean grain yield of 2.7 t ha^{-1} in these experiments) is probably reasonable, if not of the magnitude sometimes associated with hybrids in this crop.

Basis of grain yield heterosis

It would be reasonable to expect that significant heterosis would be more common in traits which are expressed poorly in the pollinator, and that such heterosis would have a greater effect on hybrid yields than would heterosis in traits which express strongly in the pollinator. Because ICMV 221 is early flowering and produces few fertile tillers and a modest biomass (Table 3), yield heterosis would be expected to be more strongly associated with heterosis for these traits rather than in harvest index and individual panicle yield. Positive grain yield heterosis in the Patancheru environment was associated with positive heterosis for biomass alone in four cases, with positive heterosis for both biomass and harvest index in five cases, and with positive heterosis for harvest index alone in one case (Table 3). Therefore the expectation that positive heterosis for biomass would result in positive heterosis for grain yield was generally true, but the expectation that positive heterosis for harvest index either would not be common or that it would not contribute to positive yield heterosis was not. Among the basic yield components measured, there was positive heterosis for panicle number (poorly expressed in ICMV 221) in five cases, of which three were associated with positive grain yield heterosis, and positive heterosis for grain yield per panicle (strongly expressed in ICMV 221) in eight cases, of which six were associated with positive grain yield heterosis (Table 3). Therefore, the expectation of positive heterosis in poorly expressed yield components resulting in positive yield heterosis (and vice-versa) was also not supported by the data. The likely explanation is that the phenotype of the seed parents (which had a range in yield component expression) mattered as much or more than that of ICMV 221 itself in the expression of yield component heterosis and its association with grain yield heterosis in the topcross hybrids.

The associations of a positive grain yield heterosis with specific trait heterosis under end-of-season drought stress varied with the timing of the stress. In the late-onset treatment, a positive grain yield heterosis was associated with a positive grain number heterosis in six of eight cases and with a positive grain mass heterosis in only two of eight cases (Table 4). In the more severe early-onset treatment, a positive grain yield heterosis was associated with a positive grain mass heterosis, rather than a positive grain number heterosis, in three of four cases, and with a zero heterosis for both components in the other case (Table 4). The ability to maintain a high grain number during a stress commencing around flowering would intuitively seem to be a necessary component of stress tolerance (Fussell *et al.*, 1991). This seemed clearly to be the case with the late-onset treatment, in which grain number should have been largely determined before the stress became severe. In the early-onset treatment, however, only one hybrid (ICMA 92444 × ICMV 221) was able to maintain a positive grain number heterosis, but it did not translate to a positive grain yield heterosis because of a (linked?) negative individual grain mass heterosis (Table 4). The severity of the early-onset stress treatment was apparently such that there was little possibility of positive heterosis for grain number to be expressed (whereas it was in both the control and late on-set treatments), with the result that the only opportunity for a positive grain yield heterosis in the early-onset treatment was through positive individual grain mass heterosis. The drought nursery is conducted during the hottest time of the year, subjecting the crop to significant atmospheric stress during grain filling, as maximum temperatures are 37–40 °C and open pan evaporation rates are of the order of 8–10 mm d^{-1}. Grain filling duration is shortened to approximately 20 days under such temperatures, and crop maximum (midday) photosynthesis rates may be reduced, even without an added soil moisture deficit. Therefore the ability to maintain a positive heterosis for individual grain mass under the combined atmospheric and edaphic stress of this environment does suggest a genuine tolerance of terminal drought stress. These results point out the complexity of genotype × environment interactions in stress environments, and underline the need for extensive field evaluation of potential new cultivars intended for environments in which stress is a common feature. The fact, however, that four of the topcross hybrids (those on ICMA 863, ICMA 97111, ICMA 98222, and ICMA 98333) did have a positive yield heterosis in both stress environments indicates that topcross hybrids with good stress tolerance do exist, even if the mechanisms are not obvious.

Individual topcross hybrid performance

Seven topcross hybrids had significant positive grain yield heterosis across all seven evaluation environments, with yield advantages over ICMV 221 ranging from 9 to 19% (Table 5). Of these however, only one hybrid (ICMA 863 × ICMV 221) had a positive yield heterosis in each of the three groups of environments. Two hybrids had positive heterosis in both sets of rainy seasons environments (Patancheru and eastern Africa) but not in the drought environments, and three had a positive yield heterosis in both Indian environments (Patancheru and the drought nursery) but not in the African one (Tables 2, 3 and 4). The eastern African environments all had less rainfall than the Patancheru environments, but the two groups of rainy season environments were otherwise more similar to each other than either was to the dry season terminal stress environments. Topcross hybrids that would be candidates for further evaluation should have positive grain yield heterosis over all of the rainy season environments to ensure that they possess sufficiently wide adaptation to the range of conditions that they will likely experience if released. Therefore the topcross hybrids on ICMA 97111 and ICMA 98333, which had a positive heterosis in Patancheru and the drought nursery, but not in the eastern African environments, are likely to be of lesser interest than the others with a positive overall yield heterosis. The hybrids on ICMA 97333 and ICMA 96222 had a positive yield heterosis in both sets of rainy season environments but not in both the drought nursery environments, although their yield was on a par with that of ICMV 221 in the drought nursery (Tables 2, 3 and 4). Heterosis in the drought nursery was always more problematic than in the rainy season environments as the component lines from which ICMV 221 was made were selected under terminal stress conditions (Witcombe *et al.*, 1997) and this variety is normally classed as tolerant to a terminal stress (F. R. Bidinger, unpublished data). Therefore positive yield heterosis in the absence of stress combined with a similar yield to that of ICMV 221 under terminal stress should be an acceptable criterion for further testing in all but target environments where severe terminal stress is a regular feature. For such environments ICMA 863 × ICMV 221, which was the only hybrid to maintain a positive yield heterosis in both the stress environments, as well as in both rainy season environments, would be the obvious choice, as the ability to maintain a positive yield heterosis over ICMV 221 under terminal stress is a good indication of terminal stress tolerance.

Topcross hybrids as a basis for a hybrid seed industry

The key question is whether the level of grain yield achieved in the best of the topcross hybrids is sufficient to justify these as a starting point for a hybrid seed industry. The answer depends partly upon the basis of comparison, i.e. the varieties farmers are already growing or the alternative new varieties to which they have access. In India, where the majority of the millet area is already sown to modern hybrids, it is generally considered that a 15% advantage in grain yield (all other things, e.g. disease resistance, being equal) is necessary for farmers to change from an existing hybrid to a superior one (C. T. Hash, ICRISAT, personal communication). By this criterion, the yield advantage of even the best of the ICMV 221 topcross hybrids would be marginal for these to replace ICMV 221 itself. However, the general absence of a seed industry in eastern Africa means that farmers do not even have ready access to seed of improved open-pollinated varieties such as ICMV 221 (except where seed of this cultivar is being multiplied by NGOs, government extension services or aid projects, examples which are rarely sustained beyond the life of the project). Therefore, the comparison that most farmers have is between the topcross hybrids and their local cultivars.

The fact that ICMV 221 has already been tested and released in several countries in the region suggests that it has significant advantages over local cultivars. This is supported by the data in this experiment, in which ICMV 221 yielded nearly 50% more than the local control in the eastern African trials (Table 1). Although the best of the topcross hybrids on ICMV 221 did have an additional 17% yield advantage, it is clear that either ICMV 221 or one of its better hybrids would be an attractive potential starting point for a pearl millet seed industry. The decision facing an interested entrepreneur would involve the trade-off between the advantages and disadvantages of each type of cultivar. Open-pollinated varieties have the advantage of greater ease and much lower cost of seed production, but a likely lower, or largely one-time, demand for seed, as farmers need not renew seed of open-pollinated varieties more than once in 3–5 years (Andrews and Harinarayana, 1984), and farmer-to-farmer spread of seed of an open-pollinated variety is likely to be as common as purchase of seed. In contrast, topcross hybrid seed is more complex and costly to produce than open-pollinated variety seed, (as breeder and foundation seed stocks of the male-sterile line, its maintainer and the pollinator have to be maintained), seed production requires considerably more supervision, and seed yields on the male-sterile seed parent will be lower than on an open-pollinated variety. To balance this, however, the seed producer is guaranteed a regular demand for seed of successful hybrids, as farmers quickly lose the advantages of heterosis if they replant seed from the previous year's harvest (F. R. Bidinger, unpublished data), or purchase or borrow seed from their neighbours. It is mainly this latter factor, plus the ability to protect a successful hybrid from competitors (which is almost impossible in the case of an open-pollinated variety) which has traditionally limited private sector interest to the production of seed of hybrids rather than open-pollinated varieties.

One additional advantage to the topcross hybrid alternative is the future option of inbreeding desirable plants in the topcross pollinators to produce inbred pollinators. Topcross pollinators, as open-pollinated varieties, are genetically heterozygous, implying that there is very likely variation within them for both general and specific combining ability. Conducting one or more cycles of recurrent selection for specific combining ability in the pollinator of a promising topcross hybrid (with the seed parent of that hybrid as the tester) is a logical next step. This will likely lead to the development of inbred restorer lines which will produce single cross hybrids with yield advantages over the original topcross hybrid. Topcross hybrids thus provide the new seed company an additional future means of producing hybrids that can replace the topcross hybrids identified in the initial evaluation exercise.

APPENDIX: BEGINNING A TOPCROSS HYBRID SEED PROGRAMME

Topcross hybrids are a very attractive proposition to an entrepreneur or company interested in entering the pearl millet hybrid seed business because of the rapidity with which an acceptable proprietary hybrid can be identified, and seed production and marketing begun. It is possible to produce seed and initiate field evaluations of experimental topcross hybrids with adapted, widely accepted open-pollinated varieties as pollinators in a single year for a very modest cost, because of the public availability of male-sterile lines from the ICRISAT pearl millet breeding programmes in both India and Africa. The most acceptable of the first experimental hybrids can be identified in a staged programme of farmer participatory evaluation in as little as two additional years. Commercial seed production, generally by contract to larger farmers or companies with irrigation facilities, can be initiated on a pilot scale in the third year and on a commercial scale in the fourth year. The first product can thus be in the market as early as the fourth year. One scenario for doing this is presented in Table 6 and discussed in detail in the following paragraphs. Further details on the mechanics of hybrid seed production in pearl millet can be found in Chopra *et al.* (1999).

Year 1

Dry season: Produce (by hand pollination) limited quantity of seed (300 g) of sets of experimental topcross hybrids, each set based on a locally adapted open-pollinated variety as pollinator and all available/appropriate A-lines. Discard A-line × pollinator combinations for which the flowering of the parents is not well enough synchronized to permit seed production to be done in isolation by open pollination. The objective is to produce seed of a wide range of experimental hybrids for farmer evaluation/selection.

Table 6. Time schedule for the evaluation and production of topcross hybrids based on locally adapted cultivars as pollinators, using a largely participatory selection approach to identify marketable hybrids, and a contract system for actual commercial seed production. (Main season is the normal cropping season and the breeding season is the dry or short rainy season used for breeding operations and seed production).

Year	Season	Location	Activity (seed production, evaluation and marketing)
1	Breeding	Station	Seed production of sets of experimental topcross hybrids based on locally adapted varieties for initial evaluation
1	Main	Station	Farmer participatory selection of preferred topcross hybrids for wider evaluation
2	Breeding	Station	Seed production of selected experimental topcross hybrids for on farm, participatory evaluation
2	Main	Farm/Station	Farmer managed, on-farm evaluations, plus on-station replicated trials, of selected topcross hybrids
3	Breeding	Station	Pilot scale commercial seed production of best of the preferred hybrids
3	Main	Farm	Second year of on-farm and on-station evaluations topcross hybrids, plus initial commercial sale of seed of to interested farmers participating in earlier evaluations
4	Breeding	Farm	First commercial seed production of best hybrid(s) under contract with larger farmers/organization
4	Main	Farm	Marketing of seed of topcross hybrid(s) to farmers and development agencies, NGOs etc.

Rainy season: Plant these experimental topcross hybrids as demonstrations in millet-growing areas that are possible markets for hybrid seed. Group all topcross hybrids based on the same pollinator together, in 2-row plots, with a plot of the pollinator planted after every five plots. Invite groups of local farmers to select those hybrids within each pollinator group that they think are the best, and that they would be interested in evaluating on their own farms during the next year. The objective is to narrow quickly the choice of potential hybrids down to a best-bet list, based on farmers' initial evaluations, for more intensive evaluation in the following year. In addition, a minimum of five panicles in each plot should be self-pollinated to evaluate the fertility restoration in the hybrid; those with very poor seed set are likely to produce low hybrid seed yields and should be eliminated.

Year 2

Dry season: Develop a consensus list of the five most-preferred topcross hybrids on each pollinator for further evaluation. Produce larger quantities of seed (5 kg) of these for more extensive, on-farm evaluation in the following rainy season. Seed production can serve as a first test of commercial production, by exploiting natural cross pollination in pearl millet, using one isolation field (to exclude pollen from local fields) per pollinator, plus the selected A-lines. Also, begin seed multiplication of the seed parents and pollinators of the selected hybrids to have sufficient stocks of parental lines for larger scale topcross hybrid seed production in the following year.

Rainy season: (i) Conduct a few well-replicated evaluations of the selected topcross hybrids, in several key locations. Record data on as many characteristics as possible to both fully describe each topcross hybrid, and to develop a data base on hybrid characteristics to improve understanding of farmers' preferences for specific phenotypic traits. (ii) Conduct large-scale, unreplicated, on-farm evaluations of the best five topcross hybrids on each pollinator, in comparison to the farmers' own variety. Have farmers manage the demonstrations themselves, if possible, so that the evaluations are done under realistic production conditions. Use appropriate participatory evaluation methods to obtain broad-based farmer assessment of the topcross hybrids, including acceptability of the local food products made from them.

Year 3

Dry season: Select the best two topcross hybrids, based on farmers' evaluations, to initiate pilot-level commercial seed production, by contract with larger farmers or organizations with irrigation and the ability to provide adequate isolation from other millet fields. Use this exercise to identify potential seed producers for future commercial seed production, as well as to produce seed for use in the next rainy season. Expand seed production of the seed parents of these hybrids to have sufficient stocks to begin commercial seed production in the following year.

Rainy season: (i) Continue large-scale, on-farm evaluations of the best topcross hybrids, in comparison to the farmers' own variety. (ii) Offer small quantities of seed (1–3 kg) of the best hybrids for sale in villages/ areas in which the on-farm demonstrations of the previous year generated farmer interest in trying the topcross hybrids on larger areas in their own regular production fields. Use these farmers' fields as demonstrations for staff of non-governmental and governmental development agencies, as well as for farmer groups.

Year 4

Dry season: Scale-up the commercial seed production of the most preferred topcross hybrid(s), based on the response of farmers in the demonstrations conducted on-farm during years 2 and 3.

Rainy season: Begin commercial sale of seed of the selected topcross hybrid(s), Target both individual farmers and development agencies, co-operatives, etc. in areas where the hybrids have been previously tested.

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