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Determination of Parents and Cross Combinations for Rice Hybrids via Line 'X' Tester Analysis

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ABSTRACT: *Twenty five hybrid combinations were produced using five CMS lines (BgCMS4A, IR68902A, IR70369A, IR68902A, IR78354A) and five restorers R147(IR72998-93-3-3-2R), R156(IR73885-1-4-3-2-1-10R), R317(IR183325-66-2-1-NPT), R160(IR75282-58-1-2-3), SN290 10-2071 following Line x Tester mating design. Twenty five cross combinations, ten parents and three standard checks namelyBg300, Bg 357 and Bg379-2 were field evaluated in Maha2014/2015 and Yala 2015 at Rice Research and Development Institute, Batalagoda to find out the better parents having high general combining ability (GCA) and, good hybrid combinations having high specific combining ability (SCA). BgCMS4A was identified as the best tester for the grain yield and has also shown significantly higher SCA for Y/P in one of the cross combinations. Number of plants per hill (P/H) has highly contributed to achieve high grain yield of BgCMS4A. IR78354A also could be considered as a comparatively better tester as it has recorded significantly higher SCA for Y/P and P/H in one of the cross combinations. IR70369A also was a tester that showed significantly higher GCA for TGW in both seasons. The crosses 22(IR78354A/IR73885-1-4-3-2-1-10R) and 2(BgCMS4A/IR73885-1-4-3-2-1-10R) were identified as the best cross combinations to be used as hybrids in the low country intermediate zone.*

Keywords: *Heterosis, GCA, SCA, Line, Tester, Genotype, Hybrid Rice*

INTRODUCTION

Rice is one of the world's most important food crops. More than half of the world's population, especially in South and Southeast Asia, depends on rice for calories and proteins. About ninety percent of all rice in the world is produced and consumed in Asia where 70% of the world's poor live (Maclean et al., 2002). Thus Asia is the rice production center in the world which determines the future of the global rice production. The population growth rate in Asia is relatively higher than the rate of increase in average rice yield (Dawe, 2007). By the year 2025, about 758 million tons of rough rice which is 70% more than current production will be needed to meet the growing demand in future. Development of high yielding varieties having multiple resistances to biotic and a- biotic stresses is important to cater to the present and future changing environment. Hybrid rice technology, formulated in China, assures the rice farmers with an increased yield over improved conventional varieties by 15 to 20% and thus, is one of the options to enhance the productivity (Tran, 2002) and income. Hybrid rice area is now rapidly increasing outside China as well as China released

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the first set of hybrids in 1976 (Yaun, 1998). Presently, many countries other than China, Vietnam, India, Indonesia, Iran, Philippines, United States of America, Bangladesh, Sri Lanka, Myanmar and Egypt are engaging to develop hybrid rice technology to suit their local conditions (Dorosti, 2014). In Sri Lanka,

the research and development (R&D) programme on hybrid rice began in late 1994 at the Rice Research & Development Institute (RRDI) and its breeders have been able to identify several hybrids with 1.0-1.5t/h yield advantage over the best inbred grown under similar environments (Iqbal, 2009).

However, limited availability of better Parents, Cytoplasmic Genetic Male Sterile lines (CMS), Maintainers (B) and Restorers (R) of hybrid rice programme was one of the constraints at present to produce good hybrids and it directly affects the strength of the hybrid rice development programme. Identification of superior parental lines, having high general combining ability (GCA) to develop better hybrid combinations with high specific combining ability (SCA) and standard heterosis (SH) is important to overcome the above constrains. Therefore, the objective of the present study was to identify better parental lines and superior hybrid combinations.

MATERIALS AND METHODS

a).Experimental Methods and Design:

The study was carried out at the Rice Research and Development Institute (RRDI), Batalagoda in the Low Country Intermediate Zone of Sri Lanka over two consecutive seasons, namely Maha 2014/2015 and Yala 2015. The experiment comprised 25 hybrid combinations developed by crossing five CMS lines with five elite restorers following the Line x Tester mating design. All 38 entries: Crosses, Lines (Restorers), Maintainers (testers), representing CMS and three standard checks in 3, 3 ½, and 4 months maturity groups are presented in Table 1.

Table 1. Cross combinations, Testers, Lines and Standard checks, used in the experiment

Entries		
1.BgCMS4A/ IR72998-93-3-3	14.IR70369A/IR75282-58-1-2-3	27.IR68902A
2.BgCMS4A/IR73885-1-4-3-2-1	15.IR70369A/SN10-2071	28.IR70369A
3.BgCMS4A/IR183325-66-2-1	16.IR78356A/IR729998-93-3-3-2R	29.IR78356A
4.BgCMS4A/IR75282-58-1-2-3	17.IR78356A/IR73885-1-4-3-2-1	30.IR78354A
5.BgCMS4A/SN10-2071	18.IR78356A/IR183325-66-2-1	31.R147IR72998-93-3-3-2R
6.IR68902A/IR72999-93-3-3-2R	19.IR78356A/IR75282-58-1-2-3	32.R156 (IR73885-1-4-3-2-1)
7.IR68902A/IR73885-1-4-3-2-1	20.IR78356A/SN10-2071	33.R317 (IR183325-66-2-1)
8.IR68902A/IR183325-66-2-1(NPT)	21.IR78354A/IR72998-93-3-3-2R	34.R160 (IR75282-58-1-2-3)
9.IR68902A/IR75282-58-1-2-3	22.IR78354A/IR73885-1-4-3-2-1	35.SN29010-2071
10.IR68902/SN10-2071	23.IR78354A/IR183325-66-2-1	36.Bg 300 (3month)
11.IR70369A/IR72998-93-3-3-2R	24.IR78354A/IR75282-58-1-2-3	37.Bg357 (3 1/2 month)
12.IR70369A/IR73885-1-4-3-2-1	25.IR78354A/SN10-2071	38.Bg379-2 (4 month)
13.IR70369A/IR183325-66-2-1(NPT)	26.BgCMS4A	

1- 25 Cross combinations, 26 – 30 Testers, 31 -35 Lines and 36-38 Standard checks

Experiment was established in in a Randomized Complete Block Design (RCBD) with two replications in each season. All entries (treatments) were planted in three raw progenies. Each progeny included 60 individuals (20 plants per line with one plant per hill) and spacing within plants and between lines were 15cm and 20cm, respectively. Spacing between two progenies was 40cm. All cultural practices were applied according to the recommendations of the Department of Agriculture (DOA).

b) Data recording:

Data were collected from ten plants randomly selected from middle raw of the progeny of the each treatment from maximum tilling stage to harvesting stage. Grain yield per plant(Y/P), panicles per hill (P/H), filled grain per panicle (FG/P), 1000 grain weight (TGW) were recorded.

c) Data analyses:

Line x Tester analysis was performed for all the characteristics studied. Data were analyzed using ANOVA procedure. When the interactions were present they were further studied using response curves and bar charts. The statistical analyses were done using statistical package software SAS 9.1.

RESULTS AND DISCUSSION

a) Grain yield:

Analysis of variance for grain yield/plant (Y/P) over seasons for genotypes, testers, lines and crosses and interactions of above traits is presented in Table2.

Table 2. Line x Tester analysis of variance for grain yield/plant (Y/P) for Genotypes, Testers, Lines and crosses over seasons

Source of Variance (SV)	Degree of freedom (df)	Y/P Means sum square (MS)
Total	139	
Season(S)	1	1569.19**
Reps/S	2	179.26**
Genotypes(G)	34	102.78**
Parents(P)	9	281.98**
P Vs Crosses	1	148.17
Crosses(C)	24	33.69*
Testers(T)	4	86.31**
Lines(L)	4	38.37ns
L x T	16	19.37ns
G x S	34	50.64*
P x S	9	105.3ns

P Vs C x S	1	1.45ns
C x S	24	32.19*
T x S	4	37.67ns
L x S	4	50.19*
L x T x S	16	26.32ns
Pooled error	**68**	**30.22**

** Significant at 1% probability level   *Significant at 5% probability level

Genotypes (G) and parents (P) had significant (p=0.01) differences for grain yield per plant. Crosses also showed significant (p=0.05) differences for grain yield per plant. P x S interaction effect was not significant but C x S interaction effect was significant and accordingly, T x S and L x S interaction effects were investigated. However, within crosses T x S interaction effect was not significant and L x S interaction effect was significant (p=0.05) while L x T x S three way and L x T two way interaction effects were not significant, thus comparing among average effects of testers over lines and seasons was valid. Average grain yield of testers over seasons are presented in Table 3. Tester 26 was the best and all the other testers were similar in grain yield/plant.

Table 3. Average grain yield of testers over seasons.

Tester	Grain yield/plant (g)
26 -BgCMS4A	39.18a
27-IR68902A	19.16b
28-IR70369A	13.09b
29 -IR78359A	11.31b
30-IR78354A	19.18b
CV %	46.65
LSD	14.65

As the two way interaction effect of Line x season was significant it was further studied using response curves. Grain yield response of different lines to Maha and Yala seasons is shown in Fig.1

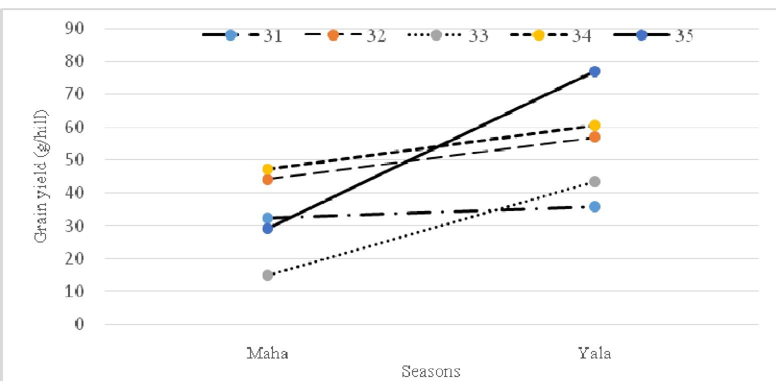


Fig 1. Grain yield response of different lines in Maha and Yala seasons.

Line 34 and 32 were stable over seasons with a higher yield level thus they can be identified as the best restores. The line 35 was highly unstable over seasons and line 33 was also unstable. Though line 31 gave poor yield it was stable over season. Accordingly, these three lines cannot be identified as good restores with respect to grain yield per plant.

Parents vs. crosses were not having significant differences for grain yield per plant. Therefore, in general, crosses have not been effective or vigorous. However C x S interaction effect was significant while P vs. C x S was not significant. Thus, C x S interaction effect

was further studied using a bar chart to improve clarity which can not be expressed in response curves due to improvement of large numbers of cross combinations (Fig. 2).

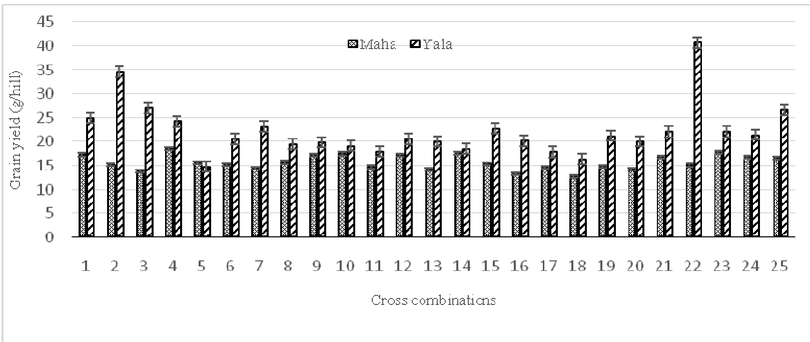


Fig 2. Grain yield response of crosses in Maha and Yala seasons.

All the crosses have given similar grain yields among them in Maha but in Yala season significant differences could be found. Cross combinations 22 and 2 gave higher grain yields in Yala season than others and, consequently those two cross combinations could be identified as the best performing crosses in the experiment. Restorer of the above two cross combinations was 32 which was identified as one of the best restores. BgCMS4A had shown significantly higher grain yield than other testers (Table 3) and it has contributed to produce the high yielding cross 2. Although the testerIR78354A was found to be similar in yield to other testers, it contributed to produce significantly high yielding cross 22 in Yala season. Therefore, tester 26 and 30 could be found as the best testers based on their performance.

Line x tester interaction effect was not significant indicating that all the lines combined equally with all the testers hence specific line for pacific tester could not be identified.

b) Yield components:

Line x tester analyses of variance for panicles/hill (P/H), filled grains/panicle (FG/P) and 1000 Grain weight (TGW) over season are presented in Table 4. Genotype were significant at (p=0.01) level for P/H, FG/P and TGW indicating that there were significant differences among genotypes for P/H, FG/P and TGW.

Table 4. Line x Tester analysis of variance for panicles /hill, filled grains/panicle and 1000 grain weight over seasons

SV	df	P/H MS	FG/P MS	TGW(g) MS
Total	139			
Season(S)	1	27.19**	35065.98**	69.86**
Reps/S	2	3.48ns	2122.38**	0.57ns
Genotypes(G)	34	9.86**	1605.13**	24.98**
Parents(P)	9	22.90**	1919.04**	51.67**
P Vs Crosses	1	23.25	16431.58	96.19
Crosses (c)	24	4.11**	869.65**	12.0**
Testers(T)	4	11.01**	1438.79**	52.07**
Lines(L)	4	5.23**	2181.22**	11.41*
L x T	16	2.56**	399.46ns	2.13ns
G x S	34	3.08**	543.63ns	3.14ns
P x S	9	4.49ns	424.41ns	5.96ns
P Vs C x S	1	18.79	18.79	0.07
C x S	24	1.89*	765.17*	2.22ns
T x S	4	2.61*	706.36ns	6.86ns
L x S	4	3.57**	1786.81**	2.06ns
L x T x S	16	1.30ns	524.46ns	3.35ns
Pooled error	**68**	**1.23**	**343.32**	**3.92**

*Significant at 5% probability level **Significant at 1% probability level

Both parents and crosses also exhibited significant differences (p=0.01) for P/H, FG/P and TGW. indicating that these were highly heritable traits.

P x S two way interaction effect was found to be not significant for P/H, FG/P and TGW so that all the parents responded evenly over seasons. As C x S interaction effect was significant for number of P/H and FG/P, T x S and L x S interaction effects were looked into. T x S interaction effect was found to be significant (p=0.05) only for P/H but not for FG/P and TGW. L x S interaction effect was significant (p=0.01) only for P/H and FG/P. In addition, L x T x S three way interaction effect was not significant for all the yield components.

The responses of testers to different seasons are presented in Fig.3. Tester 26 and 30 appeared unstable over seasons. However, these two testers had given significantly higher P/H in both seasons so that instability of these testers can be disregarded. Other three testers

were stable over seasons. Out of these three testers, tester 28 was found to be giving the lowest P/H

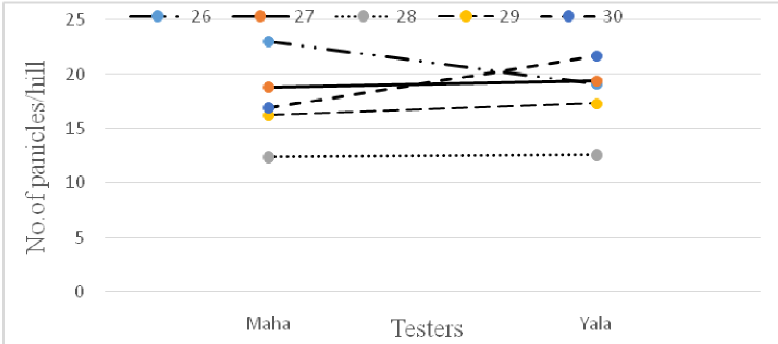


Fig 3. Response of testers for panicles/hill (P/H) in Maha and Yala seasons.

The responses of lines in different seasons are presented in Fig.4. All the lines have given similar P/H in Maha season while in Yala season significant differences could be found among lines. The line 31 was unstable while other four lines were stable over seasons. However, the line 31 became unstable just because it had given significantly higher P/H in Yala season while it had given similar P/H to other lines in Maha season and therefore, line 31is not favorable due to its instability.

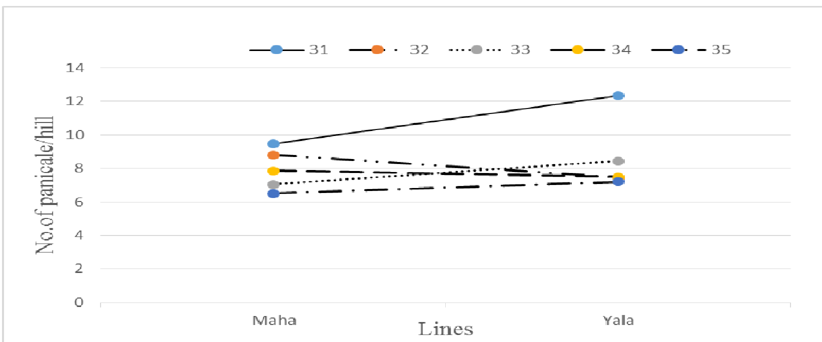
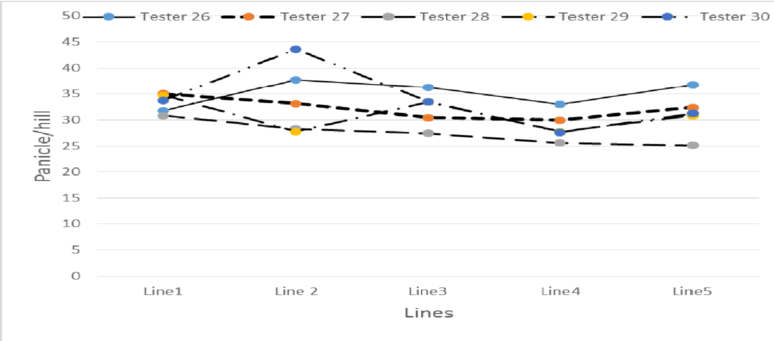


Fig 4. Response of lines for panicles/hill in Maha and Yala seasons.

L x T interaction effects also were significant (p=0.05). Although T x S two way interaction was found to be significant (p=0.05) and L x S and L x T interaction effects were significant (p=0.01), the three way interaction effect of L x T x S was not significant. Thus averaging P/H over seasons to study L x T interaction was valid and it is presented in Fg.5.



Line1-31.R147IR72998-98-3-3-2R**Line2**-32.IR73885-1-4-3-2-1 **Line3**-33.IR183325-66-2-1
Line 4-34. R160IR75282-58-1-2-3 **Line 5** -35.SN290-2071

Fig 5. Response of testers with lines for P/H.

All the testers had given similar P/H with line 1. A similar response was also found with line 3 and line 4**.** However, testers showed significant differences when crossed with line 2 and 5.

Tester 26 produced high P/H with all the lines except line 1 so that tester 26 could be considered as a better tester for P/H. In addition tester 30 produced higher P/H with line 2 and, accordingly tester 30 could be considered as a specific tester to hybridize with line 2 to achieve maximum P/H.

As L x S interaction effect was significant (p=0.01) for FG/P it is presented in Fig. 6. All lines were unstable for FG/P over seasons but line 35 and 34 showed high FG/P than other lines in both seasons thus, these two lines could be considered as the best restores for FG/P.

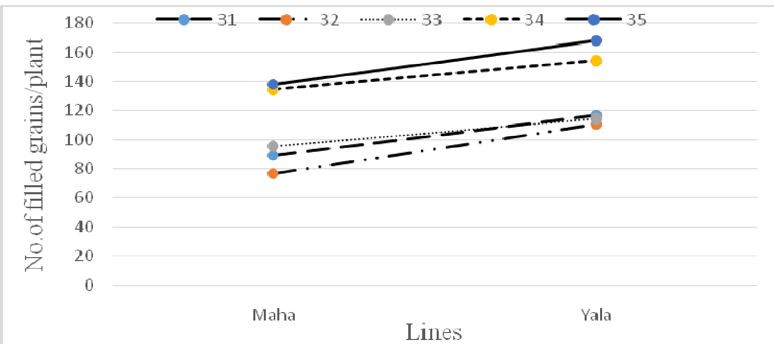


Fig 6. Response of lines for FG/P in Maha and Yala seasons.

C x S interaction was significant (p=0.05) for P/H and FG/P and they were further studied using bar chart. Response of crosses to different seasons for P/H is presented in Fig.7. Cross combination 22 produced higher P/H than others in Yala season and it has ability to produce higher yield than other crosses in Yala season. The C x S interaction response curve for grain yield indicates this effect clearly (Fig 2). Thus, P/H had highly contributed to improve grain yield in cross 22 in Yala season.

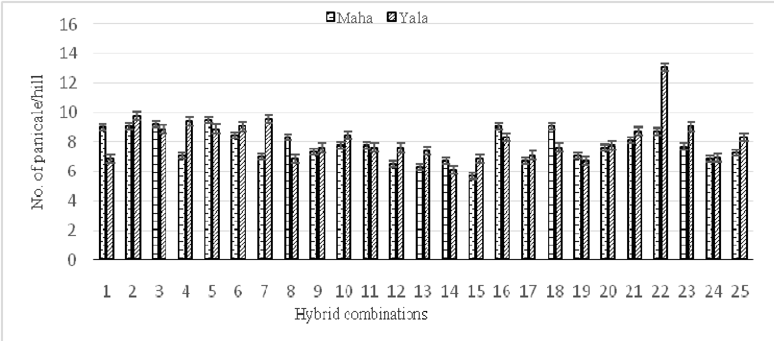


Fig 7. Response of crosses for P/H in Maha and Yala seasons.

Response of cross combinations for FG/P in different seasons is presented in Fig.8.Although cross 9 has recorded the highest FG/P in Yala season, it has not performed well in Maha season. However, crosses 15 and 10 have performed well over both seasons showing high stability over seasons. Therefore, these two cross combinations could be selected as the best with respect to FG/P. However, these two cross combination did not contribute to overall high grain yield because they had recorded comparatively less F/G. Cross combination 2 and 22 had recorded the highest grain yield per hill. However their FG/P was low while recording the highest P/H.

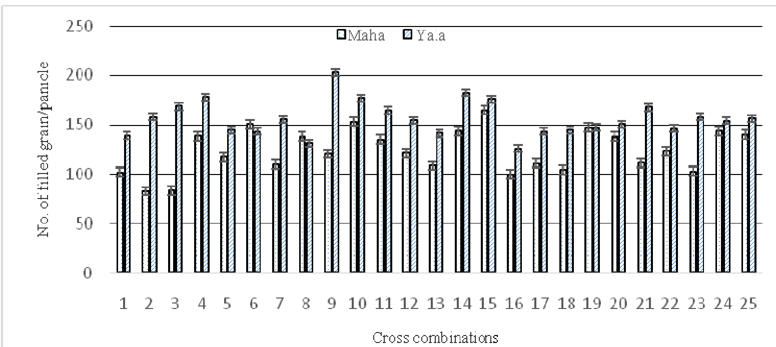


Fig 8. Response of crosses for FG/P in Maha and Yala seasons

No interaction effect with season was significant (p=0.05) with respect to TGW for testers as well as lines and accordingly average main effects were studied. Testers and lines had significant difference for TGW. TGW of testers and lines are presented in Table 5 and Table 6, respectively. Tester 28 had the highest TGW. Therefore, tester 28 could be considered as the best tester with respect to TGW. The tester 30 showed the second highest TGW. Line34 and 35showed the highest TGW and they could be considered as better lines for TGW.

Table 5. Average TGW of testers over seasons.

Tester	TGW (g)
26-BgCMS4A	26.2bc
27-IR68902A	24.57c

28-IR70369A	28.87a
29-IR78356A	25.52c
30-IR78354A	26.80b
CV %	3.89
LSD	0.93

Table 6. Average TGW of lines over seasons.

Line	TGW (g)
31-R147(IR72998-98-3-3-2R)	25.21c
32-R156 (IR73885-1-4-3-2-1)	25.77bc
33-R317 (IR183325-66-2-1)	26.55ab
34-R160 (IR75282-58-1-2-3)	27.46a
35-SN290 10-2071	26.96a
CV %	3.89
LSD	0.93

No interaction effect with season was significant (p=0.05) with respect to TGW for crosses and accordingly average main effects were studied. Crosses were significant for TGW. Only the TGW of cross combinations that are only significantly higher are presented in Table.7. Cross combinations derived from tester 28 and five lines in the test have recorded higher values for TGW.

Table 7. Average TGW of crosses over seasons.

Crosses		
Tester	**Lines**	**TGW (g)**
28-IR70369A	31-R147(IR72998-93-3-3-2R)	27.6bc
	32-R156(IR73885-1-4-3-2-1)	28.2ab
	33-R317(IR183325-66-2-1)	29.67a
	34-R160(IR75282-58-1-2-3)	29.65a
	35-SN290 10-2071	29.22a
	CV %	**3.98**
	LSD	**2.17**

GCA and SCA effects:

As interaction effects were significant for all the characteristics studied including grain yield, estimation of GCA for testers and lines were not done. However, as TGW was the only exception where interaction effects were not found, GCA effect for lines and testers for TGW was calculated (Table 8).

The tester 28 had the highest (positive) GCA while the tester 27 had the lowest GCA (negative) for TGW. The line 34 showed highest GCA (positive) for TGW. All the other had non-significant values for TGW. Assessing the GCA of parental lines is extremely useful in a hybrid breeding programme, particularly when a large number of positive parental lines and the most promising ones to be identified on the basis of their ability to give superior hybrids are available (Virmani et al., 1997).

Table 8. GCA for 1000 grain weight of lines and testers in Maha and Yala Seasons

Genotype	GCA for TGW (g)	
	Maha 2014/2015	Yala 2015
Tester (Female)		
26-BgCMS4A	0.4	-0.77
27-IR68902A	-2.01*	-1.55
28-IR70369A	2.76*	2.28**
29-IR78356A	-0.96	-0.7
30-IR78354A	-0.19	0.75*
SE	**0.6**	**0.46**
Line (Male)		
31-R147 (IR72998-93-3-3-2R)	-1.28	-1.06
32-R156 (IR73885-1-4-3-2-1)	-0.99	-0.19
33-R317 (IR183325-66-2-1)	0.22	-0.05
34-R160 (IR75282-58-1-2-3)	1.78*	1.04*
35-SN290 10-2071	0.87	0.27
SE	**0.37**	**0.71**

**Significant at 1% probability level, * Significant at 5% probability level
TWG- Thousand Grain Weight, SE-Standard error, GCA-General Combining Ability

SCA effect is the index to determine the usefulness of a particular cross combination in the exploitation of heterosis (Dhaliwal and Sharma, 1990). SCA effects of cross combinations for yield and yield related traits in Maha and Yala seasons are presented Table 9. SCA of all cross combinations were not significant for Y/P, P/H and TGW in Maha season. Cross combination 18 has shown significantly higher SCA for FG/P and indicated that this specific combination has increased FG/P in Maha season.

Crosse combination 22 and 2 had significantly high (p=0.05) SCA than others in Yala season with respect to Y/P. In addition, SCA of cross 22 was significant for (p=0.01) P/H in Yala season though all cross combinations were not significantly different in Maha season. Cross combination 2 and 22were identified as the best cross combinations with significantly positive SCA in the test. High SCA of cross combination 22 for grain yield is well supported by its high SCA for P/H.

Table 9. SCA of tested cross combinations for yield and yield related traits in Maha2014/2015and Yala 2015 seasons

Cross	YPP		P/H		FG/P		TGW	
Combinations	Maha	Yala	Maha	Yala	Maha	Yala	Maha	Yala
1	1.41	1.04	-0.5	-1.84**	0.65	-10.45	-0.454	-0.79
2	-0.49	7.63*	0.45	-0.24	-8.41	5.34	-1.444	0.19
3	-1.39	2.52	0.09	0.32	-5.67	18.71	-0.604	1.2
4	1.21	0.12	-0.95	1.47	18.3	3.83	2.236	12.82*
5	-0.73	3.7	0.89	0.28	-6.59	-17.33	0.266	11.99*
6	-0.54	1.71	-0.04	0.81	19.69	-10.43	0.661	0.34
7	-1.19	-3.51	-0.64	0.01	-10.97	-1.45	0.571	-0.03
8	0.55	-0.38	0.2	-1.23	19.27	-23.07	-11.288	0.33
9	-0.11	0.46	0.31	0.12	-28.96	24.55*	-1.399	-0.51
10	1.29	1.14	0.34	0.28	-0.75	10.09	-1.344	-0.14
11	-0.87	-0.82	0.44	0.59	4.01	9.07	-0.259	0.06
12	1.77	-5.68	0.09	-0.76	0.15	-3.95	0.051	-0.21
13	-0.73	0.67	-0.67	0.5	-10.21	-14.67	1.941	12.47*
14	0.36	-0.44	0.84	-0.2	-6.44	1.95	-0.769	0.11
15	-0.54	5.34	-0.72	-0.14	10.77	7.69	-0.964	0.53
16	-0.35	2.28	0.42	0.85	-69.65*	-7.71	14.01	-0.36
17	1.03	-7.61*	-1.03	-1.65**	6.46	5.69	-0.479	0.77
18	-0.43	-2.17	0.76	0.36	59.65*	9.77	-0.889	-1.32
19	-0.36	3.13	-0.08	0.06	13.42	-11.41	-0.149	0.29
20	0.11	3.45	-0.09	0.37	0.18	3.33	1.106	0.61
21	0.36	-3.22	-0.34	-0.42	-15.23	19.59	-0.359	0.74
22	-1.12	7.81*	1.11	2.63**	12.85	-7.04	1.301	-5.48*
23	2	-3.86	-0.4	0.04	-5.25	9.35	-1.959	0.28
24	-1.1	-4.17	-0.14	-1.46**	3.76	-18.83	0.081	-0.01
25	-0.13	2.51	-0.25	-0.8	-3.53	-4.49	0.936	-0.29
SE	**0.19**	**0.77**	**0.12**	**2.5**	**4.39**	**0.25**	**0.74**	**1.43**

** Significant at 1% probability level, *Significant at 5% probability level,

Y/P- Yield per plant, FG/P- Fill grain per plant, P/H- Panicle per hill, TWG- Thousand Grain Weight

Standard Heterosis (SH):

SH of cross combinations giving significantly high SCA values for grain yield were calculated against standard checks and are presented Table10. Cross combinations 2 and 22 showed well over 70% higher SH compared to all standard checks in Yala season.

Table.10. Standard heterosis of selected specific crosses.

Cross combinations	SHBg 300		SH Bg 357		SH Bg 379-2	
	Maha	Yala	Maha	Yala	Maha	Yala
2.BgCMS4A/IR73885-1-4-3-2-2	8.45	88.59	1.94	70.37	24.96	42.26
22.IR78354A/IR73885-1-4-3-2-1	10.05	129.57	1.20	102.9	24.05	70.2

But, in Maha season these cross combinations showed just over 20% SH compared to Bg 379-2 and comparatively even less SH over Bg300 and Bg357. Therefore, above two cross combinations are especially suitable for Yala season.

CONCLUSION

BgCMS4A could be considered as the best tester. It recorded high grain yield over seasons and high P/H in Maha season. BgCMS4A also produced comparatively high P/H in combining with all lines. BgCMS4A had shown significantly higher SCA for Y/P. IR78354A also has shown significantly higher SCA for Y/P and P/H. Therefore, IR78354A also could be considered as a comparatively better tester.IR70369A was also a good tester as it has shown significantly high GCA for TWG in both seasons. All cross combinations produced even yield with no significant difference in Maha season while cross combinations 22 (IR78354A/IR73885-1-4-3-2-1-10R) and 2 (BgCMS4A/IR73885-1-4-3-2-2-10R) recorded significantly higher yield than that of others in Yala season. Therefore, these two cross combinations may have the potential to be used for hybrids in the Low Country Intermediate Zone.

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