A Study on Sources of Cytoplasmic Genetic Male Sterility, Heterosis, and Combining Ability in Pearl Millet

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#### **Abstract**

Because of its great genetic diversity and the availability of an effective cytoplasmic genetic male sterility system, pearl millet has been a popular crop in recent years. Due to the protogynous character of its flowers; Pearl millet is a highly cross-pollinated crop with an out crossing extent of more than 85%, making it very heterozygous and diverse. The authors of this work investigate the genetic bases for male sterility, heterosis, and combining ability in pearl millet by looking at its cytoplasm.

**Keywords**: Pearl Millet, Male Sterility, Heterosis, Combining Ability.

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#### 1. Introduction

When it comes to warm-season cereal crops, pearl millet has the highest drought tolerance. Millions of people who live in areas with unpredictable rainfall and nutrient-poor soil rely on it as a staple food grain and a source of feed, fodder, and food. The efficient utilisation of soil moisture and greater degree of heat tolerance than sorghum and maize make it the sole acceptable and effective crop for dry and semiarid tropic environments. Farmers choose this crop not just out of personal preference, but also out of economic need . Nutritionally, pearl millet outshines both sorghum and wheat in terms of protein, fat, calcium, phosphorus, and iron.<sup>1</sup>

In addition to its use as a food crop, pearl millet is grown as a green fodder crop in certain areas. Green feed is prefered over sorghum because it has no HCN. Pearl millet is an essential cereal crop because of its adaptive and nutritional qualities, as well as its production potential, which may help mitigate the effects of climate change, water scarcity, land degradation, and food-related health problems.<sup>2-3</sup>

Improving yield and its associated qualities in every crop requires the recombination of numerous desired features spread out across different distinct genotypes. Methods like Mahalanobis's D2 statistics for analysing genetic diversity in a population have been shown to be beneficial. It has been shown that offspring of genetically heterogeneous parents have the best possible probability of being isolated from their strays. <sup>4-5</sup>

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It helps find the most promising recombinants in the populations. The purpose of this research is to examine the genetic variety of the samples. Understanding the genetic structure of pearl millet and the anticipated prepotency of parents in hybrid combinations is essential for developing a successful heterosis breeding scheme. <sup>6-8</sup>

Hybrid breeding does not provide desirable outcomes when selection is based only on phenotypic performance. Therefore, research into the combined abilities of prospective parents is crucial. There are a number of biometric methods now in use for assessing cognitive abilities, including the parents' combined capacity to select for heterosis in breeding is often investigated via line x tester analysis. It helps to establish the worth of source populations and the best practises for implementing a crop improvement strategy. Having this information will aid with the commercialisation of heterosis.<sup>9-10</sup>

#### 2. Material And Methods

## • Experiment-I

Assessing the potential of germplasm to reverse cytoplasmic male sterility in a variety of animal and plant species.

Three isonuclear female parents were obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in Patancheru, India, and 50 eliterestorers were developed at the in-house Pearl millet breeding programme at Nuziveeduseeds in Hyderabad, India, for this study.

#### **Crossing Programme**

The 2014 kharif and rabi seasons were used for the crossover project. In order to ensure that there would be enough seeds, sowings of the parents were staggered. For each of the three CMS sources (A1, A4, and A5), and fifty hybrids were created.

In 2015's summer and kharif, farmers sowed 150 hybrid seeds (50 from each of three different cytoplasms). The hybrids were planted in a single 4-meter-long row with 15-centimeter-wide intra-row and 50-centimeter-wide inter-row gaps. The whole set of best practices was implemented.

#### Collection of data

Prior to blooming, we sel-fed five plants in each condition using parchment paper bags, and we utilized the same plants to make the following observations.

#### Plant height (cm)

The mature height of a plant was measured from the soil line to the top of the main stem in centimetres (cm).

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## Percentage of seed setting

When the seeds were mature, they were counted as a proportion of the total number of spikelets in the ear head.

Distance between clusters i and j, shown by the notation "Di2," is the sum of the squares of all conceivable combinations (ninj) of the cluster i and j's elements.

ni= number includes i entries

nj= entry count includes j

## • Experiment 2

Studieson Combining Ability and Heterosis

The line x tester analysis was used to determine heterosis and combining capacity.

#### Material

The purpose of this study was twofold: (1) to determine the impact of various cytoplasmon quantitative characteristics, and (2) to investigate the use of several sources of male sterility in the creation of hybrids. There were a total of 3 females and 50 males used in this investigation. Females were derived from three male sterile lines (A1, A4, and A5) that represented distinct cytoplasms against the nuclear background of Tift 23A and had a wide range of dissimilar characteristics. In order to restore fertility, each of these three female parents were crossed with 50 pollen parents with genetic heterogeneity. In Tables 1 and 2, we see a complete breakdown of the parental setup. The experimental material consisted of 150 F1s, 53 parent, and 50 male parents from a crossing programme conducted in Kharif, Rabi, and the summer of 2014 in LxTashion.

#### Method

During kharif 2015, a RBD with three replicates was used to conduct an experiment with 150 F1s, 53 parents, and 86M64 checks. Each entry's seed was planted in a 4-meter-long row. The distance between rows was 50 cm, and there was 15 cm between individual rows. The parents grew up on an adjacent block. Only the hybrids (F1) and their parents were randomly assigned. The crop was grown using the recommended package of practises. Operations for weed control, cross-cultural work, and plant protection were performed as required.

#### Collection of data

Five competing plants each replication were randomly labelled, and their performance was recorded. The parameters given in the preceding experiment were used to calculate treatment means for these plants.

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## Combining ability

The Line x Tester approach proposed by Arunachalam (1989) and Kempthorne (1957) was used to calculate the combining ability variances necessary for testing the combining ability impacts of parents (gca) and crosses (sca).

The following mathematical model was used in the analysis:

$$Yijk = \mu + mi fj + Sij + eijk$$

Where,

μ= population mean

mi = Impact of the Father Gene on Offspring

fj = gca effect to fj th(female) parent

Sij= gca of (Ixj)th cross

eijk= Error-Prone Randomness Associated with the (ij)th Observation (kj)th.

For both hybrids and their parents, we performed an RBD study of cross-pollination using a Linex Tester analysis.

## • Anova for Combining Ability

## Combiningability

The following effects were computed using a two-way table of lines versus testers, with each figure representing the combined results of all three tests..

$$\mu = x.../ltr$$

$$gi = xi ../tr - x.../ltr$$

$$gj = x.j./ltr - x.../ltr$$

$$Sij = xij/r - xi../tr - x.j./lt + x.../ltr$$

x =Hybrid Number

xi..= Total number of females on the internet

x.j. = the sum of all male j-testers

xij=The ijth possible permutation

1 = Amount of broadcast lines

t=Numberoftester

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r= uantity of Repeatations

## Estimated standard deviations

The following formula was used to determine the uncertainties in the estimations.

The square root of the variances is the standard error of the estimations.

S.E.(gca forline) =  $(M_4/rt)^{1/2}$ 

S.E.(gcafortester) =  $(M_4/rl)^{1/2}$ 

S.E. (sca effect) =  $(M_4/r)^{1/2}$ 

Where,

M4 = error variance

r = replication

l =lines

t=testers

Standard errors were multiplied by the table't'value at the 5% and 1% probability levels for error degrees of freedom to determine the important differences.

## Estimation of heterosis

It was determined how much heterosis there was in comparison to the check value, the better parent, and the average parent. These were determined by comparing the values of the F1 to those of the MP, BP, and Ch using the formulas developed by Turner (1953) and Hayeset al. (1955).

(a) Heterosisovermidparent(H<sub>1</sub>)

$$\% H_1 = \frac{\overline{F_1} - \overline{MP}}{\overline{MP}} X 100$$

(b) Heterosisoverbetterparent(H2)

$$\% H_2 = \frac{\overline{F_1} - \overline{BP}}{\overline{BP}} X 100$$

(c) Heterosisovercheck(H3)

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$$\% H_3 = \frac{\overline{F_1} - \overline{Check}}{\overline{Check}}$$

#### 3. Results

# • Investigations on the Genotype-Specific Reproductive Response to Multiple Cytoplasmic Male Sterility Systems

Thirty elite genotypes were tested for their potential to reverse male sterility caused by three different kinds of cytoplasm (A1, A4, and A5). The proportion of seeds produced by F1 plants when selfed was used as the primary criterion for evaluating restoration potential; the results are shown in Table 3. The achieved outcomes are summarised below.

## 1. Percentage of seeds that are set depends on the season.

## Hybrids based on A1 cytoplasm

There were 32 hybrids with a high seed setting percentage (>90%), 6 hybrids with a high seed setting percentage (>80%), 5 hybrids with a decent seed setting percentage (>60%), and 7 F1's with an inadequate seed setting percentage (60%). Restoration on the A1 cytoplasmic source was shown for 86% of genotypes. The average seed set value disclosed by distinct fertile F1's was computed, and it was found to be 85.04 percent of 50 fertile F1's during the inkharif season (as opposed to the summer, when it was only 79.88 percent). Over the course of two seasons, 82.46 percent of seed sets delivered viable F1 offspring.

There was a large variation in the amount of seed set on A1 cytoplasm from one restorer to the next, both within each season and between seasons. Some genotypes as well as some others, exhibited greater values of seed set % throughout two seasons. In general, kharif has a greater seed set than summer.

## A4 cytoplasm based hybrids

Thirty different genotypes had seed set on a self-pollinated panicle, with 24 hybrids recording above 60% and 6 hybrids recording less than 60%.

On A4 male infertility sources, only 48% of genotypes were shown to be effective restorers. No seeds were formed in twenty different hybrids. 50 viable hybrids had a higher mean seed set percentage during kharif (82.63 percent) than during summer (74.93 percent). Over the course of two growing seasons, the average seed set disclosed by fertile F1's was 78.77 percent.

In hybrids developed from the NB525 and NB600 pollen sources, the highest documented seed set was 99.12 percent during the kharif season and 92.15 percent during the summer.respectively.

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## A5cytoplasmbasedhybrids

On A5 cytoplasm, 19 of the 30 genotypes demonstrated seed set, with 14 showing more than 60% seed setting. There were 31 items with a maintainer reply. In all, 28% of the genotypes were effective restorers. The average percentage of seeds that 19 fertileF1s produced during kharif was 76.83 percent and during summer it was 68.61 percent. Over the course of two growing seasons, the average seed yield from fertileF1s was 72.71 percent.

Table 1: The proportion of F1 hybrids developed from CMS lines that set seed

Sl.	FemalePar		A1			A4			A5	
No.	ent									
	Season	Kha	Summ	Mea	Khar	Summ	Mea	Khar	Summ	Mea
		rif	er	n	if	er	n	if	er	n
	MaleParent	•		•	•			•		•
1	NB 525	97.7	93.40	95.5	99.1	88.23	93.6	-	-	
		0		5	2		8			
2	NB 526	93.0	82.95	88.0	97.3	89.93	93.6	-	-	1
		9		2	3		3			
3	NB 527	96.6	89.90	93.2	95.1	88.93	92.0	-	-	1
		7		8	3		3			
4	NB 528	97.4	94.21	95.8	98.7	89.76	94.2	-	-	-
		0		0	0		3			
5	NB 530	96.6	93.40	95.0	96.0	92.05	94.0	93.7	80.15	86.9
		7		3	5		5	0		2
6	NB 580	93.7	83.71	88.7	-	-	-	-	-	-
		9		5						
7	NB 590	20.7	10.53	15.6	-	-	-	-	-	-
		3		3						
8	NB 595	74.1	63.91	69.0	-	-	-	-	-	-
		1		1						
9	NB 600	87.8	80.05	83.9	-	-	-	-	-	-
		6		6						

The A5 cytoplasm restorers varied in the proportion of seeds they were able to establish. In contrast to A4 and A5, the percentage of lines recovering fertility on A1 was higher in a comparison of seed-setting observations recorded on different cytoplasms. Overall, 45 were successful on A1 cytoplasm, 24 on A4, and 14 on A5 cytoplasm out of a total of 30 genotypes. Regardless of cytoplasmic sources or restorer genotypes, the average seed set % was highest during the kharif season.

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## • Effect of seasonon other plant characters

## Plant height

Different A1-based hybrids reached different maximum heights depending on the season: in kharif, 173.0 cm (A1 x NB789, A1 x NB782) to 246 cm (A1 x NB670), and in summer, 165 cm (A1 x NB789) to 263 cm (A1 x NB737). In terms of average length, the ightinkharif was 203 cm and the summer was 193 cm. The average was 198 cm.

The summer height range for A4 cytoplasm-based plants was 91 cm (A4 x NB612) to 274 cm (A4 x NB590), while the harif range was 166 cm (A4 x NB803) to 231 cm (A4 x747). Themeanplantheightduring kharifands ummer was 194 cm and 179 cm respectively. The average (over both seasons) was 187 cm. In the kharif season, A5 x NB627 and A4 x NB580 produced plants that reached a height of 151 cm, whereas in the summer, A5 x NB527 produced a plant that reached a height of 249 cm. Plants grew an average of 198 centimetres tall during kharif, compared to 186 centimetres during summer. The average height over the course of two seasons was 192 cm.

Table 2: Evaluation of the F1 hybrids derived from CMS lines for plant height

Sr.	FemalePar		A1			A4			A5	
No.	ent									
	Season	Kha	Summ	Mea	Khar	Summ	Mea	Khar	Summ	Mea
		rif	er	n	if	er	n	if	er	n
	MaleParent		l			l			<u> </u>	
1	NB 525	210	198	204	189	177	183	216	205	211
2	NB 526	210	194	202	209	193	201	231	214	223
3	NB 527	220	208	214	210	190	200	249	233	241
4	NB 528	235	224	230	213	199	206	246	235	241
5	NB 530	230	225	228	203	191	197	236	225	231
6	NB 580	193	177	185	183	170	177	151	140	146
7	NB 590	208	202	205	193	274	234	186	175	181
8	NB 595	197	188	193	185	169	177	211	200	206
9	NB 600	193	182	188	201	190	196	196	190	193

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10	NB 611	200	193	197	210	190	200	200	188	194
11	NB 612	220	200	210	200	91	146	201	182	192
12	NB 614	207	199	203	204	192	198	201	195	198
13	NB 616	212	201	207	200	189	195	196	183	190
14	NB 619	205	200	203	200	180	190	191	183	187
15	NB 624	218	209	214	220	204	212	196	181	189
16	NB 627	203	194	199	180	169	175	151	131	141
17	NB 645	185	178	182	201	180	191	211	200	206
18	NB 647	242	226	234	210	190	200	211	199	205
19	NB 648	197	184	191	208	189	199	206	197	202
20	NB 652	184	175	180	197	185	191	216	208	212
21	NB653	213	206	210	204	189	197	196	175	186
22	NB 654	203	191	197	189	177	183	196	187	192
23	NB 656	212	203	208	200	179	190	206	194	200
24	NB 657	234	228	231	201	189	195	191	179	185
25	NB 667	204	192	198	220	206	213	181	176	179
26	NB 670	246	229	238	189	177	183	191	177	184
27	NB 693	191	171	181	186	167	177	194	181	188
28	NB 714	183	170	177	179	163	171	186	181	184
29	NB 717	191	198	195	175	162	169	211	195	203
30	NB 718	200	186	193	184	188	186	206	194	200
31	NB 720	196	185	191	191	180	186	186	174	180
32	NB 732	198	187	193	201	190	196	191	184	188
33	NB 733	231	223	227	221	210	216	229	209	219
34	NB 734	186	174	180	186	175	181	226	210	218

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	Source	of Varia	tions	df	Days	to	Plan	t heigh	t	Plant	
					Maturity	7				height	
35	NB 737	221	263	242	221	20	4	213	216	5 196	206
36	NB 747	192	184	188	231	21	1	221	191	184	188
37	NB 782	173	167	170	196	17	6	186	181	169	175
38	NB 785	197	185	191	192	17	7	185	200	5 193	200
39	NB 788	183	171	177	171	16	0	166	170	5 164	170
40	NB 789	173	165	169	186	17	2	179	171	164	168
41	NB 797	210	199	205	166	15	5	161	186	5 170	178
42	NB 799	214	202	208	181	16	2	172	191	180	186
43	NB 803	194	177	186	166	14	5	156	176	5 160	168
44	NB 805	204	184	194	171	15	9	165	183	3 171	177
45	NB 809	183	167	175	166	14	9	158	200	) 180	190
46	NB 812	185	174	180	189	17	6	183	192	2 186	189
47	NB 815	182	171	177	171	15	9	165	176	5 167	172
48	NB 816	192	187	190	196	18	5	191	221	210	216
49	NB 826	189	180	185	181	16	6	174	181	169	175
50	NB 827	192	180	186	166	15	0	158	181	172	177
	Mean	203	193	198	194	17	9	187	198	3 186	192
	SD	17.2	19.94	18.1	16.1	24	.31	18.1	20.	4	20.1
		8		3	7			5	8	20.09	8
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## Variance analysis

## Character variance analysis

Table 3 displays the results of a statistical analysis of variation for 53 parental and 150 hybrid samples. There was a large amount of genetic diversity and difference between the entries, as seen

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Replicates	2	15.45	23.86	1.05
Treatments	202	69.82	2813.86	1.06
Parents	52	78.07	1075.33	1.90
Line	2	2.12	573.38	0.86
Testers	49	64.58	1070.84	1.65
Lines vs Testers	1	890.86	2299.30	16.31
Parents vs Crosses	1	6253.14	369740.18	0.29
Crosses	149	25.44	958.01	0.77
Line Effect	2	430.89	2599.81	7.90
Tester Effect	49	34.41	1721.88	0.95
Line x Tester Eff.	98	12.68	542.56	0.53
Error	404	0.16	0.6	0.82

the large amount of variance observed between the treatments. Except for 1000-grain weight, all other characteristics showed statistically significant variation across lines, testers, crosses, lines vs. testers, and parents vs. crosses.

## Analysis of variance for combining ability

Except for the influence on grain production, all other characteristics in the analysis of variance for combining ability showed statistically significant differences. All of the characters investigated had statistically significant interaction effects.

# Table 3: Nine characteristics of pearl millet were compared between the parents and the F1 hybrids

The results of integrating ability estimations of variances for several attributes. Non-additive genes had a role in all nine investigated traits, since the SCA variance was larger than the GCA variance. For all variables included in this analysis, dominance variance (2D) was shown to be larger than additive genetic variance (2A).

## Combining ability effects (gcaandsca)

Three lines and fifty testers' general combining ability (gca) effects and the specific combining ability (sca) impacts of 150 crossings across nine qualities are shown in tables. The effects of gca

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and sca are briefly described (in character order) below.

## Days to maturity

Lines with cytoplasm derived from A1 or A4 exhibited a negative gcaeffect, whereas lines derived from A5 showed a positive one. There was a statistically significant negative gcaeffect for NB580, NB590, NB670, NB718 and NB803, whereas a positive gcaeffect was seen for NB527, NB526, NB528 and NB619. 26 of the 50 tests found significant positive results, whereas 24 found significant negative results. Significant negative scaeffects for days to maturity were found in 23 crosses from the A1, 17 from the A4, and 21 from the A5 source. The most negative scaeffects were seen in the crosses ICMA05666 x NB619 (-5.78), ICMA07999 x NB667 (-4.73), ICMA05666 x NB530 (-3.68), and ICMA05666 x NB624 (-3.57).

## Plant height

Both the A1 and A4 cytoplasmic origins were positively associated with gca effects across all three lineages. When comparing to A4 sources, a negative gcaeffect was found. Fifty participants were tested, and twenty-five showed a significant positive gcaeffect and twenty-five showed a significant negative gcaeffect. The significant gcaeffect varied from - 22.95 (NB580) to 32.26 (NB528) percentage points among the test subjects. Good general-purpose blenders were determined to be testersNB528, NB527, NB733, NB647, NB530, and NB526.

A statistically significant scaeffect was found in 117 out of 150 crossings. Twenty A1 crossovers, twenty-seven A4 crosses, and seventeen A5 crosses showed statistically significant positive scaeffects. The most favourable scaeffect (32.28) was found in the cross ICMA 99444 x NB670. The cross ICMA07999 x NB627 had the largest negative sca impact (-28.45) and was thus the worst particular combining cross.

#### Productive tillers per plant

A1 had the most negative gcaeffect (-0.26) out of the three cytoplasmically different male sterile sources, whereas A4 and A5 had the largest positive gcaeffects (0.15 and 0.10). Twenty-two of the fifty testers showed a significant positive gca impact, whereas twenty-eight showed a significant negative gca effect. NB734 had the highest gcaeffect (0.74), whereas NB805 had the lowest (-0.74).

Twenty-two different cross combinations yielded statistically significant positive scaeffects for this character, eight of which included crossing A1 and A4, and six of which involved crossing A5. The greatest values were found in the CMA07999 x NB816 cross and the ICMA07999 x NB797 cross.

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Table 4: Pearl millet sca affects for nine character types over 30 crossings

Crosses	Days to Maturity	Plant height	Plant height
1.ICMA99444xNB525	-0.362	-0.38	0.097
2.ICMA99444xNB526	-0.582	-6.490	-0.126
3.ICMA99444xNB527	-1.026	-12.933	0.097
4.ICMA99444xNB528	-1.696	1.957	-0.292
5.ICMA99444xNB530	0.088	1.400	0.152
6.ICMA99444xNB580	0.304	13.177	0.374
7.ICMA99444xNB590	1.414	5.733	0.430
8.ICMA99444xNB595	-0.142	-4.603	-0.181
9.ICMA99444xNB600	-0.029	-8.603	-0.403
10.ICMA99444xNB611	0.088	-7.827	0.430
11.ICMA99444xNB612	-1.029	3.400	-0.237
12.ICMA99444xNB614	-0.472	-2.823	0.486
13.ICMA99444xNB616	-1.696	3.400	0.041
14.ICMA99444xNB619	1.304	8.397	0.041
15.ICMA99444xNB624	-0.802	8.507	-0.014
16.ICMA99444xNB627	-0.359	21.950	-0.348
17.ICMA99444xNB645	-1.249	-18.827	0.541
18.ICMA99444xNB647	-1.476	18.290	-0.07
19.ICMA99444xNB648	-1.579	-12.600	0.319
20.ICMA99444xNB652	-1.136	-13.160	-0.07
21.ICMA99444xNB653	-1.472	4.173	-0.181
22.ICMA99444xNB654	0.861	5.287	-0.292

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23.ICMA99444xNB656	3.084	2.400	-0.07
24.ICMA99444xNB657	2.754	20.060	0.263
25.ICMA99444xNB667	3.751	1.620	0.152
26.ICMA99444xNB670	1.974	32.287	0.041
27.ICMA99444xNB693	-0.469	-8.713	0.263
28.ICMA99444xNB714	-0.139	-4.710	0.263
29.ICMA99444xNB717	0.084	9.177	-0.403
30.ICMA99444xNB718	1.311	-12.380	-0.403

## Heterosis Studies

Tables show the results of the thirty hybrids in comparison to the average parental value (standard heterosis), the superior parental value (heterobeltiosis), and the mid-parental value (relative heterosis). Below is a summary, organised by trait, of the character-altering effects of heterosis.

## Days to maturity

Fifty hybrids were produced from each source, and substantial negative heterosis over MP for early maturity was seen in 43 hybrids from A1, 45 hybrids from A4, and 26 hybrids from A5. Source A4 (ICMA05666 x NB619) -12.69 and A1 (ICMA99444 x NB816) -9.38 were found to have very substantial and negative mid parent heterosis. Mid-parent heterosis was negative and statistically significant across all three sources in crosses when NB816 pollen parents were used. Heterosis performance was best on an A4 source (-4.81), next A1 (-3.89), and finally A5 (-1.28).

Table5: Percentage of heterosis predicted for days until maturity

Crosses	M	В	Ch	Crosses	M	В	Ch	Crosses	MP	В	Ch
	P	P	eck		P	P	eck			P	eck
ICMA9944	-	5.	-	ICMA9944	-	1.	-	ICMA0566	-	3.	-
4xNB525	2.	58	1.6	4xNB670	3.	72	5.2	6xNB525	5.03	51	5.5
	00		0		46		0				9
ICMA9944	ī	6.	-	ICMA9944	1	ı	-	ICMA0566	-	7.	1

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4xNB526	4.	44	0.7	4xNB693	5.	0.	7.6	6xNB526	4.48	46	1.9
	25		9		33	86	0				9
ICMA9944	-	6.	-	ICMA9944	-	0.	-	ICMA0566	-	7.	-
4xNB527	4.	87	0.4	4xNB714	3.	43	6.4	6xNB527	4.28	89	1.6
	05				11		0				0
ICMA9944	_	4.	_	ICMA9944		1.	_	ICMA0566	_	7.	
4xNB528			2.4	4xNB717	-		5.2			46	1.0
4XIND)28	3.	72		4XIND/1/	5.	72		6xNB528	2.19	40	1.9
	56		0		39		0				9
ICMA9944	-	5.	-	ICMA9944	-	0.	_	ICMA0566	-	0.	-
4xNB530	4.	15	1.9	4xNB718	7.	00	6.7	6xNB530	9.67	43	8.4
	29		9		54		9				0
ICMA9944	-	-	-	ICMA9944	-	-	-	ICMA0566	-	-	-
4xNB580	3.	2.	8.8	4xNB720	5.	1.	7.9	6xNB580	3.19	0.	9.1
	80	14	0		35	28	9			43	9
ICMA9944	_	_	_	ICMA9944	_	6.	_	ICMA0566	_	_	-
4xNB590	6.	0.	7.2	4xNB732	1.	87	0.4	6xNB590	12.4	5.	13.
IAIADJJO	46	43	0	IAI (D) JZ	78	3/	0.1	UAL AD J J U	2	70	99
	10	13			/ 0				2	/ 0	
ICMA9944	-	0.	-	ICMA9944	-	3.	-	ICMA0566	-	0.	-
4xNB595	4.	85	6.0	4xNB733	5.	87	3.1	6xNB595	5.74	88	7.9
	67		0		09		9				9

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ICMA9944	_	0.	_	ICMA9944	1.	2.	-	ICMA0566	_	_	-
4xNB600	4.	85	6.0			17		6xNB600	6.81		
4XIND000		0)		4xNB734	73	1/	5.5	OXINDOUU	0.81	0.	9.6
	09		0				9			88	0
ICMA9944	-	0.	-	ICMA9944	-	0.	-	ICMA0566	-	1.	-
4xNB611	5.	01	6.7	4xNB737	3.	43	6.4	6xNB611	5.13	31	7.6
	28		9		51		0				0
ICMA9944	-	1	-	ICMA9944	-	5.	1	ICMA0566	-	0.	1
4xNB612	5.	2.	8.8	4xNB747	2.	15	1.9	6xNB612	3.96	88	7.9
	79	14	0		77		9				9
					' '						
ICMA9944	-	_	-	ICMA9944	-	_	-	ICMA0566	-	1.	1
4xNB614	8.	0.	7.2	4xNB782	2.	1.	7.9	6xNB614	7.60	31	7.6
4XIND014				4XIND/02				OXIND014	7.00	31	
	13	43	0		95	28	9				0
ICM (A OO / /				ICM (A OO / /				101440566	0.07		
ICMA9944	-	0.	-	ICMA9944	-	6.	-	ICMA0566	0.84	6.	-
4xNB616	3.	85	6.0	4xNB785	1.	87	0.4	6xNB616		14	3.1
	10		0		00						9
TOLING				TO STORY				TOLINA			
ICMA9944	-	8.	1.2	ICMA9944	-	0.	-	ICMA0566	-	-	-
4xNB619	3.	58	0	4xNB788	2.	85	6.0	6xNB619	12.6	0.	9.1
	62				90		0		9	43	9
ICMA9944	-	2.	-	ICMA9944	-	-	-	ICMA0566	-	_	1
4xNB624	4.	58	4.3	4xNB789	4.	2.	9.1	6xNB624	8.69	0.	9.6
	40		9		01	57	9			88	$\begin{vmatrix} \cdot \cdot \cdot \\ 0 \end{vmatrix}$
	10				01	)/				00	

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ICMA9944	- -	_	-	ICMA9944	0.	2.	_	ICMA0566	_	0.	-
4xNB627	5.	2.	8.8	4xNB797	21	58	4.3	6xNB627	4.20	00	8.8
	20	14	0	,			9	,			0
ICMA9944	-	-	-	ICMA9944	-	0.	-	ICMA0566	-	0.	-
4xNB645	6.	0.	7.6	4xNB799	4.	85	6.0	6xNB645	5.74	88	7.9
	29	86	0		09		0				9
ICMA9944	-	0.	-	ICMA9944	-	-	-	ICMA0566	-	2.	-
4xNB647	5.	85	6.0	4xNB803	5.	0.	7.6	6xNB647	5.67	20	6.7
	82		0		52	86	0				9
				1015400//				1011110			
ICMA9944	-	1.	-	ICMA9944	-	-	-	ICMA0566	-	3.	-
4xNB648	5.	30	5.5	4xNB805	5.	0.	7.6	6xNB648	4.43	95	5.2
	78		9		33	86	0				0
ICMA9944	_	0.	_	ICMA9944	_	1.	_	ICMA0566	_	0.	-
4xNB652	5.	00	6.7	4xNB809	0.	72	5.2	6xNB652	5.35	88	7.9
4x1\100)2	09	00	9	4x1\10007	21	/ 2	0	OXINDO J2	).3)	00	9
	09		9		21		0				9
ICMA9944	-	0.	-	ICMA9944	-	2.	-	ICMA0566	_	2.	_
4xNB653	7.	43	6.4	4xNB812	1.	58	4.3	6xNB653	6.40	63	6.4
	33		0		85		9				0
ICMA9944	-	3.	-	ICMA9944	-	2.	-	ICMA0566	-	5.	-
4xNB654	2.	43	3.6	4xNB815	1.	58	4.3	6xNB654	1.84	70	3.6
	83		0		44		9				0

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ICMA9944	1.	5.	-	ICMA9944	-	-	-	ICMA0566	-	0.	-
4xNB656	44	58	1.6	4xNB816	9.	0.	7.2	6xNB656	5.00	00	8.8
			0		38	43	0				0
ICMA9944	-	7.	0.4	ICMA9944	-	-	-	ICMA0566	-	4.	-
4xNB657	4.	73	1	4xNB826	3.	1.	8.4	6xNB657	8.64	38	4.8
	56				99	72	0				0
ICMA9944	1.	8.	0.8	ICMA9944	-	5.	-	ICMA0566	-	4.	-
4xNB667	20	15	0	4xNB827	2.	58	1.6	6xNB667	3.04	83	4.3
					96		0				9
				Mean	-	1.	-				
					3.	96	4.9				
					89		9				

Midparent heterosis was found to have an extent of between -12.69 (ICMA0566A4 xNB619) and 4.22 (ICMA07999 xNB624) percent. There was considerable midparent heterosis in 36 of the 150 crossings, early maturity heterosis in 114, and late maturity heterosis in the remaining 36. Heterosis between the superior parents ranged from -16.70% (ICMA05666 x NB590) to 16.20% (ICMA07999 x NB619). Significant heterosis over check was seen in 144 crosses, while heterosis in the opposite direction was seen in 136 crosses.

## Plant height

The hybrids have considerable positive mid-parent heterosis across all three cytoplasmic sources. After A5 (ICMA07999xNB 527) and A4 (I CMA05666xNB611), the hybrid (ICMA99444xNB670) on A1 source reported positive and much increased heterosis.

Table shows that A1 (52.0) has the greatest mean mid-parent heterosis, followed by A4 (50.72) and A5 (40.03). From 9.75 percent (ICMA07999 x NB580) to 90.14 percent (ICMA99444 x NB670), the heterosis over the midparent varied. From -3.97 percent (ICMA07999xNB657) to 83.25 percent (ICMA99444 xNB670), the range of superior parents was wide. The ICMA07999 x NB527 cross showed the highest levels of heterosis (41.27 percent) compared to the gold standard check (86M64).

## Productive tillers per plant

Six hybrids exhibited strong and positive mid-parental heterosis, but 68 hybrids passed the

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conventional hybridization test, out of a total of 150 hybrids. Sources A4 (ICMA05666 x NB737) and A5 (ICMA07999 x NB627) showed quite substantial positive heterosis.

Table 6: Height heterogeneity (in %) estimate

Crosses	M	BP	Che	Crosses	M	BP	Ch	Crosses	M	BP	Che
	P		ck		P		eck		P		ck
ICMA9944	59	53	16.	ICMA9944	90	83	37.	ICMA0566	47	36.7	3.9
4xNB525	.7	.0	32	4xNB670	.1	.2	52	6xNB525	.5	9	4
	9	9			4	5			4		
ICMA9944	64	54	21.	ICMA9944	35	23	3.3	ICMA0566	62	48.5	17.
4xNB526	.3	.7	95	4xNB693	.0	.8	8	6xNB526	.9	7	07
	5	6			5	3			3		
ICMA9944	76	75	21.	ICMA9944	42	37	2.8	ICMA0566	76	71.6	18.
4xNB527	.1	.2	95	4xNB714	.1	.0	2	6xNB527	.7	7	20
	5	0			6	1			2		
ICMA9944	73	58	33.	ICMA9944	47	29	19.	ICMA0566	59	41.3	18.
4xNB528	.3	.4	21	4xNB717	.7	.8	32	6xNB528	.4	0	76
	8	9			4	0			5		
ICMA9944	63	47	27.	ICMA9944	37	23	7.6	ICMA0566	49	31.0	13.
4xNB530	.6	.8	58	4xNB718	.3	.4	9	6xNB530	.6	9	13
	6	3			2	4			3		
ICMA9944	49	43	8.4	ICMA9944	35	19	9.0	ICMA0566	46	35.6	2.8
4xNB580	.1	.0	4	4xNB720	.7	.7	1	6xNB580	.1	4	2
	6	7			5	9			4		
ICMA9944	52	42	14.	ICMA9944	45	33	10.	ICMA0566	46	32.3	6.0
4xNB590	.3	.3	07	4xNB732	.1	.9	32	6xNB590	.1	2	0
	8	9			9	4			8		
ICMA9944	57	56	10.	ICMA9944	39	12	27.	ICMA0566	52	46.2	3.1
4xNB595	.6	.6	50	4xNB733	.5	.5	77	6xNB595	.3	8	9
	9	5			5	6			6		
ICMA9944	56	56	8.6	ICMA9944	36	26	2.2	ICMA0566	69	63.7	13.
4xNB600	.2	.0	3	4xNB734	.0	.7	5	6xNB600	.2	9	70
	8	6			8	5			8		

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ICMA9944	66	61	12.	ICMA9944	58	47	19.	ICMA0566	82	81.4	19.
4xNB611	.7	.9	76	4xNB737	.5	.6	14	6xNB611	.4	3	14
	1	9			5	8			7		
ICMA9944	60	47	21.	ICMA9944	42	33	7.3	ICMA0566	60	43.1	18.
4xNB612	.2	.7	95	4xNB747	.8	.0	2	6xNB612	.3	8	20
	9	3			2	3			1		
ICMA9944	69	68	17.	ICMA9944	36	31	-	ICMA0566	75	70.9	17.
4xNB614	.5	.1	07	4xNB782	.8	.1	0.3	6xNB614	.5	5	07
	6	9			5	1	8		3		
ICMA9944	60	52	18.	ICMA9944	51	47	9.1	ICMA0566	57	44.5	12.
4xNB616	.8	.3	58	4xNB785	.9	.3	9	6xNB616	.6	8	57
	2	0			5	4			9		
ICMA9944	59	48	20.	ICMA9944	38	31	2.2	ICMA0566	53	38.3	11.
4xNB619	.8	.8	07	4xNB788	.6	.3	5	6xNB619	.3	7	63
	0	4			8	3			5		
ICMA9944	72	68	23.	ICMA9944	25	12	-	ICMA0566	65	56.4	14.
4xNB624	.9	.7	45	4xNB789	.2	.7	1.8	6xNB624	.7	1	44
	3	2			6	1	8		6		
ICMA9944	59	54	15.	ICMA9944	45	27	17.	ICMA0566	46	36.2	2.2
4xNB627	.7	.0	57	4xNB797	.1	.5	26	6xNB627	.1	6	5
	9	0			8	5			2		
ICMA9944	51	49	3.7	ICMA9944	43	23	18.	ICMA0566	70	67.2	12.
4xNB645	.2	.0	5	4xNB799	.1	.1	95	6xNB645	.5	2	95
	9	5			1	0			4		
ICMA9944	78	61	38.	ICMA9944	49	43	8.8	ICMA0566	59	40.6	20.
4xNB647	.4	.9	28	4xNB803	.4	.2	2	6xNB647	.8	5	07
	5	8			8	1			0		
ICMA9944	50	44	9.1	ICMA9944	57	52	13.	ICMA0566	64	52.4	15.
4xNB648	.1	.0	9	4xNB805	.7	.9	32	6xNB648	.2	7	57
	9	6			0	0			6		
ICMA9944	44	35	7.6	ICMA9944	29	14	3.7	ICMA0566	54	40.4	12.
4xNB652	.2	.0	9	4xNB809	.3	.2	5	6xNB652	.8	7	01
	1	5			5	6			6		

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ICMA9944	49	31	20.	ICMA9944	42	36	4.6	ICMA0566	48	26.5	16.
4xNB653	.5	.4	64	4xNB812	.8	.0	9	6xNB653	.2	8	13
	3	9			9	9			6		
ICMA9944	70	66	15.	ICMA9944	35	25	2.2	ICMA0566	61	59.7	5.8
4xNB654	.7	.5	95	4xNB815	.2	.2	5	6xNB654	.3	7	2
	1	7			4	9			7		
ICMA9944	58	48	18.	ICMA9944	23	2.	8.2	ICMA0566	56	41.8	13.
4xNB656	.7	.7	58	4xNB816	.2	12	5	6xNB656	.4	8	13
	9	0			9				2		
1011100//		1.0	2.1	1011100//	16		/ 1	101110566	20	0.00	- /
ICMA9944	44	16	31.	ICMA9944	46	44	4.1	ICMA0566	28	0.99	14.
4xNB657	.0	.1	89	4xNB826	.8	.1	3	6xNB657	.5		64
	5	9			3	6			0		
ICMA9944	65	64	14.	ICMA9944	48	43	7.3	ICMA0566	78	72.9	20.
4xNB667	.1	.9	82	4xNB827	.3	.0	2	6xNB667	.7	7	07
	8	6			8	1			7		
				Mean	52	42	13.				
					.0	.2	95				
						5					

The average of the six hybrids was much higher than the average of either parent. None of the hybrids showed a substantial heterosis in the superior parent's direction. The CMA05666xNB737 cross showed the greatest amount of heterosis (38.46 percent). Significant positive heterosis was found in 68 cross-checks.

#### Contribution of lines, tester and their interactions

In table we see the nine characters, the lines they contributed, the testers that worked on them, and the relationships between them. The findings showed that the contribution of line was highest for the number of days it took to reach 50% blooming and 100% maturity. The testers' greatest input was for the plant's height, followed by the panicle's diameter and finally its length. Panicle weight, grain production per plant, 1000 grain weight, panicle girth, and productive tiller count per plant were all significantly influenced by the L x T interaction.

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Table 7: Lines', testers', and interactions' contributions

S		Contributed by					
No.	Characters	Lines (%)	Testers (%)	Interaction (LxT)(%)			
1	Days toMaturity	22.73	44.47	32.79			
2	Plantheight (cm)	3.64	59.11	37.25			
3	Productivetillersperplant	13.77	40.59	45.63			

#### Conclusion

In order to increase the seed set % on various sources of male sterile lines, it was recommended that the population improvement programme be initiated based on the findings of the restoration investigations. Better 'B' and 'R' lines on various cytoplasmic sources may be created by using divergent pairings in different groups. Hybrids developed from the A4 source may be safely employed for commercial production, and the restoration development project based on the A4 source should be enhanced. Alloplasmic and isonuclear hybrids may be created by crossing common restorers on these different sources, and they can be used to study the impact of cytoplasm on different phenotypes. It is possible to test the consistency of the A4 source's productive crosses by growing them in different environments and at different times of year.

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