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## Society For Advancement of Rice Research

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- To advance the cause of rice research and development in the country
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- To provide consultancy in rice production and development
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# **Inter-relationship among Quality Traits in Fine Grain Scented Rice Mutants Derived through Recurrent Mutagenesis**

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## **Abstract**

Seventy five fixed mutant lines derived from recurrent mutagenesis (EMS, NG and their combinations) of PB 1, Pusa Sugandha 2 and a popular local scented rice variety Ketakijoha were assessed for seed yield and sixteen physical and cooking quality traits. Grain yield was unique to exhibit significant negative correlation with Kernel L/B ratio, but positive significant correlation with gel consistency among all 16 physico-chemical quality features. Volume expansion ratio had no significant correlation with yield and quality features except hulling percentage having significant negative correlation. Grain length had significant positive correlation with kernel length before and after cooking. So, it may serve as an ideal parameter to the consumers for better cooking quality. Hulling percentage was

found to have significant positive correlation with gelatinization temperature but correlated negatively with gel consistency and alkali spreading value. This envisaged that the genotypes with high hulling percentage would separate upon cooking and develop less splitting of kernels which are desirable. Alkali Spreading Value (ASV) had perfect negative relationship with gelatinization temperature and significant positive correlation with gel consistency. Aroma had significant negative correlation with gel consistency indicating that the varieties which do not split upon cooking would have high aroma. The above information would be of immense value for breeding of basmati type genotypes for export value.

**Key words:** Fine grain scented rice mutants, inter-relationship, grain yield, physical and cooking quality traits.

India is an exporter of world class aromatic rice. Basmati rice is a resident of India by

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birth. Indian basmati - the soft cooking aromatic rice is acclaimed as the best quality rice in the world market. It has potential to contribute sizeable foreign exchange in the national economy. India is the largest producer and exporter of basmati rice in the world. The annual production in the country hovers at around 10-15 lakh tones a year, of which around two-third is exported. Self-sufficiency in rice production and enhanced purchasing power have resulted changed life style and awareness among consumers to improve and diversify diets including quality of rice consumed. Aroma and cooked kernel elongation are the most important quality traits, which differentiate the highly valued aromatic rice from the other rice types. Although genes/ QTLs of these two traits are linked and present on chromosome 8 (Jain *et al.*, 2006), in reality, there seems to have inconsistent correspondence between these two important traits (Golam *et al.*, 2010) owing to environmental influence particularly temperature for expression of the characters. The temperature of 25°C at day time and 21°C at night during the ripening stage has been found to have favorable effect in aroma as well as kernel elongation.

Rice being a major staple food, pleasant flavour in cooked rice becomes a

highly valued quality attribute. More than 100 volatile aromatic compounds have been detected in fragrant rice among which biochemical basis of aroma is mainly attributed to 2-acetyl-1-pyrroline (2AP) (Tanchotikul and Hsieh 1991) and the fragrance gene (*frg*) was successfully identified by Bradbury *et al.*, (2005) in the aromatic rice. Betaine aldehyde dehydrogenase (BADH-2) is the key enzyme in the biochemical path way for synthesis of 2AP. The *frg* gene has eight non-functional alleles and a few functional alleles among which a single recessive allele *badh 2.1* is predominant in virtually all fragrant rice varieties today including Basmati and Jasmine types. More recently, three SNPs and an eight base pair-deletion in exon 7 of the gene encoding betaine aldehyde dehydrogenase 2 (BAD2) on chromosome 8 of rice were identified as the probable cause of aroma enzyme in aromatic rice (Bradbury *et al.*, 2005). Kibria *et al.*, (2008) reported three SSR markers RM 223, RM 342A and RM 515 linked to fragrance gene (*fgr*) locus on chromosome 8.

Lengthwise elongation of cooked kernel without or minimum increase in girth is the characteristic feature of high quality basmati rice (Khush *et al.*, 1979). The inheritance pattern of rice kernel elongation

is controlled by a single gene and is influenced by some modifier genes (Golam *et al.*, 2004). However, Sood *et al.*, (1983) reported the involvement of both non-additive and additive types of gene affects with the former playing a predominant role in kernel elongation.

Keeping in view the consumer's perspective and export value, high grain yield along with genetic amelioration of cooking quality of rice assumes an important objective in current breeding programme. However, the crosses so far made involving Pusa Basmati-1, Taraori Basmati and Basmati 370 have not rendered any remarkable breakthrough in genetic enhancement for productivity in basmati type rice. Besides, many often the delicate genetic background conforming to the basmati standard is disturbed in the segregating population derived from recombination breeding. Better understanding of inter- relationship among quality features and seed yield can be helpful to breed superior aromatic rice genotypes. Therefore, an attempt was taken to investigate the nature of inter-relationship among physico-chemical quality features and grain yield in a set of fixed mutant lines derived from a few well

adapted fine grain Indian scented rice varieties.

## Materials and Methods

The experimental materials comprised 75 fixed mutant lines ( $M_8$  generation) derived through recurrent mutagenesis with EMS (0.2, 0.4 and 0.6%), NG (0.01, 0.015 and 0.02% and a combination of 0.4% EMS and 0.015% NG (following a pre-soaking period of 10.5 h in distilled water), their parents (Pusa Basmati-1, Pusa Sugandha -2 and a popular land race of Odisha "Ketakijoha local") and two standard check varieties (Geetanjali and Pusa Sugandha-4). The experiment was laid out in randomized block design with three replications. Freshly harvested grains of the test genotypes were assessed for grain yield and 16 quality parameters. The quality tests e.g., gel consistency (GC) was based on the method described by Cagampang *et al.*, (1973), The varieties with gel consistency > 60 mm, between 40-60 mm, and < 40 mm were considered soft, medium and hard gel genotypes, respectively. Alkali spreading value (ASV) and Gelatinization temperature (GT) scores ranging from 1- 7 were assessed as per Little *et al.* (1958). GT is defined as the temperature at which 90% of the starch granules would swollen irreversibly in hot water and it is scored indirectly as an inverse measure of ASV. In the present investigation, the cooking qualities e.g., cooked kernel length, cooked kernel length/breadth ratio, elongation ratio

upon cooking were calculated as per Verghese (1950), Volume Expansion Ratio (VER) were determined as per method of Juliano and Perez (1984). Presence or absence of aroma was scored (0-3 scale) after 10 min. (Sood and Siddiq 1978) during alkali digestion of milled kernels soaked in 1.7 % KOH for 23 hrs for determination of ASV at 30°C. Assessment of all these quality features was repeated thrice to minimize experimental error. Routine statistical procedures were followed for analysis of variance and covariance as per Singh and Choudhary (1976). The correlation coefficient for each pair of characters were computed following Al-Jibouri *et al.* (1958) and the significance of correlation coefficients was tested by 't'-test at n-2 degrees of freedom.

## Results and Discussion

Genetic relationship of different quality traits is very important for selection of genotype for export value. Kernel length of more than 7 mm., L/B ratio more than 3.5, intermediate ASV, moderate GT, intermediate AC, intermediate GC, moderately high volume expansion after cooking and pleasant aroma are the minimum quality features of basmati types.

In the present investigation, efforts have been taken to study and implicate the relationship of seed yield with some easily observable quality traits that determine

cooking qualities. Grain yield was unique to exhibit significant negative correlation (-0.50) with Kernel L/B ratio but had significant positive correlation (0.47) with gel consistency only (to be discussed later) (Table 1). Grain yield was also observed to have moderate positive correlation with kernel breadth but negative relationship with most of the quality features including grain length, kernel length and kernel L/B. Long fine grain and kernel types attract consumer's preference and have export value. *GS3*- an evolutionarily important gene controls grain length in rice. An association study revealed that a C to A mutation in the second exon of *GS3* (A allele) was associated with enhanced grain length in *Oryza sativa*, but was absent in other species of *Oryza* (Takano-kai *et al.*, 2009). Pusa 1121(Pusa Sugandha-4), an Indian basmati type rice is acclaimed as world's longest grain rice variety with exceptionally high cooked kernel elongation (Singh and Singh 2002). Besides, ORM 250-3 and ORM 228-3: two mutants of PS-4 included in this pursuit, had more longer kernel (9.12 mm) than even PS-4 (9.04 mm) (Tripathy *et al.*, 2012). Grain length exhibited significant positive correlation with grain L/B ratio, kernel length, kernel L/B ratio and kernel length after cooking as

similar to the findings of Vivekanandan and Giridharan (1998). Besides, positive significant association of kernel length with kernel L/B ratio and cooked kernel length observed in this pursuit was in agreement with Lin (1978) and Somrith (1979). Hence, length of grain and kernel become ideal parameters to the consumers for better cooking quality. However, Chouhan (1996) reported significant positive association of kernel length with kernel L/B ratio but negative association with kernel breadth in some crosses of aromatic x non-aromatic varieties.

Genetic variation is a common feature for kernel recovery after hulling in any set of test material. The recovery of whole kernel varies between genotypes mainly due to extent of compactness of the starch grains in the endosperm, air space between hull and kernel, and hull thickness. About 20-25 per cent of grain weight is reduced due to hull after hulling depending upon the genotype. Besides, the whole kernel in which endosperm with aleurone layer and embryo remain intact, reflects the real nutritional value of kernel in terms of starch, vitamins and protein content. Hence, relationship of hulling percentage with other quality traits and yield *per se* is of great importance. In the present investigation, hulling percentage

is found to have significant positive correlation with GT but correlated negatively with GC and ASV. This envisaged that the genotypes with high hulling percentage would separate upon cooking and develop less kernel splitting which is highly desirable.

Volume expansion ratio is the measure of relative increase in volume of kernel after cooking and thus, the genotypes having high volume expansion are of great value to the consumers. But, results indicated that there is no significant correlation of Volume Expansion Ratio (VER) with any of the quality features except hulling percentage with significant negative correlation which is undesirable. In contrast, it had considerable degree of positive association with grain yield indicating recovery of a few high yielding genotypes with high volume expansion. Chouhan (1995) indicated significant positive association of volume expansion with raw kernel length and kernel L/B ratio. However, Nayak *et al.* (2003) reported that genotypes with high kernel elongation after cooking resulted less volume expansion.

Kernel elongation ratio was shown to have positive correlation with cooked kernel length ( $r=0.523$ ) and cooked kernel L/B

ratio ( $r=0.567$ ) (Table 1). Sadhukhan and Chattopadhyay (2000) observed positive association of kernel elongation ratio with kernel length after cooking. In contrast, elongation after cooking was also reported to be negatively correlated with kernel length (Kumari and Padmavati 1991) and positively with kernel breadth (Bocevskaa *et al.*, 2009).

Gel consistency (GC) separates high amylose rices into hard gel (26-40 mm) and low amylose rices into soft gel (>61mm). Rice varieties with medium gel consistency (41-60 mm) are preferred as the cooked rice remains separate (non-sticky) and soft (palatable). GC measures the tendency of cooked rice to harden when it cools down and therefore, it serves as a good index of cooked rice texture. The differences between hard and soft, hard and medium, and medium and soft gel consistency are under monogenic control and that modifiers affect the expression of the trait. Multiple alleles in the same locus designated as *geca* for medium gel consistency and *gecb* for soft gel consistency, were recessive to the wild type allele (GEC) for hard gel consistency; and *geca* was dominant over *gecb* (Tang *et al.*, 1991). In the present investigation, grain yield/plant was observed to have moderate positive significant correlation (0.47) with

gel consistency only. High gel consistency (>61mm) is the indication of low AC which is undesirable leading to inferior cooking qualities; particularly, the kernels become soft, sticky and intermingled after cooking and thus, not suitable for table purpose. Hence, high yielding genotypes are qualitatively poor and these have limited scope for export purpose. Sadhukhan and Chattopadhyay (2000) reported positive association of grain length with AC. In contrast, Samal *et al.* (2014) observed negative correlation of AC with kernel length in a set of aromatic germplasm.

ASV, GT and GC had shown to have typical *inter se* correlation which are usually taken as indicators for AC of a genotype and hence the cooking quality. ASV had perfect negative relationship with GT and significant positive correlation with GC. GT refers to the range of temperature within which starch granules start swelling irreversibly in hot water. In other words, GT determines the time taken to cook rice. Rice genotypes with high gelatinization takes longer time to cook, expand more, elongate less and many often remain uncooked under standard cooking procedure (Singh *et al.*, 2002) and hence, least preferred. The GT of rice varieties are classified as low (55-69°C), intermediate (70-74°C) and high (75-79°C).



In the present investigation, GT was numerically scored 1-7 for low to high in relation to high or low ASV value. In general, varieties with low alkali spreading value and gel consistency are expected to harbor high AC. But, a genotype should have an intermediate AC of 20-25% to fulfill the minimum standard of basmati types. Hence, selection of a genotype with delicate balance of ASV, GT and GC at intermediate or moderate level is indispensable for their suitability for export purpose.

Aroma had significant negative correlation with GC indicating that, in the present set of material, the varieties which do not split upon cooking would have high aroma. This association is desirable in basmati types and a priority consideration for breeding of genotypes for export value. In contrast, the local types, barring a few do not bear such favorable association. For instance, Leelavati - a short bold grain local land race gives high scent upon chewing and cooking, but its kernels become sticky and split upon cooking due to high gel consistency making it not suitable for table rice. The present study on analysis of physical and quality traits of aromatic rice will be of value in breeding high yielding Basmati type rice.

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Table 1: Correlation among grain yield/ plant and sixteen quality characters in 20 selected rice genotypes

Chara- cters	G.B	G.L/B	K.L	K.B	K.L/B	Hull. %	C.L	C.B	C.L/B	E.R	V.E.R	A.S.V	G.T	G.C	Aroma	GY/P
G.L	0.32	0.74**	0.85**	-0.25	0.70**	-0.08	0.61**	0.26	0.32	-0.18	-0.06	0.09	-0.09	0.11	0.37	-0.41
G.B		-0.39	0.20	0.36	-0.08	-0.31	0.16	0.05	0.04	-0.05	-0.17	0.12	-0.12	0.05	0.09	-0.20
G.L/B			0.69**	-0.49*	0.74**	0.13	0.46*	0.23	0.26	-0.17	0.06	0.03	-0.03	0.10	0.29	-0.23
K.L				-0.19	0.76**	0.17	0.63**	0.22	0.38	-0.33	-0.27	0.01	-0.01	0.01	0.22	-0.35
K.B					-0.78**	-0.25	-0.22	-0.20	-0.05	-0.05	-0.09	0.11	-0.11	0.12	-0.33	0.40
K.L/B						0.27	0.55*	0.24	0.29	-0.17	-0.13	-0.04	0.042	-0.06	0.38	-0.50*
Hull. %							0.17	0.02	0.14	0.03	-0.36	-0.53*	0.53*	-0.52*	0.39	-0.07
C.L								-0.05	0.81**	0.52*	-0.19	-0.08	0.08	-0.11	0.25	-0.42
C.B									-0.60**	-0.30	0.06	-0.13	0.13	0.35	-0.18	0.03
C.L/B										0.56**	-0.09	0.01	-0.01	-0.25	0.28	-0.27
E.R											0.07	-0.10	0.10	-0.14	0.05	-0.13
V.E.R												0.10	-0.10	0.28	-0.23	0.37
A.S.V													-1.0**	0.51*	-0.34	-0.01
G.T														-0.51*	0.34	0.01
G.C															-0.47*	0.47*
Aroma																-0.34

\*- significant at P<sub>0.05</sub>, \*\*-significant at P<sub>0.01</sub>

# Stability Analysis of Grain Yield and its Components in Rice (*Oryza sativa* L.) Genotypes

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## Abstract

The Genotype (G) x Environment (E) interaction and stability for grain yield and associated traits were studied for 13 rice genotypes in five environments from *kharif* 2007 to *kharif* 2011. The stability analysis showed significant differences among genotypes for all the traits studied including grain yield. The linear component of environment was significant for all the characters and the pooled deviation was significant for plant height, productive tillers/plant, panicle length and test weight. Based on the stability parameters, BPT 2411 followed by BPT 2409 and BPT 2295 showed higher grain yield over the mean with regression coefficient near unity and non significant deviation from regression. Thus they found to be stable and may be recommended for commercial cultivation in this region.

**Key words:** Stability, regression, yield components

Rice is one of the main sources of food in the world where the increased demand for rice is expected to enhance production in many parts of Asia, Africa and Latin America (Subathra Devi *et al.*, 2011). Yield is a complex character which is dependent on a number of other characters and is highly influenced by many genetic factors as well as environmental fluctuation. For stabilizing yield, it is necessary to identify the stable genotypes suitable for wide range of environments. Stability of a cultivar refers to its consistency in performance across environments and is affected by the presence of genotype and environment interaction (Sharma *et al.*, 1987). Wider adaptability and stability are prime considerations in formulating efficient breeding programme. Stability analysis is a good technique for measuring the adaptability of different crop varieties to varying environments (Morales *et al.*, 1991). Therefore, the present study was undertaken to estimate the G X E interactions through stability parameters and performance of

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some other characters of 13 rice genotypes across environments and to identify suitable genotypes for future breeding programme.

### **Materials and Methods**

The experimental material comprised of 13 rice genotypes including five released varieties *viz.*, Samba Mashuri (BPT 5204), Sona Mashuri (BPT 3291), Bapatla Sannalu (BPT 1768), Bhavapuri Sannalu (BPT 2270) and Akshaya (BPT 2231) along with eight pre-released cultures (BPT 2295, BPT 2403, BPT 2405, BPT 2406, BPT 2408, BPT 2409, BPT 2411 and BPT 2425) developed at Rice Research Unit (RRU), Bapatla. The pedigree and the physical grain quality characters of the genotypes were given in Table 1. The genotypes were evaluated in low land situation during five consecutive *kharif* seasons from 2007 to *kharif* 2011 at Rice Research Unit, Bapatla. The experiment was laid out in Randomised Block Design with three replications. Seedlings aged 25-30 days were transplanted at a spacing of 20 x15 cm between and within the rows respectively and the plot size was 7.2 m<sup>2</sup>. All the recommended cultural practices were adopted to raise the crop. Observations on days to 50 % flowering, plant height, productive tillers/plant, panicle length, test weight and grain yield/plant were recorded

at maturity. The mean values for all the traits across the seasons were subjected to stability analysis (Eberhart and Russel, 1966).

### **Results and Discussion**

The results of the combined analysis of variance after Eberhart and Russell model are presented in Table 2. Partitioning of mean sum of squares into that of genotypes, environment + (genotypes x environment) and pooled error revealed that genotypes were highly significant for all the characters studied indicating the presence of genetic variability in the experimental material under study. Mean sum of squares due to environment were significant for days to 50% flowering, plant height, productive tillers/plant and test weight. The linear component of environment is significant for all the characters studied indicating the existence of variation among the environments tested and the linear component of genotype x environment interaction was significant for plant height, productive tillers/plant, panicle length and test weight. The linear component of genotype x environment interaction was highly significant than the non-linear component of genotype x environment for the characters *viz.*, productive tillers/plant and test weight. This indicated significant

differences among the genotypes for linear response to environments. Similar findings were previously reported by Ramya & Senthilkumar (2008) and Sreedhar *et al.* (2011).

The estimates on the three stability parameters, mean performance ( $\bar{X}_i$ ), regression coefficient ( $b_i$ ) and deviation from regression ( $S^2_{di}$ ) for different yield attributing traits are presented in Table 3. The deviation from the regression for grain yield was significant in the genotypes BPT 2406 (22.15) and BPT 2425 (31.78). Among the genotypes tested, BPT 2411, BPT 2409, BPT 2295 and BPT 2405 showed unit regression and non significant deviation from regression for grain yield. The deviation from regression for days to 50% flowering was significant for the genotypes BPT 2231 and BPT 2408. However, BPT 5204, BPT 2409 and BPT 2270 showed unit regression and less deviation from regression for this trait. All the genotypes except BPT 2409, BPT 2425 and BPT 2403 exhibited non significant deviation from regression for plant height. A unit regression and non significant deviation from regression was observed in plant height for the genotypes BPT 2411, BPT 3291, BPT 2231 and BPT 2406. The rice variety Bapatla Sannalu (BPT 1768) recorded more

number of days for 50% flowering while the variety Bhavapuri Sannalu (BPT 2270) manifested maximum plant height. The trait namely productive tillers/plant recorded significant values for deviation from regression in BPT 2231, BPT 2403 and BPT 2408. Among all the genotypes tested, BPT 2408 and BPT 2411 showed unit regression and less deviation from regression for productive tillers/plant. The  $S^2_{di}$  value for panicle length was significant for only one genotype BPT 2403 where as the genotypes BPT 1768, BPT2411 and BPT 2425 exhibited unit regression and non significant deviation from regression for panicle length. Among the 13 test entries, seven genotypes manifested significant value for  $S^2_{di}$  while BPT 1768 and BPT 2403 showed unit regression and less deviation from regression for test weight. Any generalization regarding the stability of a genotype for all the traits is quite difficult. Some of the genotypes used in the present study did not exhibit uniform stability and response pattern for different traits.

In addition to grain yield, the genotype BPT 2411 also manifested regression coefficient nearer to unity for plant height, productive tillers/plant and panicle length and thus finally exhibited stability for grain yield. Based on the individual stability

parameters, the genotypes BPT 2411 and BPT 2295 recorded higher grain yield over the general mean with regression coefficient near unity and non significant deviation from regression indicating their average stability (Amirthadevarathinam, 1987 and Dushyantha Kumar *et al.*, 2010). Hence, the above genotypes could be recommended for cultivation with stable performance, Moreover, BPT 2295 completed three years of minikit testing in Krishna zone of Andhra Pradesh and BPT 2411, a cross between BPT 5204 and BPT 4358 was also given for minikit testing in Krishna zone to test its suitability over locations.

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**Table 1: The pedigree and physical grain quality characters of the genotypes**

S.No	Designation/ Name of the variety	Cross combination	Kernel length (mm)	Kernel breadth (mm)	L/B ratio	Grain type
1	BPT 5204	GEB24/TN(1)//Mashuri	5.12	1.79	2.86	MS
2	BPT 3291	Sona/Mashuri	5.05	1.83	2.76	MS
3	BPT 1768	BPT3301/Mashuri mutant	4.6	1.8	2.56	MS
4	BPT 2270	BPT5204/CRM1523	5.21	1.81	2.88	MS
5	BPT 2231	BPT4358/IR64	5.4	2.02	2.67	MS
6	BPT 2295	BPT 1768/NLR 33641	5.21	1.81	2.87	MS
7	BPT 2403	BPT5204/NLR33641// BPT5204	5.02	1.85	2.71	MS
8	BPT 2405	BPT5204/NLR33641	5.2	2.01	2.59	MS
9	BPT 2406	BPT5204/NLR33641	5.15	1.83	2.81	MS
10	BPT 2408	BPT5204/BPT3291	5.03	1.81	2.78	MS
11	BPT 2409	BPT5204/IR64// MTU4870/MTU1001	5.11	1.78	2.87	MS
12	BPT 2411	BPT5204/BPT4358	5.76	2.19	2.64	MS
13	BPT 2425	BPT5204/IR64// MTU4870/MTU1001	5.32	2.10	2.53	MS

L/B ratio: Length/breadth ratio; MS: Medium slender

**Table 2: Analysis of variance for stability performance for grain yield and component traits**

Sources	df	Mean sum of squares					Grain yield (kg/ha)
		Days to 50%flowering	Plant height	Productive tillers/plant	Panicle length	Test weight	
Varieties	12	259.10***	245.22***	6.88***	7.56***	19.25***	754.2***
Environment	4	18.67**	67.24**	14.31***	1.10	0.69*	644.6
Variety x environment	48	4.73	17.51	1.79*	0.61	0.46*	548.9
Env.+ (Var*Env.)	52	5.80	21.33*	2.76**	0.65	0.48*	556.3
Env. (linear)	1	74.67***	268.96***	57.23***	4.42**	2.77**	2578.5*
Var.*Env.(Lin.)	12	4.37	30.08*	3.80**	0.99*	1.01***	2117.3
Pooled deviation	39	4.48	12.30***	1.04*	0.44**	0.25***	24.1
Pooled error	120	3.52	3.59	0.67	0.21	0.06	546.8

\*Significant at 10% level: \*\*Significant at 5% level: \*\*\*Significant at 1% level



**Table 3: Estimates of different stability parameters for grain yield and it's component traits**

Varieties	Days to 50% flowering			Plant height			Productive tillers/plant			Panicle length			Test weight			Grain yield		
	X	Bi	S <sup>2</sup> di	X	Bi	S <sup>2</sup> di	X	Bi	S <sup>2</sup> di	X	Bi	S <sup>2</sup> di	X	Bi	S <sup>2</sup> di	X	Bi	S <sup>2</sup> di
BPT 5204	113.7	1.14	-2.7	90.33	-0.63	-0.29	9.6	0.32	0.18	20.89	-3.0*	0.08	15.17	-0.31	0.30**	5.2	-0.75	-5.89
BPT 3291	104.3	0.64	-2.5	92.95	1.09	-0.59	8.1	-0.19*	-0.39	20.64	-0.25*	-0.16	19.34	7.0*	0.31***	4.9	-0.141*	-8.10
BPT 1768	130.1	0.46	-3.3	104.4	0.21	1.03	10.0	0.23*	-0.34	23.08	1.01	-0.07	19.62	1.29	0.0425	5.7	0.543	-2.32
BPT 2270	128.6	1.27	-1.4	110.6	0.31	3.57	11.1	-0.05*	-0.56	24.35	2.13	-0.08	14.69	-1.92*	-0.05	6.1	-0.066*	-8.22
BPT 2231	127.1	0.29	20.4***	104.6	0.98	-0.36	10.8	0.67	3.29***	23.89	0.57	0.11	19.33	1.80	0.12*	6.2	0.336*	-8.15
BPT 2295	123.8	0.49	-1.0	106.1	-0.01	-0.36	11.2	-0.01*	-0.34	24.22	0.29	-0.13	15.66	-0.99*	0.01	6.3	0.753	-4.37
BPT 2403	129.1	2.79	1.3	91.42	2.72	17.96**	11.0	1.37	2.37**	24.32	4.09	0.36*	15.10	0.78	0.15	4.9	-3.445	5.39
BPT 2405	125.5	1.43	-3.0	90.64	3.08	-2.43	10.2	1.68	0.06	23.79	0.31	0.26	14.87	1.74	0.15*	6.0	1.524	4.25
BPT 2406	124.0	0.26	0.2	94.27	0.96	-0.51	9.9	2.0	0.58	23.97	2.34	0.00	14.47	0.62	0.88***	5.6	4.912	22.15*
BPT 2408	123.3	2.61	9.4*	92.68	-0.09*	-3.99	10.2	1.48	0.32	24.27	2.71	-0.10	13.94	2.53	0.39***	5.3	5.153*	-1.78
BPT 2409	124.9	1.20	-2.6	99.53	0.34	50.67***	11.5	1.66	0.47	23.58	0.66	0.26	15.91	0.67	0.29**	5.9	0.806	7.22
BPT 2411	120.1	0.23*	-3.3	91.70	1.12	-2.56	10.3	1.55	0.11	23.78	1.09	0.15	15.82	0.25	0.01	6.3	1.120	5.55
BPT 2425	117.1	0.18	0.8	101.9	2.93	45.14***	9.5	2.29	4.65***	23.62	1.12	2.41	16.31	-0.44	-0.05	5.5	2.26	31.78**

# Effect of Mutagens on Quantitative and Qualitative Characters in M<sub>3</sub> Generation of Rice Variety 'Akshaya' (BPT 2231)

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## Abstract

The mutagenic effects of gamma rays (10, 20, 30 and 40 kr) and EMS (0.1, 0.2, 0.25 and 0.3%) singly and their combination treatments were studied in rice variety Akshaya (BPT 2231) to find out the effectiveness of these mutagens on various quantitative and qualitative characters, heritability and genetic advance in M<sub>3</sub> generation. Macro mutations such as dwarf and tall plants, early and late flowering along with variation in hull colour and grain type were also observed. Early flowering mutants were isolated from 30 kr + 0.3 per cent EMS combination treatment. Combination treatments of EMS with 40kr and 30 kr doses reduced the plant height considerably. Moderate to high GCV and PCV coupled with high heritability (broad sense) and moderate to high genetic advance as percent of mean were observed for plant height, fertile grains per panicle, grain yield per plant and alkali spreading value.

The remaining characters under study manifested low GCV, PCV, high heritability estimates along with low to moderate genetic advance as percent of mean indicating the operation of both additive and non-additive gene action in the inheritance of these traits.

**Key words:** Macro mutations, quantitative and qualitative traits, heritability, genetic advance.

Mutation breeding has already made significant contribution to crop improvement all over the world. This is amply evident from the fact that more than 2250 varieties of different crops had been released that were derived as direct mutants or from hybridization involving desirable mutants Ahloowalia *et al.* (2004). Induced mutation which increases genetic variability is one of the traditional but still relevant, highly effective, economic and recognized methods for enhancing natural genetic resources and developing improved cultivars of cereals, fruits and other crops Lee *et al.* (2002). Information regarding the effect and efficiency of physical and chemical

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mutagens and their combination doses was available in many crops but such information is meager in rice. Hence, an attempt was made in the present to study the effect of single and combination doses of physical and chemical mutagens on rice variety Akshaya (BPT 2231) with an aim to uncover the various mutagenic treatment effects and to estimate the genetic parameters on various quantitative and qualitative characters in M<sub>3</sub> generation.

### Material and Methods

The material for the present study comprised of 24 mutagenic treatments along with the control of Akshaya rice variety (BPT 2231) treated with gamma rays (10kr, 20kr, 30kr & 40kr), ethyl methane sulfonate (0.1%, 0.2%, 0.25% & 0.3%) and their combinations. The M<sub>1</sub> and the M<sub>2</sub> generations were grown during *kharif* 2009 and 2010 respectively at Rice Research Unit, Bapatla. Twenty randomly selected plants per treatment in M<sub>2</sub> generation were advanced to M<sub>3</sub>. The nursery in M<sub>3</sub> was sown as panicle to row method on raised beds at RRU, Bapatla during *kharif* 2011-12. 25 treatments were grown in a randomized block design with three replications. Thirty day old seedlings were transplanted with a spacing of 20x15 cm between and within the rows respectively. Each progeny consisted of 33 plants/replication and each treatment was

represented by 20 rows of 5m length in each replication. Normal recommended cultural practices were followed for raising the crop. Observations were recorded on 10 single plants selected from each progeny at random. Thus, 200 single plants were studied in each treatment for recording observations on yield and yield attributing traits. The quality parameters *viz.*, kernel length, kernel breadth, L/B ratio, amylose content, protein content and alkali spreading value were estimated replication wise on plot basis as per the standard procedures delineated by Murthy and Govinda Swamy (1967), Juliano (1971) and Little *et al.* (1958) and Piper (1966). The mean data was used for analysis of various genetic parameters as per the standard statistical procedures described by Allard (1960) and Burton and Dewane (1952).

### Results and Discussion

The spectrum of macro mutations of varying magnitudes recorded in M<sub>3</sub> generation were grouped character wise. These were found to occur at different stages of crop growth in varying frequencies under different treatments. In M<sub>3</sub> generation of Akshaya, eight different types of morphological mutations were observed (Table 1). Among all types of visible morphological mutants, dwarf plant type occupies the major share followed by tall plants and early types. The

combination treatments of EMS, particularly with 30kr and 40kr series induced majority of dwarf plant types. The minimum plant height in dwarf mutant was below 90 cm. The maximum frequency of dwarf mutants was observed in 30kr + 0.1% EMS followed by 40kr + 0.25% EMS treatment. Chang *et al.* (1985) also reported similar findings. The combination treatments of EMS with 30kr series showed more number of tall plants compared to dwarf ones. The tallest mutant (155cm) was observed in 0.25 % EMS treatment followed by a mutant with 131 cm in 30kr+0.25% EMS while the parent Akshaya possess 100-110cm height. The results were in accordance with the findings of Satyanarayana *et al.* (1993). The earliest flowering mutant was observed in the treatment of 30kr + 0.3% EMS, which flowered in 84 days. The maximum frequency of late flowering mutants (days to heading was delayed by one week to fifteen days than the parent) were identified from the treatment of 40kr followed by 20kr + 0.3% EMS. Rao and Reddi (1986), Chakraborty and Kole (2009) also reported late flowering mutants in rice by induced mutation. Mutants possessing long bold grain type were observed in 20kr and 0.25% EMS treatments while from 30kr + 0.25% EMS and 30kr + 0.3% EMS treatments, mutants with long slender grain type were isolated. The hull colour of the seed

obtained from three treatments viz., 10kr+0.25%EMS, 30kr + 0.30% 0.25% EMS and 40kr + 0.25% EMS was changed to straw colour whereas the untreated control had brown colour hull (Fig. 1& 2). From 30kr+0.3%EMS treatment, mutants possessing dark brown hull colour were also isolated. Domingo *et al.* (2007) and Luzi-Kihupi1 *et al.* (2008) also reported mutants with changed hull color and grain type than their respective parents in rice. Among all treatments studied, 10kr + 0.25% EMS, 30kr+0.3%EMS and 0.25% EMS treatments produced high yielding mutant genotypes with 5-10 per cent yield advantage over the parent Akshaya. The high grain yield recorded in these mutants was not only due to one trait but the cumulative effects of a number of yield attributing traits resulted in the manipulation of high yield. Shashidhar (2001) and Singh and Singh (2003) also reported high yielding mutants in rice through induced mutation.

The analysis of variance for 14 yield components and quality parameters revealed that the mean sum of squares of mutant genotypes were highly significant for all the characters studied indicating the presence of genetic variability among the experimental material. The range, mean and the estimates of genetic parameters for 14 quantitative and qualitative parameters were presented in

Table 2. The results of genetic parameters revealed slight differences between genotypic and phenotypic coefficients of variation values reflecting the minimum environmental influence and consequently greater role of genetic factors on the expression of these traits. High heritability estimates obtained in the present study for all the characters suggest high component of heritable portion of variation that can be exploited by breeders in the selection of superior genotypes on the basis of phenotypic performance. The maximum value for genotypic coefficient of variation and the phenotypic coefficient of variation were observed for alkali spreading value (45.88 and 46.42) respectively followed by grain yield per plant, number of fertile grains per panicle and plant height. Moderate GCV and PCV were manifested for number of productive tillers, days to 50 per cent flowering, test weight, kernel breadth, L/B ratio and protein content while the characters *viz.*, panicle length, kernel length, spikelet fertility and amylose content recorded low estimates. Yusuff Oladosu *et al.* (2014) also reported high GCV and PCV for grain yield/hectare and low GCV and PCV for panicle length in mutant population of MR219 paddy variety by gamma irradiation. Moderate GCV and PCV for days to 50% flowering and test weight Vijaya Lakshmi *et al.* (2008) in upland rice

varieties; and low estimates for kernel length and amylose content were reported by Krishna Veni *et al.* (2006) in aromatic parents and hybrids.

All the fourteen characters studied recorded high heritability estimates ranging from 70.3 (L/B ratio) to 99.8 (No. of fertile grains per panicle). Almost similar results for heritability from studies on induced mutants in aromatic rice have been reported for plant height (Hasib and Kole, 2004); days to flower, test weight and grain yield (Kole and Hasib, 2003). Krishna Veni *et al.* (2006) also reported high heritability estimates for kernel length, kernel breadth, L/B ratio, amylose content and alkali spreading value in a study involving 10 parents and 25 hybrids of aromatic rice. The estimates of genetic advance as per cent of mean were high for alkali spreading value followed by grain yield per plant and number of fertile grains per panicle, moderate for plant height, number of productive tillers, days to 50 per cent flowering, test weight and kernel breadth while the remaining traits manifested low estimates of genetic advance. High genetic advance as per cent of mean has been reported by Sharma and Sharma (2007) for No. of fertile grains per panicle and grain yield per plant. Uttamchand *et al.* (2001) and Mamta Singh *et al.* (2007) also reported

moderate estimates for plant height and test weight. Similar findings for kernel breadth and L/B ratio were previously reported by Krishna Veni *et al.* (2006).

The overall results of genetic parameters revealed that moderate to high GCV, PCV coupled with high heritability and moderate to high genetic advance were observed for plant height, fertile grains per panicle, grain yield and alkali spreading value suggesting the predominance of additive type of gene action in controlling these traits. Thus apparently important contribution of additive genetic variance is involved in the expression of these traits. Hence, good response to selection may be attained in early generations in improving these traits. The remaining characters under study *viz.*, No. of productive tillers, days to 50% flowering, spikelet fertility, test weight, panicle length, kernel length, kernel breadth, L/B ratio, amylose content and protein content manifested low GCV, PCV, high heritability estimates along with low to moderate genetic advance indicating the operation of both additive and non-additive action in the inheritance of these traits.

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**Table 2: Estimates of genetic parameters for yield components and quality traits in M<sub>3</sub> generation of Akshaya variety**

S.No.	Character	Mean	Range	PCV	GCV	Heritability (%)	Genetic advance as percent of mean (%)
1	Plant height (cm)	109.81	96.67-155.33	11.50	11.43	98.7	29.98
2	No. of productive tillers	17.52	14.64-20.67	9.8	9.13	86.7	22.44
3	Days to 50% flowering	115.55	84-124.3	7.98	7.96	99.5	20.96
4	No. of fertile grains/panicle	161.44	121.33-244.0	16.63	16.61	99.8	43.82
5	Spikelet fertility (%)	87.2	75.29-95.54	5.35	5.33	99.3	14.02
6	Panicle length (cm)	25.5	23.12-27.92	4.05	3.49	74.2	7.94
7	Test weight (g)	18.96	15.69-23.40	9.81	9.62	96.1	24.88
8	Grain yield/plant(g)	29.64	22.19-38.72	17.85	17.66	97.9	46.11
9	Kernel length (mm)	5.67	5.35-6.03	3.71	3.67	97.5	9.56
10	Kernel breadth (mm)	2.15	1.95-2.25	8.10	7.79	92.5	19.79
11	L/B ratio		2.51-3.01	8.50	7.13	70.3	15.77
12	Amylose content (%)	24.43	22.40-25.44	3.30	2.85	74.5	6.49
13	Protein content (%)	8.4	7.22-9.26	7.86	6.69	72.5	15.04
14	Alkali spreading value	3.93	1.0-6.83	46.42	45.88	97.7	119.74



**Table 1: Spectrum and frequency of morphological mutants in M<sub>3</sub> generation of Akshaya (BPT 2231) by different mutagenic treatments**

Treatment	Total population	Mutation spectrum										Total morphological mutants	Mutation frequency (%)
		Plant height		Flowering		Hull colour		Grain type					
		Dwarf	Tall	Early flowering	Late flowering	Straw colour	Dark brown	Long slender	Long bold				
<b>Gamma rays</b>													
10kr	1980	-	5	3	-	-	-	-	-			8	0.40
20kr	1980	-	3	-	-	2	-	-	4			9	0.45
30kr	1980	-	2	-	-	-	-	-	-			2	0.10
40kr	1980	-	4	-	12	-	-	-	-			16	0.81
<b>EMS treatments</b>													
0.1%	1980	-	-	-	6	-	-	-	-			6	0.30
0.2%	1980	-	-	-	3	-	-	-	-			3	0.15
0.25%	1980	-	1	-	3	-	-	-	32			36	1.82
0.3%	1980	7	-	-	-	-	-	-	-			7	0.35
<b>Combination treatments</b>													
10kr+0.1%EMS	1980	5	-	-	-	-	-	-	-			5	0.25
10kr+0.2%EMS	1980	5	3	-	-	-	-	-	-			8	0.40
10kr+0.25%EMS	1980	12	5	-	2	35	-	-	-			54	2.73
10kr+0.3%EMS	1972	2	-	-	1	-	-	-	-			3	0.15
20kr+0.1%EMS	1974	-	-	5	3	-	-	-	-			8	0.41
20kr+0.2%EMS	1980	-	-	-	4	-	-	-	-			4	0.20
20kr+0.25%EMS	1978	3	-	-	7	-	-	-	-			10	0.51
20kr+0.3%EMS	1980	-	-	-	11	-	-	-	-			11	0.56
30kr+0.1%EMS	1980	22	-	-	-	-	-	-	-			22	1.11
30kr+0.2%EMS	1970	-	16	25	-	-	-	-	-			41	2.08
30kr+0.25%EMS	1973	-	26	-	7	-	-	22	-			55	2.79
30kr+0.3%EMS	1980	5	18	35	-	5	3	21	2			89	4.49
40kr+0.1%EMS	1980	13	-	-	-	-	-	-	-			13	0.66
40kr+0.2%EMS	1980	-	2	-	-	-	-	-	-			2	0.10
40kr+0.25%EMS	1975	21	-	-	-	15	-	-	-			66	3.34
40kr+0.3%EMS	1980	-	-	-	3	-	-	-	-			3	0.15
<b>Total</b>		<b>95</b>	<b>85</b>	<b>68</b>	<b>62</b>	<b>57</b>	<b>3</b>	<b>43</b>	<b>38</b>			<b>451</b>	



**Figure 1: Variation in hull colour and grain shape in Akshaya mutant population**



**Figure 2: Panicles of Akshaya (left) and Akshaya mutant**

# Molecular Screening for Fertility Restorer Genes *Rf3* and *Rf4* of WA -CMS and Evaluation of F<sub>1</sub> hybrids in Rice (*O. sativa* L.)

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## Abstract

In rice WA (Wild Abortive) CMS system is commercially used for hybrid seed production. In WA-CMS fertility restoration is governed by two independent and dominant genes namely *Rf4* and *Rf3*. Conventionally, the process of screening for the trait of fertility restoration is by tedious testcross progeny evaluation. In this study, earlier reported SSR markers RM6100 and RM 10313 linked to *Rf4* and *Rf3*, respectively have been utilized to screen one hundred breeding lines and identified that 61 lines to carry both *Rf3* and *Rf4* genes and these lines can be utilized in hybrid rice breeding as restorers. A set of eighteen restorer lines with different combination of *Rf4* and *Rf3* were selected for crossing with five CMS lines viz., APMS6A, Pusa 5A, IR58025, IR68897, IR79156 and IR68888 and seventy test cross progenies

were evaluated for their fertility restoration based on pollen and spikelet fertility. The hybrids viz., APMS6A X GQ-86, IR 79156A X IR-55778R, APMS 6A X VG-269 and IR 68888A X BR-827-35 were observed to have more than 90% spikelet fertility. In this study observed that restoration ability varied with different CMS lines hence CMS lines also playing major role achieving higher heterosis.

**Key words:** Hybrid Rice, Molecular markers, Fertility restoration, *Rf4*, *Rf3*.

Rice is a staple food for more than half of the world's population. Hybrid rice have clearly shown a standard heterosis of 15–20% in commercial cultivation mainly in the *indica* genotypes (Hussain *et al.*, 2010). The magnitude of heterosis depends on the choice of appropriate parental lines. Rice being self pollinated crop, use of male sterility system is a prerequisite for commercial exploitation of heterosis in rice.

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The WA cytoplasm is the most widely used since it is a most stable system and the pollen sterility is almost nearly complete (Shinjyo and Omura 1966). Pollen abortion in WA-CMS is sporophytic, forming typical abortive pollen (Huang *et al.*, 2003). CGMS system/ Three-line system has been widely used for developing rice hybrids. This system involves a CMS or 'A' line, a maintainer or 'B' line and a restorer or 'R' line. Since three lines are required for the production of a hybrid, this is popularly called as three line system. Cytoplasmic male sterility (CMS) is a maternally inherited trait that results in inability of the plant to produce fertile pollen. Pollen fertility is restored by nuclear-encoded genes called fertility restorer (*Rf*) gene. For developing high yielding heterotic hybrids, the first step is to identify restorers that can efficiently restore the fertility of CMS lines. Earlier investigations confirmed that fertility restoration is governed by two independent dominant nuclear genes with one gene being stronger in action than the other (Young and Virmani 1984; Virmani *et al.*, 1986). Different studies also indicated different types of gene interaction like recessive epistasis, (Govinda Raj and Virmani 1988) semi-epistasis (Pradhan and Jachuck 1999), epistasis with incomplete dominance

(Govindaraj and Virmani 1988; Sarkar *et al.*, 2002), epistasis with complete dominance (Sohu and Phul 1995) or no interaction (Li and Yuan 1986). Huang *et al.* (1986), Anandakumar and Subramaniam (1992) reported that a major dominant gene controls fertility restoration of WA-cytoplasm. However most of the genetic studies of fertility restoration for the WA CMS system have suggested that fertility restoration is governed by two genes namely *Rf4* and *Rf3* have been mapped to chromosomes 10 and 1 respectively (Yao *et al.*, 1997; Zhang *et al.*, 1997; Ahmadikhah and Karlov 2006; Ahmadikhah and Alavi 2009). The use of molecular markers linked to *Rf* genes can enhance the selection efficiency, save time and avoid the complications associated with phenotype-based screening. The genetic linkage analysis indicated that the SSR markers RM6100 reported by Singh *et al.* (2005), on the long arm of chromosome 10, linked with the *Rf4* gene at distance of 1.2 cM and RM10313 reported by Neeraja (2008), on the short arm chromosome 1, linked with *Rf3* gene at a distance of 4.2 cM have been utilized to screen one hundred breeding lines for the identification of restorers. Among these breeding lines eighteen lines have been selected for test crossing to study the relative

role of *Rf3* and *Rf4* genes in fertility restoration of WA-CMS system.

## Materials and Methods

### Plant material

The leaf samples of one hundred breeding lines were collected from 15-20 days old seedlings grown at Directorate of Rice Research, Rajendranagar, Hyderabad, during early hours (8am to 9am) and stored at -20°C for DNA isolation.

### Molecular analysis

DNA was isolated from young leaves by CTAB method reported by Dellaporta *et al.* (1983). With respect to the SSR markers, polymerase chain reaction was carried out using 15–20 ng of template DNA, 250 µM of dNTPs (Eppendorf, USA), 5 pmoles of each F and R primer, 1 unit of Taq DNA polymerase (Bangalore Genei, India), 1X PCR reaction buffer (Bangalore Genei, India) in a total volume of 10 µl. The cycling conditions were an initial denaturation at 94°C for 5 min followed by 35 cycles of PCR amplification under the following parameters: 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C, followed by a final extension at 72°C for 7 min. The sequences for the SSR primers are presented in (Table 1).

Amplified PCR products were resolved in 3% agarose gel, stained with ethidium bromide and visualized under UV light using the Alpha Imager® 1220 gel documentation system (Alpha Innotech Corporation San Leandro, CA, USA).

### Spikelet fertility was calculated by:

$$\text{Spikelet fertility \%} = \frac{\text{Number of fertile spikelets in the panicle}}{\text{Total number of spikelet in the panicle}} \times 100$$

## Results and Discussion

In rice, after the deployment of semi- dwarf varieties, hybrid rice technology has been the major strategy for raising further the ceiling of genetic yield. In hybrid seed production using three line system, the combination of a CMS line, a maintainer line and a restorer line carrying the fertility restorer gene (*Rf*) to restore fertility is indispensable for the development of hybrids (Virmani *et al.*, 2003). Wild abortive (WA) type cytoplasmic male sterility (CMS) is commercially used for production of hybrid seeds in Asia.

### Screening for fertility restorer genes *Rf4* and *Rf3*

The one hundred breeding lines have been screened for the presence of fertility restorer gene *Rf4* (Table 2) located on chromosome 10, with the help of SSR marker RM6100

reported by Singh *et al.* (2005). Figure 1 shows the amplification pattern of *Rf4* gene. Out of one hundred, seventy lines showed the presence of *Rf4* by amplifying 175- bp size fragment and twenty three lines showed the absence of *Rf4* by amplifying 165-bp size and seven showed the heterozygous amplification pattern. Based on these results we can confirm that out of one hundred breeding lines seventy are restorers, twenty three are non- restorers and seven lines may be partial restorers. In same way breeding lines were screened with the help of SSR marker RM10313 linked to *Rf3* gene reported by Neeraja (2008). Out of one hundred screened, seventy seven showed the presence of *Rf3* by amplifying 215- bp size fragment and twenty three showed the absence by amplifying 200- bp product size (Figure 2). Based on molecular screening results we can assume that out of one hundred breeding lines, seventy seven are restorers, twenty three are non- restorers and the identified restorer lines could be effectively utilized in hybrid rice breeding program.

### **Evaluation of rice hybrids**

To confirm the fertility restoration of identified restorer lines, eighteen lines with different combinations of *Rf4*, *Rf3* and

without *Rf* genes were selected for test crossing with known five CMS lines (Table 3) and seventy F<sub>1</sub> hybrids were produced. Of the seventy hybrids with or without fertility restorer genes *Rf4* & *Rf3*, ten hybrids were identified to have more than 90% spikelet fertility. The results of F<sub>1</sub> spikelet fertility is presented in Table 4. The F<sub>1</sub> hybrids which are identified as restorer with high spikelet fertility (>90%) are APMS6A x VG 269, Pusa5A x BR-827-35, IR 79156 A x (IR55778R, KMR 3R and GQ 86) and partial restorers (< 70%) are IR 68897A x KMR<sub>3</sub>R and IR 79156A x IBL 57 and partial sterile (<50%) are IR 68897A x C-20R and IR 68897A x EPLT 109 and maintainers (<20%) are IR 68897 A x GQ 37-1 & Pusa 5A x BR 827-35. But presence or absence the *Rf* genes under study were not showing a significant influence on spikelet fertility of the F<sub>1</sub> hybrids. According to Govind *et al.* (1988) the fertility restoration is governed by two independent and dominant genes, and one of the genes appeared to be stronger in action than the other. Cai *et al.* (2013) studied allelic differentiations and effects of the *Rf3* and *Rf4* genes on fertility restoration in rice and explained allelic differences, interactions and background effects are influencing the fertility than presence of these genes. These

two fertility restorer genes are additive in their inheritance and the effect of *Rf4* appeared to be larger than that of *Rf3* (Yao *et al.* 1997, Zhuang *et al.* 2001, Sattari *et al.* 2008).

The mode of action of the two genes varied in different CMS/restorer combinations. The present study also confirms that mode of action of fertility restorer genes are different in different CMS/restorer combination. Although all the CMS lines derived from WA source, restorer lines performance varied with the different CMS lines hence CMS diversification may have direct influence on improving grain yield heterosis.

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**Table 1: Primer sequence**

Name of the Primer	Gene tagged	Primer Sequence (5' - 3')	Amplification Product Size (bp)	AT(° C)
RM 6100	<i>Rf4</i>	F:TTCCCTGCAAGATTCTAGCTACACC R:TGTTTCGTCGACCAAGAACTCAGG	175 Restorer 165 Non Restorer	55
RM 10313	<i>Rf3</i>	F: ACTTACACAAGGCCGGGAAAGG R: TGGTAGTGGTAACTCTACCGATGG	215 Restorer 200 Non restorer	55

**Table 2: Screening results of *Rf4* and *Rf3***

S. No.	Genotype	RM 6100	RM 10313	<i>Rf3</i> & <i>Rf4</i>
1.	BCW-56	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
2.	EPLT-109	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
3.	EPLT-104	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
4.	RPHR-612-1	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
5.	RPHR-111-3	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
6.	RPHR-1096	<i>Rf4</i>	No	<i>Rf4</i>
7.	KMR-3	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
8.	RPHR-619-2	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
9.	RPHR-1009	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
10.	RPHR-1004	H	<i>Rf3</i>	<i>Rf3</i>
11.	RPHR-1005	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
12.	SC5 2-2-1	No	<i>Rf3</i>	<i>Rf3</i>
13.	GQ-37-1	No	<i>Rf3</i>	<i>Rf3</i>
14.	RPHR-611-1	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
15.	SALIVAHANA	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
16.	RPHR-1124	No	<i>Rf3</i>	<i>Rf3</i>
17.	SC5 22-2-3-1	No	<i>Rf3</i>	<i>Rf3</i>
18.	GQ-102	No	<i>Rf3</i>	<i>Rf3</i>
19.	GQ-70	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
20.	GQ-58	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
21.	GQ-54	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
22.	RPHR-998	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>

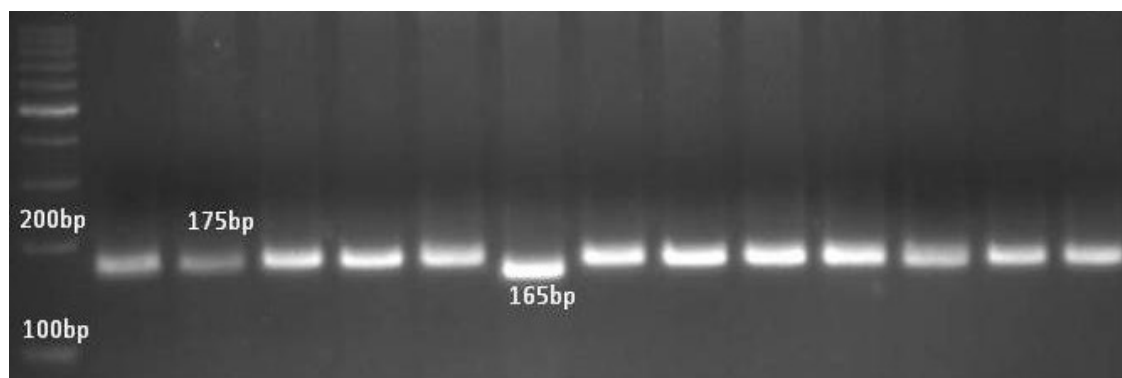
23.	GQ-64-1	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
24.	IRCD 16-9-2-1	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
25.	IRCD 16-1-4-2-1	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
26.	DR 714-1-2R	<i>Rf4</i>	No	<i>Rf4</i>
27.	RPHR-945-1-2	No	<i>Rf3</i>	<i>Rf3</i>
28.	SG22-289-3	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
29.	IBL-52-1	No	<i>Rf3</i>	<i>Rf3</i>
30.	VG-13	No	No	No
31.	VG-58	<i>Rf4</i>	No	<i>Rf4</i>
32.	VG-175	No	No	No
33.	VG-269	No	No	No
34.	VG-294	No	No	No
35.	IR-40750R	No	No	No
36.	MTU-9992	No	<i>Rf3</i>	<i>Rf3</i>
37.	C-20R	<i>Rf4</i>	No	<i>Rf4</i>
38.	UPRI-92-133	<i>Rf4</i>	No	<i>Rf4</i>
39.	BR-827-35	No	No	No
40.	IR-66	<i>Rf4</i>	No	<i>Rf4</i>
41.	NDR-3026	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
42.	AJAYA-R	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
43.	PNR-3158	No	No	No
44.	SC5 9-3	No	No	No
45.	TCP-3699	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
46.	IR-55178R	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
47.	SG-27-105	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
48.	SG-27-131	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
49.	SG-27-175	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
50.	SG-27-177	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
51.	RPHR-255	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
52.	IBL-57	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
53.	RPHR-517	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
54.	SG-17-118-3	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>

55.	RPHR-118	<i>Rf4</i>	No	<i>Rf4</i>
56.	GQ-25	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
57.	GQ-25-74	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
58.	RPHR-124	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
59.	SG-26-120	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
60.	SG-22-23-1	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
61.	NRI-38P2	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
62.	RPHR-972P1	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
63.	SHRABANI	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
64.	SC5 28-4-1-1	No	<i>Rf3</i>	<i>Rf3</i>
65.	RPHR-628-2	No	<i>Rf3</i>	<i>Rf3</i>
66.	PNR-2-49	No	<i>Rf3</i>	<i>Rf3</i>
67.	RPHR-695-1	No	<i>Rf3</i>	<i>Rf3</i>
68.	TG-70P1	No	<i>Rf3</i>	<i>Rf3</i>
69.	TG-64P4	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
70.	TG-23P4	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
71.	B-95-12	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
72.	376	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
73.	524-2	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
74.	541-2	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
75.	1163	H	<i>Rf3</i>	<i>Rf3</i>
76.	BR-22	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
77.	SN-199	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
78.	SN-230	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
79.	SN-234	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
80.	SN-241	No	<i>Rf3</i>	<i>Rf3</i>
81.	SN-247	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
82.	SN-257	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
83.	R-42	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
84.	R-43	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
85.	R-57	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
86.	AYT-1(APO)	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>

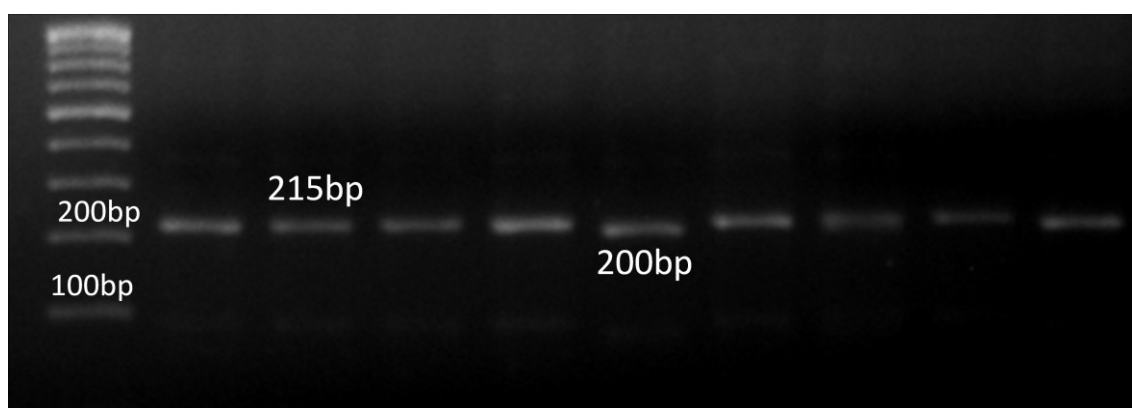
87.	AYT-3(IR-72667-16-1-B-B-3	H	<i>Rf3</i>	<i>Rf3,Rf4(H)</i>
88.	IR-78877-181-B-1-2	H	No	<i>Rf4(H)</i>
89.	IR-79956-B-60-2-3	H	No	<i>Rf4(H)</i>
90.	CR-691-58	No	No	<i>NO</i>
91.	IRRI-7	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
92.	IRRI-10	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
93.	IRRI-37	<i>Rf4</i>	No	<i>Rf4</i>
94.	VIBHAV	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
95.	VIKRAMARYA	H	No	<i>Rf4(H)</i>
96.	PHALYUNA	<i>Rf4</i>	No	<i>Rf4</i>
97.	ADHITYA	H	No	<i>Rf4(H)</i>
98.	IET-19367	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>
99.	AJAYA	<i>Rf4</i>	No	<i>Rf4</i>
100.	R-38	<i>Rf4</i>	<i>Rf3</i>	<i>Rf3/Rf4</i>

**Table 4: Spikelet fertility of hybrid**

S. No.	Hybrid and <i>Rf</i> gene	SPF (%)
1	APMS6A/VG-269 (no <i>Rf</i> gene)	91.0
2	APMS6A/IR66 ( <i>Rf4</i> )	73.7
3	PUSA5A/RPHR-1124 ( <i>Rf3</i> )	79.5
4	PUSA5A/VG-269 (no <i>Rf</i> gene)	66.1
5	PUSA5A/EPLT-109 ( <i>Rf3</i> & <i>Rf4</i> )	50.0
6	PUSA5A/GQ-86 ( <i>Rf3</i> & <i>Rf4</i> )	90.8
7	PUSA5A/IBL-57 ( <i>Rf3</i> & <i>Rf4</i> )	64.5
8	IR58025A/SG27-105 ( <i>Rf3</i> & <i>Rf4</i> )	77.4
9	IR58025A/GQ-70 ( <i>Rf3</i> & <i>Rf4</i> )	63.7
10	IR58025A/BR827-35 (no <i>Rf</i> gene)	70.8
11	IR68897A/KMR-3 ( <i>Rf3</i> & <i>Rf4</i> )	58.0
12	IR68897A/C-20R ( <i>Rf4</i> )	42.7
13	IR68888A/BR827-35 (no <i>Rf</i> gene)	92.1
14	IR79156A/GQ37-1( <i>Rf3</i> )	90.9
15	IR79156A/IR55778R ( <i>Rf3</i> & <i>Rf4</i> )	92.8
16	IR79156A/RPHR1096 ( <i>Rf4</i> )	62.3
17	IR79156A/KMR-3 ( <i>Rf3</i> & <i>Rf4</i> )	90.3
18	IR79156A/GQ86 ( <i>Rf3</i> & <i>Rf4</i> )	73.9
19	IR79156A/C-20R ( <i>Rf4</i> )	42.7
20	IR79156A/IBL-57( <i>Rf3</i> & <i>Rf4</i> )	59.9



**Figure 1: Screening of fertility restorer gene (*Rf4*)**



**Figure 2: RM 10313 Screening for *Rf3* gene**

**Table 3: Selected parents for crossing**

PARENT	RM6100	RM 10313
RPHR-1124	-	P
SG-27-105	P	P
VG-269	-	-
EPLT-109	P	P
RPHR-118	P	-
GQ-25	P	P
GQ-37-1	-	P
IR-55178	P	P
RPHR 1005	P	P
GQ -70	P	P
BR 827-35	-	-
RPHR 1096	P	-
KMR 3	P	P
GQ 86	P	P
IR 66	P	-
C 20 R	P	-
IBL 57	P	P
IR 24	P	P

# Impact of Low Temperature Tolerance at Seedling Stage on Rice Genotypes

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## Abstract

The experiment was carried out at the quality laboratory of Genetics and Plant Breeding department, IGKV, Raipur. The experimental material comprised of 50 rice genotypes, received from IRRI, Philippines, along with four local checks. The experiment was laid out in RCBD with two replications and 54 treatments. The results of experiment explained that root length varies from 0.1cm to 0.8 cm. One of the genotype HHZ 5-DT1-DT1 had 0.8 cm root length at 15° C and its germination percentage is also 86.66 %, therefore this genotype is supposed to be good for summer paddy since it has cold tolerance. It was also observed that the root and shoot length were similar in most of the genotypes at 15° C as well as 28 °C. The maximum shoot length recorded was 9.3 cm in HHZ 12-SAL 8-Y1-SAL1 genotype. The genotypes which showed

100 per cent germination were IR 10C132 followed by IR 10C153, HHZ 8-SAL6-SAL3-Y2 and HHZ 17-DT6-Y1-DT1. These genotypes exhibited tolerance for cold temperature for germination. The genotypes which come under 40 to 80 per cent germination can be considered as tolerant for cold temperature. The genotypes HHZ 5-Y3-Y1-DT1, HHZ 5-DT20-DT2-DT1 and HHZ 12-SAL 8-Y1-Y2 recorded germination percentage below 40 per cent are considered as susceptible for germination under cold and not recommended for growing.

**Key words:** Germination, cold temperature, rice, genotype.

Rice (*Oryza sativa* L.) is an important food crop of India. The cultivated rice originated in the South East Asia. Rice is the most important cereal crop because of its use as prime food in many countries of world. Rice is mainly grown during *kharif*,

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but in some areas it is also grown during *rabi*. *Rabi* paddy is usually sown in the month of November to January that coincides with winter season in North India, and the cold adversely affect the *rabi* rice germination, growth at seedling stages, tillers formation and fertility and ultimately affecting yield.

In North India, rice is grown as second crop during summer also known as summer paddy. The sowing of summer paddy are taken up during January, that coincides with very low atmospheric temperature as winter season in this region is at peak during this period. During this period, germination of rice is drastically affected due to low temperatures. Rice plants are susceptible to low temperature during the young microspore primordial stage, which occurs 10–12 days before heading. Low temperature during seedling and vegetative growth stage of summer rice crop affects germination, establishment of seedling, seedling chlorosis, mortality and prolongs the duration of the crop and it effects the transplanting of subsequent autumn rice crop. Low temperature stress is an important factor affecting the growth and development of rice during germination and seedling stage mainly on *rabi* (summer rice). Cold temperature during juvenile phase affects yield contributing

traits and spikelet fertility. Low temperature at this stage increases spikelet sterility which can cause massive yield loss. The spikelet sterility may be the cause of improper development of male and female organs, which ultimately affects the economic produce and farmer's income.

## Materials and Methods

The experimental material comprised of 50 rice genotypes, received from IRRI, Philippines, along with four local checks. The experiment was conducted under lab condition in the germinator under different temperature.

About 30 quality seeds of 50 rice genotypes and 4 local checks were placed on wet filter papers in Petri dishes at 15 °C for germination. After a cold treatment for two weeks, the germination percentage (Gper15), shoot length (SL15) and root length (RL15) of the seedlings were measured. Then temperature was increased to 28°C to initiate the growth of seedling recovery. One week later, the germination percentage (Gper28), shoot length (SL28) and root length (RL28) of the seedlings were again recorded. The experiment was conducted in two replications to observe the germination percentage and seedling growth at 15°C as well as at 28 °C. Root and shoot lengths

were measured for growth at both temperatures under controlled conditions (Sun *et al.*, 2012).

### Results and Discussion

Highest 100 per cent seed germination was recorded in varieties named IR 10C132 followed by IR 10C153, HHZ 8-SAL6-SAL3-Y2 and HHZ 17-DT6-Y1-DT1. These genotypes exhibited tolerance for cold temperature for germination whereas genotypes which showed germination of 40 to 80 per cent can be considered as tolerant for cold temperature. The genotypes HHZ 5-Y3-Y1-DT1, HHZ 5-DT20-DT2-DT1 and HHZ 12-SAL 8-Y1-Y2 recorded germination percentage below 40 percent are considered as susceptible for germination under cold and not recommended for summer paddy (Table 1 & Fig. 1-7).

The root length development at 15°C was very poor in many varieties. It ranged from 0.1 to 0.8 cm. Genotype HHZ 5-DT1-DT1 had 0.8 cm root length at 15° C and its germination percentage is also 86.66 per cent, therefore this genotype is supposed to be good for summer paddy. This is followed by HHZ8-SAL6-SAL3-Y2 having 0.7 cm root length at 15° C with 100 per cent germination, IR 10G103 with 0.7 cm root length with 96.66 per cent germination and IR 10C157 with 0.7 cm

root length and 90 per cent germination (Table 1 & Fig. 1-7).

It is observed that the root and shoot development is similar in most of the genotypes at 15°C as well as at 28°C. Genotypes HHZ 12-Y4-Y1-DT1, HHZ 5-SAL 14-SAL 2-Y2, IR 64197-3B-15-2 and HHZ 5-SAL 14-SAL 2-Y1 exhibited 0.1cm shoot expansion at 15°C whereas in 28°C they have shown 8.5cm shoot length. The maximum shoot length recorded was 9.3 cm in HHZ 12-SAL 8-Y1-SAL1 genotypes (Table 1 & Figs. 1-7). Ye *et al.* (2008) revealed that the cold tolerance at germination stage was correlated with those at seedling, booting and flowering stage and the cold tolerance at seedling stage was also correlated with those at booting stage. This suggests that selection at germination and early seedling stage may be useful in rice breeding programs focused on cold tolerance. Similar studies were reported by Xiong *et al.* (1990), Nilanjaya *et al.* (2003), Cruz and Milach (2004) and Xia *et al.* (2006).

### Conclusion

The results of experiment explained that one of the genotypes HHZ 5-DT1-DT1 had 0.8 cm root length at 15° C and its germination percentage is also 86.66 per cent, therefore this genotype is supposed to be good for summer paddy since it has



cold tolerance. It was also observed that the root and shoot length were similar in most of the genotypes at 15° C as well as 28 °C. The maximum shoot length recorded was 9.3 cm in HHZ 12-SAL 8-Y1-SAL1 genotype. The genotypes which come under 40 to 80 per cent germination can be considered as tolerant for cold temperature. The genotypes HHZ 5-Y3-Y1-DT1, HHZ 5-DT20-DT2-DT1 and HHZ 12-SAL 8-Y1-Y2 recorded germination percentage below 40% are considered as susceptible for germination under cold and not recommended for growing.

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**Table 1: Observations were recorded at 15°C and 28°C**

S.NO	I.C.NO. / DESIGNATION (TEMPERATURE)	GERMINATION (%)	ROOT LENGTH (cm)		SHOOT LENGTH (cm)	
		15 °C	15 °C	28°C	15 °C	28°C
1.	IR 10C103	93	0.2	8.5	0.4	7.0
2.	IR 10C173	83	0.2	8.3	0.3	8.0
3.	IR 10C179	83	0.4	8.2	0.3	7.5
4.	HHZ 5-Y3-SAL2-SUB 1	83	0.3	8.5	0.3	7.8
5.	IR 10C136	63	0.3	8.7	0.3	7.7
6.	HHZ 12-Y4-Y1-DT1	50	0.2	8.8	0.1	8.5
7.	HHZ 5-SAL 14-SAL 2-Y2	70	0.1	8.3	0.1	8.5
8.	HHZ 8-SAL 6-SAL-3-SAL 1	63	0.1	8.1	0.3	8.5
9.	HHZ 8-SAL 6-SAL 3-Y 1	56	0.1	8.5	0.2	6.0
10.	HHZ 5-Y3-Y1-DT1	20	0.3	7.5	0.5	6.5
11.	IR 10C174	80	0.4	8.6	0.3	9.0
12.	HHZ 12-SAL 8-Y1-SAL 1	70	0.4	8.8	0.5	9.3
13.	N 22	80	0.5	8.8	0.2	8.9
14.	IR 83141-B-32-B	80	0.2	8.2	0.3	7.5
15.	HHZ 5-DT20-DT2-DT1	23	0.4	5	0.2	8.7
16.	HHZ 12-SAL 2-Y3-Y2	63	0.5	6.8	0.3	8.5
17.	IR 10C132	100	0.2	8.5	0.4	8.8
18.	HHZ 5-SAL10-DT3-Y2	46	0.4	8.2	0.4	8.6
19.	HHZ 17-DT 16-Y3-Y1	86	0.3	8	0.4	7.8
20.	IR 64197-3B-15-2	76	0.3	6.3	0.1	8.4
21.	IR 10C113	66	0.4	8.5	0.3	8.8
22.	IR 10C153	100	0.4	8.4	0.5	8.7
23.	HHZ 8-SAL 6-SAL 3-Y 2	100	0.7	8.4	0.5	8.3
24.	HHZ 11-DT7-SAL 1-SAL 1	86	0.5	8.2	0.4	8.0
25.	IR 10C110	50	0.6	8.5	0.5	8.8
26.	HHZ 8-SAL 12-Y2-DT 1	83	0.6	8.8	0.4	7.8
27.	IR 10C167	66	0.5	7.5	0.3	8.0
28.	HHZ 5-SAL14-SAL2-Y1	80	0.4	8	0.1	8.5
29.	HHZ 12-SAL 8-Y1-Y2	33	0.4	8.5	0.3	8.5
30.	IR 10C108	46	0.5	8	0.3	8.0
31.	IR 10C139	73	0.7	8.6	0.4	8.5
32.	LOCAL CHECK	76	0.7	7	0.8	9.0
33.	IR 10C137	76	0.6	8.5	0.5	8.0
34.	IR 83143-B-51-B	93	0.6	8.5	0.7	8.6
35.	IR 10G103	96	0.7	7.7	0.7	8.6
36.	HHZ 5-DT1-DT1	86	0.8	8.2	0.7	8.7
37.	DULAR(ACC 32561)	83	0.3	8.2	0.5	8.5
38.	IR 10C114	86	0.4	8.7	0.3	8.7
39.	HHZ 17-DT6-Y1-DT1	100	0.4	8.8	0.7	8.0
40.	IR 83142-B-36-B	90	0.6	8.8	0.4	8.4
41.	IR 10C161	96	0.6	8.8	0.6	8.5
42.	HHZ 12-Y4-DT1-Y2	76	0.4	6.6	0.4	7.8

43.	IR 10C157	90	0.7	8.5	0.4	8.8
44.	IR 10C126	93	0.6	8.4	0.6	7.5
45.	IR 10C172	93	0.8	8	0.4	8.3
46.	IR 10C138	70	0.8	8.3	0.8	8.9
47.	HHZ 5-SAL8-DT2-SAL 1	53	0.7	8.1	0.3	8.9
48.	HHZ 17-Y16-Y3-Y2	86	0.7	6.5	0.5	8
49.	HHZ 12-Y4-DT1-Y3	76	0.8	8.8	0.6	8.3
50.	HHZ 8-SAL 14-SAL 1- SUB 1	83	0.8	8.5	0.5	7.9
C-1	POORNIMA	60	0.7	8	0.8	8.7
C-2	KARMA MAHSURI	73	0.8	8.7	0.5	4.8
C-3	IGKV-R1	90	0.6	8.7	0.8	7
C-4	MAHESHWARI	76	0.6	8.2	0.8	5.5
	<b>AVERAGE</b>	<b>75.37</b>	<b>0.49</b>	<b>8.16</b>	<b>0.43</b>	<b>8.13</b>

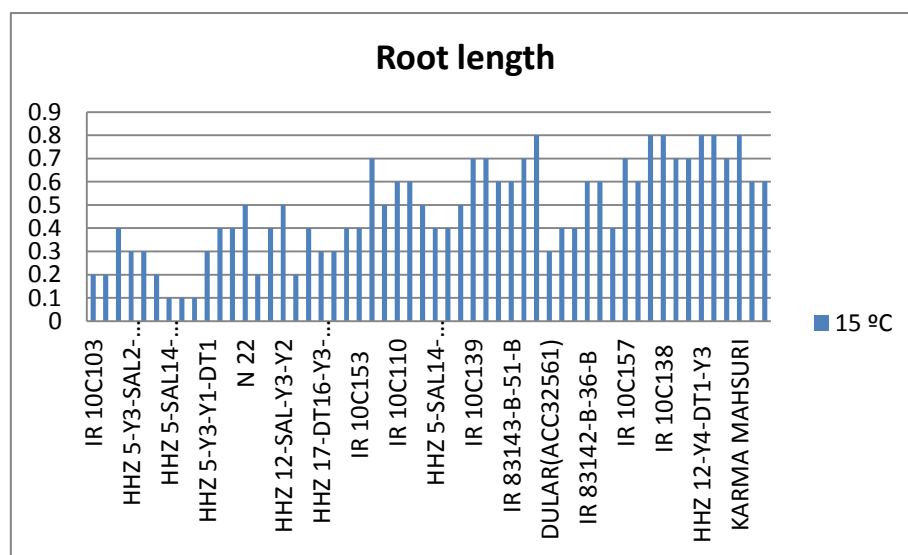
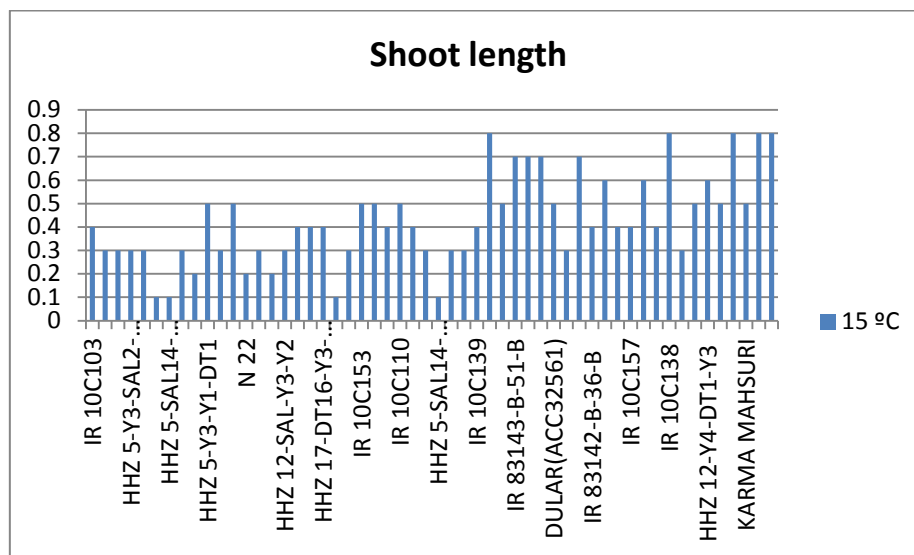
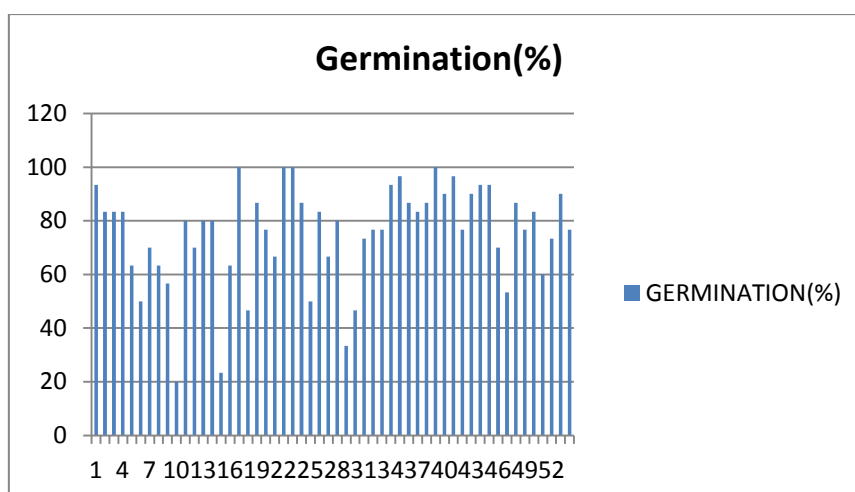


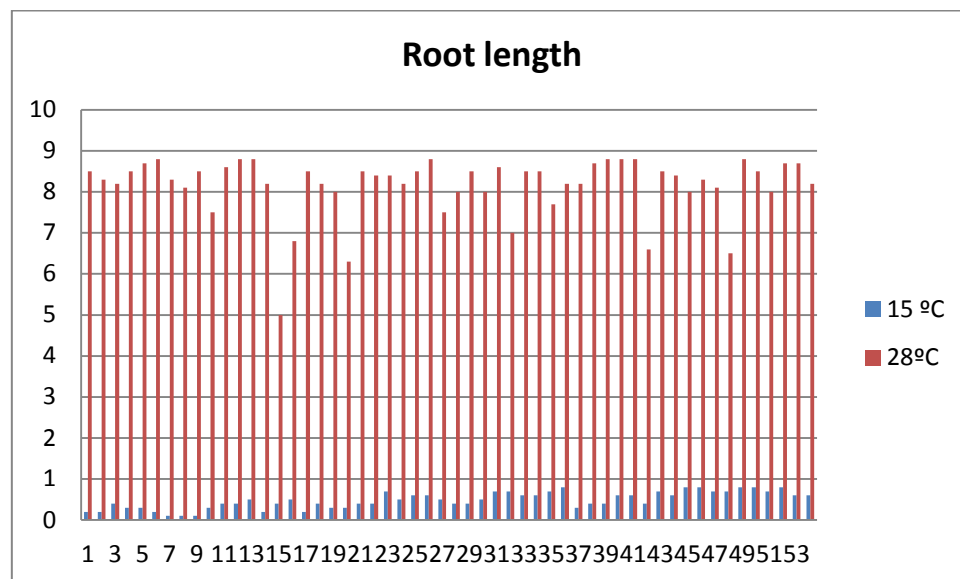
Figure 1: Root length at 15 °C for 2 weeks



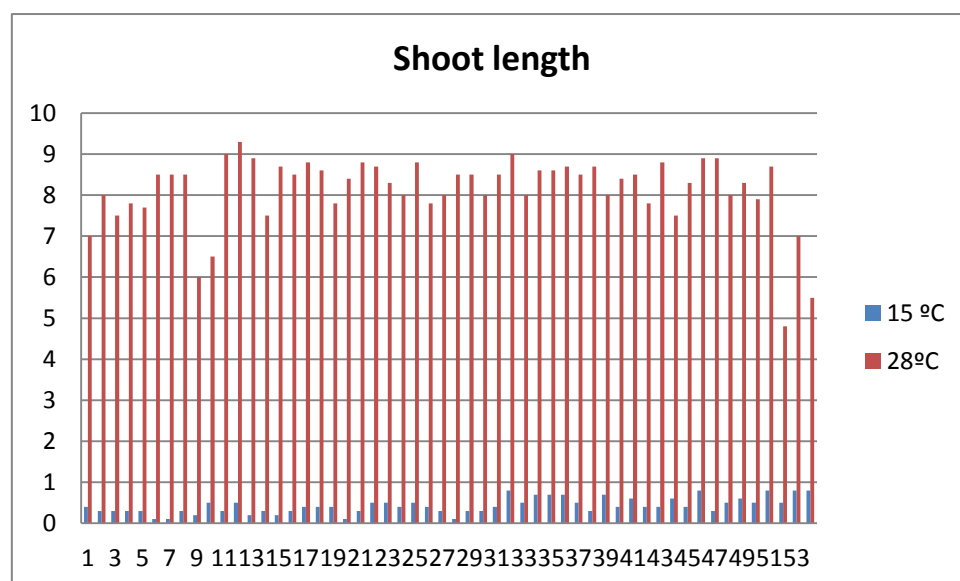
**Figure 2: Shoot length at 15 °C for 2 weeks**



**Figure 3: Germination (%) after 2 weeks**



**Figure 4: Root length at 15 °C for 2 weeks and then at 28°C for a week**



**Figure 5: Shoot length at 15 °C for 2 weeks and then at 28°C for a week**



**Figure 6: Germination of seedlings after 2 weeks**



**Figure 7: Maximum root and shoot length**

# **Assessment of Grain Zinc and Iron Variability in Rice Germplasm using Energy Dispersive X-ray Fluorescence Spectrophotometer (ED - XRF)**

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## **Abstract**

**Micronutrient and vitamin deficiency diseases are prevalent among the rice eating population and particularly poor people who are unable to afford nutrient rich supplementary food sources. Consequently, biofortification project has been initiated to identify or develop varieties having high iron and zinc. In the present study, rice germplasm was collected from various sources and cultivated at one location. Considering the advantages of grain micronutrient content analysis through non-destructive method, grain iron and zinc content were analyzed with energy dispersive X-ray fluorescence spectrophotometer (ED - XRF). Among the various germplasm studied, promising lines for zinc alone were identified in IRRI and North Eastern Land Races to further enhance zinc through conventional breeding. Effect of sample weight and moisture were also studied and the results indicate that 5 g optimum sample weight is essential for ED - XRF and all the samples should**

**contain similar moisture level to identify the best lines.**

**Key words:** XRF, biofortification, micronutrient, rice, iron and zinc.

Half of the global population consumes rice as staple food and poor people in developing countries solely eat rice and they are rarely accessible to nutrient rich food sources to supplement rice. In fact, rice is consumed in polished form (white rice) and it constitutes starch as chief component followed by proteins, lipids, minerals and negligible levels of vitamins and thus, rice supplies more energy than essential nutrients leading to micronutrient deficiency which is also known as “hidden hunger”. The recommended dietary allowance (RDA) of iron and zinc for human population in the age group of 25-50 years are 10-15 and 12-15 mg respectively (FAO/WHO, 2000). In developing countries zinc, iron and vitamin A deficiencies were reported in human population.

Prevalence of micronutrient deficiency diseases among the rice consuming population prompted in initiating biofortification programme

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which has been identified as an efficient means to develop as well as transfer the genetically improved high micronutrient containing rice grains to the poor people who depend on rice for both energy and nutrients. Further, seeds with increased micronutrients also perform better in micronutrient deficient soils by producing longer roots leading to better absorption and yield Graham *et al.* (2001) and improved disease resistance and stress tolerance (Welch, 1999).

Since nutrient quality of rice varies among the varieties, screening of rice germplasm having one or more desired micronutrients at higher concentrations is prerequisite prior proceeding to biofortification. Hence, in this study, rice germplasm was collected from different places and cultivated under the same conditions. Subsequently, grain iron and zinc contents were analyzed and the generated information will be useful for selecting parents/donors for biofortification programme. The effects of grain moisture content and sample weight on grain micronutrient analysis with ED-XRF are also included.

### **Materials and Methods**

Rice germplasm comprising of Landraces collected from North East Land Races (NELR), Aromatic Short Grain (ASG) types from DRR germplasm collection and other germplasm from IRRI were grown at

Ramachandrapuram farm of DRR, ICRISAT campus (17.53 °N latitude and 78.27 °E longitude, 545 m altitude, with mean temperature of 31.2 °C and mean annual precipitation of 988.3 mm), Hyderabad, India during *Kharif* 2012 under irrigated rice ecosystem. Before planting, soil pH was in the range of 8.52 to 8.57 and soil iron and zinc in the samples were 2.74 to 3.48 and 3.55 to 3.66 ppm respectively. After harvesting, all the samples were processed using non-iron and zinc husker and miller Ravindra Babu *et al.* (2014).

Micronutrient content can be estimated by both destructive and non-destructive methods. Iron and zinc were estimated using non-destructive ED - XRF machine which is quite useful to select promising lines from large number of samples and further these values may be confirmed with destructive methods. Brown or polished rice grain can be used as sample in ED - XRF. Rice varies in grain size and moisture content and in addition, sample amount is generally less in genetic studies. Therefore, the effects of moisture and sample size on micronutrient estimation were also studied by taking short, medium and long grain rice samples and these parameters were considered while screening the germplasm.

### **Optimization of sample weight**

Thirty rice genotypes of different grain types - short, medium and long grain



samples were separately analyzed for grain iron and zinc content in triplicate using ED-XRF with manufacturer recommendations except for sample weight. As the objective of the present study is to standardize sample weight, for each genotype, samples of one to ten grams were made and used for analysis.

### **Effect of moisture**

Grain iron and zinc content were analyzed with ED-XRF and volume of the fixed amount of rice grain was followed using 10 ml measuring jar before and after incubation in hot air oven.

### **Micronutrient estimation**

Grain iron and zinc contents were estimated with ED - XRF in brown and polished rice samples by taking the optimum weight identified during the above experiment. Based on these values, separate equations were also developed to predict the iron and zinc content in the polished samples using values of the brown rice.

## **Results and Discussion**

### **Sample weight optimization**

Manufacturer recommended filling rice sample in ED - XRF sample cups to a mark which is roughly  $3/4^{\text{th}}$  volume of the total space and around 20 g of sample is required to fill up to this mark. As per the results obtained (Figure 1), a minimum of

3 and 5 g sample is required for iron and zinc respectively in all the samples tested and therefore, 5 g sample is necessary for simultaneous estimation of both iron and zinc. Nicholas *et al.* (2012) reported 4 g sample mass is required for both rice and pearl millet. The determined minimum sample weight will enhance the life of specially designed sample processing non-contaminating machines as well as significantly reduces the sample processing time.

### **Effect of moisture**

In general, dried samples exhibited more iron and zinc values (Table 1) than samples with moisture. This was due to decrease in the size (Figure-2) of individual grains and thus more number of dried grains was distributed in the inner space of the sample cup leading to increase in the values. Therefore, it is important to store all the samples in uniform conditions to identify the best lines while screening with ED - XRF. Improper moisture content increases grain breakage which in turn affect micronutrient analysis with ED - XRF.

### **Grain iron and zinc content**

Iron content is less in soft rices whereas the range and means of iron are similar in the other collections (Table 2). Highest zinc content was found in IRRI collection followed by NELR germplasm while it was similar in soft and ASG collections.

Among the analyzed, ASG group is smaller in size with more grain surface area, however, the present results doesnot indicate any advantage due to variation in surface area. Range of both iron and zinc is well within the reported Graham *et al.* (1999) range of iron (7.5 to 24.4 ppm) and zinc (13.5 to 58.4 ppm).

### **Correlation between iron and zinc**

In this study, significant positive correlation between iron and zinc was observed in IRRI (Table 3) and soft rice whereas negligible positive association was observed in ASG and NELR germplasm. Correlation analysis between the estimated and predicted values indicates that equation for zinc alone is useful to predict the values in polished rice from the estimated values of brown rice samples. Graham *et al.* (1999) reported positive correlation between iron and zinc in rice, wheat and beans and Stangoulis *et al.* (2007) reported significant positive correlation in double haploid rice population indicating co-segregation of concerned factors. In contrast, Vijay *et al.* (2009) could not find significant correlation in recombinant inbred line populations of wheat except Xgwm473-Xbarc29 and also opined that correlation might be possible with some loci.

### **Effect of polishing**

After polishing, large variation in iron and zinc levels was observed among the varieties. Compared with zinc (~20 to 40%), loss of iron (~60 to 80%) is nearly twice after 10% polishing across the grain shapes. Gregorio (2002) also observed more loss of iron than zinc during polishing. This could be due to partial or complete loss of both embryo and aleurone regions during polishing, more iron is distributed in the embryo followed by aleurone layer and endosperm (Gregorio, 2002), variation in the thickness of the aleurone layer or embryo size or both, etc. In addition to the loss of iron during polishing, another 10 per cent is lost during washing before cooking and ultimately around 20 per cent remains in the cooked food. Whereas, Sanjeeva Rao *et al.* (2014) reported that maximum loss of zinc during polishing is around 40 % and loss during washing before cooking is almost negligible and this indicates that around 60 per cent zinc remains in the cooked food.

Apart from genotypic differences, grain micronutrient content is also dependent on location (Ravindra Babu *et al.*, 2014). Considering this, losses during polishing as well as washing and international threshold values of 7 ppm for iron and 24 ppm for zinc, varieties having  $\geq 30.0$  ppm zinc / iron in brown rice can be considered as potential donors for breeding

programme for enhancing zinc/iron. Promising high zinc containing lines are more in IRRI germplasm (Table 4) than NELR (Table 5). Using the brown and polished values, regression equations were developed separately for iron and zinc to predict the values in polished samples from brown rice values.

However, world average per capita rice consumption is 65.0 Kg, European Union-27 is least with 5.7 Kg, Cambodia is highest with 292 Kg and India with 76.7 Kg (Eric and Eddie, 2012). Hence, in India the average daily intake of rice is around 220 g and polished rice having 45.5 to 68.2 ppm ( $\text{mg Kg}^{-1}$ ) iron and 54.5 to 68.2 ppm zinc can only meet the RDA (FAO/WHO, 2000) without considering their assimilation (bioavailability) from the digested food in the alimentary tract. These values are much higher than the present international threshold values considered for grain iron and zinc. Bioavailability is a complex phenomenon governed by various dietary components. Anti-nutrient like phytic acid binds to these ions and makes them unavailable for absorption and in contrary, citric acid being a pro-nutrient promotes iron absorption. The composition and availability of these components varies among the varieties and thus, a part of the available iron and zinc in the cooked food enter the blood stream. Hence, bioavailability studies on high zinc lines

are prerequisite to plan for further enhancement of zinc in rice varieties.

## Conclusion

Optimum sample weight of 5 g is required to analyze grain micronutrient content in ED - XRF. This is the first report which indicates that ED - XRF underestimates grain micronutrient content in samples with grain moisture content and therefore, moisture should be either uniform across the samples or it should be removed before analysis while selecting the best lines among the germplasm. ED - XRF is quite useful to screen large number of samples and to select promising lines. Variability observed in the present study offers scope for further enhancement of zinc through conventional breeding whereas transgenic approach appears inevitable for iron.

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**Table 1: Effect of grain moisture on grain micronutrient content (in ppm) with ED - XRF**

S. No.	Before oven treatment (A)		After oven treatment (B)		Difference (A-B)	
	Fe	Zn	Fe	Zn	Fe	Zn
1	13.5	16.7	12.7	18.7	0.8	-2
2	19.4	23.8	15.4	26.6	4.0	-2.8
3	18.5	18.1	19	19.6	-0.5	-1.5
4	12	25.8	11	25.4	1.0	0.4
5	39.6	36	42.5	43.6	-2.9	-7.6
6	48	22.7	50.1	23.5	-2.1	-0.8
7	19.8	21.8	16.9	25	2.9	-3.2
8	15.4	26.4	15.6	26.4	-0.2	0
9	12.8	24.2	13.7	24.5	-0.9	-0.3
10	19.2	38.6	18.9	42	0.3	-3.4
11	20.3	28.9	20.4	30.9	-0.1	-2
12	17.8	44.4	19.8	40.5	-2.0	3.9
13	19.4	37.5	18.1	39.7	1.3	-2.2
14	18.9	46.1	18.9	42.8	0	3.3
15	27.1	35.2	27.9	36	-0.8	-0.8
16	22.6	48	20.7	49.1	1.9	-1.1
17	30.1	36.6	31.9	36.3	-1.8	0.3
18	56.9	29.6	60.1	33.1	-3.2	-3.5
19	38.3	32.8	35.5	36.2	2.8	-3.4

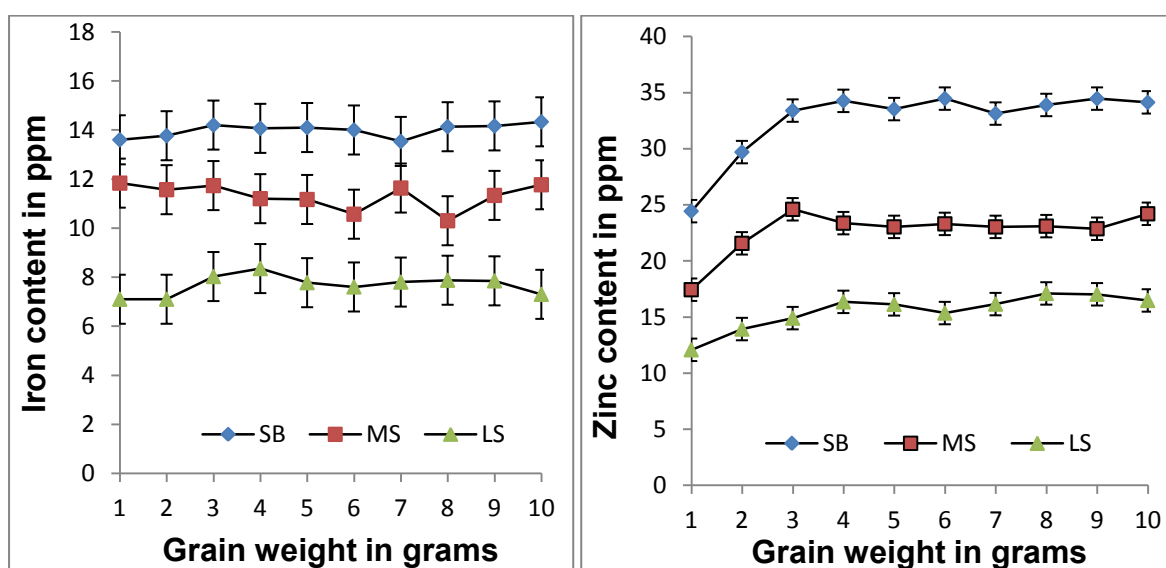
**Table 2: Micronutrient contents in the brown rice of some germplasm collections**

Germplasm (number)	Range of micronutrient in ppm (mean $\pm$ 1.0)		No. of potential donors/entries	
	Iron	Zinc	Iron	Zinc
NELR (230)	7.4-22.7 (10.67)	16.5-33.0 (22.67)	Nil	06
Soft rices (30)	2.7-6.9 (3.98)	17.3-26.9 (21.44)	Nil	Nil
ASG (236)	6.1-14.2 (10.01)	12.7-27.4 (19.12)	Nil	Nil
IRRI (306)	7.2-16.6 (10.29)	18.6-50.0 (31.74)	Nil	164

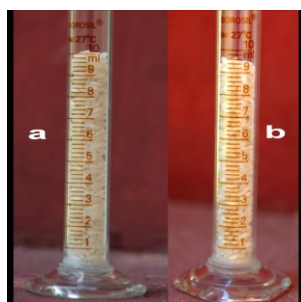
**Table 3: Correlation between iron and zinc in IRRI rice germplasm**

	Iron brown	Iron Polished	Zinc brown	Zinc Polished
Iron brown	1			
Iron Polished	<b>0.25*</b>	1		
Zinc brown	<b>0.46*</b>	0.12	1	
Zinc Polished	0.22	<b>0.45*</b>	<b>0.43*</b>	1

\*Significant at 95.0 %



**Figure 1: Relationship between iron and zinc content with sample weight. SB - short bold, MS - medium slender and LS - long slender**



**Figure 2: Decrease (0.5 mm) in the height of rice sample a) before and b) after the removal of grain moisture content**

**Table 4: List of promising rice varieties from IRRI germplasm for zinc (in ppm)**

Variety designation	Zinc (ppm)	Variety designation	Zinc (ppm)	Variety designation	Zinc (ppm)	Variety designation	Zinc (ppm)
IR91158-133-2-1-2-3	50.0	IR 91184-219-3-3	36.9	IR 92937-237-3-3	34.5	IR 92963-85-1-3	32.4
IR91159-65-2-2-3-3	48.2	IR 91159-11-3-3-2-3	36.8	IR 92943-58-3-3	34.4	IR 91949-111-1-1-3	32.3
IR 91916-3-3-1-3	47.5	IR 91167-157-1-1-3-3	36.7	IR 92966-95-1-3	34.2	IR 92960-87-3-3	32.3
IR 91158-85-3-2-3-3	45.2	IR 92977-170-1-3	36.7	IR 91167-99-1-1-1-3	34.1	IR 91909-53-3-1-3	32.2
IR 90210-123-2-1-1-3	45.1	IR 92937-163-2-3	36.6	IR 91169-14-2-2-2-3	34.1	IR 91949-14-3-3-3	32.2
IR 92937-119-3-3	44.2	IR 91173-81-2-3-1-3	36.5	IR 92937-168-2-6	34.0	IR 92947-140-1-3	32.2
IR 92966-86-1-3	43.8	IR 92971-70-3-3	36.4	IR 92947-26-1-3	34.0	IR 92972-9-2-3	32.2
IR 90210-106-2-1-2-3	42.8	IR 91158-16-1-1-2-3	36.3	IR 90241-72-1-1-3-3	33.8	IR 91166-113-2-3-3-3	32.1
IR 91175-27-1-3-1-3	42.5	IR 91917-6-1-1-3	36.1	IR 92966-30-3-3	33.8	IR 92938-73-1-3	32
IR 92945-68-2-3	42.0	IR 91957-44-2-2-3	36.1	IR 92969-127-2-3	33.8	IR 91173-35-2-3-1-3	31.9
IR 91173-104-1-1-3-3	41.7	IR 92957-147-1-3	36.0	IR 91949-87-1-3-3	33.7	IR 91189-10-1-1-1-3	31.9
IR 91175-50-1-2-1-3	41.6	IR 90210-199-2-1-1-3	35.9	IR 92937-189-1-3	33.7	IR 91896-83-3-1-3	31.9
IR 92956-97-2-3	41.5	IR 92965-82-2-3	35.9	IR 92966-106-2-6	33.6	IR 92969-40-3-3	31.8
IR 91188-63-3-3-1-3	41.2	IR 91175-65-1-2-2-3	35.8	IR 91949-78-2-3-3	33.5	IR 92978-131-2-3	31.8
IR 92957-99-2-3	41.2	IR 91964-143-2-2-3	35.8	IR 92960-75-1-3	33.5	IR 92963-110-2-3	31.7
IR 92967-101-1-3	41.2	IR 91937-213-2-3	35.8	IR 92963-23-1-6	33.5	IR 91189-39-1-2-1-3	31.6
IR 92934-39-1-6	40.7	IR 91184-162-1-3	35.8	IR 91167-133-1-1-2-3	33.4	IR 91922-9-3-1-3	31.6
IR 92937-235-2-3	40.7	IR 91967-25-1-1-3	35.7	IR 92953-60-2-3	33.4	IR 92934-79-1-3	31.6
IR 92947-88-2-3	40.4	IR 92937-166-1-3	35.7	IR 92969-229-1-3	33.4	IR 91949-21-1-1-3	31.5
IR 92937-168-2-3	39.6	IR 92935-29-3-3	35.6	IR 90240-31-2-2-2-3	33.3	IR 91967-30-1-1-3	31.5
IR 91167-106-2-1-3-3	39.3	IR 92937-178-2-3	35.6	IR 91906-4-3-1-3	33.3	IR 90241-197-2-2-1-3	31.4
IR 91171-104-1-2-1-3	39.0	IR 92963-19-2-3	35.5	IR 92947-43-1-3	33.3	IR 9191182-3-2-3	31.4
IR 91182-36-3-3-3-3	38.9	IR 91167-31-3-1-3-3	35.3	IR 91167-46-2-1-2-3	33.2	IR 91949-52-3-1-3	31.4
IR 92937-215-3-3	38.8	IR 91949-48-3-1-8	35.3	IR 91967-88-1-1-3	33.2	IR 92937-116-2-3	31.4
IR 92953-3-2-3	38.7	IR 91175-61-1-2-2-3	35.2	IR 92972-85-3-3	33.2	IR 91184-129-3-6	31.4
IR 91184-122-3-3	38.6	IR 90241-7-1-1-2-3	35.1	IR 92978-5-1-3	33.2	IR 92968-88-1-3	31.4
IR 92966-106-2-3	38.5	IR 92937-205-1-3	35.1	IR 91917-12-1-1-3	33.1	IR 92971-109-1-3	31.4
IR 91149-26-1-3	38.5	IR 92969-162-2-3	35.1	IR 91953-141-2-1-3	33.0	IR 90240-81-3-3-1-3	31.3
IR 91159-99-1-3-1-3	38.4	IR 92937-226-1-3	35.0	IR 92970-111-1-3	33.0	IR 92942-57-3-3	31.2
IR 92937-85-2-3	38.4	IR 92969-227-3-3	35.0	IR 92969-61-2-3	32.9	IR 91184-3-3-3-2-3	31.1
IR 91149-23-3-3	38.2	IR 92963-49-1-3	34.9	IR 91186-39-3-3-2-3	32.8	IR 92969-201-1-3	31.1
IR 92978-79-2-3	37.7	IR 91913-115-3-1-3	34.8	IR 91967-64-1-1-3	32.8	IR 92947-12-1-3	31
IR 92956-129-1-3	37.6	IR 92956-146-3-3	34.8	IR 92934-39-1-3	32.8	IR 92957-123-1-3	31
IR 91180-80-3-2-1-3	37.5	IR 92957-32-3-3	34.8	IR 91172-75-1-2-3-3	32.7	IR 92972-170-1-3	31
IR 92952-144-1-3	37.5	IR 91899-145-1-2-3	34.7	IR 92977-145-1-3	32.6	IR 92978-74-1-3	31
IR 91181-96-1-1-1-3	37.4	IR 91916-35-2-1-3	34.7	IR 90241-2-1-2-1-3	32.5	IR 92978-90-2-3	30.9
IR 91949-97-1-2-3	37.4	IR 91184-159-2-3	34.7	IR 92965-23-2-3	32.5	IR 91962-54-2-1-3	30.8
IR 91166-195-2-1-2-3	37.2	IR 91184-198-1-6	34.6	IR 92969-24-2-3	32.5	IR 91964-172-3-2-3	30.8
IR 91171-103-1-1-1-3	37.0	IR 90210-123-2-1-1-6	34.5	IR 91184-32-2-3-2-3	32.4	IR 91906-99-3-1-3	30.7
IR 92947-57-2-3	36.9	IR 91171-66-3-2-1-3	34.5	IR 92957-88-1-3	32.4	IR 91184-198-1-3	30.7
IR 92972-92-1-3	30.7	IR 92937-56-2-3	30.6	IR 91184-158-2-3	30.6	IR 92958-39-3-3	30.5

**Table 5: List of promising rice varieties from NELR germplasm for zinc**

Entry name	Zinc (ppm)
VPB/GP 61	33.0
VPB/GP 73	32.5
VPB/GP 229	31.6
VPB/GP-114	29.6
VPB/GP 204	29.5
VPB/GP 50	29.3
VPB/GP 105	29.1

# Evaluation of Rice Genotypes for Phosphorus use Efficiency under Soil Mineral Stress Conditions

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## Abstract

In order to identify the superior rice genotypes with higher P use efficiency, and also those tolerant to low soil P status, twenty eight pre-release promising rice varieties and hybrids were evaluated for their grain yield, and response to graded levels of applied phosphorus in a low soil-P fertility status calcareous vertisol (Olsen P: 2.04 ppm P) located, during *kharif* seasons of 2004 and 2005 at DRR farm, Rajendranagar, Hyderabad.

Among rice cultures, four distinct patterns in grain yield response were observed with eight rice cultures at 0 P-level, six rice cultures at medium P-fertility level (20-30 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) were exhibiting higher grain yield response; while five recorded higher grain yields and yield response only at higher P-levels of 50 – 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (65 – 93 kg grain/kg P<sub>2</sub>O<sub>5</sub>) compared to others (16 –

66 kg grain kg<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>). The other cultures IET 17190, Sumati and Rajavaddu did not show any grain yield response either at 0 – 10 or 50 – 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>; indicating the existence of genetic variability for P-use efficiency trait, which in conjunction with unraveling of physiological mechanisms underlying the observed genetic variation can be utilized for breeding elite rice culture with superior grain yield stability (either by conventional or by molecular breeding techniques) under soil minimal nutrient availability and / or nutrient stress conditions, with minimal dependence on chemical fertilizer inputs.

**Key words :** Low soil-P fertility status, phosphorus-use efficiency, rice genotypes, molecular breeding

Enhancement and sustainability of crop production in different rice growing system have become issues of national importance to meet the food and calorie energy requirements of Indian population.

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Although, this had been achieved during past four decades by adopting appropriate crop production practices, like adoption of suitable semi-dwarf HYVs and other agro-inputs, the present scenario indicates that during last six years, there had been a declining trend in rice grain yields mainly due to wide spread soil health problems, macro and micro-nutrient deficiencies, biotic and abiotic stresses. Among Indian rice growing soils, among major nutrients the Phosphorus (P) deficiency is widespread and it has become the most limiting nutrient next to nitrogen (N). Additionally, the farmers are not applying adequate quality of P fertilizer, in intensively rice cropped areas, and also in rice based cropping systems, mainly due to economic reasons, resulting in serious imbalance not only in soil fertility status, but also causing an erosion of soil organic base with resultant degradation of soil physical properties.

While application of mineral fertilizers at optimum dosages in conjunction with blend of organics and inorganic amendments may bring about an amelioration of the over- exploited soil fertility system; evolving superior rice cultivars which can utilize the soil nutrients present at sub-optimal levels, understanding of molecular

basis of P nutrition and concerted efforts towards genetic manipulation of nutrient acquisition mechanism from soil nutrient pool have been elucidated as prime research priorities for long term sustainability of rice production.

Based on above research perspectives, in order to identify superior rice genotypes with higher P-use efficiency, and also those tolerant to low soil-P status., various pre-release mini-kit rice varieties and rice hybrids have been evaluated for their grain yield, response to graded levels of applied phosphorus, in a low soil-P fertility status calcareous vertisol located at DRR farm, Rajendranagar, Hyderabad.

### **Materials and Methods**

In order to identify higher P-use efficient rice genotypes, thirteen high yielding varieties and three rice hybrids during *kharif*, 2004 viz. PRH-122, HRI-126, MPH-5401, Dhanarasi, Nidhi, IET 14554, IET 15358, IET 15420, IET 17020, IET 17278, IET 17430, IET 17467, IET 17475, IET 17476, IET 17544; Suraksha and twelve rice cultures during *kharif* 2005 viz. Pant Sankar Dhan-1, PHB-71, PA6444, PA6201, IET9691, Sagar Samba, IET 11768, IET 13652, IET 17190, Early Samba, Sumati



and Rajavadlu were evaluated at graded levels of P from 0 – 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> i.e. 0, 10, 20, 30, 40, 40, 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, for their grain yield response to applied P, in a calcareous vertisol of low soil phosphorus fertility status. The soil type of the experimental site is a calcareous vertisol with pH 7.94, available nitrogen of 214 kg/ha, available phosphorus of 2.04 ppm P, available potassium of 624 kg K<sub>2</sub>O ha<sup>-1</sup> and organic carbon of 0.61%.

After raising the nursery with recommended package of practices, in the main field 30-day old seedlings were planted with a spacing of 20x10 cm, in a split-plot design with P-levels as main plot and varieties as sub-plot treatments. At the time of planting, during last harrowing 40 kg Nitrogen, and 40 kg K<sub>2</sub>O ha<sup>-1</sup> were applied; while only nitrogen @ 40 kg N ha<sup>-1</sup> was applied each time, at tillering and panicle initiation stages. After planting, the plots were kept saturated for first 6 days after transplanting and the field was flooded with 2-3 cm depth of water after 6<sup>th</sup> day. The water level was gradually raised to 5-10cm and maintained till crop maturity.

## Results and Discussion

The grain yield data indicated that during both the seasons, the treatment differences due to varieties, P-levels and their interaction effects were found to be significant. During *kharif* 2004, among varieties IET 14554 recorded higher grain yield even at low soil P level (*i.e.*, at 0 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) of 3.63 t ha<sup>-1</sup>, followed by PRH-122, Dhanarasi, IET 15358, IET 17467 and IET 17475 which recorded grain yields of 3.05 – 3.26 t ha<sup>-1</sup>. However, at higher P - levels of 30 – 40 and 50 – 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, PRH-122 was found to be superior, recording higher grain yields of 5.75 – 6.63 and 6.89 – 7.30 t ha<sup>-1</sup>, respectively; compared to IET 14554 (4.95 – 5.65 and 6.23 – 6.80 t ha<sup>-1</sup>, respectively). (Tables 1-2). Among other cultures, IET 15358, IET 17467 and IET 17475 although exhibited low-P tolerance and higher yields at low-P levels of 10 and 20 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (3.35 – 4.27 t ha<sup>-1</sup>); at higher P-levels of 50 – 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, they recorded low grain yields (5.54 – 5.85 t ha<sup>-1</sup>); while Dhanarasi which exhibited higher yields at low-P, also recorded marginally higher yields at 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (6.33 t ha<sup>-1</sup>) over above three cultures but lower than PRH-122 and IET 14554.

The rice hybrids HRI-126 and MPH-5401, although were found to be not tolerant at 0 P kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> level (2.46 – 2.64 t ha<sup>-1</sup>), they exhibited superior grain yields of 6.08 – 6.50 t ha<sup>-1</sup> at 50 – 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> levels, indicating that they need higher initial-P to achieve similar grain yield as that of PRH-122 or IET 14544. The other cultures IET 17020, IET17278 and Nidhi were found to be neither low-P tolerant nor P-responsive at higher-P levels (1.18-4.66 t ha<sup>-1</sup>).

During *kharif* 2005, IET 9691 and PA6201 exhibited higher grain yields of 1.58 – 3.15 t ha<sup>-1</sup> at 0 – 10 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> of applied P; followed by PHB-71, PA6444 and Sagar Samba (1.04 – 1.11 and 2.00 – 2.88 t ha<sup>-1</sup>). However, at higher P-levels, PHB-71, PA6201 and PA6444 were found to be superior and recorded highest grain yields of 4.78 – 5.52 and 5.97 – 6.66 t ha<sup>-1</sup> at 50 – 60 P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>; while IET 9691 recorded lower grain yields of 4.59 – 5.15 t ha<sup>-1</sup>. (Tables 3-4). Sagar Samba, although recorded higher grain yields at 0 – 10 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 20 – 30 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (1.11 – 2.88 and 3.24 – 3.36 t ha<sup>-1</sup>) and higher yield response (107 – 178 and 61 – 75 kg grain kg<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>) at higher P levels, it recorded only marginal grain yields of 3.56 – 3.83 t ha<sup>-1</sup> and response of 45 – 49 kg grain kg<sup>-1</sup>

P<sub>2</sub>O<sub>5</sub>. Similar marginal grain yield response was observed with Early Samba at 0 – 10 and 50 – 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (1.30 – 1.65 and 2.69 – 3.35 t ha<sup>-1</sup>). IET 13652 and IET 11768, although did not exhibit any low-P tolerance at lower P levels of 0 – 10 kg P<sub>2</sub>O<sub>5</sub> (0.36 – 1.85 t ha<sup>-1</sup>), they exhibited higher yield potential than the second group Sagar Samba and Early Samba at 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (4.73 – 4.94 t ha<sup>-1</sup>) and grain yield response of 65 – 66 kg grain kg<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>. The other cultures IET 17190, Sumati and Rajavadlu did not show any grain yield response either at 0 – 10 or 50 – 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (0.30 – 0.69 and 1.53 – 3.24 t ha<sup>-1</sup>).

In India, phosphorus deficiency is wide-spread in nearly 85 per cent of the soils and in-sufficient plant-available soil phosphorus can be a major constraint for rice production. While in highly acidic, P-fixing soils upland rice growing soils, this is a common problem, under lowland conditions also, P deficiency is becoming the main factor limiting performance of modern rice varieties under intensive rice production (De Datta *et al.* 1990). This is due to lack of locally available P-sources and the high cost of water soluble P-fertilizers and because of these reasons, resource-poor rice farmers are not applying

adequate quantities of P. Additionally, some rice soils can quickly fix up to 90% of the added P fertilizer into less soluble forms (Dobermann *et al.* 1998). Therefore, an attractive, cost-effective and alternative strategy is to develop rice cultivars capable of extracting higher proportion of native as well as applied P.

In the present investigation, four distinct patterns in grain yield response was observed with eight rice cultures *viz.* IET 14554, PRH-122, IET 15358, IET17467, IET17473, IET15358, IET17476 and IET17475 recording significant low soil-P response and higher grain yields at 0-10 kg P<sub>2</sub>O<sub>5</sub> /ha level ; six rice cultures *viz.* IET11768, IET17430, IET17544, IET 15420, Pant Sankar Dhan-1 and Sagar Samba exhibiting higher grain yields at medium P- fertility level of 20-40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>; while five *viz.* PHB -71, PA6444, PA6201, HRI-126 and MPH 5401 recorded higher grain yields and yield response only at higher P-levels of 50 – 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (65 – 93 kg grain kg<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> ) compared to others (16 – 66 kg grain kg<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> ).The other cultures IET 17190,IET 17020, IET17278, Sumati and Rajavadlu did not show any grain yield response either at 0 – 10 or 50 – 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. Similar genetic

variability among lowland and upland rice cultivars in their ability to exploit soil and fertilizer P were reported by (Wissuwa and Ae 2001b) and (Fageria *et al.* 1988); and rice varietal differences to produce higher grain yields under sub-optimal phosphorus conditions by Koyama *et al* (1973) and Ponnampereuma (1976).

Earlier studies on low P tolerance mechanisms, indicate that as P does not move freely into the rhizosphere, (in high P-fixing soil, as soil mineral constituents easily bind applied P), plants with better P utilization-ability may acquire P by expanding their root system, thereby exploring a greater soil volume (Loneragan, 1978). Low P tolerant plants may acquire hardly soluble P by excreting organic compounds capable of releasing soil-bound P and resultant P solubilization due to organic-anion excretion may be responsible for the bulk of P uptake by rice from a P-deficient soil (Kirk *et al.*, 1999); while higher root metabolic activity and longevity of the root systems had been reported to be responsible, for better P utilization of two rice cultures, compared to their susceptible counterpart ( Krishnamurthy *et al.*, 2004 ). Since genetic variation in tolerance to P-deficiency could effectively be exploited for rice improvement; it is postulated that

efforts should be intensified to screen available varieties as well as traditional land races, and identify the morphological and physiological mechanisms underlying the low P-tolerance or sensitivity under field conditions.

### Conclusions

Summerised over two seasons, the results indicated that genetic variability exists among rice cultures in utilization of applied P and grain yield responses. Assuming that a minimum level of soil-P availability could be maintained in the rice fields by adapting suitable agronomic practices, this trait can be utilized for breeding elite rice cultures with superior grain yield stability and sustainability, under low available soil-P and high P-fixing soil conditions. Adaptability of certain varieties like IET 14554, PRH-122, IET 15358, IET17467, IET17473, IET15358, IET17476, IET17475, IET 9691 and PA 6201, for specific mineral stresses in the soil is an added quality and mere substitution of the variety itself is going to be a paying proposition under marginal soil fertility farming conditions, with minimal dependence on chemical fertilizer inputs.

Additionally, unraveling of bio-chemical and physiological mechanisms underlying the observed genetic variation could lead to

further advances in identifying genes for tolerance to P deficiency, which may then be manipulated to attain levels of tolerance that are presently not achievable. As tolerance to P-deficiency is quantitatively inherited with both additive and dominant effects, progress through conventional breeding approaches may be slow; and more rapid progress in this regard can be achieved by molecular breeding. Studying of the phosphate deficit-inducible promoters; and in particular, the identification of unique regulatory elements that can be used for engineering phosphorous uptake or other traits, could provide a significant benefit, in molecular breeding program, in evolving superior rice cultivars with wider adaptation to abiotic stresses under different soil and agro-climatic conditions.

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**Table 1: Effect of P levels on Grain yield (t ha<sup>-1</sup>) of rice genotypes, *kharif* 2004.**

S. No.	Variety	P –Levels ( Kg P <sub>2</sub> O <sub>5</sub> /ha.)							
		0	10	20	30	40	50	60	Mean
1	PRH122	3.27	4.05	5.15	5.75	6.63	6.89	7.30	<b>5.58</b>
2	HRI126	2.46	3.52	3.95	4.56	5.03	6.08	6.51	<b>4.59</b>
3	MPH-5401	2.64	3.70	4.53	5.18	5.59	6.08	6.56	<b>4.90</b>
4	Dhanarasi	3.11	3.39	4.24	5.02	5.66	6.02	6.34	<b>4.83</b>
5	Nidhi	1.80	3.49	3.03	3.26	3.87	4.17	4.39	<b>3.43</b>
6	IET 14554	3.63	3.98	4.47	4.91	5.65	6.24	6.80	<b>5.10</b>
7	IET 15358	3.20	3.79	4.27	4.67	5.38	5.86	5.74	<b>4.70</b>
8	IET 15420	1.82	2.27	2.93	3.50	4.77	5.31	5.61	<b>3.74</b>
9	IET 17020	1.18	1.73	2.60	3.05	3.55	3.93	4.69	<b>2.96</b>
10	IET 17278	1.71	2.42	3.28	3.57	3.69	4.15	4.61	<b>3.35</b>
11	IET 17430	0.94	2.39	3.30	3.74	4.30	4.98	5.48	<b>3.59</b>
12	IET 17467	3.09	3.57	4.15	4.78	5.12	5.58	5.86	<b>4.59</b>
13	IET 17475	3.05	3.35	3.58	4.11	4.78	5.54	5.84	<b>4.32</b>
14	IET 17476	2.04	3.31	3.64	4.11	4.70	5.28	5.71	<b>4.11</b>
15	IET 17544	2.28	3.45	3.73	4.2	5.22	5.58	5.86	<b>4.33</b>
16	Suraksha	1.65	2.56	3.99	4.24	4.67	4.92	5.48	<b>3.93</b>
	<b>Mean</b>	<b>2.37</b>	<b>3.19</b>	<b>3.80</b>	<b>4.29</b>	<b>4.91</b>	<b>5.41</b>	<b>5.80</b>	<b>4.25</b>

**C.D. (0.05):** P- levels: 0.07  
Varieties: 0.12

P at same V: 0.26  
V at same P: 0.27

**C. V. (%):** P- levels: 3.16  
Varieties: 3.74

**Table 2: Grain yield response of rice genotypes (kg grain/kg P<sub>2</sub>O<sub>5</sub>) to P application ( *kharif* , 2004 )**

Varieties		P –Levels ( Kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )					
		10	20	30	40	50	60
1	PRH 122	78.9	94.4	82.7	84.1	72.5	67.2
2	HRI 126	105.9	74.7	70.1	64.3	72.4	67.5
3	MPH 5401	106.1	94.3	84.5	73.6	68.7	65.2
4	Dhanarasi	28.9	56.8	64.0	63.8	58.3	53.8
5	Nidhi	168.2	61.3	48.7	51.6	47.2	43.1
6	IET 14554	35.1	42.5	42.7	50.7	52.2	53.0
7	IET 15358	59.0	53.3	48.8	54.4	53.2	42.4
8	IET 15420	45.8	55.5	56.1	73.8	69.9	63.3
9	IET 17020	54.5	71.1	62.3	59.3	54.9	58.0
10	IET 17278	70.9	78.9	62.1	49.7	48.8	48.3
11	IET 17430	145.6	118.1	93.4	84.1	81.0	46.1
12	IET17467	48.1	53.2	56.3	50.8	49.7	46.1
13	IET 17475	29.7	26.4	35.5	43.3	49.8	46.6
14	IET 17476	126.5	79.7	68.9	66.4	64.7	61.2
15	IET 17544	117.1	72.8	64.1	73.5	66.1	59.6
16	Suraksha	90.4	116.6	86.3	75.4	65.4	63.7

**Table 3: Effect of P-levels on grain yield of rice genotypes (t ha<sup>-1</sup>), *kharif* 2005.**

Varieties		P –Levels ( t P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> .)							Mean
		0	10	20	30	40	50	60	
1	Pant Sankar Dhan-1	0.37	1.33	2.25	3.26	3.28	3.42	4.09	2.57
2	PHB-71	1.04	2.69	3.24	3.69	4.22	4.79	5.97	3.66
3	PA6444	1.05	2.00	3.29	4.38	4.76	5.29	6.60	3.91
4	PA6201	1.58	2.90	3.94	4.69	5.28	5.52	5.99	4.27
5	IET 9691	1.83	3.15	3.37	3.44	4.29	4.59	5.15	3.69
6	Sagar Samba	1.11	2.88	3.24	3.37	3.55	3.56	3.83	3.08
7	IET 11768	0.82	1.85	2.14	2.87	3.42	3.97	4.73	2.83
8	IET 13652	0.95	1.76	1.96	2.55	3.42	3.58	4.94	2.74
9	IET 17190	0.30	0.51	0.60	0.71	0.92	1.08	1.53	0.81
10	Early Samba	1.30	1.65	1.92	2.37	2.36	2.69	3.35	2.23
11	Sumati	0.56	1.22	1.33	1.90	2.39	2.44	2.91	1.82
12	Rajavadlu	0.69	1.83	2.10	2.63	3.04	3.26	3.24	2.40
	Mean	0.97	1.98	2.45	2.99	3.41	3.68	4.36	2.83

**C.D. (0.05):** P- levels: 0.20

P at same V: 0.54

**C.V.(%) :** P- levels :0.06

Varieties: 0.16

V at same P: 0.54

Varieties: 9.53

**Table 4 : Grain yield response of rice genotypes to applied- P (Kg grain/Kg P<sub>2</sub>O<sub>5</sub>).  
*kharif*, 2005.**

Varieties		P –Levels ( Kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> .)					
		10	20	30	40	50	60
1	Pant Sankar Dhan-1	96.5	94.1	96.5	72.8	61.1	62.0
2	PHB-71	164.6	110.1	88.4	79.5	74.9	82.2
3	PA6444	95.1	111.8	110.9	92.7	84.8	92.6
4	PA6201	132.0	118.1	103.5	92.4	78.8	73.4
5	IET 969	132.0	76.8	53.7	61.5	55.1	55.3
6	Sagar Samba	177.6	106.5	75.4	61.1	49.1	45.4
7	IET 11768	102.8	66.0	68.3	65.1	62.9	65.2
8	IET 13652	80.6	50.4	53.3	61.7	52.5	66.5
9	IET 17190	21.5	14.9	13.6	15.6	15.6	20.5
10	Early Samba	34.7	31.3	35.6	26.5	27.7	34.2
11	Sumati	66.6	38.9	44.7	45.8	37.8	39.2
12	Rajavadlu	113.2	70.5	64.4	58.8	51.3	42.4

# **Influence of Planting Methods and Integrated Nutrient Management on Growth, Yield and Economics of Rice**

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## **Abstract**

A field experiment was conducted during *kharif* season of 2010-11 and 2011-12 at AICRP on Rice, Agricultural Research Institute (ARI), Rajendranagar, Hyderabad to evaluate different planting methods (SRI, Yangi - China, Kobota transplanter and farmers method) and Integrated Nutrient Management (INM). The experiment was comprising four different planting methods (SRI method of planting (M1), Yangi-China transplanter (M2), Kobota transplanter (M3) and farmer's method (M4)) as main plots and four INM practices (I<sub>1</sub>, (100% RDF + FYM @ 5 t ha<sup>-1</sup>), I<sub>2</sub> (100% RDF + FYM @ 5 t ha<sup>-1</sup>), I<sub>3</sub> (100% RDF + BF @ 7.5 kg/ha (*Azospirillum* @ 2.5 kg/ha + PSB @ 5 kg ha<sup>-1</sup>) and I<sub>4</sub> only RDF (100% RDF 150:60:40 kg NPK ha<sup>-1</sup>) as sub plots in split plot design replicated thrice. The plant height and biomass production of rice at the time of harvest during both the years was higher with Kobota transplanter method among planting methods and with integrated use of

100% RDF + FYM @ 5 t ha<sup>-1</sup> among Integrated Nutrient Management (INM) practices. The maximum mean grain, straw yield and B:C ratio (Average of two years) was recorded with machine (Kobota) transplanting (7.0, 16.02 t ha<sup>-1</sup> and 2.20) followed by farmers method (manual) of planting (6.30, 13.32 t ha<sup>-1</sup> and 2.00) that was on par with SRI method (5.8, 11.40 t ha<sup>-1</sup> and 1.60). The lowest mean grain, straw yield and B:C ratio was obtained with Yangi - China transplanter (5.20, 8.42 t ha<sup>-1</sup> and 1.30). Among the INM treatments significantly superior mean grain, straw yield and B:C ratio were registered with application of 100% RDF + FYM @ 5 t/ha (6.90, 15.60 t ha<sup>-1</sup> and 2.00) respectively over the remaining, INM treatments. Application of GLM @ 5 t ha<sup>-1</sup> + RDF produced higher mean grain and straw yield (6.40 and 13.30 t ha<sup>-1</sup>) and B:C ratio (1.80) followed by RDF + BF 7.5 kg/ha). Significant interaction effect was obtained in straw yield and B:C ratio (Pooled mean). Transplanting with Kobota transplanter along with application of FYM @ 5 t ha<sup>-1</sup> + RDF (M<sub>3</sub>I<sub>1</sub>) was found best with significantly

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**highest straw yield over the other combinations and was at par with farmers method of planting along with RDF + FYM @ 5 t ha<sup>-1</sup> (M<sub>4</sub>I<sub>1</sub>). The average of two years (pooled mean) of B:C ratio was significantly higher with Kobota transplanter along with RDF+FYM @5 t ha<sup>-1</sup> (2.40) and was at par with RDF+GLM @ 5 t ha<sup>-1</sup> (2.23) and only RDF (2.10) with the same planting method (M<sub>3</sub>I<sub>2</sub>, M<sub>3</sub>I<sub>4</sub>) and with farmers method of transplanting with RDF +FYM @ 5 t ha<sup>-1</sup> (2.37) and RDF+GLM @5 t ha<sup>-1</sup> (2.10).**

**Key words:** SRI, Transplanter, methods, RDF, B:C ratio.

Rice is the staple food crop in Asia including India. In the year 2011, the area under rice in India 44.41 m ha with a production of 104 m ha with productivity of 2.2 t ha<sup>-1</sup> CMIE, 2011). In Andhra Pradesh the area was 4.0 lakh ha and production is 12.8 lakh tonnes. Rice is mainly grown in canal command areas with assured irrigation facilities. Rice is traditionally planted as transplanting method but in recent years, because of scarce labour coupled with higher wages during the peak period of farm operations invariably lead to delay in transplanting. This was aggravated by untimely release of water from canals and delayed monsoon showers. This lead to indenting alternate methods of rice cultivation without

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reduction in yield. Among them, transplanting using mechanical transplanter and SRI method of cultivation gained significance among farmers because of easy adoptability and on par yield with that of conventional transplanting method. Rice transplanters are available from different companies. Mechanization (both for planting and harvesting) in rice cultivation revolutioned the rice cultivation by decreasing cost of cultivation. In Andhra Pradesh, which is called as “rice bowl of India”, rice growing has become burden on farmers as the cost of labour increased many fold with similar yield. On the other side, mechanical transplanting has its own disadvantages like special nursery, non uniformity in number of seedlings hill<sup>-1</sup> and main field preparation. Introduction of high yielding varieties responsive to chemical nutrients brought a spectacular increase in use of chemical fertilizers in rice. Nutrient mining by high yielding varieties was usually more than that applied through chemical fertilizers. This type of nutrient mining over years led to impoverishment of soil fertility and decline in crop productivity (Nambiar, 1992). Integrated use of chemical fertilisers with manures and green manure crop is important for sustainable rice production. The increased prices of fertilizers also intensified the problem by increasing cost of inputs. Hence the mechanical transplanters and INM

practices were evaluated with an aim to reduce the cost of cultivation.

### Materials and Methods

A field experiment was conducted during *kharif* season of 2010-11 and 2011-12 at AICRP on Rice, Agricultural Research Institute, Rajendranagar, Hyderabad. The Soil of the experiment field was Sandy clay loam in texture with pH 7.7, organic carbon 0.67% and available N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O as 245.61, 38.5 and 301 kg/ha respectively during *kharif* season of 2010-11 and during *kharif* season of 2011-12 with pH 7.82, organic carbon 0.65 and available N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O as 215.1, 31.6 and 288.2 kg ha<sup>-1</sup> respectively. The total rain fall received during *kharif* 2010 [1 st July- 30 October (120 days)] was 485.9 mm in 31 rainy days and during 2011 [July –October (127)] the crop period recorded 615.8 mm in 39 rainy days. The experiment was replicated thrice with 16 treatmental combinations comprising four different planting methods (SRI method of planting (M1), Yangi-China transplanter (M2), Kobota transplanter (M3) and farmer's method (M4)) as main plots and four INM practices I<sub>1</sub> (100% RDF + FYM @ 5 t ha<sup>-1</sup>), I<sub>2</sub> (100% RDF + FYM @ 5 t ha<sup>-1</sup>), I<sub>3</sub> (100% RDF + BF @ 7.5 kg ha<sup>-1</sup> (*Azospirillum* @ 2.5 kg ha<sup>-1</sup> + PSB @ 5 kg ha<sup>-1</sup>) and I<sub>4</sub> only RDF (100% RDF 150:60:40 kg NPK ha<sup>-1</sup>) as sub plots in split plot design. The two types of

transplanter's transplanted by using mat tray nursery at 15 days old seeding with fixed inter row spacing 30 cm for Kobota and 22.5 cm for Yangi-China transplanter with varying intra row spacing (can be adjusted as per requirement) 12 and 14 cm respectively maintained (fixed) in mechanical transplanters. For SRI method used markers (25 x 25 cm) and transplanted with women labour at 10 days old seedlings and for farmers method of transplanting 25-30 days old seedlings were used and transplanted using labour. FYM and GLM were incorporated one week before transplanting and 100 g SSP was added to GLM treatment for quick decomposition. Rice variety "Satya" was used for the experiment. Organic manures *i.e* FYM and (GLM) @ 5 t ha<sup>-1</sup> were applied one week before transplanting 1/3 nitrogen, total phosphorus and 2/3 potassium were applied as basally at the time of transplanting. 1/3<sup>rd</sup> N was applied at maximum tillering stage and remaining 1/3<sup>rd</sup> N and 1/3 potassium were applied at panicle initiation stage. Nitrogen, phosphorus and potassium were applied in the form of urea, single super phosphate (SSP) and muriate of potash (MOP) respectively. The crop was sown on 1<sup>st</sup> July in both the years. Biofertilizers *i.e* *Azospirillum* @ 2.5 kg ha<sup>-1</sup> and PSB @ 5 kg ha<sup>-1</sup> were applied 3 days after transplanting. In SRI method, cono weeder was operated for 3-4 times for control of

weeds and aeration but for remaining planting methods, pre-emergence application of butachlor @ 1.5 kg ai ha<sup>-1</sup> was applied 2 days after transplanting followed by one hand weeding at 30 DAT for weed control. The yield attributes, grain and straw yield were recorded at the time of harvest. Economics was calculated based on the cost of prevailing market rate of inputs and yield of rice crop.

Plant height is a direct index to measure the growth and vigour of the plant. The plant height in all planting methods at the time of harvest were insignificant indicating that all the tested planting methods showed at par effect on growth of rice after establishment (Table 1). The effect of INM practices on plant height at harvest revealed that application of FYM @ 5 t ha<sup>-1</sup> + RDF had registered taller plants over the other treatments during both the years. Application of biofertilizer @ 7.5 kg ha<sup>-1</sup> produced lesser plant height than FYM but significantly at par with green leaf manuring of Dhaincha @ 5 t ha<sup>-1</sup> along with RDF during *kharif* 2010-11. The mineralisation during the decomposition of organic manures (FYM and green manure) due to integrated use of inorganic fertilisers might have enhanced nitrogen availability in the rhizosphere resulting in increased nitrogen uptake by the crop which in turn promoted the increase in plant height in the above treatments. Further, synchronised

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availability of essential plant nutrients to the crop for a longer period with use of organic manures along with inorganic fertilisers has increased plant height as the crop growth advanced Harish *et al.* (2011). The interaction effect of planting methods and INM practices was found insignificant during both the years.

Dry matter production at harvest varied between 1.21 and 2.46 kg ha<sup>-1</sup> irrespective of treatments. Transplanting with Kobota transplanter has registered highest dry matter production over (2.14, 2.46 kg m<sup>-2</sup>) the other planting methods during both the years. This is because of optimum plant population and tillering ability of the crop. Followed to this, significantly higher dry matter production was observed in farmers method followed by SRI method during both the years. Transplanting with Yangi-China transplanter produced lowest dry matter production during both the years. This may be attributed to non uniform transplanting and less number of tillers hill<sup>-1</sup>. This was supported by Anbumani *et al.* 2004. Among the INM practices, application of FYM @ 5 t ha<sup>-1</sup> along with RDF has accounted to significantly higher dry matter production (2.09 and 2.41 kg m<sup>-2</sup>) over the other treatments during both the years followed by RDF + GLM @ 5 t ha<sup>-1</sup> and RDF + BF@ 7.5 kg ha<sup>-1</sup>. Application of RDF alone registered lowest dry matter production during both the years. This may

be attributed that combined application of inorganic fertiliser and organic manure could have helped in balanced availability of nutrients till harvesting time Siddarama *et al.* (2011). Similar findings of higher dry matter accumulation with application of FYM, green manuring and biofertilisers was reported by Jagadish Kumar *et al.* (2010), Anchal Dass *et al.* (2009) and Balaji Naik and Yakadri, (2004). Interaction between planting methods and INM practices (Table 2.a) revealed that transplanting with Kobota transplanter along with the application of FYM @ 5 t ha<sup>-1</sup> + RDF was noted to be the best combination with highest dry matter production 2.77 and 2.50 kg m<sup>-2</sup> in pooled analysis. However, it was followed RDF + FYM @ 5 t ha<sup>-1</sup> along with farmers method (M<sub>4</sub> I<sub>1</sub>) and significantly at par with RDF + GLM @ 5 t ha<sup>-1</sup> along with Kobota transplanter (M<sub>3</sub> I<sub>2</sub>) .

Panicles m<sup>-2</sup> of rice was significantly influenced by planting methods and INM practices during both the years except that INM practices were insignificant during 2011-12. Among the planting methods, transplanting with Kobota transplanter was found best with maximum no. of panicles m<sup>-2</sup> (498.9 and 566.2) over the other treatments in both the years. However, manual transplanting in farmer's method was found at par to machine transplanting with Kobota with respect to panicle number m<sup>-2</sup> during both the years. The Journal of Rice Research 2014, Vol. 7 No. 1 & 2

increase in panicles m<sup>-2</sup> with Kobota transplanter was mainly due to optimum plant population and plant geometry (30 x 12cm) that resulted in even distribution of light, moisture and nutrients among rice plants in a unit area leading to manifestation of ideal growth and yield attributes. This was supported by Anbumani *et al.* (2004) and Singh *et al.* (2009). Followed to this, farmer's method followed by SRI method produced more number of panicles m<sup>-2</sup>. Transplanting with Yangi-China has putforth crop with lowest number of panicles during both the years.

Effect of INM practices on panicles/m<sup>2</sup> was insignificant during 2011-2012. Of the different INM practices, application of FYM @ 5 t ha<sup>-1</sup> + RDF registered highest panicle number m<sup>-2</sup> over the other treatments during both the years. But in 2010, green leaf manure treatment along with RDF was found at par to the above treatment with comparably higher panicles m<sup>-2</sup>. Increase in panicles m<sup>-2</sup> through FYM was supported by Barik *et al.* (2006) and Mirza *et al.* (2005) and through green manuring was supported by Vaiyapuri and Sri Ramachandra Sekaran, (2002). Application of bio fertilizers + RDF produced less number of panicles m<sup>-2</sup> than above treatments but higher than only RDF. The beneficial effect of biofertilizers was reported by Anchal Dass *et al.* (2009) and Jagdish Kumar *et al.* (2010). Application of RDF alone without any

supplementation of organic manures recorded least number of panicles  $\text{m}^{-2}$ . The interaction was found insignificant during both the years.

Significantly highest number of grains/panicle was noted due to transplanting by Kobota transplanter, compared to other transplanting methods during both the years. However, it was at par with farmers method of planting during 2011-12. This may be due to more light interception because of wider spacing (30 x 12 cm), that resulted in more dry matter accumulation and partitioning in to sink (panicles). Farmers method of transplanting produced next higher number of grains panicle<sup>-1</sup>, but was at par with SRI method during both the years and its pooled mean and also with Yangi- China transplanter during 2010-11 only. Lower number of grains/panicle was reported with Yangi- China transplanter method. Among the INM practices, application of RDF + FYM @ 5t ha<sup>-1</sup> produced more number of grains/panicle during both the years. However it was at par with RDF + GLM @ 5 t ha<sup>-1</sup> during 2011-12. Nutrients available from decomposing FYM to the rice crop during the reproductive stage were utilised for grain formation and grain filling leading to higher no. of grains per panicle. This was supported by Mirza *et al.* (2005). Application of RDF + GLM @ 5t ha<sup>-1</sup> attained next level of grains /panicle during 2010-11 but was at par with RDF

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+ BF @ 7.5 kg ha<sup>-1</sup> during 2010-11. Biofertilizers + RDF gave rise to next level of number of grains/panicle but significantly higher than only RDF application except during 2011-12. Interaction was not significant during 2010-11. During 2011-12 (Table 2.b), farmers method along with RDF + FYM @ 5 t ha<sup>-1</sup> (M<sub>4</sub> I<sub>1</sub>) produced more number of grains/panicle (381.5) and was at par with same planting method along with application of RDF + GLM @ 5t ha<sup>-1</sup> (M<sub>4</sub>I<sub>2</sub>), RDF + BF @ 7.5 kg/ha (M<sub>4</sub>I<sub>3</sub>); Kobota transplanter method along with RDF + FYM @ 5 t ha<sup>-1</sup> (M<sub>3</sub>I<sub>1</sub>), RDF + GLM @ 5 t/ha(M<sub>3</sub> I<sub>2</sub>) and only RDF application (M<sub>3</sub> I<sub>4</sub>). Better supply of macro and micro nutrients by FYM and green manure besides better establishment and growth conditions due to Kobota transplanting and farmer's method might have helped for more enzymatic activity and physiological process of plant, resulting in better translocation of photosynthates and hence apportioning of sink reflected in no. of grains/panicle. These results are supported by Harish *et al.* (2011).

Grain yield of rice was significantly influenced by planting methods, integrated nutrient management (INM) practices and their interaction during both the years. Among different planting methods, Kobota transplanter recorded highest grain yield which was significantly superior to all crop

establishment methods during both the years (6.50 (2010) & 7.50 (2011) t ha<sup>-1</sup>) and their pooled mean (7.00 t ha<sup>-1</sup>). However, during 2011-12, transplanting by farmer's method (6.91 t ha<sup>-1</sup>) was found at par to Kobota transplanting. Better vegetative growth with efficient dry matter accumulation and effective partitioning to the panicles resulting in more no. of panicles m<sup>-2</sup> and grains/panicle, reflected in grain yield of above treatments. The increase in grain yield in machine transplanting was in agreement with the results reported by Anoop Dixit *et al.* (2007), Manjunath *et al.* (2009) and Venkateshwarlu *et al.* (2011). Followed to this, farmer's method of transplanting produced higher yield and was at par with SRI method of transplanting during 2011-12. Kobota transplanter method gave an yield increase of 11.1, 20.7 and 34.6 per cent (on pooled mean) over farmers method, SRI and Yangi China transplanter respectively. All the tested methods (Kobota, SRI and Yangi - China transplanter) showed an yield increase / decrease of 11.1, -8.0 and -17.5 over farmers method respectively. The lowest yield on the other side was recorded with Yangi-China transplanter (4.61 t ha<sup>-1</sup>) as it is a heavy machine compared to Kobota and sank in the field resulting in uneven planting, higher depth of planting and inturn less number of tillers m<sup>-2</sup>, less number of panicles m<sup>-2</sup>, number of

grains/panicle and inturn yield. Among the integrated nutrient management practices, the higher grain yield (6.41, 7.42 and 6.9 t ha<sup>-1</sup>) was obtained with RDF+FYM@5 t ha<sup>-1</sup> which was significantly superior to all the other practices during 2010-11 and pooled mean, however during 2011-12, the grain yield exhibited by GLM @ 5 t ha<sup>-1</sup> + RDF (6.90 t ha<sup>-1</sup>) was found statistically at par with above treatment. The grain yield with application of RDF + GLM @ 5t/ha produced 5.87 and 6.4 t ha<sup>-1</sup> during 2010-11 and pooled mean respectively and it was followed by RDF + BF @ 7.5 kg/ha and only RDF treatment respectively. Where as during 2011-12, RDF – GLM @ 5 ha<sup>-1</sup> was at par with RDF + BF @ 7.5 kg ha<sup>-1</sup>. The increase in yield (pooled mean) was 32.7, 23.1 and 9.6 per cent with FYM, GLM and BF over only RDF treatment. The higher yield with integrated use of organic and inorganic fertilisers might be attributed to increased availability of major and minor nutrients by improving physical and chemical environment of the soils. The superiority of INM practices over sole chemical fertilisation might be due to the presence of humic acid compounds which helps in dissolution of minerals and chelation of micronutrients and enhanced microbial activity. The superiority of FYM in increasing the yield was supported by Barik *et al.* (2006) and Sudhakar (2010). The superiority of green leaf manuring was

supported by Rajbir Garg *et al.* (2007), Anchal Dass *et al.* (2009) and Balaji Naik and Yakadri (2004). The superiority of biofertilizers was reported by Jagdish Kumar *et al.* (2010) and Mihilal Roy *et al.* (2011). The interaction effect between planting methods and integrated nutrient management practices was significant only during 2010-11 and 2011-12. During 2010-11 (Table 2.c), Kobota transplanter method along with RDF + FYM @ 5 t ha<sup>-1</sup> (M<sub>3</sub>I<sub>1</sub>) produced significantly higher grain yield over other treatment combinations but was at par with same transplanter method along with RDF + GLM @ 5t ha<sup>-1</sup> (M<sub>3</sub>I<sub>2</sub>). Followed to this, farmer's method and SRI method of transplanting along with RDF + FYM @ 5t / ha. (M<sub>4</sub>I<sub>1</sub> & M<sub>1</sub>I<sub>1</sub>) exhibited higher grain yield. During 2011-12 (Table 3.a), Kobota transplanter along with FYM @ 5 t ha<sup>-1</sup> (M<sub>3</sub>I<sub>1</sub>) exhibited higher grain yield but at par with M<sub>3</sub>I<sub>2</sub> (Kobota + RDF + GLM @ 5 t ha<sup>-1</sup>), M<sub>3</sub>I<sub>4</sub> (Kobota + RDF), M<sub>4</sub>I<sub>1</sub> (Farmers method + RDF + FYM @ 5 t ha<sup>-1</sup>), M<sub>4</sub>I<sub>2</sub> (Farmers method+ RDF + GLM @ 5 t ha<sup>-1</sup>), M<sub>4</sub>I<sub>3</sub> (farmers method + RDF + BF @ 7.5 kg ha<sup>-1</sup>) and M<sub>1</sub>I<sub>1</sub> (SRI method + RDF + FYM @ 5 t ha<sup>-1</sup>). The better performance of crop in the above combinations was the outcome of enhanced growth measured in terms of plant height, hastened development, improved yield attributes that resulted in higher yield. (Shekhar *et al.*, 2009).

Straw yield of rice varied significantly due to planting methods and INM practices. The crop transplanted with Kobota transplanter has maintained comparably higher number of tillers hill<sup>-1</sup> and accumulated greater biomass during both the years of study and hence their pooled mean. The increase in stalk yield due to Kobota transplanting was to the tune of 20.3 per cent than manual transplanting. On the other side, Yangi-China transplanter method reported lowest straw yield compared to the other methods. This might be due to heavy weight of machine with no adjustment to different puddle conditions that resulted in uneven planting that was expressed through seedlings hill<sup>-1</sup> and no of hills m<sup>-2</sup>. Farmers method of planting attained straw yield next to Kobota transplanter and was followed by SRI method of planting during both the years and hence pooled mean. Similar increase was reported by Anbumani *et al.* (2004) and Singh *et al.* (2009). The demonstrated effect of FYM along with RDF on grain yield has been repeated once again with stalk yield as adequate stalk production is obligatory for effective photosynthesis and steady transport of nutrients and metabolites required for grain production Ramesh *et al.* (2007). The stalk yield registered in this treatment was found directly proportional to tiller formation and dry matter accumulation in the crop. Application of GLM @ 5 t ha<sup>-1</sup> + RDF

expressed next level of straw yield followed by RDF + bio fertilizer @ 7.5 t ha<sup>-1</sup>. The increase in stalk yield (pooled mean) was 67.6, 42.8 and 18.1 per cent with FYM, GLM and BF over only RDF treatment. As where, application of RDF alone has resulted in production of lowest stalk yield compared to the other INM practices during both the years and pooled mean. The interaction between planting methods and INM practices on straw yield was significant during both the years and pooled mean. Pooled mean of straw yield (Table 3.b) found that transplanting with Kobota transplanter along with application of FYM @ 5 t ha<sup>-1</sup> + RDF (M<sub>3</sub>I<sub>1</sub>) was found best with significantly highest stalk yield over the other combinations and was followed by farmers method of planting along with RDF + FYM @ 5 t/ha (M<sub>4</sub>I<sub>1</sub>).

The benefit cost ratio obtained from transplanting rice through different methods, INM practices and their interaction was significant during both the years and pooled mean (Table 3b). Irrespective of treatments, B:C ratio in II year was higher than I year. Among the planting methods, Kobota transplanter method produced highest benefit cost ratio (2.0, 2.4 and 2.20) during both the years and its pooled mean but was at par with farmer method of transplanting during 2011(2.3) and pooled mean (2.0). The higher benefit cost ratio was attributed to higher net returns with reduced cost of

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cultivation as there is a labour saving of about 11 man days per hectare. The cost of mechanical transplanting was Rs. 25095 ha<sup>-1</sup> with only 56 labour whereas manual transplanting costs Rs.23289 ha<sup>-1</sup> with 67 labour. This was supported by Manjunath *et al.* (2009) and Anoop Dixit *et al.* (2007). Followed by this, SRI method of planting attained benefit cost ratio of 1.4, 1.8 and 1.6 during both the years and pooled mean and significantly higher than Yangi- China transplanter except at 2011-2012. The higher B:C ratio in SRI method was in accordance with Jayadev and Prabhakara Shetty, (2006) and Hugar *et al.* (2009). Lowest B: C ratio was recorded with Yangi-China transplanter and was attributed to lower grain yield.

Among the INM practices, application of RDF+ FYM @5 t/ha attained significantly higher B:C ratio (1.83, 2.20 and 2.0) during both the years and pooled mean owing to higher grain yield and in turn higher gross and net returns in this treatment. However, it was on par with application of RDF+GLM @5 t ha<sup>-1</sup> (2.0) during 2011. Application of RDF + GLM @ 5 t/ha fetched next higher B : C ratio (1.6, 2.0 & 1.8) and significantly higher than RDF+BF @7.5 kg ha<sup>-1</sup> except pooled mean. The beneficial effect of FYM and GLM in improving the net returns and B:C ratio was also supported by Balaji Naik and Yakadri, (2004), Vikas Gupta and Sharma (2007) and Bali and Vani



(2004). Lowest B:C ratio of 1.22, 1.81 and 1.52 was reported by application of only RDF treatment during both the years and pooled mean and was at par with RDF + BF @7.5kg ha<sup>-1</sup> during 2011.

The pooled mean of B:C ratio (Table 3.C) was significantly higher with Kobota transplanter along with RDF+FYM @5 t ha<sup>-1</sup> and was at par with RDF+GLM @ 5 t ha<sup>-1</sup> (2.40) and only RDF (2.10) with the same planting method (M<sub>3</sub> I<sub>2</sub>, M<sub>3</sub> I<sub>4</sub>) and with farmers method of transplanting with RDF + FYM @ 5 t ha<sup>-1</sup> (2.37) and RDF+GLM @ 5 t ha<sup>-1</sup> (2.10).

### Conclusions

The present study had shown that the mechanical transplanting with Kobota transplanter resulted in higher growth, yield attributes and yield in turn B:C ratio in black soils of Andhra Pradesh compared to Yangi-China transplanter. Complimenting RDF with FYM or GLM @5 t ha<sup>-1</sup> recorded higher growth, yield attributes, yield and economics of rice. Incorporation of FYM, GLM and bio-fertilisers being socially acceptable, economically viable and environmentally sustainable sources of nutrient application help in improving and maintain sustainability of soil and crop productivity.

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**Table 2.a: Interaction effect on Dry matter production (kg) at harvest of paddy as influenced by planting methods and INM practices of pooled**

Interaction	Dry matter production (kg) – harvest				
	M <sub>1</sub> - SRI	M <sub>2</sub> - Yangio china	M <sub>3</sub> - Koboto	M <sub>4</sub> - Farmer's method	Mean
I <sub>1</sub> - RDF+FYM@ 5 t ha <sup>-1</sup>	2.10	1.67	2.77	2.50	<b>2.26</b>
I <sub>2</sub> - RDF+GLM @ 5 t ha <sup>-1</sup>	1.77	1.50	2.47	2.13	<b>1.96</b>
I <sub>3</sub> - RDF+BF @ 7.5 kg ha <sup>-1</sup>	1.60	1.20	2.03	1.87	<b>1.68</b>
I <sub>4</sub> - RDF (150 N:60 P <sub>2</sub> O <sub>5</sub> : 60 K <sub>2</sub> O kg ha <sup>-1</sup>	1.43	1.07	1.97	1.33	<b>1.45</b>
<b>Mean</b>	<b>1.73</b>	<b>1.35</b>	<b>2.31</b>	<b>1.96</b>	
		<b>M x I</b>	<b>I x M</b>		
<b>S.Em ±</b>		0.06	0.08		
<b>CD (P=0.05)</b>		0.18	0.25		

**Table 2.b: Interaction effect of grains panicle<sup>-1</sup> of paddy as influenced by planting methods and INM practices during 2011-2012**

Interaction	Grains panicle <sup>-1</sup>				
	M <sub>1</sub> - SRI	M <sub>2</sub> - Yangio china	M <sub>3</sub> - Koboto	M <sub>4</sub> - Farmer's method	Pooled
I <sub>1</sub> - RDF+FYM@ 5 t ha <sup>-1</sup>	344.10	331.33	378.83	381.53	<b>358.94</b>
I <sub>2</sub> - RDF+GLM @ 5 t ha <sup>-1</sup>	327.00	305.17	376.93	369.03	<b>344.53</b>
I <sub>3</sub> - RDF+BF @ 7.5 kg ha <sup>-1</sup>	315.37	302.93	317.63	363.90	<b>324.96</b>
I <sub>4</sub> - RDF (150 N:60 P <sub>2</sub> O <sub>5</sub> : 60 K <sub>2</sub> O kg ha <sup>-1</sup>	308.57	293.23	362.83	245.33	<b>302.49</b>
<b>Pooled</b>	<b>323.75</b>	<b>308.17</b>	<b>359.06</b>	<b>339.95</b>	
		<b>M x I</b>	<b>I x M</b>		
<b>S.Em ±</b>		18.0	52.6		
<b>CD (P=0.05)</b>		16.9	18.8		

**Table 2.c: Interaction effect of grain yield t ha<sup>-1</sup> of paddy as influenced by planting methods and INM practices during 2010-2011**

Interaction	Grain Yield				
	M <sub>1</sub> - SRI	M <sub>2</sub> - Yangio china	M <sub>3</sub> - Koboto	M <sub>4</sub> - Farmer's method	Mean
I <sub>1</sub> - RDF+FYM@ 5 t ha <sup>-1</sup>	6.08	5.42	7.52	6.63	<b>6.41</b>
I <sub>2</sub> - RDF+GLM @ 5 t ha <sup>-1</sup>	5.22	5.36	7.07	5.82	<b>5.87</b>
I <sub>3</sub> - RDF+BF @ 7.5 kg ha <sup>-1</sup>	4.97	4.39	5.96	4.99	<b>5.08</b>
I <sub>4</sub> - RDF (150 N:60 P <sub>2</sub> O <sub>5</sub> : 60 K <sub>2</sub> O kg ha <sup>-1</sup> )	4.86	3.26	5.42	4.89	<b>4.61</b>
<b>Mean</b>	<b>5.28</b>	<b>4.61</b>	<b>6.50</b>	<b>5.58</b>	
		<b>M x I</b>	<b>I x M</b>		
<b>S.Em ±</b>		0.21	0.21		
<b>CD (P=0.05)</b>		0.60	0.65		

**Table 3.a: Interaction effect of grain yield t ha<sup>-1</sup> of paddy as influenced by planting methods and INM practices during 2011-2012**

Interaction	Grain Yield				
	M <sub>1</sub> - SRI	M <sub>2</sub> - Yangio china	M <sub>3</sub> - Koboto	M <sub>4</sub> - Farmer's method	Mean
I <sub>1</sub> - RDF+FYM@ 5 t ha <sup>-1</sup>	6.91	6.56	8.21	8.00	<b>6.41</b>
I <sub>2</sub> - RDF+GLM @ 5 t ha <sup>-1</sup>	6.51	5.77	7.82	7.51	<b>5.87</b>
I <sub>3</sub> - RDF+BF @ 7.5 kg ha <sup>-1</sup>	6.07	5.33	6.70	7.36	<b>5.08</b>
I <sub>4</sub> - RDF (150 N:60 P <sub>2</sub> O <sub>5</sub> : 60 K <sub>2</sub> O kg ha <sup>-1</sup> )	5.71	5.60	7.28	4.75	<b>4.61</b>
<b>Mean</b>	<b>7.50</b>	<b>5.82</b>	<b>6.30</b>	<b>6.91</b>	
		<b>M x I</b>	<b>I x M</b>		
<b>S.Em ±</b>		0.38	0.42		
<b>CD (P=0.05)</b>		1.10	1.32		

**Table 3.b: Interaction effect of Straw yield (t ha<sup>-1</sup>) of paddy as influenced by planting methods and INM practices (pooled)**

Interaction	Straw yield (t ha <sup>-1</sup> )				
	M <sub>1</sub> - SRI	M <sub>2</sub> - Yangio china	M <sub>3</sub> - Koboto	M <sub>4</sub> - Farmer's method	Mean
I <sub>1</sub> - RDF+FYM@ 5 t ha <sup>-1</sup>	14.30	10.77	19.67	17.57	<b>15.57</b>
I <sub>2</sub> - RDF+GLM @ 5 t ha <sup>-1</sup>	11.73	9.50	17.13	14.67	<b>13.25</b>
I <sub>3</sub> - RDF+BF @ 7.5 kg ha <sup>-1</sup>	10.50	7.30	13.90	12.33	<b>11.00</b>
I <sub>4</sub> - RDF (150 N:60 P <sub>2</sub> O <sub>5</sub> : 60 K <sub>2</sub> O kg ha <sup>-1</sup> )	9.00	6.17	13.40	8.70	<b>9.31</b>
<b>Mean</b>	<b>11.38</b>	<b>8.42</b>	<b>16.02</b>	<b>13.32</b>	
		<b>M x I</b>	<b>I x M</b>		
<b>S.Em ±</b>		0.40	0.42		
<b>CD (P=0.05)</b>		1.17	1.28		

**Table 3.c: Interaction effect of B:C Ratio of paddy as influenced by planting methods and INM practices (pooled)**

Interaction	B:C Pooled				
	M <sub>1</sub> - SRI	M <sub>2</sub> - Yangi china	M <sub>3</sub> - Koboto	M <sub>4</sub> - Farmer's method	Mean
I <sub>1</sub> - RDF+FYM@ 5 t ha <sup>-1</sup>	1.77	1.47	2.40	2.37	<b>2.00</b>
I <sub>2</sub> - RDF+GLM @ 5 t ha <sup>-1</sup>	1.50	1.33	2.23	2.10	<b>1.80</b>
I <sub>3</sub> - RDF+BF @ 7.5 kg ha <sup>-1</sup>	1.53	1.17	1.97	2.07	<b>1.70</b>
I <sub>4</sub> - RDF (150 N:60 P <sub>2</sub> O <sub>5</sub> : 60 K <sub>2</sub> O kg ha <sup>-1</sup> )	1.47	1.03	2.10	1.47	<b>1.52</b>
<b>Mean</b>	<b>1.57</b>	<b>1.25</b>	<b>2.18</b>	<b>2.00</b>	
		<b>M x I</b>	<b>I x M</b>		
<b>S.Em ±</b>		0.09	0.10		
<b>CD (P=0.05)</b>		0.27	0.32		



# **Influence of Weed Management Practices on Nutrient Uptake and Productivity of Rice under Different Methods of Crop Establishment**

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## **Abstract**

An experiment was conducted during *kharif* season of 2010 and 2011 at College Farm, Rajendranagar, Acharya N.G. Ranga Agricultural University, Hyderabad. The experiment was laid out in split plot design with three replications. The treatments consisted of three rice establishment methods (direct sowing of sprouted seeds under puddled condition, System of Rice Intensification and transplanting) as main plot treatments and four weed management practices (bensulfuron methyl 60 g a.i ha<sup>-1</sup> + pretilachlor 600 g a.i ha<sup>-1</sup> fb mechanical weeding at 30 DAS/T, bispyribac sodium @ 25 g a.i ha<sup>-1</sup>, farmer's practice and weedy check) as sub plot treatments. The results of the experiment indicated that farmer's practice (hand weeding twice at 20 and 40 DAS/T in direct sown rice and transplanted rice and cono weeding thrice from 20 DAT with 10 days interval in SRI) of weeding resulted in

significantly lower weed density, weed dry weight and lower removal of nutrients by weeds resulting in superior grain yield and higher uptake of nutrients by rice and it was on par with bensulfuron methyl 60 g + pretilachlor 600 g a.i ha<sup>-1</sup> fb mechanical weeding at 30 DAS/T due to better control of weeds leading to lower removal of nutrients by weeds and higher nutrient uptake by grain. Among the establishment methods, transplanting method of establishment resulted in significantly higher grain yield due to lower weed density and as well as lower weed dry weight and it was comparable with SRI.

**Key words:** Weed density, weed dry weight, grain yield and uptake.

Rice (*Oryza sativa* L.) crop suffers more from weed competition unlike other cereal crops. The degree of competition and extent of yield losses vary greatly with rice cultures. Weeds compete with crop plants for moisture, nutrients, light, space and other growth factors and in the absence of an

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effective control measures, remove considerable quantity of applied nutrients resulting in a significant yield losses. Weeds cause substantial losses in yield through production of growth inhibiting compounds a phenomenon referred as allelopathy (Yaduraju *et al.*, 2005). Weed infestation and weed competition are more in direct seeded rice as compared to transplanted rice and SRI because the land is exposed till the initial seedling establishment in direct seeded rice. Crop establishment and weed management techniques are critical in rice farming. So, present investigation to study the weed infestation and nutrient removal by weeds in different crop establishment methods of rice, their influence on productivity of rice and nutrient uptake by rice was taken up.

### **Materials and Methods**

Field experiment conducted was conducted during *kharif* season of 2010 and 2011 at College Farm, College of Agriculture, Rajendranagar, Hyderabad. The soil of the experimental site was sandy loam in texture with pH of 7.8 and available nitrogen (234.5 kg ha<sup>-1</sup>), available phosphorus (28.9 kg ha<sup>-1</sup>) and potassium (271.6 kg ha<sup>-1</sup>). The experiment was laid out in a split plot design with three crop establishment methods as

main plots i.e. SRI (M<sub>1</sub>), Direct sowing of sprouted seeds under puddled condition (M<sub>2</sub>) and transplanting (M<sub>3</sub>) and four weed management practices as sub-plots i.e. bensulfuron-methyl 60 g + pretilachlor 600 g a.i ha<sup>-1</sup> applied on followed by mechanical weeding at 30 DAS/T (S<sub>1</sub>), bispyribac sodium @ 25 a.i ha<sup>-1</sup> (S<sub>2</sub>) as early post emergence, famer's practice (hand weeding twice at 20 and 40 DAS in direct seeded rice and transplanted rice, conoweeding thrice from 20 DAT with 10 days interval in SRI) (S<sub>3</sub>) and unweededcheck (S<sub>4</sub>) replicated thrice. The crop was fertilized with 120 kg N, 60 kg P<sub>2</sub>O<sub>5</sub> and 40 kg K<sub>2</sub>O ha<sup>-1</sup>. Half dose of N (60 kg ha<sup>-1</sup>) and full dose of phosphorus (60 kg ha<sup>-1</sup>) and potassium (40 kg ha<sup>-1</sup>) was applied basal before sowing. The remaining half nitrogen (60 kg ha<sup>-1</sup>) was top dressed in two equal splits at tillering and panicle initiation stages. Bensulfuron ethyl + pretilachlor mixture @ 60 + 600 g a.i ha<sup>-1</sup> was applied at 3 DAS/T (S<sub>1</sub>) by mixing with sand and followed by a mechanical weeding with push hoe at 30 DAS/T. Bispyribac sodium (S<sub>2</sub>) @ 25 g a.i ha<sup>-1</sup> was applied when, weeds were at 2-3 leaf stage. A thin film of water is maintained at the time of herbicide application. Farmer's practice (S<sub>3</sub>) comprises hand weeding twice at 20 and 40



DAS/T was carried out in normal transplanting and direct seeding of sprouted seeds, conoweeding thrice from 20 DAT with 10 days interval in SRI. The un-weeded control as weedy check (S<sub>4</sub>) was kept undisturbed for the entire cropping period. Weed density and weed dry weight was recorded and their original values transformed using  $\sqrt{x} + 2$  and for nutrient depletion by weeds also square root transformation was done. Nursery sowing for SRI and transplanting was done on the day of direct sowing of sprouted seeds. Direct sowing and nursery sowing for normal transplanting and system of rice intensification were done simultaneously on same day in both years.

## Results and Discussion

### Weed density (m<sup>-2</sup>) and Weed dry weight (g m<sup>-2</sup>)

Crop establishment methods exerted significant influence on the weed count (m<sup>-2</sup>) and weed dry weight (g m<sup>-2</sup>) recorded at 60 DAS (Table 1). Total weed density recorded in transplanting (57.00 and 52.52 m<sup>-2</sup>) and SRI (64.48 and 57.43 m<sup>-2</sup>) were at par and in turn were significantly lower compared to direct seeded rice (75.63 and 66.69 m<sup>-2</sup>) under puddle condition. Similar trend was

noticed with respect to total weed dry weight. The total weed density and dry weight of weeds were higher (43.49 and 39.47 g m<sup>-2</sup>) under direct seeded rice (sprouted seeds) under puddle condition compared to transplanting (33.73 and 32.20 g m<sup>-2</sup>) and SRI (37.67 and 33.67 g m<sup>-2</sup>) which might be due to failure to maintain flooded conditions in field and non-submergence of crop in the initial stages, crop and weeds germinate simultaneously so competition exists. These results are in conformity with those of Subramanayam *et al.* (2007).

Weed management practices had significant influence on the total weed count and total weed dry weight. Farmer's practice of weeding (hand weeding twice at 20 and 40 DAS in direct seeded rice and transplanted rice and conoweeding thrice from 20 DAT with 10 days interval in SRI) recorded significantly lower weed count (29.31 and 25.65 m<sup>-2</sup>) and weedy weight (11.25 and 8.74 g m<sup>-2</sup>) and it was on par with bensulfuron-methyl 60 g + pretilachlor 600 g a.i ha<sup>-1</sup> fb mechanical weeding at 30 DAS/T (34.68 and 31.45 m<sup>-2</sup> and weed dry weight 15.32 and 11.86 g m<sup>-2</sup> respectively during 2010 and 2011) and in turn significantly lower compared to other treatments. This is due to frequent removal

of broad spectrum of weeds and similar observations were reported by Bali *et al.* (2006). Weedy check recorded significantly higher weed count and weed dry weight during both the years. Interaction between rice crop establishment methods and weed management practices was found to be non significant during both the years.

### **Grain yield (kg ha<sup>-1</sup>)**

Grain yield of rice influenced significantly by rice establishment methods and weed management practices. Transplanting method recorded significantly higher grain yield (4408 and 4593 kg ha<sup>-1</sup>) and it was on par with SRI (4266 and 4438 kg ha<sup>-1</sup>) and both were registered significantly superior grain yield over direct seeded rice (3894 and 4075 kg ha<sup>-1</sup>) under puddle condition. Submerged conditions in transplanted rice facilitate availability of more mineralized form of N, P and K uptake in transplanted rice than that of direct sowing which encouraged tiller production in addition contributed to higher dry matter production and grain yield. Similar findings were observed by Shashikumar (1990).

Among weed management practices, higher grain yield (5601 and 5857 kg ha<sup>-1</sup>) and was recorded in S<sub>3</sub> *i.e.* farmer's practice

(two hand weeding at 20 and 40 DAS in direct seeded rice and transplanted rice and con weeding in SRI) was at par with bensulfuron methyl 60 g + pretilachlor 600 g a.i ha<sup>-1</sup> fb mechanical weeding at 30 DAS/T (5326 and 5585 kg ha<sup>-1</sup>) and in turn these two treatments were significantly superior over other treatments during both the years. The higher grain yield with bensulfuron methyl is due to decreased weed competition and minimum nutrient removal by weeds which might have increased the capacity of nutrient uptake and enhanced the source and sink sizes which in turn increased the yield attributes *viz.*, panicle number per hill, panicle length and filled grains per panicle. Saha and Rao (2010) and Sunil *et al.* (2010) found similar type of findings in their study. Significant interaction was not found between rice establishment methods and weed management practices.

### **Straw yield (kg ha<sup>-1</sup>)**

Transplanting method of establishment recorded significantly higher straw yield (5579 and 5811 kg ha<sup>-1</sup>) compared to SRI (5364 and 5697 kg ha<sup>-1</sup>) and direct sowing of rice (sprouted seeds) under puddle condition (4949 and 5300 kg ha<sup>-1</sup>). This is due to less crop weed competition and led to taller plants, more number of tillers and dry

matter production which in turn resulted in higher straw yield. Subramanyam *et al.* (2007) also reported similar results. Treatment farmer's practice of weeding (hand weeding twice at 20 and 40 DAS resulted in significantly higher straw yield (6766 and 7134 kg ha<sup>-1</sup>) and it was on par with bensulfuron-methyl 60 g + pretilachlor 600 g a.i ha<sup>-1</sup> fb mechanical weeding at 30 DAS/T (6489 and 6824 kg ha<sup>-1</sup>) and both were significantly superior to bispyribac sodium @ 25 g a.i ha<sup>-1</sup> (5203 and 5433 kg ha<sup>-1</sup>) and weedy check (2911 and 3019 kg ha<sup>-1</sup>). Higher straw yield was attributed to weed management treatments provided conducive environment and enhanced the growth of rice crop which in turn was reflected in terms of straw yield. These results are in confirmation with the findings of Sanjay *et al.* (2006). Interaction between rice crop establishment methods and weed management practices was found to be non significant during both the years.

### **Nutrient Removal by Weeds (kg ha<sup>-1</sup>)**

Nutrient removal by weeds in direct seeded rice (sprouted seeds) under puddle condition (3.90, 3.53 kg ha<sup>-1</sup> respectively during both the years) nitrogen, (2.57 and 2.04 kg ha<sup>-1</sup>) phosphorus and (8.83 and 4.50 kg ha<sup>-1</sup>) potassium was significantly higher

compared to transplanting (2.90 and 2.67 N kg ha<sup>-1</sup>; 1.65 and 1.46 P kg ha<sup>-1</sup> and 6.43 and 3.46 K kg ha<sup>-1</sup> respectively during both the years) and SRI (3.15 and 2.84 N kg ha<sup>-1</sup>; 1.98 and 1.65 P kg ha<sup>-1</sup> and 6.61 and 3.89 K kg ha<sup>-1</sup>). Shan *et al.* (2012) opined this could be due to the reason that the crop could not suppress the weeds initially due to poor establishment which resulted in more depletion of nutrients by the weeds.

Among weed management practices, S<sub>3</sub> i.e. farmer's practice of weeding (hand weeding twice at 20 and 40 DAS in direct seeded rice and in transplanted rice and cono weeding thrice in SRI) (0.92 and 0.70 N kg ha<sup>-1</sup>; 0.41 and 0.32 P kg ha<sup>-1</sup> and 0.95 and 0.86 kg ha<sup>-1</sup> during both the years respectively) and bensulfuron-methyl 60 g + pretilachlor 600 g a.i ha<sup>-1</sup> fb mechanical weeding at 30 DAS/T (1.24 and 0.96 kg N ha<sup>-1</sup>; 0.69 and 0.45 kg P ha<sup>-1</sup> and 1.39 and 1.20 K kg ha<sup>-1</sup> respectively during 2010 and 2011) were recorded significantly lower removal of nitrogen, phosphorus and potassium compared to other treatments. This may be due to control of broad spectrum of weed control in turn resulted in lower biomass accumulation of weeds. The findings of the present study are in conformity with the results obtained by

Jacob and Syriac, (2005). Weedy check recorded significantly higher nitrogen (8.10 and 7.83 kg ha<sup>-1</sup>), phosphorus (5.31 and 4.54 kg ha<sup>-1</sup>) and potassium (22.91 and 10.62 kg ha<sup>-1</sup>) removal by weeds compared to other treatments during two years of experimental study. Similar results were reported by Puniya *et al.* (2007). Nutrient removal by weeds was not influenced significantly due to interaction effect of crop establishment methods and weed management practices.

### **Nutrient Uptake by Rice grain (kg ha<sup>-1</sup>)**

The data on nutrient uptake by rice grain at harvest indicated that nitrogen phosphorus and potassium uptake by rice (62.84 and 65.44 N kg ha<sup>-1</sup>; 12.40 and 12.92 P kg ha<sup>-1</sup>; 12.13 and 12.65 K kg ha<sup>-1</sup>) in transplanting method and SRI(59.96 and 62.38 N kg ha<sup>-1</sup>; 11.96 and 12.44 P kg ha<sup>-1</sup> and 12.13 and 12.65 K kg ha<sup>-1</sup> respectively during both the years) significantly higher compared to direct seeded rice (sprouted seeds) under puddle condition (54.27 and 56.77N kg ha<sup>-1</sup>; 10.19 and 10.66 P kg ha<sup>-1</sup> and 10.01 and 10.48 K kg ha<sup>-1</sup> respectively ) and it was due to decreased weed competition in transplanted rice might have augmented the uptake of applied nutrients as well as soil nutrients. Similar effects were reported earlier by Chander and Pandey (1997).

Among weed management practices, treatment farmer's practice recorded significantly higher uptake of nitrogen (80.35 and 83.96 kg ha<sup>-1</sup>) phosphorus (16.07 and 16.79 kg ha<sup>-1</sup>) and potassium (15.51 and 16.21 kg ha<sup>-1</sup>). This was at par with bensulfuron-methyl 60 g + pretilachlor 600 g *a.i* ha<sup>-1</sup> *fb* mechanical weeding at 30 DAS/T (75.92 and 79.60 N kg ha<sup>-1</sup>; 15.10 and 15.83 P kg ha<sup>-1</sup> and 14.82 and 15.55 kg ha<sup>-1</sup>) and in turn was significantly superior over other treatments during both the years. Higher nutrient uptake is due to better control of weeds leading to lower depletion of nutrients by weeds and higher nutrient uptake by rice. The results are in conformity with the findings of Sanjay *et al.* (2006). Weedy check registered significantly the lowest nutrient uptake by crop. Interaction effect was not found between crop establishment methods and weed management practices.

### **Nutrient Uptake by Rice Straw (kg ha<sup>-1</sup>)**

Significantly higher nutrient uptake (42.94 and 44.71N kg ha<sup>-1</sup>; 7.31 and 7.62 P kg ha<sup>-1</sup> and 48.53 and 50.55 K kg ha<sup>-1</sup>) by rice straw was observed with transplanted rice and it was comparable (40.01 and 42.49 N kg ha<sup>-1</sup>; 6.55 and 6.95P kg ha<sup>-1</sup> and 46.24 and 49.11 kg ha<sup>-1</sup>) with SRI. The lowest uptake

of nutrients (36.51 and 39.10 N kg ha<sup>-1</sup> ; 5.55 and 5.95 P kg ha<sup>-1</sup> and 42.15 and 45.15 K kg ha<sup>-1</sup>) was registered with direct seeded (sprouted seeds) rice under puddle condition. Farmer's practice of weeding recorded significantly higher nutrient uptake by straw (52.04 kg ha<sup>-1</sup> and 54.85; 8.81 and 9.29 P kg ha<sup>-1</sup> and 58.93 and 62.14 K kg ha<sup>-1</sup>) and it was comparable with bensulfuron-methyl 60 g + pretilachlor 600 g a.i ha<sup>-1</sup> fb mechanical weeding at 30 DAS/T (49.47 and 52.02 N kg ha<sup>-1</sup>; 8.25 and 8.66 P kg ha<sup>-1</sup> and 56.61 and 59.52 kg ha<sup>-1</sup>) in turn was significantly superior over bispyribac sodium. Weedy check resulted in significantly lower uptake of nitrogen (20.82 and 21.60 kg ha<sup>-1</sup>), phosphorus (2.94 and 3.05 kg ha<sup>-1</sup>) and potassium (24.14 and 25.04 kg ha<sup>-1</sup>) by rice straw. Interaction effects were found non significant between crop establishment methods and weed management practices.

### Conclusion

The data revealed that transplanting method of establishment resulted in significantly higher grain yield and it was comparable with SRI and among weed management practice, farmer's practice of weeding recorded significantly higher grain yield of rice and it was on par with bensulfuron-

methyl 60 g + pretilachlor 600 g a.i ha<sup>-1</sup> fb mechanical weeding at 30 DAS/T due to better control of weeds leading to lower removal of nutrients by weeds and higher nutrient uptake by grain.

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**Table 1: Total weed density, weed dry weight, grain yield and straw yield as influenced by crop establishment methods and weed management practices**

Treatments	Weed density (m <sup>-2</sup> )		Weed dry weight (g m <sup>-2</sup> )		Grain yield (kg ha <sup>-1</sup> )		Straw yield (kg ha <sup>-1</sup> )	
	2010	2011	2010	2011	2010	2011	2010	2011
<b>Main treatments</b>								
M <sub>1</sub> – SRI	8.14(64.48)	7.69 (57.43)	6.29 (37.67)	5.97 (33.67)	4265	4438	5364	5697
M <sub>2</sub> – Direct sown rice	8.81 (75.63)	8.30 (66.69)	6.75 (43.49)	6.44 (39.47)	3894	4075	4948	5300
M <sub>3</sub> – Transplanting	7.68 (57.00)	7.23(52.62)	5.98 (33.73)	5.85 (32.20)	4408	4593	5579	5811
SEm±	0.17	0.15	0.12	0.10	91	90	99	97
CD (5%)	0.66	0.60	0.45	0.41	356	354	387	381
<b>Sub treatments</b>								
S <sub>1</sub> – Bensulfuron methyl + Pretilachlor fb mechanical weeding 30 DAS/T	6.06(34.68)	5.78(31.45)	4.16(15.32)	3.72 (11.86)	5326	5585	6489	6824
S <sub>2</sub> – Bispyribac sodium	8.48 (69.92)	7.92(60.80)	5.84(32.13)	5.57 (29.06)	3975	4158	5023	5433
S <sub>3</sub> – Farmer's practice	5.60 (29.31)	5.26(25.65)	3.64 (11.25)	3.28 (8.74)	5601	5857	6766	7134
S <sub>4</sub> – Weedy check	11.44(128.92)	10.94(117.73)	9.82(94.48)	9.63 (90.78)	1854	1874	2911	3019
SEm±	0.17	0.21	0.18	0.19	95	107	106	109
CD (5%)	0.50	0.64	0.54	0.57	283	318	316	323

Values in parenthesis ( ) are original values

**Table 2: Nutrient removal by weeds at 60 DAS as influenced by crop establishment methods and weed management practices during 2010 and 2011**

Treatments	N uptake (kg ha <sup>-1</sup> )		P uptake (kg ha <sup>-1</sup> )		K uptake (kg ha <sup>-1</sup> )	
Main treatments	2010	2011	2010	2011	2010	2011
M <sub>1</sub> – SRI	2.27 (3.15)	2.19 (2.84)	1.99 (1.98)	1.91 (1.65)	2.93 (6.61)	2.43 (3.89)
M <sub>2</sub> – Direct sown rice	2.43 (3.90)	2.36 (3.53)	2.14 (2.57)	2.01 (2.04)	3.29 (8.83)	2.56 (4.50)
M <sub>3</sub> – Transplanting	2.21 (2.90)	2.16 (2.67)	1.91 (1.65)	1.86 (1.46)	2.90 (6.43)	2.34 (3.46)
SEm±	0.04	0.04	0.03	0.02	0.06	0.03
CD (5%)	0.15	0.16	0.14	0.09	0.23	0.12
Sub treatments						
S <sub>1</sub> – Bensulfuron methyl + Pretilachlor <i>fb</i> mechanical weeding at 30 DAS/T	1.80 (1.24)	1.72 (0.96)	1.64 (0.69)	1.57 (0.45)	1.84 (1.39)	1.79 (1.20)
S <sub>2</sub> – Bispyribac sodium	2.17 (2.72)	2.10 (2.43)	1.89 (1.59)	1.84 (1.40)	2.43 (3.91)	2.26 (3.12)
S <sub>3</sub> – Farmer's practice	1.71 (0.92)	1.64 (0.70)	1.55 (0.41)	1.52 (0.32)	1.72 (0.95)	1.69 (0.86)
S <sub>4</sub> – Weedy check	3.18 (8.10)	3.14 (7.83)	2.70 (5.31)	2.56 (4.54)	4.99 (22.91)	3.55 (10.62)
SEm±	0.05	0.04	0.05	0.03	0.07	0.05
CD (5%)	0.15	0.13	0.14	0.09	0.21	0.15

Values in parenthesis are original values

**Table 3: Nutrient uptake by rice grain (kg ha<sup>-1</sup>) at harvest as influenced by rice crop establishment methods and weed management practices**

Treatments	N uptake (kg ha <sup>-1</sup> )		P uptake (kg ha <sup>-1</sup> )		K uptake (kg ha <sup>-1</sup> )	
	2010	2011	2010	2011	2010	2011
<b>Main treatments</b>						
M <sub>1</sub> – SRI	59.96	62.38	11.96	12.44	11.48	11.96
M <sub>2</sub> – Direct sown rice	54.27	56.77	10.19	10.66	10.01	10.48
M <sub>3</sub> – Transplanting	62.84	65.44	12.40	12.92	12.13	12.65
SEm±	1.42	1.44	0.33	0.33	0.32	0.35
CD (5%)	5.59	5.66	1.28	1.29	1.26	1.37
<b>Sub treatments</b>						
S <sub>1</sub> – Bensulfuron methyl+ Pretilachlor <i>fb</i> mechanical weeding at 30 DAS/T	75.92	79.60	15.10	15.83	14.82	15.55
S <sub>2</sub> – Bispyribac sodium	55.21	57.74	10.37	10.84	10.20	10.67
S <sub>3</sub> – Farmer's practice	80.35	83.96	16.07	16.79	15.51	16.21
S <sub>4</sub> – Weedy check	24.62	24.82	4.53	4.56	4.31	4.36
SEm±	1.47	1.54	0.37	0.33	0.26	0.31
CD (5%)	4.37	4.58	1.10	0.98	0.78	0.91

**Table 4: Nutrient uptake by rice straw (kg ha<sup>-1</sup>) as influenced by crop establishment methods and weed management practices**

Treatments	N uptake (kg ha <sup>-1</sup> )		P uptake (kg ha <sup>-1</sup> )		K uptake (kg ha <sup>-1</sup> )	
	2010	2011	2010	2011	2010	2011
<b>Main treatments</b>						
M <sub>1</sub> – SRI	40.01	42.49	6.55	6.95	46.24	49.11
M <sub>2</sub> – Direct sown rice	36.51	39.10	5.55	5.95	42.15	45.15
M <sub>3</sub> – Transplanting	42.94	44.71	7.31	7.62	48.53	50.55
SEm±	0.86	0.79	0.19	0.20	1.02	0.85
CD (5%)	3.36	3.12	0.76	0.80	4.00	3.32
<b>Sub treatments</b>						
S <sub>1</sub> – Bensulfuron methyl + Pretilachlor <i>fb</i> mechanical weeding at 30 DAS/T	49.47	52.02	8.25	8.66	56.61	59.52
S <sub>2</sub> – Bispyribac sodium	36.94	39.93	5.88	6.35	42.88	46.37
S <sub>3</sub> – Farmer's practice	52.04	54.85	8.81	9.29	58.93	62.14
S <sub>4</sub> – Weedy check	20.82	21.60	2.94	3.05	24.14	25.04
SEm±	1.09	1.03	0.21	0.22	1.11	0.96
CD (5%)	3.22	3.07	0.63	0.66	3.31	2.86



# On Farm and Multi Location Performance of Indigenous Sex Pheromone Lure against Rice Yellow Stem Borer

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## Abstract

**A series of supervisory male annihilation on-farm and multi locations field trials conducted with YSB lure having 3 mg of (Z)-11-hexadecenal and (Z)-9-hexadecenal at 3:1 ratio loaded in PVC dispensers installed @ 20 traps/ha clearly indicated that the indigenous YSB lure as an effective tool for the management of YSB problem in rice. Reduction in insect pest incidence was also reflected with increase yield and C: B ratio.**

**Key words:** Indigenous, lure, sex pheromone, trap, YSB

Rice is the major staple food crop in India with annual production of 106.29 million tones during 2013-14 (www.oryza.com). Among different crop pests, the Yellow Stem Borer (YSB), *Scirpophaga incertulas* (Walker) (Pyralidae: Lepidoptera) with country wide distribution is the most

dominant and destructive. It causes yield loss up to 38 to 80 per cent (Dale, 1994). Since stem borer problem extends up to maturity phase of the crop, over dependence on insecticide is considered as undesirable. In the absence of resistant rice varieties against stem borer, there is a need for eco-friendly alternatives for stem borer management.

Insect sex pheromone is a promising tool for the management of YSB because of their natural occurrence, lack of toxicity, high bioactivity, species specificity, long potency and compatibility with other IPM components. Though the use of sex pheromone is a well established technique in rice IPM for monitoring and mass trapping of the insect, so far there has been out sourcing of knowledge and skill that leads to dependence on foreign expertise in molecule synthesis and formulation development. Absence of indigenous formulation has also adds to higher price and non availability of quality lures in sufficient quantity especially in pest out break situation.

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Considering the above issues in view, the present studies were undertaken to develop and demonstrate cost effective indigenous pheromone lure for the management of YSB in rice, so that the technique can be made available in the public system for use against rice stem borer in the country.

### **Materials and Methods**

Designing indigenous pheromone blend for the management of local geographic population of YSB was achieved through different steps which involved synthesis of molecules, screening for biological activity, designing of dispenser and trap, quality control and bio efficacy. The indigenous lure was also aimed at cost effectiveness and timely availability of quality lure in sufficient quantity for the ultimate adoption by the farmers.

The sex pheromone molecule for YSB, (Z)-11-hexadecenal and (Z)-9-hexadecenal were synthesized using the expertise of pheromone laboratory, IICT, Hyderabad. Based on bioactive studies the blend combination having (Z)-11-hexadecenal and (Z)-9-hexadecenal at 3:1 ratio was designed as indigenous YSB lure and was loaded to the pre treated PVC dispenser @ 3mg/lure.

The lures were placed in to the polythene sleeve trap and deployed in the field.

Performance of indigenous YSB lure was evaluated for their efficacy in monitoring and mass trapping (male annihilation) of the target pest. The bio efficacy experiments were conducted as, on farm trial during *Kharif* 2009 at farmer's field in Kabisthalam village of Kumbakonam, Tamilnadu. The lure were also tested across different location in the state through Multi Location Trial (MLT) which includes Rice Research Station, Tirur (TIR), Agriculture Research Station, Thirupathisaram (TPS), Plant Breeding Station, Coimbatore (CBE), Anbil Dharmalingam Agricultural College and Research Institute (ADAC&RI), Trichy (TRY) and TRRI, Aduthurai (ADT). In all the trials, for monitoring the lures were used @ 8 traps/ha. The traps were installed at 10 DAT with an inter trap distance of 60 m in a triangular pattern. Trap height was maintained at 0.5 m above the crop canopy. The trap catch threshold of 8 moths/trap/day recommended by Krishnaiah *et al.* (2004) was used for deploying mass trapping. The mass trapping has been done by increasing the number to 20 traps/ha with 20 m x 25 m spacing. Lures were changed at every 21 days till crop harvest. In all the field trials,

care was taken not to use any insecticides. The trap catches were recorded at weekly interval and analyzed statistically. Further, the stem borer damage in terms of Dead Heart (DH) was assessed at 30 and 45 DAT from 100 randomly selected tillers in 1 x1m micro plot. For White Ear (WE) damage, the samples were drawn at 15 days after flowering synchronizing with milk filling and dough stage of the crop. Yield estimation was also made in three 5x5m micro plot in each treatment and reported as kg ha<sup>-1</sup>. The efficacy of the treatment was assessed by comparing the crop damage, pheromone trap catches and grain yield with untreated control. By adopting partial budgeting procedures the cost benefit ratio was also worked out.

## **Results and Discussion**

### **On farm performance of indigenous YSB lure**

The performance of indigenous YSB lure at the farmer field at Kabisthalam in *kharif* 2009 season revealed low incidence of dead heart in pheromone intercepted plot. Incidence of dead heart at 30 and 45 DAT was 3.20 and 1.70 per cent. In the plot without any pest control, a much higher damage of 8.43 and 7.00 per cent DH was

observed at 45 and 30 DAT (Table 1). White ear incidence of 3.90 per cent was observed in pheromone trap installed plot against 10.20 per cent in the control. The highest grain yield of 4567 kg ha<sup>-1</sup> was recorded in the pheromone trap installed than control plot of 3240 kg ha<sup>-1</sup>. Returns per additional cost involved in mass trapping were 14 fold and the yield gain was 1327 kg ha<sup>-1</sup> in pheromone intercepted field.

### **Performance of indigenous YSB lure under multi location**

The mean trap catches in the interception period in the tested multi locations ranged from 19.8 moths/trap in Thirupathisaram (TPS) to a maximum of 34.5 moths/ trap in Coimbatore (CBE) (Table 2). In tillering stage, reduction in dead heart observed was 32.80 per cent in Tirur (TIR) followed 22.40 per cent in Coimbatore. In Thirupathisaram, Aduthurai (ADT) and Trichy (TRY) reduction in dead heart percentage due to indigenous lure installation was 18.60, 4.62 and 14.80 per cent respectively over the untreated control. In Aduthurai at reproductive stage of the crop up to 80.52 per cent reduction in white ear damage was observed. In terms of yield gains, maximum yield of 225 kg ha<sup>-1</sup> was realized. Following two time mass trapping a yield gain of 208

kg ha<sup>-1</sup> was realized in Coimbatore and 252 kg ha<sup>-1</sup> was realized in Trichy (Table 2).

The results of the field trials conducted in on-farm and multi locations trials clearly indicated that the indigenous YSB lure as an effective tool for the management of YSB problem in rice. Reduction in insect pest incidence was also reflected in increase in yield and C:B ratio. The above said findings were in line with the findings of Varma *et al.* (2000), Krishnaiah *et al.* (2004), Tiwary (2004), and Ravi *et al.* (2008) who employed male annihilation technique for the management of YSB in rice.

### Conclusions

It was concluded from the study that YSB lure with 3 mg of (Z)-11-hexadecenal and (Z)-9- hexadecenal @ 3:1 impregnated in PVC dispensers recommended @ 20 traps/ha can be used for mass trapping of *S. incertulas*. The technique can reduce the damage level and there by dependence on chemical spray may either be avoided or reduced.

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**Table 1: On farm performance of indigenous YSB lure**

Treatment	Dead Heart (%)		WhiteEar (%)	Yield (Kg ha <sup>-1</sup> )	CB ratio
	30 DAT	45 DAT			
Pheromone trap installed plot (20 traps ha <sup>-1</sup> )	3.20 (11.09)	1.70 <sup>a</sup> (8.53)	3.90 (12.11)	4567	2.04
Control plot (without any plant protection)	7.00 (15.79)	8.43 <sup>b</sup> (17.46)	10.20 (19.00)	3240	1.51
CD (P= 0.05)	NS	0.55	NS	NS	

Figures in parentheses are transformed values

**Table 2: On station multi location performance of indigenous YSB lure**

Trap Efficacy Parameters	Locations				
	ADT	TRY	CBE	TPS	TIR
Mean trap catches in the interception period	22.67 (3-32)	22.67 (1-26)	34.50 (0- 32)	19.80 (0- 42)	24.00 (2-26)
Per cent reduction in Dead Heart	14.80	4.62	22.40	18.60	32.80
Per cent reduction in White ear	80.52	25.68	44.50	22.80	44.50
No. of lure replacement (interception at 25,46,57 DAT)	Three	Two	Two	Three	Three
Cost of interception ha <sup>-1</sup> (@Trap Rs 20; lure Rs 6)	760	640	520	760	760
Yield gain kg ha <sup>-1</sup>	225	252	208	124	241

Figures in parentheses indicates range in catches (minimum - maximum)



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