

**IDENTIFICATION OF STABLE TWO-LINE RICE HYBRIDS  
(*ORYZA SATIVA* L.) USING ADDITIVE MAIN EFFECTS AND  
MULTIPLICATIVE INTERACTION (AMMI) MODEL**

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**ABSTRACT**

A multi-location trial was conducted with ten-two line of thermo sensitive genic male sterile (TGMS) rice hybrids with five standard checks in three locations and stability was tested. Seven TGMS hybrids performed well and they gave high yield performances in all locations. In AMMI I, five hybrids fell under class 1 with higher mean and positive Interaction Component Principal Analysis (ICPA I) values. In the AMMI 2 three environments had differed in both main and interaction effects. The Coimbatore (E<sub>2</sub>) and Bhavanisagar (E<sub>3</sub>) environments were exhibiting moderate interaction effect and E<sub>1</sub> had high interaction effect. The projection line of the genotypes in AMMI Biplot indicated that three two-line hybrids and three check entries had least influenced by the environments. Four stable two-line rice hybrids identified of which two mid-early hybrids gave productivity of 85.13 and 81.38 kg/ha/day whereas two medium duration hybrids gave 93.96 and 99.6 kg/ha/day. The two-line rice hybrids gave additional per day productivity ranging from 3.2 to 44.5 % increase than three-line hybrids. The stable hybrid TNAU60S x IET21009 belonging to medium duration group had a maximum of 53.5 % increase in per day productivity over the standard varieties.

**KEYWORDS:** *Oryza sativa*, Rice, Stability AMMI model, TGMS, Two-line hybrids.

**INTRODUCTION**

A specific genotype does not always exhibit the same phenotypic characteristics under different environments and different genotypes respond differently to a specific environment. Yield is a complex quantitative characteristic and is greatly influenced by environmental fluctuations; hence, the selection for superior genotypes based on yield at a single location in a year may not be very effective. Thus, evaluation of genotypes for stability of performance under varying environmental conditions for yield has become an essential part of any breeding programme (Gurdeep Singh *et al.*, 2001). Plant breeding is the exploitation of genetic variability which is, however, not directly measurable by itself and has to be inferred from the phenotype. It is commonly observed that the relative performance of different genotypes varies in different environments due to genotype-environment interactions (GEIs). An understanding of the causes of GEI can help in identifying traits and environments for better genotype evaluation. Numerous methods

have been used in the search for an understanding of the causes of GEI (Van Eeuwijk *et al.*, 1996).

Several methods used in stability analysis of rice genotypes so far are grouped under one strategy that involves factorial regression analysis of the GE matrix (*i.e.* the yield matrix after the environment and genotypic main effects are removed) against environmental factors, genotypic traits or combinations thereof (Basil *et al.*, 1995). Another strategy involves correlation or regression analysis which relates the genotypic and environmental scores derived from principal component analysis of the GEI interaction matrix to genotypic and environmental covariates. This strategy is associated with the use of the Additive Main Effects and Multiplicative Interaction Model (AMMI). The first example of using this model was provided by Zobel *et al.* (1988) who studied the interaction between the maturity of the genotypes and the day length of the locations in soybean Multi Evaluation Trial (MET).

Biplot from AMMI parameters provided the comprehensive understanding of the pattern of the data and is a useful tool in analyzing yield data (Zobel *et al.*, 1988). Varietal adaptability to environmental fluctuations is important for the stabilization of crop production over locations and years. Information on GEI helps to identify stable genotypes, which could be developed and recommended for specific regions or for general cultivation. Following the landmark finding of rice genotypes reversibly turn male fertile to male sterile and *vice versa* with changes in environmental factors, such as temperature and/or day length during critical phases of plant growth, the concept of two-line breeding emerged as an alternative to the three-line approach in China (Yuan, 2001). The main advantages of two-line heterosis breeding include the ability to use a wide range of genotypes as male parents, absence of negative effects associated with sterility-inducing cytoplasm and no need for maintainer lines (Virmani *et al.*, 1982.) Male sterility in thermo sensitive genic male sterile (TGMS) lines is heritable. Higher temperature (>30 °C) results in sterility while lower temperature (<23 °C) results in fertility. These characteristic features of TGMS ease out the hybrid seed production and subsequently, it was demonstrated that the TGMS was more effective in increasing grain yield and seed production efficiency.

The International Rice Research Institute (IRRI) has shown that two-line hybrids derived from TGMS lines had higher frequency of heterotic combinations than the three-line hybrids derived from CMS lines (Yan, 2001). Virmani *et al.* (1982) reported that there was an occurrence of variable yield in *indica* hybrid rice grown in the tropics both in wet and dry seasons. One of the practical approaches to enhance the productivity and

mitigating possible bottle necks in three-line rice hybrids to achieve food security is the development and use of two-line rice hybrids with high yield and desirable grain quality for different environments. Therefore, an investigation was carried out to test the promising two-line rice hybrids for stability across multiple environments and also identify superior two-line hybrids for commercialization.

## MATERIALS AND METHODS

Seedlings of ten selected two line hybrids *viz.*, TNAU18S x IET21009, TNAU18S x IET21044, TNAU18S x IET21508, TNAU18S x IET 21044, TNAU18S x IET20921, TNAU60S x IET21493, TNAU60S x IET21513, TNAU60S x CB-09-106, TS-29-150GY x CB-09-106, TS-29-150GY x DRR3306, and five standard checks *viz.*, TNRH174, TNRH180, CORH 3, CO(R)50 and ADT45 were raised in a nursery at Paddy Breeding Station, Coimbatore, India. The seedlings were then transplanted in three locations *viz.*, Coimbatore (E<sub>1</sub>), Bhavanisagar (E<sub>2</sub>) and Sirugamani (E<sub>3</sub>) representing three diverse rice growing environments. Randomized Complete Block Design (RCBD) was followed in three replications with plots having dimensions of 0.75 m x 3.8 m in order to accommodate 90 plants/plot at the spacing of 20 cm x 15 cm between and within the rows, respectively. Single seedlings were planted in hills. Standard package of cultural practices were followed. At the time of maturity the biometrical observations *viz.*, plant height, maturity days, number grains per panicle, grain weight, 1,000 grain weight and plot yield were recorded. The trials were harvested manually at the physiological maturity of the grains and record data for grain mass (kg)/plot and all the plots were counted to get the final stand/plot. Data were analysed using WINSTAT package developed by ICRISAT, Hyderabad, India. To study the underlying interaction components, more advanced techniques such as principal component analysis (PCA) are required. So this study aimed to use this valuable method to identify the best performing two line rice hybrids using AMMI model. The AMMI model is a hybrid model involving both additive and multiplicative components of two way data structure. The AMMI model separates the additive variance from the multiplicative variance and then applies PCA to the multiplicative interaction portion to extract a new set of coordinate axes which explain in more detail the interaction pattern. The estimation is accomplished using the least squares principle (Bradu and Gabriel, 1978).

### **Biplot analysis**

Biplot analysis is the most powerful interpretive tool of AMMI models. Biplots are graphs where aspects of both genotypes and environments are plotted on the same

axis so the inter-relationships can be visualised. There are two basic AMMI Biplots, the AMMI Biplot where the main effects (genotype mean and environments) are plotted against each other and the AMMI Biplot where scores for IPCA 1 and IPCA 2 are plotted.

## RESULTS AND DISCUSSION

The ANOVA for grain yield in three environments and combined ANOVA using AMMI model for genotypes is presented in Table 1. Yield performances of 15 genotypes over three environments are shown in Figure 1. Interaction Biplots (AMMI 1 and IMMI 2 of the genotypes with three environments are given in Figures 2 and 3, respectively. Estimates of stability parameters for grain yield are presented in Table 2. Categorization of genotypes using AMMI model is presented in Table 3. Grain yield and maturity duration data in three locations are presented in Table 4 whereas comparison of per day productivity is given in Table 5. The analysis of variance of multi-location trials is useful for estimating variance components related to different sources of variation, including genotypes and GEI (Martin, 2004). In general, variance component methodology is important in multi-location trials, as errors in measuring the yield performance of a genotype arise largely from GEI. In the present investigation, analysis of variance for AMMI analysis of GxE interaction indicated that genotypes, environments and Genotype x Environment interaction (GEI components) were significant for the four traits studied.

**Table 1.** Combined ANOVA over genotypes and environments for grain yield (kg/ha) using AMMI model.

Source of variation	DF	Values		
		SS	MS	% SS
Genotypes	14	90,183,806	644,700	7.14
Environment	2	6,195,293	3,097,646	49.99
GXE interaction	28	23,242,331	830,083	3.57
IPCA 1	15	19,080,825	1,272,055	6.66
IPAC2	13	4,161,713	320,131	7.69

The highest and positive ICPA 1 value (Table 3) was observed in E<sub>1</sub> over the rest of the two environments with negative values. Highest and positive ICPA 2 value was also recorded in E<sub>1</sub> while negative values were observed in other two environments with negative values. LSD comparison (see Figure 1) revealed that the E<sub>2</sub> (Bhavanisagar) followed by E<sub>3</sub> (Sirugamani) were well suited and most preferred environments for these genotypes which had a little edge over the E<sub>1</sub> (Coimbatore). The hybrids *viz.*, TNAU60S x IET21009, TNAU60S x CB-09-106, TNAU18S x IET27044 and TNAU18S x

IET20921 ranked above all the genotypes in multi-location testing and notably, TNRH 174, the check hybrid also ranked above other hybrids in its yield performance.

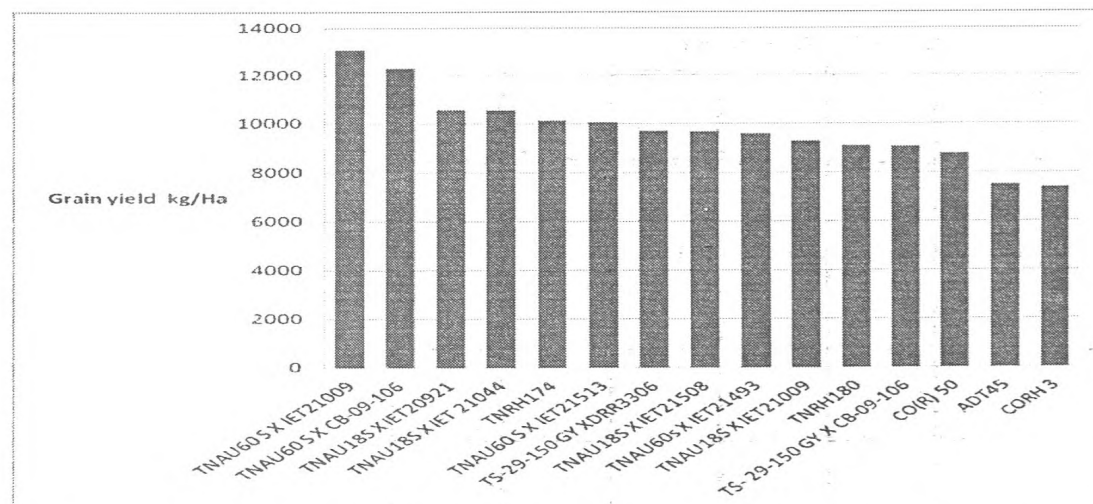


Figure 1. Yield performances of 15 genotypes over three environments.

In AMMI 1, five hybrids *viz*, TNAU60S x IET21009, TNAU60S x CB-09-106, TNAU18S x IET20921, and TNAU18S x IET21004 and TNAU60S x IET21513 fell under class I with comparatively higher mean and positive ICPA 1 values. Check hybrid TNRH 174 was grouped under class II. All other entries had relatively lesser mean values than the average and fell under other two classes of low mean values with respective IPCA indicators. Therefore, the above mentioned five hybrids could be rated as stable entries as per AMMI 1 model (Table 4).

### AMMI 1 Biplot for grain yield

The AMMI 1 Biplot for grain yield clearly indicated that the three environments differed in both main and interaction effects (Figure 2). Among three environments, the  $E_1$  environment was far away from the origin. The environments  $E_2$  and  $E_3$  were exhibiting moderate interaction effect whereas the environment  $E_1$  had high interaction effect with the other two environments. However, these three environments showed only a little variation in main effect with each other.

The genotypes *viz*, TNAU60S (10), TNAU60S x CB-09-106 (7), TNAU18S x IET21044 (3), TNAU18S x IET20921 (4), TNAU60S x IET21513 (6) and TNRH 174 (11) (number in the parenthesis indicates the corresponding genotype's number in AMMI Biplots) showed positive IPCA 1 score close to zero with high main effects indicating that these hybrids were relatively less influenced by environments (Figures 1 and 2). Hence, the above said hybrids are stable and had general adaptability over all the

environments. As the environment E1 had positive IPCA 1 score, it had positive interaction with above six genotypes and this environment is considered as the stable environment for these hybrids.

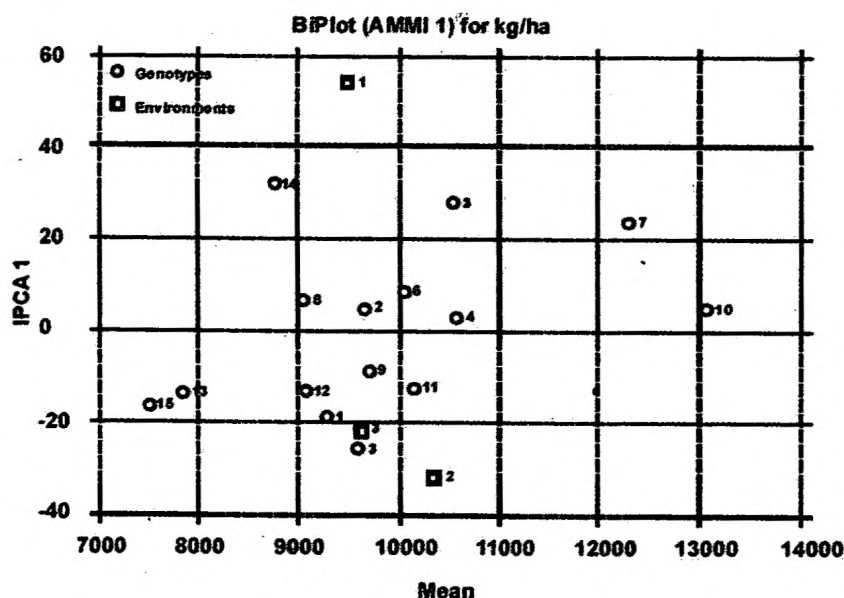


Figure 2. IPCA positions of the genotypes in AMMI 1.

Note: 1- 15 are the genotypes tested referred in Table 3.

### AMMI 2 Biplot for grain yield

In AMMI 2 Biplot, the environment scores are joined to the origin by site lines. Sites with short spikes do not exert strong interactive forces. Those with long spikes exert strong interaction. An example of this is shown in Figure 3 where the points representing the environments E1, E2 and E3 are connected to the origin. The environment E1 had a long spoke and hence it exerts strong interaction while the E2 and E3 had short spikes and they do not exert strong interactive forces. The interaction of genotypes with sites can be read directly from the Biplot by projecting genotype marks on the site lines as indicated by Martin (2004). If the projection falls on to a site line then genotype is positively adapted to that site. In Figure 3, the projection line of the genotype TNAU18 x IET21009 (1), TNAU18S x IET20921 (4), TNAU60S x IET21513 (6), TS29-150GY x CB-09-106 (7) and TNAU18S x IET27044 (3) and CO(R)50 (14) falls on the site line of environment E<sub>1</sub> indicating these hybrids have positive interaction with the environment.

The direction of the genotypes from the centre relative to each other is indicative of the correlations between them (Raju, 2002). For instance, the genotypes present within a class (class I to IV) tend to have a positive correlation between their GEI effects while negatively correlated with that of genotypes present in the opposite plots *i.e.*, between genotypes of class I and class IV (positive-positive and negative-negative) and between the genotypes of class II and class III (positive-negative and negative-positive).

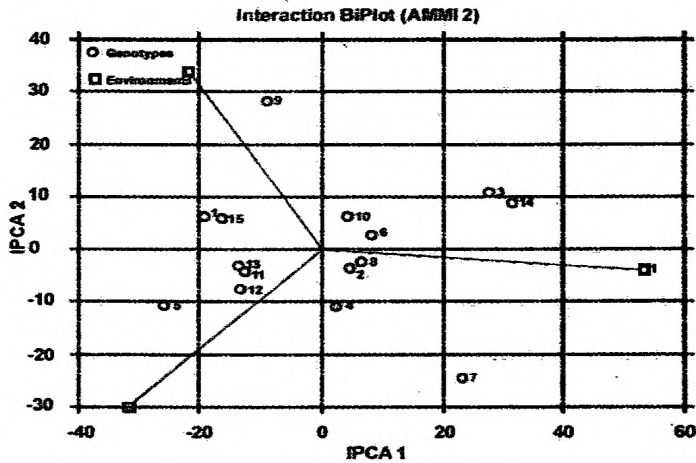


Figure 3. Interaction Biplot (AMMI2) of the genotypes with three environments.

Note: 1- 15 are the genotypes tested referred in Table3.

Table 2. Estimates of stability parameters based on grain yield (kg/ha) for genotypes using AMMI model.

Genotype number	Genotypes	Grain yield (kg/ha)		
		Mean	IPCA 1	IPCA 2
1	TNAU18S x IET21009	9,267	-19.01	6.28
2	TNAU18S x IET21508	9,643	4.72	-3.75
3	TNAU18S x IET 21044	10,531	27.74	10.71
4	TNAU18S x IET20921	10,556	2.60	-10.89
5	TNAU60S x IET21493	9,574	-25.77	-10.76
6	TNAU60S x IET21513	10,037	8.24	2.89
7	TNAU60S x CB-09-106	12,308	23.27	-24.62
8	TS- 29-150GY x CB-09-106	9,043	6.50	-2.74
9	TS-29-150GY x DRR3306	9,691	-0.94	28.01
10	TNAU60S x IET21009	13,082	4.62	5.96
11	TNRH174	10,133	-12.58	-4.38
12	TNRH180	9,064	-13.23	-7.73
13	CORH 3	7,387	-13.59	-3.25
14	CO(R)50	8,754	31.75	8.57
15	ADT45	7,499	-16.30	2.69
	Coimbatore (E <sub>1</sub> )	9,475	53.65	2.98
	Bhavanisagar (E <sub>2</sub> )	10,320	-31.87	-1.77
	Sirugamani (E <sub>3</sub> )	9,609	-21.77	-1.21
	Grand mean	9,801.33		

Note: Values given in parenthesis followed by genotype indicate the number used for particular genotype in Biplot analysis.

**Table 3. Categorization of genotypes tested over multilocation yield trials based on AMMI 1 model.**

<i>Category</i>	<i>Genotypes</i>
High mean and positive IPCA	TNAU 60 S X IET 21009 (10); TNAU 60 S X CB-09-106(7); TNAU 18 S X IET 20921(4); TNAU 18 S X IET 21044(3); TNAU 60 S X IET 21513(6)
High mean and negative IPCA	TNRH 174(11)
Low mean and positive IPCA	TNAU 18 S X IET 21508 (2); TS- 29-150 GY X CB-09-106(8); CO (R) 50(14)
Low mean and negative IPCA	TS-29-150 GY XDRR3306(9); TNAU 60 S X IET 21493(5); TNAU 18 S X IET 21009(1); TNRH 180(12); CORH 3(13); ADT45(15)

Note: Value given in parenthesis represents genotype number.

The genotypes that will have similar origin will result in similar yields in all environments while genotypes apart may either differ in mean yield or show pattern of response over the environments (Shinde *et al.*, 2002). Genotypes near the origin are non sensitive to environmental interactive forces and those distant from the origins are sensitive and have large interaction. Accordingly, the two-line hybrids *viz.*, TNAU 60 S (10), TNAU60S x IET21513 (6), TS29-150-GY x CB-09-106 (8), TNAU18S x IET21508 (2) and TNAU18S x IET20921 (4) and the check entries *viz.*, TNRH 174 (11), TNRH 180 (12) and CORH 3 (13) were close to the origin in interaction Biplot (AMMI 2). Therefore, the above mentioned two line of rice hybrids had proven as stable along with the check entries. Two of checks, CORH3 and TNRH 175 had already been proven stable in the earlier studies conducted by several workers in Tamil Nadu Agricultural University and have been released already for commercial cultivation.

As the hybrids, TNAU60S x IET21009, TNAU60S x CB-09-106, TNAU18S x IET20921, TNAU18S x IET27044, TNAU60S x IET21513 out yielded one check hybrid, TNRH 174 and these were positioned closer to the origin in AMMI I Biplots and these hybrids have only less influence by the environment effects. Other check hybrids, TNRH 180 and CORH 3 were also positioned in the same direction - one of them has already been proven stable and had been released for commercial cultivation in Tamil Nadu. Therefore, this analysis clearly indicates that at least four two-line rice hybrids *viz.*, TNAU60S x IET21009, TNAU60S x CB-09-106, TNAU18S x IET20921 and TNAU60S x IET21513 outperformed the checks and also showed a considerable stability over the tested locations based on AMMI model.

**Table 4.** Grain yield, maturity duration and per day productivity of rice genotypes in different environments.

Genotype Number	Genotype	Grain yield (kg/ha)			Maturity duration(days)			Pooled mean	Maturity duration group	PDP (kg/ha/day)
		E1	E2	E3	E1	E2	E3			
1	TNAU18S x IET21009	7,946	10,579	9,277	110	108	109	109	E	85.02
2	TNAU18S x IET21508	9,555	9,900	9,474	125	123	122	123	ME	78.19
3	TNAU18S x IET21044	11,736	10,485	9,374	125	123	123	124	ME	85.16
4	TNAU18S x IET20921	10,326	10,669	10,675	126	124	122	124	ME	85.13
5	TNAU60S x IET21493	7,822	10,594	10,306	132	131	132	132	M	72.72
6	TNAU60S x IET21513	10,164	10,379	9,568	125	123	122	123	ME	81.38
7	TNAU60S x CB-09-106	13,132	11,353	12,440	132	131	130	131	M	93.96
8	TS-29-150GY x CB-09-106	9,055	9,274	8,802	111	110	109	110	E	82.21
9	TS-29-150GY x DRR3306	8,997	11,329	8,748	110	109	108	109	E	88.91
10	TNAU60S x IET21009	13,027	13,631	12,588	132	130	132	131	M	99.61
11	TNRH174	9,115	10,923	10,363	130	131	132	131	M	77.36
12	TNRH180	7,998	9,775	9,421	120	122	121	121	ME	74.92
13	CORH 3	6,769	8,693	8,051	110	109	110	110	E	71.47
14	CO(R) 50	10,165	8,516	7,581	135	134	135	135	M	65.01
15	ADT45	6,321	8,707	7,470	110	108	107	108	E	69.23
	<b>Mean</b>	<b>9,475</b>	<b>10,321</b>	<b>9,609</b>	<b>122</b>	<b>121</b>	<b>121</b>	<b>121</b>		<b>81.00</b>
	<b>CD (5 %)</b>	<b>1290.32</b>	<b>1654.21</b>	<b>1332.24</b>	<b>05</b>	<b>05</b>	<b>04</b>	<b>05</b>		

Note: E1=Coimbatore; E2=Bavanisagar; E3=Sirugamani; PDP=Per day productivity (kg/ha/day); E=Early duration; ME=Mid early duration; M=Medium duration.

**Table 5.** Comparison of per day productivity of two line hybrids with their corresponding check entries with similar duration.

Genotype No	Genotype	Yield (kg/ha)	Maturity Duration (days)	PDP (kg/ha/day)	Comparison of per cent yield increase over the standard check	
					CORH3	ADT45
<i>Early</i>						
9	TS-29-150GY x DRR 3306	9,691	109	88.91	24.4	28.4
1	TNAU18S x IET21009	9,267	109	85.02	19.0	22.8
8	TS-29-150GY x CB-09-106	9,043	110	82.21	15.0	18.7
13	CORH 3 (standard check)	7,837	110	71.47	-	3.2
15	ADT 45 (standard check)	7,500	108	69.23	-	-
<i>Mid early</i>						
<i>TNRH180</i>						
2	TNAU18S x IET21508	9,643	123	78.19	4.4	
3	TNAU18S x IET21044	10,532	124	85.16	13.7	
4	TNAU18S x IET20921	10,557	124	85.13	13.6	
6	TNAU60S x IET21513	10,037	123	81.38	8.6	
12	TNRH180 (standard check)	9,065	121	74.92	-	-
<i>Medium</i>						
<i>TNRH174</i> <i>CO(R) 50</i>						
5	TNAU60S x IET21493	9,574	132	72.72	-5.8	11.9
7	TNAU60S x CB-09-106	12,308	131	93.96	21.5	44.5
10	TNAU60S x IET 21009	13,082	131	99.61	28.8	53.2
11	TNRH174 (standard check)	10,134	131	77.36	-	19.0
14	ADT 45 (standard check)	8,754	135	65.0	-	-

Each new cultivar must have a yield equal or better than the previous releases. Yield data of a few rice hybrids and the high yielding varieties (HYV) in several Asian countries showed that 15-20 % yield advantage of rice hybrids over inbred HYVs with 70 to 80 kg/ha/day (Virmani and Zaman,1998). In the present study, among the stable genotypes, two mid early duration hybrids *viz.*, TNAU60S x IET21513 and TNAU18S x IET20921 gave 85.13 and 81.38 kg/ha/day of per day productivity, respectively (Table 5). Two medium duration hybrids, TNAU60S x CB-09-106 and TNAU60S x IET 21009 had 93.96 and 99.6 kg/ha/day of productivity.

On comparison of the two-line rice hybrids with their check entries of similar maturity groups belonging to CGMS system and HYVs, obviously the two-line rice hybrids gave additional per day productivity ranging from 3.2 to 44.5 %. The stable hybrid belonging to medium duration group notably had maximum of 53.5 % increase in per day productivity and the mid early group hybrids which were also stable in their *per*

se performances had maximum of 33 % increase over their checks of similar maturity duration. This trend of high per day productivity might be attributed to their greater dry matter production. The reasons for the greater rate of biomass production and high yield potentials of these hybrids might be attributed to increased growth rate during early vegetative stage, more efficient sink formation relative to the rate of dry matter accumulation that results in both greater biomass, a larger panicles at flowering stage and a relative high grain filling percentage despite the large number of spikelets (Peng, 1999).

Recent approaches in breaking yield gap by super rice suggests that the new varieties should out yield local widely grown check varieties by 10 % with acceptable grain quality and pest resistance. Another goal of super rice (hybrid) is to produce 100 kg grain yield/ha/day (Yuan, 2001). This is a plausible criterion because it eliminates the approach of improving yield potential by increasing crop growth duration so that cropping intensity could be maintained in the cropping system. In Sri Lanka, diverse environments are prevailing due to agro-ecological variations with low and high temperature regimes. Therefore, this technique could be a valuable tool for the production of two-line rice hybrids in future.

### CONCLUSIONS

Stability analysis using AMMI model showed that hybrid TNAU60S x IET21009 showed more stable performance in all three environments *viz.*, Coimbatore, Bhavanisagar, and Sirugamani than all other checks; CGMS hybrids and varieties. This particular hybrid falls under medium group maturity duration with 131 days giving the highest grain yield of 13,082 kg/ha with 99.6 kg/ha/day productivity. Three other two-line hybrids *viz.*, TNAU60S x CB-09-106, TNAU18S x IET20921 and TNAU60S x IET21513 also performed well over three environments. This illustrates that TGMS hybrid rice technology can be successfully commercialized in Tamil Nadu.

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