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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.
- To disseminate knowledge on latest developments in rice research through publications, seminars, lectures and training programmes.
- To provide consultancy in rice production and development.
- To facilitate research and industry collaboration and public private partnership at national level.
- To honour outstanding achievers in rice research and development.
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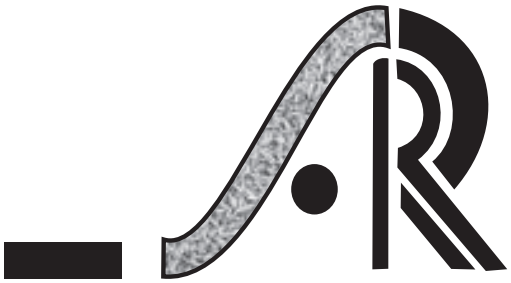

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Variability and correlation of yield traits in BIL x BIL populations derived from Swarna x *Oryza nivara*

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Abstract

Wild introgression lines have wider genetic diversity than normal cultivars and contribute for crop improvement. Segregating populations consisting of 161 families at F_3 and F_4 generations derived from back cross introgression line (BIL) parents were used in the present study. Field evaluation for yield traits were carried out during two seasons of *rabi* and *kharif* in 2017. Correlation analysis was performed to determine associations among yield traits and highly significant association was observed for single plant yield (SPY) with total dry matter (TDM) and harvest index (HI) in F_3 and F_4 generations. Significant positive association of tiller number (TN), productive tiller number (PTN) with SPY and also highly significant negative association between SPY and thousand grain weight (TGW) were observed in F_4. All the traits in F_3 and F_4 showed positively skewed distribution except for the traits, plant height and TGW. Significant lines were identified through pair-wise mean comparisons with parents 166S, 148S and Swarna. Four lines C_3-53, C_3-38, C_3-70, and C3-96 exhibited positively significant values for TGW compared with parents. These lines can be used further for yield improvement and genetic dissection of target traits.

Key words: Correlation, yield, *Oryza nivara*, back cross introgression lines

Introduction

Increase in yield potential of crop varieties is a challenging task in modern plant breeding. It is estimated that 60% increase in agricultural production needs to be achieved by 2050 to feed the increasing population (Alexandratos and Bruinsma., 2012). Yield is a complex trait which is influenced by different contributing traits and their polygenic inheritance and environment interaction effects (Usman *et al*., 2017). So, we need to evaluate all yield component traits and their contribution in a holistic manner (Oladosu *et al*., 2018). The correlation analysis showing interrelationship among yield and its components, is also very helpful to carry out efficient selection process (Rasel *et al*., 2018). The present study was conducted with following objectives i) to study the variability in the population for all the yield traits ii) to study interrelationships among yield component traits and iii) to identify significantly different lines for each trait by comparing with control/ parents. The study will be useful for identifying traits and genotypes for yield improvement in advanced generations.

Materials and methods

The parents 166S and 148S (BC_2F_8 BILs) derived from Swarna x *O. nivara* (Swamy *et al*., 2014) were crossed for generating F_1. Both F_3 and F_4 population with 161 plants were forwarded by single panicle selection from F_2 generation (Kavitha *et al*., unpublished). The parental BILs, 166S and 148S were detected as stable lines using multi environment data (Divya *et al*., 2016). 166S is a potential donor for improving yield traits like single plant yield, grain number, productive tiller number, panicle weight and germination percentage (Kavitha *et al*., 2019), while it is also reported to be

drought and salinity tolerant (Kota *et al*., 2012). Field experiment was carried out during *kharif* 2017 and *rabi* 2017 at Indian Institute of Rice Research (IIRR) farm, Rajendranagar, Hyderabad, India at latitude of 17° 19′ N and longitude of 78° 29′ E. The experiment was conducted in randomized complete block design with two replications. Seedlings from the nursery bed were transplanted in field at one to two seedlings per hill with a spacing of 20 x 15 cm (plant x row) and followed with recommended agronomic practices for good crop growth.

Observations were recorded from five middle row plants from each line in two replications. Data were recorded for yield traits of plant height (PH), tiller number (TN), productive tiller number (PTN), single plant yield (SPY), biomass (BM), total dry matter (TDM), harvest index (HI) and thousand grain weight (TGW) using standard evaluation system of IRRI (SES, IRRI,2013). Statistical analysis was performed for descriptive statistics, frequency distribution and correlation using Pearson's product-moment correlation method at the significant levels of $^{*}P$ = 0.05-0.001 and $^{**}P \geq 0.001$ with PB tools (Version 1.4, http://bbi.irri.org/products) software and significant pair wise comparisons with controls were carried out using STAR v2.0.1 software.

Results and Discussion

The range, variance and standard deviation values were lower for each trait (except for PH) in F_4 generation than in F_3 and the critical value (CV) for each trait were lower in F_4 than in F_3 except for the traits BM and TDM (**Table 1)**. TGW was observed with almost equal values of range, variance, standard deviation and critical values in F_3 and F_4. All the traits in F_3 and F_4 showed positively skewed distribution except for the traits PH (Vijaya and Shailaja., 2016) and TGW which showed negatively skewed distribution. Except the traits of TN, PTN and SPY all other traits showed

Table 1: Descriptive statistics for the yield traits in F_3 and F_4 population of 166S x 148S

Trait	Generation	Min	Max	Mean	Range	Variance	Standard deviation	Critical value	Skewness	Kurtosis
PH	F_3	59.67	153.67	111.57	94	339.65	18.43	16.52	-0.75	0.29
	F_4	71.5	155.33	119.34	83.83	344.19	18.55	15.55	-0.59	-0.29
TN	F_3	4.67	28	11.76	23.33	11.04	3.32	28.26	1.1	3.27
	F_4	4.67	16.4	7.15	11.73	2.47	1.57	21.98	2.18	8.94
PTN	F_3	4.67	28	11.72	23.33	11.13	3.34	28.47	1.07	3.23
	F_4	4.67	15.4	7.13	10.73	2.29	1.51	21.21	1.85	6.54
SPY	F_3	1.65	25.57	9.49	23.92	22.38	4.73	49.84	0.85	0.71
	F_4	3.93	32.47	11.11	28.54	13.12	3.62	32.6	1.56	7.01
BM	F_3	9.37	62.9	26.44	53.53	83.57	9.14	34.57	0.68	0.8
	F_4	5.13	31.6	13.55	26.47	27.09	5.21	38.41	1.29	2.18
TDM	F_3	15.97	67.47	35.88	51.5	101.65	10.08	28.1	0.32	0.24
	F_4	11.08	53.26	24.62	42.18	59.04	7.68	31.21	0.96	1.36
HI	F_3	4.23	57.32	26.77	53.09	130.28	11.41	42.64	0.27	-0.47
	F_4	23.77	70.05	45.5	46.28	60.55	7.78	17.1	0.4	0.6
TGW	F_3	12.16	30.44	22.48	18.28	8.22	2.87	12.76	-0.33	1.28
	F_4	12.16	30.44	22.49	18.28	8.05	2.84	12.62	-0.26	1.14

PH- plant height, TN- Tiller number, PTN- Productive tiller number, SPY- Single plant yield, BM- Biomass, TDM- Total dry matter, HI- Harvest index, TGW-Thousand grain weight.

leptokurtic distribution (Raghavendra and Hittalmani., 2015) with more than three values. Remaining yield traits exhibited platykurtic distribution with less than three values (**Figure 1)**.

Correlation coefficient analysis showed highly significant association among yield traits in F_3 and F_4 (**Table 2**). In $F_{3,}$ SPY showed highly positive significant association with BM, TDM and HI. Earlier studies reported similar associations for SPY with HI (Kishore *et al*., 2018 and Archana *et al*., 2018), and SPY with BM (Bitew *et al*., 2018). In the present study, positive association was also significant among PH with BM and TDM, TN and PTN as well as BM and TDM. Highly negative significant association was observed for HI with PH, TN, PTN and BM as well as PH with TN and PTN. Similar results were reported by Sadimantara *et al*., (2018) in case of PH with TN and PTN. Archana *et al*., (2018) reported negative association between HI and PH.

The traits TN and PTN showed significant positive association with BM and significant negative association between HI and TDM; SPY and PH, TGW with TN and PTN. Sreedhar and Uma Reddy., (2019) reported similar association between SPY and PH. In F_4, highly significant association was shown by SPY with PH, BM, TDM and HI; TDM with PH and BM; PH with BM and TDM and in between TN and PTN. Highly significant negative association of PH with TN, PTN and HI; TGW with SPY, BM and TDM; HI with PH, BM and TDM was also observed. Significant

Table 2: Correlation among yield traits in F_3 and F_4 population of 166S x 148S

Trait	Generation	PH	TN	PTN	SPY	BM	TDM	HI	TGW
PH	F_3	1.00							
	F_4	1.00							
TN	F_3	-0.25**							
	F_4	-0.26**							
PTN	F_3	-0.23**	0.99**						
	F_4	-0.26**	1.00**						
SPY	F_3	-0.15*	-0.06	-0.07					
	F_4	0.28**	0.12*	0.11*					
BM	F_3	0.43**	0.12*	0.13*	-0.08				
	F_4	0.50**	0.09	0.08	0.46**				
TDM	F_3	0.33**	0.08	0.09	0.39**	0.88**			
	F_4	0.48**	0.09	0.08	0.77**	0.91**			
HI	F_3	-0.35**	-0.17**	-0.19**	0.81**	-0.57**	-0.14*		
	F_4	-0.28**	-0.05	-0.05	0.35**	-0.58**	-0.23**		
TGW	F_3	-0.01	-0.12*	-0.12*	-0.07	0.02	-0.01	-0.06	1.00
	F_4	0.01	-0.14*	-0.13*	-0.31**	-0.17**	-0.25**	-0.08	1.00

*P = 0.05-0.001, significant lines, **P ≥ 0.001, highly significant lines

Highly significant positive values in italics, highly significant negative values in bold

PH- plant height, TN- Tiller number, PTN- Productive tiller number, SPY- Single plant yield, BM- Biomass, TDM- Total dry matter, HI- Harvest index, TGW-Thousand grain weight. F_3 and F_4- Generation.

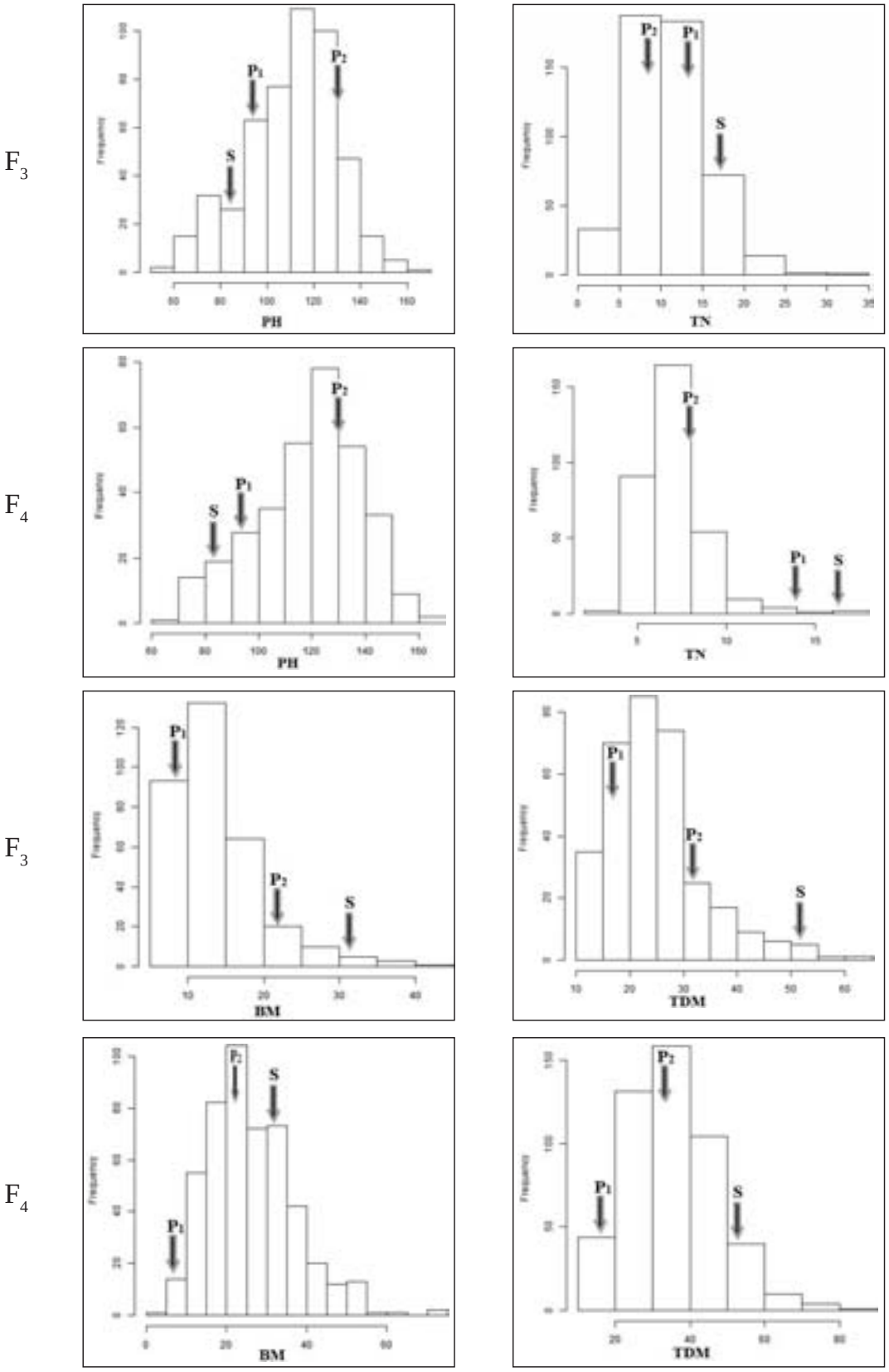

Figure1. Frequency distribution of yield traits in F3 and F4 populations of 166S x 148S

PH- plant height, TN- Tiller number, PTN- Productive tiller number, SPY- Single plant yield, BM- Biomass, TDM- Total dry matter, HI- Harvest index, TGW-Thousand grain weight. F_3 and F_4- Generation. P1- 166S, P_2- 148S, S- Swarna

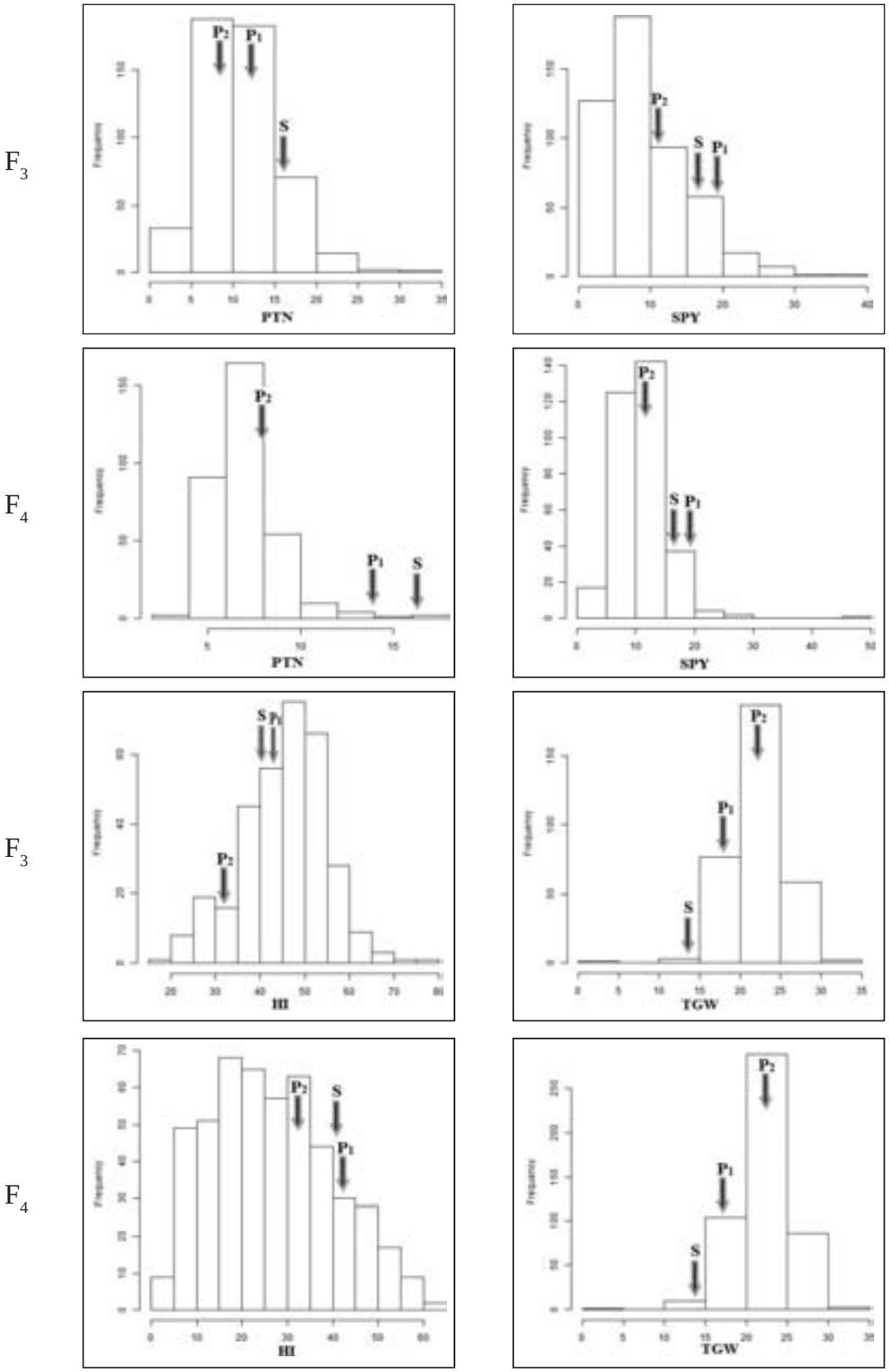

Figure1. Frequency distribution of yield traits in F3 and F4 populations of 166S x 148S

PH- plant height, TN- Tiller number, PTN- Productive tiller number, SPY- Single plant yield, BM- Biomass, TDM- Total dry matter, HI- Harvest index, TGW-Thousand grain weight. F_3 and F_4- Generation. P1- 166S, P_2- 148S, S- Swarna

positive correlation of SPY with TN and PTN and significant negative association for TGW with TN and PTN were observed. Consistent correlation among traits in both the generations included SPY with TDM and HI; PH with BM and TDM and between TN and PTN with highly significant positive association and other traits PH with TN, PTN and HI; HI with BM and PH showing highly significant negative association. TGW showed significantly negative association consistently with TN and PTN. Similar associations between PH and PTN; TGW and PTN were reported by Lakshmi *et al*., (2014), while Zahid *et al*., (2006) observed the same result of TN with PH and TGW.

Significant lines for each trait from two populations were identified through pair wise comparisons by comparing with parents 166S and 148S along with Swarna (**Table 3**). Positive significant lines compared with 166S were identified for PH (93L in F_3 and F_4), TN (3L in F_3), PTN (3L in F_3), BM (14L in F_3, 1L in F_4), TDM (3L in F_3, 1L in F_4) and for TGW (116L in F_3, 44L in F_4). In case of 148S, positive significant lines compared were identified for TN (6L in F_3), PTN (5L in F_3), SPY (1L in F_3, 2L in F_4), for BM and TDM 6L and 3L respectively in F_3, for HI (2L in F_3, 14L in F_4) and for TGW (10L in F_3, 2L in F_4) traits. Positive significant lines compared with Swarna were identified for PH (110 in F_3, 109 in F_4), for TN and PTN 1L each in F_3, SPY (1L in F_4), BM (1L in F_3) and for TGW (155L in F_3, 117L in F_4). From the results it was observed that C_3 53, C_3 38, C_3 70 and C_3 96 lines showed positive significant values over 166S, 148S and Swarna for TGW. For SPY, the line C_3 36 showed positive significance with 148S and Swarna while the lines C_3 145 and C_3 3 showed positive significance with 148S. These significant lines for TGW and SPY can be further used for yield improvement.

Table 3: 166S x 148S significant lines for 166S, 148S and Swarna

Significant lines *	PH		TN		PTN		SPY		BM		TDM		HI		TGW	
	F_3	F_4	F_3	F_4	F_3	F_4	F_3	F_4	F_3	F_4	F_3	F_4	F_3	F_4	F_3	F_4
166S (+ve)	93	93	3	-	3	-	-	-	14	1	3	1	-	-	116	44
148S (+ve)	-	-	6	-	5	-	1	2	6	-	3	-	2	14	10	2
Swarna (+ve)	110	109	1	-	1	-	-	1	1	-	-	-	-	-	155	117
Total	203	202	10	-	9	-	1	3	21	1	6	1	2	14	276	163
166S (-ve)	-	-	-	-	-	-	41	15	-	-	-	-	40	-	-	-
148S (-ve)	56	24	-	-	-	-	-	-	-	27	-	-	2	-	7	3
Swarna (-ve)	-	-	6	160	5	160	20	3	7	147	47	146	25	-	-	-
Total	56	24	6	160	5	160	61	18	7	174	47	146	67	-	7	3

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Characterization of rice genotypes for grain Fe, Zn using energy dispersive X-ray fluorescence spectrophotometer (ED - XRF)

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Abstract

Iron and zinc are the most essential micronutrients required for growth and development of all organisms including human beings. The deficiency disorders of Fe and Zn constitute a major health concern around the globe. This is majorly affecting rural populations residing in developing countries with minimum purchasing power and low access to a diverse diet. Biofortification; the enrichment of staple food crops with bioavailable micronutrients or vitamins in the edible grains, provides a potential sustainable solution towards such issues. Utilization of rice as a platform for the delivery of Fe and Zn through biofortification could greatly impact the livelihood of people dependent on rice-based agro-food systems globally. In this study, 100 rice genotypes from different mapping populations were analyzed for grain iron and zinc through the non-destructive method, energy-dispersive X-ray fluorescence spectrophotometry for three seasons. Considerable variation was observed in the micronutrient density among the germplasm assessed. Iron concentration varied from 1.6 to 15.2 ppm whereas zinc concentration ranged from 6.2 to 33.2 ppm in brown rice samples and 10 genotypes N22, 148S, 61-1B, 70S, 196M, 24K, 105B, 88B, 132Z and 185M with high grain iron and zinc concentration were identified, which have the potential to be used in rice improvement.

Key words: Biofortification, ED-XRF, micronutrient, Iron, Zinc

Introduction

Rice is one of the most important staple foods that supports the livelihood for nearly half of the world's population and more than 3.5 billion people depends on rice for more than 20% of their daily calories (Global Rice Science Partnership (GRiSP), 2013). An estimated 486.96 million metric tons of rice was consumed globally during 2018/2019 and a further increase is predicted (Rice consumption worldwide, Statista 2019). More than 90% of the rice was produced and consumed by the Asian countries (USDA 2019). India is the second largest producer of rice with an annual production of 116.5 million metric tons for 2018-2019. Globally, Fe and Zn deficiency is widespread particularly in rural and developing regions where people consume cereal-based diets and have less opportunities for diet diversification; however, its presence is also detected in prosperous areas where diets are unbalanced, contributing to 'hidden hunger' (Roohani *et al*., 2013). It is estimated that micronutrient deficiencies affect approximately 1.6 billion people globally, particularly children, pregnant and lactating women, in low and middle-income countries. Because of its high consumption, rice constitutes an ideal vehicle for delivering micronutrients such as Fe and Zn, at a large scale. And it is necessary to improve both Fe and Zn concentrations and their bioavailability in rice grain to overcome these deficiency disorders of the populations dependent on rice as their staple food. Hence bio fortification is considered as the most sustainable and cost effective approach to develop micronutrient dense cereals crops.

The pre-requisite for initiating a programme to develop micronutrient rich genotypes, is to screen the available germplasm and to identify the source of the genetic variation for the target trait which can be used further for developing crosses, detecting genetic variation, improved lines, molecular markers and to understand the basic mechanisms of micronutrient enhancement. Selecting genotypes with high efficiency of Fe and Zn accumulation in the endosperm and their bioavailability from existing germplasm collection may be an efficient and reliable way to deliver Fe nutrition benefits to farmers and local population (Prom-u-thai *et al.*, 2006). Cheng et. al., (2009) screened 113 rice landraces from 12 provinces of China. They reported that japonica rice had higher Fe than that of indica rice varieties in brown rice samples. 11,400 rice samples of brown and milled rice were evaluated for Fe and Zn during 2006-2008 by Martinez et. al., (2010). They found that brown rice had 10-11 ppm Fe and 20-25 ppm Zn while milled rice had 2-3 ppm Fe and 16-17 ppm Zn. Anuradha *et al.*, (2012) screened 126 rice lines including cultivars and wild accessions and showed that wild rice accessions have higher grain Fe and Zn concentration in brown rice samples. Both wild rice and deep water rices are known to be sources of high Fe and Zn (Sarla *et al*., 2012).

Micronutrient content can be estimated by both destructive and non-destructive methods. Perl's Prussian blue and DTZ staining method are standardized for Fe and Zn estimation to conduct the initial screening of genotypes. Although these methods are simple and inexpensive they are qualitative instead of quantitative in nature (Velu *et al*., 2008). Accurate estimation of Fe and Zn concentration is normally achieved through inductively coupled plasma-optical emission spectrophotometry (ICPOES) or atomic absorption spectroscopy (AAS) (Choi *et al*., 2007). Estimation of grain micronutrients using non-destructive energy dispersive X-ray fluorescence (ED – XRF) machine is also very useful and efficient rapid screening method to select high grain Fe and Zn lines from large number of samples and further these values may be confirmed with destructive methods. Georgia *et al*., (2017) demonstrated the application of ED-XRF for high throughput screening of Fe and Zn concentration in common bean, maize and cowpea and its advantages in bio fortification breeding programmes. Maganti et. al (2020) studied the variation of grain Fe and Zn concentration in 159 rice germplasm of both brown and polished rice samples using XRF and observed a positive correlation between the two micronutrients. Takahashi *et al.*, (2009) revealed that Fe is most abundant in the embryo and in the aleurone layer while Zn has been localized in the endosperm of rice by X-ray micro fluorescence imaging. Screening of Fe and Zn concentration using EDXRF is a convenient and cost effective method in bio fortification breeding programs (Paldridge *et al.*, 2012). The objectives of the present study were to screen rice germplasm for iron and zinc concentration in brown rice using EDXRF method and to identify lines with high Fe and Zn which can be further utilized in bio fortification programmes.

Materials and Methods

Plant material: A set of 100 rice genotypes were grown during 3 seasons - one wet season (*Kharif* 2015) and two dry seasons (*Rabi* 2016 and *Rabi* 2017) at IIRR farm, Hyderabad, India. (17.53^0N latitude and 78.27^0 E longitude, 545 MSL, with mean temperature of 31.2^0C and mean annual precipitation of 988.3 mm). Randomized Complete Block Design (RCBD) with two replications was followed to conduct the experiments. The 100 rice genotypes included N22 mutants (4), BPT x *O. rufipogon* (WR119) BILs (30), Swarna x *O.nivara* (IRGC818489(S) and 81832(K)) BILs (19) and BCRILs of 233K x 24K (5), KMR3 x *O.rufipogon* (WR120) BILs (29), Madhukar x Swarna RILs (5) and Jalmagna x Swarna RILs (5). At the time of transplanting, soil pH was in the range of 8.52 to 8.57, while soil iron and zinc concentrations were 2.74 to 3.48 ppm and 3.55 to 3.66 ppm respectively.

Iron and Zinc Concentration: Iron and zinc concentration in brown rice samples was estimated using non-destructive, energy-dispersive X-ray fluorescence spectrometry (EDXRF) instrument (model X-Supreme 8000; Oxford Instruments plc, Abingdon, UK) at IIRR, Hyderabad. 10g of well dried paddy sample from each genotype was de husked using non-metallic de-husker (Krishi international 810 de-husker) having roller made of polymer to avoid iron and zinc contamination. De-husked rice was cleaned by removing broken grains and debris and 5g of each sample was weighed and transferred to sample cups. The sample cups were gently shaken for uniform distribution of samples and kept for analysis. Concentration of Fe/ Zn was expressed in microgram/ gram (µg/g) or parts per million (ppm) grains.

Results and Discussion

The mean grain iron concentration of 3 seasons ranged from 1.6 to 15.2 ppm and most of the genotypes (67) showed 10 to 12 ppm Fe. The mean zinc concentration in 3 seasons varied between 6.2 and 33.2 ppm, 50 genotypes showed 20 to 25 ppm Zn (**Table 1**). The mean value of iron in 100 genotypes was 9.7 ppm and

Table 1: Mean grain Fe and Zn concentration in 100 rice genotypes for three seasons

S No.	Genotype	Zn (ppm)	Fe (ppm)	S no.	Genotype	Zn (ppm)	Fe (ppm)
1	**N22**	30.3	14.1	51	14K	21.3	12.5
2	NH787	13.4	7.2	52	7K	20.8	11.2
3	NH59	16.8	2.6	53	233K	14.2	3.8
4	NH686	15.2	12	54	24K	24.8	9.2
5	**BPT5204**	17.3	7.6	55	132Z	24.3	13
6	105B	24.43	12.63	56	127Z	23.4	11.2
7	88B	24.40	10.70	57	133Z	21.3	10
8	27B	23.87	12.47	58	314Z	21.3	9.7
9	161B	23.17	10.60	59	515Z	12.8	2.7
10	16-1B	22.90	12.40	60	KMR3	13.4	7.7
11	61-1B	26.73	12.17	61	50-7	6.2	1.8
12	34B	22.63	11.43	62	50-13	11.3	1.6
13	26B	22.57	11.87	63	86-18	14.2	6.8
14	76B	22.53	11.17	64	363-12	24.2	10.3
15	55B	22.17	9.83	65	90-5	17.8	6.8
16	69B	22.17	11.77	66	12-38	18.8	9.8
17	33B	22.17	11.83	67	377-24	10.5	8.3
18	71B	22.13	12.23	68	363-5	13	7.3
19	83B	21.87	10.60	69	13-5	18.5	11.3
20	77B	21.80	10.97	70	10-3	17.6	11
21	4B	20.53	11.67	71	86-1	21.4	10.3

S No.	Genotype	Zn (ppm)	Fe (ppm)	S no.	Genotype	Zn (ppm)	Fe (ppm)
22	78B	20.50	10.63	72	50	20.2	8.3
23	41B	20.50	11.70	73	381	16	8.4
24	32B	20.47	10.63	74	14	23.2	12.2
25	30B	20.43	11.57	75	495	18	8.8
26	16B	20.43	11.63	76	463	19.5	10.2
27	28B	20.40	11.47	77	407	18.8	9
28	98B	17.73	9.83	78	473	18	8.8
29	122B	17.77	10.20	79	410	11.8	9.7
30	51B	19.80	10.23	80	213	21.2	8.8
31	21B	17.80	12.00	81	198	23.6	11.8
32	112B	17.93	10.10	82	117	17.3	8.3
33	14B	17.93	12.47	83	109	16.8	8
34	148B	18.00	10.87	84	458	22.1	10.3
35	154B	17.60	9.73	85	431	21.1	15.2
36	**Swarna**	18.2	8.8	86	501	21.8	9.4
37	14S	20	11.5	87	40	18.3	9.9
38	166S	10.8	4.9	88	194	22.6	11.1
39	75S	23.2	11.8	89	**Madhukar**	24.3	11.2
40	70S	25.4	12.3	90	185M	33.2	3.5
41	148S	27.5	12.2	91	140M	21.3	7.2
42	228S	15.6	8.7	92	166M	21	4.9
43	166-30S	14.2	6.8	93	176M	23.2	7.7
44	166S	11.5	5.2	94	196M	25.2	10.1
45	14-3S	12.6	9.1	95	**Jalmagna**	20.1	9.3
46	248S	14.8	8.8	96	421J	22.3	8.3
47	65S	15.2	10	97	287J	20.9	10.2
48	250K	23.2	10.2	98	43J	19.7	9.7
49	3K	22.2	10.8	99	284J	18.5	10.1
50	236K	20.2	13.2	100	281J	18.4	10

*N22 mutants-1to 4; BPT BILs – 5 to 35; Swarna BILs -36 to 54; 233K x 24K BCRILs-55 to 59; KMR3 BILs -60 to 88; Madhukar x Swarna RILs-89 to 94; Jalmagna x Swarna RILs- 95 to 100;

that of zinc was 19.6 ppm (**Table 2 & Figure 1**). The lowest concentration of iron was observed in IL 50-13 with 1.6 ppm and that of zinc in IL 50-7 with 6.2 ppm while the highest grain Fe and Zn concentrations were observed in IL 431 with 15.2 ppm and 185M with 33.2 ppm respectively. The results showed a significant genetic diversity or variation in the existing rice germplasm. Ten genotypes, N22, 148S, 61-1B, 70S, 196M, 24K, 105B, 88B, 132Z and 185M had high grain zinc concentration of > 24 ppm. Among these 10 genotypes except 185M and 24K, all the other lines also showed high grain Fe concentration (> 10 ppm). Most of the lines with high Fe (> 10 ppm) also had high Zn (> 20 ppm) concentration, however high Zn lines did not always have high Fe. Gautami *et al*., (unpublished) observed similar results in 136 Backcross Introgression Lines (BILs) from the cross of BPT5204 x *O. rufipogon*.

Anuradha *et al*., (2012) screened 126 accessions of rice germplasm in brown rice samples for grain Fe and Zn using Atomic Absorption Spectrophotometer (AAS). Two popular varieties BPT5204, Swarna, the drought and heat tolerant line Nagina22 and deep water rice varieties Madhukar and Jalmagna were common with this study. When compared, these lines had different grain Fe and Zn concentration in the two studies. In our study, the grain Fe and Zn concentration in these five lines BPT5204, Swarna, Nagina22, Madhukar and Jalmagna was 7.6ppm, 17.6ppm; 8.8ppm, 18.2ppm; 14.1ppm, 30.3ppm; 11.2ppm, 24.3 ppm; and 9.3ppm, 20.1 ppm respectively while the grain Fe and Zn concentration of these lines reported in their study was 13.4ppm, 47.8ppm; 32.1ppm, 58.2ppm; 30.3ppm, 43.2ppm; 12.4ppm, 51ppm and 11.5ppm, 42.2ppm, respectively. Analysis of grain Fe and Zn concentration using AAS which is a destructive method requires extensive preanalysis preparation of

Table 2: Descriptive statistics of grain Fe and Zn in 100 rice genotypes for three seasons

Variable	Samples	Min	Max	Mean	Range	Vari-ance	Std_ Dev	SE_ Mean	Skew ness	Kurt osis
Fe-*Kharif*_2015	100	1.4	15.0	9.4	13.6	6.5	2.56	0.25	-1.13	1.69
Zn-*Kharif*_2015	100	5.6	33.2	18.8	27.6	20.6	4.53	0.45	-0.01	0.61
Fe-*Rabi*_2016	100	1.6	15.3	9.8	13.7	7.5	2.74	0.27	-1.02	1.17
Zn-*Rabi*_2016	100	6.2	33.3	19.8	27.1	20.7	4.55	0.45	-0.21	0.68
Fe-*Rabi*_2017	100	1.8	15.3	9.8	13.5	6.9	2.64	0.26	-1.06	1.47
Zn-*Rabi*_2017	100	6.8	33.1	20.4	26.3	21.5	4.64	0.46	-0.28	0.40

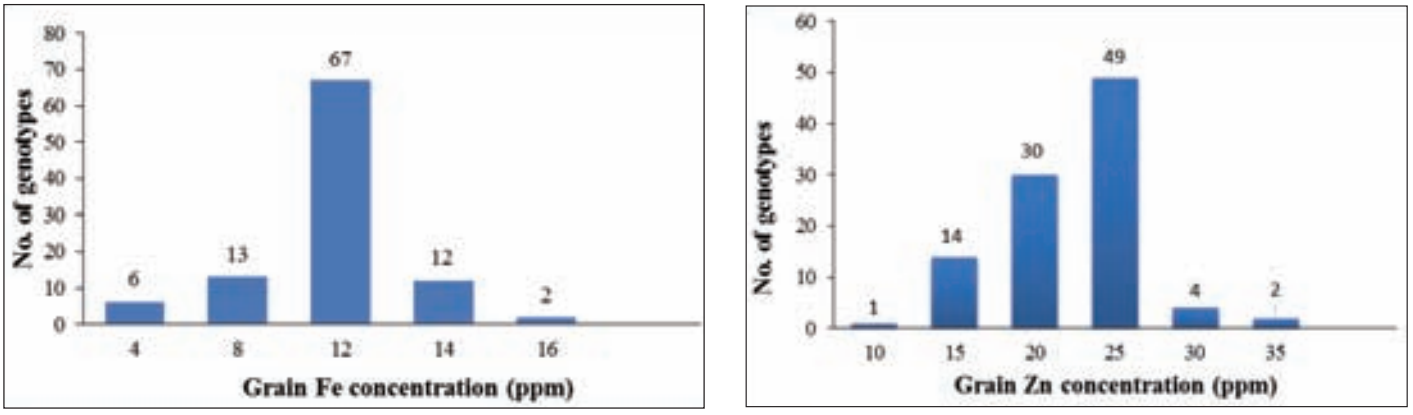

Figure1: Frequency distribution of grain Fe and Zn concentration in 100 rice genotypes

samples such as drying, cleaning, grinding, weighing, digestion and dilution compared to EDXRF method. Previous reports also suggest that the micronutrient concentration values obtained from AAS analysis were higher compared to EDXRF analysis (Singh et. al., 2017). ILs 185M (IET23814), 51B (IET24775), 24K (IET22624), 233K (IET25459), 515Z (IET27998), 61-1B (IET28715), 83B (IET28695), 88B (28706) were entered in AICRIP Bio fortification trials and their respective overall mean Fe and Zn values were 3.3, 31.69 ppm; 4.0, 19.9 ppm; 2.29, 18.7 ppm; 1.6, 18.13 ppm; 3.7, 18.7 ppm; 4.0, 20.19 ppm; 3.2, 19.6 ppm in polished samples across different locations of India (**Figure 2**). IL185M showed the highest overall mean value of 31.69 ppm zinc in polished samples out of 45 entries across 17 locations in AICRIP 2014. Bioavailability studies using the in vitro Caco-2 cell system at National Institute of Nutrition, India showed that in the presence of ascorbic acid, Fe was two times and Zn was three times more bioavailable in 185M than in its parent Swarna (Raghu *et al*., 2019). Recently, 185M was reported to show 22.6 to 40.07 ppm Zn in brown rice and showed the highest mean of 30.62 ppm Zn among 68 entries studied at 6 locations in 3 years (Naik *et al.*, 2020). IL51B with high Zn in unpolished rice was evaluated under AICRIP- Bio fortification trial for 4 years 2014-2017. In these trials, 51B showed high grain zinc (19.1ppm) in polished rice consistently compared to the two check rice varieties Kalanamak and Chittimuthyalu measured using XRF.

Figure 2: Paddy and brown rice of elite lines 185M [IET 23814] and 51B [IET24475] tested in AICRIP Bio fortification trials

It was observed from the previous work and our results that Fe and Zn content values were not consistent. The values can vary with the sample lots even from same accession and also with the analytical method used for the estimation of grain Fe and Zn. The position of grain on the panicle may also significantly affect its Fe and Zn levels. Su *et al*., (2014) reported that the heavy-weight grains, located on primary rachis and top rachis had higher mineral concentrations compared to the small-weight grains located on secondary rachis and bottom rachis, regardless of rice genotypes. Variations in Fe and Zn values in different samples of the same accession can also arise due to presence or absence of embryo in grains, variations in time of harvest or different digestion or analytical methods. This variation in iron and zinc values is also due to homeostasis regulating their absorption, translocation, and transport within the plant system (Welch *et al*., 2004). The moisture content of grain samples also influences grain iron and zinc concentration in EDXRF analysis as the instrument underestimates the grain micronutrient concentration of the samples with high moisture and hence the samples should be well dried before analysis (Rao *et al.*, 2014). Another factor contributing to difference in iron and zinc values is the phloem sap loading and unloading rates within the reproductive organs. Different seed lots of the same accession had different Fe and Zn concentration

even though they were harvested from the same plot. Thus, there is a wide range of variation in Fe and Zn concentration and are not consistent quite akin to the yield trait.

Soil properties also influence the grain Fe and Zn concentration. The pH, organic matter content and Fe/Zn levels of native soil showed significant effects on grain Fe and Zn content (Chandel *et al.*, 2010). Low soil pH and anaerobic conditions, as found in lowland rice fields, trigger the reduction of Fe^{3+} to Fe^{2+}, which ultimately enhances Fe absorption (Zhai *et al*., 2014). A relatively low concentration of $ZnSO_4 \cdot 7H_2O$ added to Fe-AA (Amino acid) significantly increased Fe and Zn accumulation in rice grain. Foliar sprays of iron and zinc fertilizers are known to be an important agronomic practice to improve Fe and Zn concentrations in rice grain (Yuan *et al*., 2013; Ram *et al*., 2016). Younes *et al*., (2016) studied the effect of bio fertilizers and foliar application of zinc on nutrient content of cultivated variety of wheat. Inoculation of plants with bio fertilizers and zinc, improved zinc content and yield in wheat under water-limited condition as well as normal irrigation.. Fe concentration is known to vary with location but Zn values appear to be more consistent (Chandel *et al*., 2010). Also, the range of variation is much more for Fe concentration than for Zn. Environment, genotype and genotype × environment interaction significantly affected Fe concentration in rice grains (Suwarto, 2011). While grain Fe content showed significant genotype × environment interaction effect, Zn content of brown rice was significantly influenced more by native soil properties (Chandel *et al*., 2010; Suwarto, 2011). Thus, in general grain zinc appears to be more consistent than grain Fe content. Sellappan *et al*., (2009) suggested that the number of aleurone layers, size of the embryo and size of the caryopsis determine the quantity of important micronutrients such as iron, zinc in the grains. The high genetic correlation between grain characteristics and some mineral element contents can be used for indirect selection of grain characteristics for mineral element content in a breeding program (Zang *et al*., 2005). All these factors influence the grain iron and zinc concentration in rice and might be responsible for the variations in the same genotype under different environmental conditions in different studies.

Conclusions

Ten high grain Fe and Zn lines, N22, 148S, 61-1B, 70S, 196M, 24K, 105B, 88B, 132Z and 185M were identified among the germplasm screened using the nondestructive ED-XRF method. Lines with high Fe invariably had high zinc but not vice versa and is supported by our subsequent unpublished work on BILs derived from BPT5204 x *O. rufipogon.* Variability in grain Fe and Zn concentrations in the introgression lines derived from wild species and crosses with deep water rices offers scope for further enhancement of Fe and Zn.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Combining ability and heterosis studies for selecting elite parents and hybrids in rice (O*ryza sativa* L.)

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Abstract

Thirty-two hybrids along with their parents (B and R lines) and standard checks *viz*., RNR 15048 and PA 6444 were evaluated for grain yield and related traits to study combining ability and heterosis in rice. The mean performance of the hybrids for most of the characters was higher than that of parents except for milling per cent. The analysis of variance revealed significant differences among parents, lines and hybrids for most of the characters studied. Degree of dominance was more than unity for all the traits except for plant height (0.53), panicle length (0.83), panicle weight (0.94),1000 grain weight (0.83), number of grains per panicle (0.73) and spikelet fertility (0.79). SCA variances were higher than GCA variances for most of the characters, which indicated the predominance of non-additive gene action. The *gca* effects revealed that among the lines JMS 14B had significant *gca* effects in desired direction for several traits including yield. Among the testers, RNR 26059and RNR 26072 were found to be good general combiner for the traits *viz.*, days to 50 per cent flowering, panicle length, head rice recovery, per day productivity and grain yield per plant. Among thirty two hybrids, JMS 14A × RNR 26083, CMS 64A × PAU 2K10-23-451-2-37-34-0-3,CMS 23A × RNR26072, JMS 14A × RNR 26084,CMS 64A × RNR 26059 and JMS 20A × RP 5898-54-21-9-4-2-2 were found good specific combiners based on grain yield per plant. Six hybrids recorded positive significant standard heterosis over variety (RNR 15048), whereas two hybrids recorded positive significant heterosis over hybrid check (PA 6444). Overall data revealed that JMS 14A × RNR 26083 and CMS 64A × RNR26059 were identified as potential hybrids with respect to all characters based on their *sca* and heterosis estimates.

Key Words: Combining ability, heterosis, gene action, rice hybrids

Introduction

Rice is one of the most important food crops in the world, especially in Asian countries. It is estimated that by 2035, global demand for rice will increase to 852 million tons, however, records have shown that annual growth in yield was close to 1% only in the past decade (Khush, 2013). But there is dire need to increase production to meet the growing population (Kumar *et al*., 2014). Theoretically, rice still has great yield potential to be tapped and there are many ways to raise rice yield, such as molecular breeding, new plant type and hybrid rice technology. However, hybrid rice technology offers the most effective solution to

enhance yield on suitable land for rice cultivation. The economical way to increase productivity is to develop hybrid varieties based on the fruitful experience gained in China (Galal Bakr Anis *et al*., 2017). The success story of hybrid rice technology in China (Lin and Yuan, 1980) as leading producer of hybrid rice in the world (Swaminathan, 2006) and some other countries along with India has been witnessed as an important and readily adoptable genetic option to increase the rice production and offers a viable solution to meet the ever increasing food challenge in different countries (Rai, 2009; Sanghera and Wani, 2008; Virmani *et al.*, 2003). Exploitation of heterosis for yield increase in rice through hybrid varieties becomes a practical option and is considered as an important breeding tool to overcome the present yield barriers. This seems to be more effective, as commercial rice hybrid has been reported to exhibit 38 % more yield in comparison with best commercial variety (Singh *et al*., 2013). The study on the magnitude of heterosis is the most important prerequisite for undertaking any heterosis breeding program (Saravanan *et al*., 2008).

For generating promising hybrids, the first step is selection of desirable parents. The contribution of parents in a cross and combining ability of parents in crosses can be assessed by biometrical methods through combining ability studies. Line × Tester analysis devised by Kempthorne (1957) is one of the effective mating designs followed to estimate gca and sca which enables the effective screening of parental lines. Combining ability analysis is one of the powerful tools available to estimate the combining ability effects and aids in selecting the desirable parents and crosses for the exploitation of heterosis (Sarker *et al*., 2002). The knowledge of combining ability is useful to assess nicking ability in self-pollinated crops and at the same time elucidate the nature and magnitude of gene actions involved. It provides the breeder an insight into nature and relative magnitude of fixable and non-fixable genetic variances *i.e.* due to dominance or epistatic components (Pratap *et al*., 2013). Keeping this in view, the present investigation was formulated to study the combining ability and magnitude of heterosis for grain yield and important yield attributes in rice.

Materials and Methods

The present investigation was conducted at Regional Agricultural Research Station, Warangal, Telangana, India, during *Kharif*, 2019. Four stable Wild Abortive cytoplasm based CMS lines and eight restorer lines were utilized in the present study (**Table 1**). Crosses

Table 1: Details of experimental material used for study

S. No	Genotype	Features
	Lines	
1.	RNR 26059	Mid late duration, Long-Slender, Resistance to BLB
2.	RNR 26072	Medium duration, Long-Slender, Resistance to BLB
3.	RNR 26074	Medium duration, Long-Slender, Resistance to BLB
4.	RNR 26083	Medium duration, Long-Slender, Resistance to BLB
5.	RNR 26084	Mid late duration, Long-Slender, Resistance to BLB
6.	Pusa 1701-10-5-8	Medium duration, Long-Slender, Resistance to BLB
7.	PAU 2K10-23-451-2-37-34-0-3	Mid late duration, Long-Slender, Resistance to BLB
8.	RP 5898-54-21-9-4-2-2	Late duration, Short-Slender, Resistance to BLB
	Testers	
1.	CMS 23B	Early duration, Long-Bold, Susceptible to BLB
2.	CMS 64B	Medium duration, Long-Slender, Susceptible to BLB
4.	JMS 14B	Medium duration, Short-Slender, Susceptible to BLB
4.	JMS 20B	Mid-early duration, Short-Slender, Susceptible to BLB

were made between these CMS lines and restorers in Line X Tester mating design during *rabi* 2018-19 at Rice Research Centre, ARI, Rajendranagar, Hyderabad and obtained 32 hybrids. These hybrids along with eight pollen parents (testers), four maintainers of corresponding CMS lines and two checks were grown in single row of 3 m length by adopting a spacing of 20 × 15 cm in RBD design with 2 replications. Recommended agronomic practices were followed to raise a good crop.

Observations were recorded on 5 randomly selected plants for estimation of different traits *viz.*, plant height (cm), number of productive tillers per plant, panicle length (cm), panicle weight (g), spikelet fertility (%) and grain yield per plant(g). However, days to 50% flowering was recorded on whole plot basis, whereas number of grains per panicle, 1000 grain weight (g), kernel length (mm), kernel breadth (mm), kernel length breadth ratio, hulling per cent, milling per cent and head rice recovery (%) were recorded on a random sample taken in each plot and per day productivity was calculated by dividing grain yield in hectare with days to maturity. The character means of each replication was subjected to analysis of variance (Panse and Sukhatme, 1967). Combining ability analysis and the testing of significance of different genotypes was estimated based on the procedure given by Kempthorne (1957) and also recorded the heterosis over the better parent, standard variety and standard hybrid (Fonseca and Patterson, 1968). Computer software Windostat version 9.1 was used for analysis of data.

Results and Discussion

The analysis of variance revealed significant differences for all the characters studied for hybrids (**Table 2**). The variance due to hybrid was partitioned into variance due to lines, testers and lines × testers for all the characters. The variance due to lines was significant for all the characters except panicle weight, number of filled grains per panicle and kernel length. Variance due to testers was significant for all traits studied. The variance due to lines × testers were significant for the five characters *viz.*, days to 50% flowering, plant height, 1000 grain weight, number of grains per panicle and spikelet fertility. Parents × hybrids showed significant variance for six characters *viz.*, plant height, panicle length, panicle weight, 1000 grain weight, number of grains per panicle and kernel length indicating superiority of hybrids and presence of heterosis for these traits studied.These results emphasized the importance of combining ability studies indicating the variability in the material and there is a good scope for identifying promising parents and hybrid combinations for improving yield through its components.

In the present investigation, the degree of dominance was more than unity for the traits *viz.*, days to 50 per cent flowering (1.05), number of productive tillers per plant (1.59), kernel breadth (1.05), kernel length breath ratio (1.32), hulling per cent (2.08), milling per cent (1.70), head rice recovery (1.57), grain yield per plant (1.25) and per day productivity (1.35) indicating predominance of non-additive gene action, while plant height (0.53), panicle length (0.83), panicle weight (0.94), 1000 grain weight (0.83), number of grains per panicle (0.73) and spikelet fertility (0.79) exhibited additive gene action (**Table 3**). SCA variances were higher than GCA variances for most of the characters, indicating the predominance of non-additive gene action. The importance of additive as well as non-additive gene effects with predominance of non-additive gene effects in inheritance of grain yield and yield components of rice were in agreement with earlier findings of Saleem *et al*., (2010), Saidaiah *et al.* (2010), Rashid *et al*., (2007), Saravanan *et al.*, (2006) and Vanaja *et al.*, (2003).

The *gca* effects revealed that among the lines JMS 14B had significant *gca* effects in desired direction for important traits *viz.*, grain yield per plant (3.95), number of grains per panicle (28.15), spikelet fertility (3.54) and panicle weight (0.35) (**Table 4**). Among the testers, RNR 26059 and RNR 26072 were good

Table 2: Analysis of variance for yield and yield components in rice

Source of variation	DF	X1	X2	X3	X4	X5	X6	X7	X8	X9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	X_{16}
Replications	1	7.10	88.76**	0.03	0.30	0.14	1.70	441.01	3.93	0.02	0.00	0.02	2.00	1.69	0.86	1.24	0.20
Treatments	43	205.24**	468.07**	3.87**	17.80**	2.58**	20.16**	3140.38**	61.57**	0.83**	0.04**	0.20**	28.67**	25.99**	43.26**	41.92**	308.88**
Parents	11	129.07**	771.59**	1.33**	24.42**	2.61**	32.26**	3256.58**	10.93**	1.27**	0.12**	0.25**	11.34**	13.86**	62.59**	7.55	38.38
Lines	3	2348.26**	1025.53**	27.83**	146.62**	0.40	19.47**	218.93	312.32**	0.02	0.13**	0.53**	10.06*	6.45*	41.12**	22.28*	1284.72**
Testers	7	163.14**	342.38**	4.00**	11.29**	2.65**	15.88**	3193.38**	71.45**	0.70**	0.01**	0.17**	35.42**	30.92**	36.47**	54.75*	373.38**
Line × Tester	1	418.39*	408.15**	5.92	2.70	1.57	39.14*	7179.04**	337.36**	1.04	0.03	0.14	22.79	35.30	46.0	127.31	465.95
Parents × hybrids	1	194.01	1111.56**	3.27	33.93**	6.98**	28.77*	6921.07**	46.13	1.37*	0.02	0.29	31.90	28.55	37.67	46.16	507.87
Hybrids	31	116.39**	76.59**	3.97**	4.97**	1.36**	8.27**	1381.44**	41.89**	0.42**	0.01**	0.13**	38.40**	31.09**	34.72**	47.24**	315.32**
Error	43	5.21	4.43	0.44	0.98	0.11	0.54	133.29	2.27	0.02	0.00	0.02	2.41	1.17	1.68	3.92	45.91

*Significant at P=0.05 level **Significant at P=0.01 level

X_1=Days to 50% flowering, X_2=Plant height (cm), X_3=No of productive tillers, X_4=Panicle length (cm), X_5=Panicle weight (g), X_6=1000 grain weight (g), X_7=No. of grains per panicle, X_8=Spikelet fertility (%), X_9=Kernel length (mm), X_{10}=Kernel breadth (mm), X_{11} =Kernel length breadth ratio, X_{12} =Hulling per cent, X_{13}=Milling per cent, X_{14}=Head rice recovery, X_{15} = grain yield per plant (g), X_{16}=Per day Productivity, DF=degrees of freedom

Table 3: Estimates of general and specific combining ability variances and proportionate gene action for different traits in rice

Character	Source of variation			Degree of Dominance $(\sigma^2sca/\ \sigma^2gca)^{1/2}$	Nature of gene action
	σ^2gca	σ^2sca	$\sigma^2gca/\ \sigma^2sca$		
Days to 50% flowering	50.164	55.586	0.902	1.053	Non additive
Plant height (cm)	125.904	36.08	3.49	0.535	Additive
Number of productive tillers per plant	0.692	1.762	0.393	1.596	Non additive
Panicle length (cm)	2.89	1.997	1.447	0.831	Additive
Panicle weight (g)	0.695	0.625	1.112	0.948	Additive
1000 grain weight (g)	5.569	3.861	1.442	0.833	Additive
Number of grains per panicle	1152.794	624.078	1.847	0.736	Additive
Spikelet fertility (%)	31.58	19.811	1.594	0.792	Additive
Kernel length (mm)	0.197	0.199	0.989	1.005	Non Additive
Kernel breadth (mm)	0.004	0.004	0.907	1.050	Non Additive
Kernel length breadth ratio	0.033	0.057	0.573	1.321	Non additive
Hulling per cent	4.156	17.994	0.231	2.081	Non additive
Milling per cent	5.126	14.962	0.343	1.708	Non additive
Head Rice Recovery (%)	6.692	16.518	0.405	1.571	Non additive
Grain yield per plant (g)	13.803	21.664	0.637	1.253	Non additive
Per day productivity (kg/ha/day)	73.499	134.705	0.546	1.354	Non additive

general combiners for the traits *viz*., panicle length, grain yield per plant and head rice recovery. For per day productivity the testers *viz.*, RNR 26059 (10.01), RNR 26072 (8.54) and RNR 26074 (7.35) were found to be good general combiners. The testers, RNR 26059 (1.65), RNR 26083 (1.41), RNR 26084 (1.24) and PAU 2K10-23-451-2-37-34-0-3 (1.92) were found good general combiners for 1000 grain weight. It was observed in certain instances that the lines and testers with good *perse* performance were not good general combiners and *vice versa*, thus the association between *perse* performance and GCA effects evident in the present study indicated that the effectiveness of choice of parents based on *per se* performance alone was not appropriate for predicting the combining ability of the parents. Similar findings were reported by Manjunath *et al.*, 2019.

The *sca* effects revealed that among thirty two hybrids, JMS 14A × RNR 26083 (10.00) recorded highest significant positive *sca* effect for grain yield per plant followed by CMS 23A × RNR 26072 (7.72), CMS 64A ×PAU 2K10-23-451-2-37-34-0-3 (6.75), JMS 14A × RNR 26084 (4.51), CMS 64A × RNR 26059 (4.36) and JMS 20A × RP 5898-54-21-9-4-2-2(4.02) and were considered as desirable (**Table 5**). Six hybrids *viz*., CMS 64A × RNR 26072 (-17.01), JMS 14A × RNR 26074 (-11.20), CMS 23A × RNR 26083 (-11.57), JMS 14 A × RNR 26084 (-7.95), JMS20A × RNR 26059 (-6.20) and CMS 23A × PAU 2K10-23-451-2-37-34-0-3 (-5.95) recording significant and negative *sca* effects for days to flowering were considered to be highly desirable for earliness.

Six hybrids *viz*., CMS 23A × RNR 26072 (9.06), CMS 64A × PAU 2K10-23-451-2-37-34-0-3 (5.47), JMS

Table 4: Estimates of general combining ability effects in lines and testers for yield and yield contributing characters in rice

Parents	Days to 50% flowering	Plant height (cm)	Number of productive tillers per plant	Panicle length (cm)	Panicle weight (g)	1000 grain weight (g)	Number of grains per panicle	Spikelet fertility (%)	Hulling per cent	Milling per cent	Head rice recovery %	Grain yield per plant (g)	Per day productivity (kg/ha/day)
LINES													
CMS 23A	4.672 **	-5.798 **	0.006	-0.342	-0.32 **	1.749 **	-23.313 **	-6.689 **	0.432	0.251	1.325 **	-2.576 **	-3.277
CMS 64A	5.766 **	0.371	-0.731 **	0.337	0.156	0.209	-2.375	0.860 *	1.437 **	2.001 **	1.524 **	-0.108	-4.202 *
JMS 14A	2.828 **	6.459 **	-0.034	0.375	0.359 **	0.099	28.125 **	3.542 **	-0.562	-1.040 **	-0.932 **	3.952 **	7.677 **
JMS 20A	-3.922 **	-1.032	0.759 **	-0.369	-0.185 *	-2.056 **	-2.438	2.286 **	-1.307 **	-1.213 **	-1.918 **	-1.268 *	-0.197
TESTERS													
RNR 26059	-1.859 *	11.171 **	-0.521 *	2.068 **	1.875 **	1.654 **	34.875 **	4.942 **	0.494	0.064	1.369 **	4.153 **	10.012 **
RNR 26072	-5.734 **	-3.733 **	0.012	2.147 **	-1.019 **	-0.830 **	-31.250 **	0.465	1.526 **	1.064 **	1.693 **	2.085 **	8.547 **
RNR 26074	-1.609	2.002 *	0.448	0.518	-0.402 **	-0.027	-11.750 **	-3.672 **	-2.552 **	-1.753 **	-1.238 *	-0.502	7.357 **
RNR 26083	8.766 **	16.162 **	-0.809 **	2.043 **	0.519 **	1.414 **	-1.500	-0.271	1.994 **	1.328 **	1.818 **	0.008	-6.478 *
RNR 26084	-1.859 *	5.946 **	0.041	0.056	-0.220	1.242 **	-13.125 **	0.054	0.938	0.939 *	0.568	-0.392	-2.468
Pusa 1701-10-5-8	-4.109 **	-22.091 **	-0.321	-2.969 **	-0.969 **	-2.743 **	-32.500 **	-0.292	0.486	1.106 **	-0.306	-3.683 **	-5.220 *
PAU 2K10-23-451-2-37-34-0-3	0.641	-4.204 **	1.271 **	-2.132 **	0.351 **	1.929 **	5.625	-1.431 *	0.744	1.189 **	0.818	0.475	0.154
RP 5898-54-21-9-4-2-2	5.766 **	-5.254 **	-0.121	-1.732 **	-0.136	-2.640 **	49.625 **	0.204	-3.631 **	-3.936 **	-4.722 **	-2.145 **	-11.902 **

* Significant at 0.05 % level, **Significant at 0.01 % level

Table 5: Estimates of specific combining ability effects in crosses for yield and yield contributing characters in rice

Hybrids	Days to 50% flowering	Plant height (cm)	Number of productive tillers per plant	Panicle length (cm)	Panicle weight (g)	1000 grain weight (g)	Number of filled grains per panicle	Spikelet fertility (%)	Hulling per cent	Milling per cent	Head rice recovery %	Grain yield per plant (g)	Per day productivity(kg/ha/day)
CMS 23A × RNR 26059	0.04	4.09 **	-0.05	1.19	-0.31	0.40	13.18	-1.47	-1.55	-0.50	0.55	1.58	1.59
CMS 23A × RNR26072	9.42 **	3.25 *	1.20 *	1.47 *	1.03 **	2.86 **	31.31 **	9.06 **	1.41	0.49	1.17	7.72 **	14.32 **
CMS 23A × RNR 26074	-1.20	-11.98 **	1.55 **	-2.65 **	-0.78 **	-0.89	-5.68	1.57	-0.01	1.31	1.60	-2.81	1.34
CMS 23A × RNR 26083	-11.57 **	-0.29	-0.51	-0.48	-0.97 **	-0.78	-24.43 **	0.79	-0.55	-0.76	-0.95	-3.22 *	-5.18
CMS 23A × RNR 26084	10.54 **	-3.97 *	1.13 *	-1.44 *	-0.24	0.50	-5.81	-3.10 **	-0.001	-0.87	-0.70	-2.80	-11.34 *
CMS 23A ×Pusa 1701-10-5-8	2.79	1.11	-1.55 **	2.03 **	1.26 **	-0.26	-7.43	-4.53 **	-1.54	-2.04 *	-3.82 **	0.68	-9.35
CMS 23A × PAU 2K10-23-451-2-37-34-0-3	-5.95 **	2.77	-1.29 *	-0.80	1.14 **	-2.24 **	23.93 **	-1.23	-1.80	-2.12 **	-3.45 **	-0.64	5.14
CMS 23A × RP 5898-54-21-9-4-2-2	-4.07 *	5.02 **	-0.45	0.69	-1.14 **	0.42	-25.06 **	-1.07	4.06 **	4.49 **	5.59 **	-0.49	3.49
CMS 64A × RNR 26059	0.60	1.92	-0.31	0.41	0.13	0.85	-14.75	1.66	1.93	0.74	-1.55	4.36 **	8.77
CMS 64A × RNR26072	-17.01 **	-1.36	-1.45 **	-2.01 **	0.11	-6.34 **	-7.12	-6.30 **	-3.59 **	-1.75 *	-1.52	0.42	13.02 *
CMS 64A × RNR 26074	7.85 **	-1.40	-1.18 *	0.36	0.33	2.14 **	-2.62	1.33	2.48 *	1.06	1.40	2.81	-4.51
CMS 64A × RNR 26083	3.48 *	-3.16 *	-0.03	1.93 **	-0.29	-0.38	23.62 **	-4.67 **	-2.06	-1.51	-2.14 *	-8.36 **	-18.04 **
CMS 64A × RNR 26084	-4.89 **	-1.14	-0.28	0.02	0.11	1.81 **	9.25	2.66 *	-2.00	-1.62 *	-0.39	-2.29	-3.99
CMS 64A × Pusa 1701-10-5-8	2.35	11.39 **	-0.61	0.25	-0.84 **	0.08	-13.37	-0.94	-1.01	-1.29	-1.52	-0.66	-3.71
CMS 64A × PAU 2K10-23-451-2-37-34-0-3	4.10 *	-0.99	3.10 **	-0.68	0.24	1.43 *	-14.50	5.47 **	2.68 *	1.62 *	1.35	6.75 **	15.10 **
CMS 64A × RP 5898-54-21-9-4-2-2	3.48 *	-5.24 **	0.78	-0.28	0.19	0.39	19.50 *	0.78	1.56	2.74 **	4.39 **	-3.03 *	-6.64
JMS 14A × RNR 26059	5.54 **	-3.35 *	-0.01	1.57 *	0.87 **	-1.15 *	-26.75 **	-0.34	-0.56	-0.71	0.75	-0.81	-1.12
JMS 14A × RNR26072	4.92 **	-4.45 **	-0.55	-0.20	-1.02 **	0.72	-33.62 **	-8.33 **	-0.96	-0.71	-1.56	-3.51 *	-14.52 **
JMS 14A × RNR 26074	-11.20 **	5.01 **	1.21 *	1.42	-0.01	-1.25 *	23.37 **	0.17	-8.51 **	-7.89 **	-8.13 **	-2.49	0.04
JMS 14A × RNR 26083	6.92 **	4.45 **	1.77 **	-1.00	0.83 **	2.08 **	42.12 **	-0.62	1.93	2.52 **	2.80 **	10.00 **	20.84 **
JMS 14A × RNR 26084	-7.95 **	-0.63	-1.67 **	-0.11	-0.22	-0.68	1.25	4.61 **	0.99	1.41	2.05 *	4.51 **	19.03 **
JMS 14A ×Pusa 1701-10-5-8	-3.70 *	1.70	0.78	-1.23	-0.77 **	-0.02	-12.87	4.33 **	-0.62	-0.08	0.93	-1.99	-0.58
JMS 14A × PAU 2K10-23-451-2-37-34-0-3	4.04 *	-2.48	-1.10 *	-0.22	-0.18	0.41	17.50 *	0.65	1.18	1.16	1.80	-5.20 **	-19.39 **
JMS 14A × RP 5898-54-21-9-4-2-2	1.42	-0.23	-0.41	-0.22	0.51 *	-0.10	-11.00	-0.47	6.56 **	4.29 **	1.34	-0.49	-4.28
JMS 20A × RNR 26059	-6.20 **	-2.66	0.39	-3.18 **	-0.69 **	-0.10	28.31 **	0.15	0.18	0.46	0.24	-5.13 **	-9.24
JMS 20A × RNR26072	2.67	2.57	0.79	0.74	-0.13	2.76 **	9.43	5.56 **	3.15 **	1.96 *	1.91 *	-4.63 **	-12.81 *
JMS 20A × RNR 26074	4.54 **	8.37 **	-1.57 **	0.86	0.46	0.003	-15.06	-3.08 **	6.04 **	5.51 **	5.12 **	2.49	3.13
JMS 20A × RNR 26083	1.17	-0.99	-1.22 *	-0.45	0.42	-0.91	-41.31 **	4.51 **	0.68	-0.24	0.29	1.58	2.38
JMS 20A × RNR 26084	2.29	5.75 **	0.82	1.53 *	0.35	-1.64 **	-4.68	-4.17 **	1.01	1.08	-0.95	0.59	-3.69
JMS 20A ×Pusa 1701-10-5-8	-1.45	-14.20 **	1.39 **	-1.04	0.35	0.20	33.68 **	1.14	3.19 **	3.42 **	4.41 **	1.97	13.65 **
JMS 20A × PAU 2K10-23-451-2-37-34-0-3	-2.20	0.70	-0.70	1.71 *	-1.20 **	0.40	-26.93 **	-4.89 **	-2.06	-0.66	0.29	-0.90	-0.85
JMS 20A × RP 5898-54-21-9-4-2-2	-0.82	0.45	0.09	-0.18	0.43	-0.70	16.56	0.76	-12.1**	-11.5**	-11.32**	4.0**	7.43

* Significant at 0.05% level, **Significant at 0.01% level

20A × RNR 26072 (5.56), JMS 14A × RNR 26084 (4.61), JMS 14A × Pusa 1701-10-5-8 (4.33) and CMS 64A × RNR 26084 (2.66) showed significant positive *sca* effects for spikelet fertility (%), an important parameter to be considered for hybrids. Six hybrids *viz*., CMS 23A × RNR 26072 (2.86), JMS 20A × RNR 26072 (2.76), CMS 64A × RNR 26074 (2.14), JMS 14A × RNR 26083 (2.08), CMS 64A × RNR 26074 (1.81) and CMS 64A × PAU 2K10-23-451-2-37-34-0-3 (1.43) having bold grains recorded significant positive *sca* effects for 1000-grain weight. JMS 20A (-2.05) line. three testers *viz.,* Pusa 1701-10-5-8 (-2.74), PAU 2K10-23-451-2-37-34-0-3 (-2.64) and RNR 26072 (-0.83) along with five hybrids *viz*., CMS 64A × RNR 26072 (-6.34), CMS 23A × PAU 2K10-23-451-2-37-34-0-3 (-2.24.), JMS 20A × RNR 26084 (-1.64), JMS 14A × RNR 26074 (-1.25), JMS 14A × RNR 26059 (-1.15) were fine grain type and recorded significant negative *gca* or *sca* effects for 1000-grain weight.

The *sca* effect was significant and positive for seven hybrids for head rice recovery and identified as desirable. Considering various parameters, six hybrids *viz*., JMS 14A × RNR 26083, CMS 64A × RNR 26059, CMS 64A × PAU 2K10-23-451-2-37-34-0-3, JMS 14A × RNR 26084, JMS 20A × RP 5898-54-21-9-4-2-2 and CMS 23A × RNR 26072 were found to be good specific combiners for grain yield and yield attributing traits. Heterosis studies showed that the heterobeltiosis over better parent ranged from -33.61 to 47.10% for grain yield (**Table 6**). Six hybrids showed significant positive heterosis for this trait. Highest significant positive heterobeltiosis was recorded by JMS 14A × RNR 26083 followed by CMS 23A × RNR 26072, CMS 64A × RNR 26059, JMS 14A × RNR 26084, JMS 14A × RNR 26059and CMS 64A × PAU 2K10-23-451-2-37-34-0-3.

For grain yield per plant, six hybrids exhibited positive significant standard heterosis over variety (RNR 15048). Highest significant positive heterosis was recorded in JMS 14A × RNR 26083 followed by CMS 64A × RNR 26059, JMS 14A × RNR 26084, JMS 14A × RNR 26059, CMS 23A × RNR 26072 and CMS 64A × PAU 2K10-23-451-2-37-34-0-3. Two hybrids *viz*., JMS 14A × RNR 26083 and CMS 64A × RNR 26059 showed positive significant heterosis over standard hybrid check (PA 6444). Among these, JMS 14A × RNR 26083 and CMS 64A × RNR 26059 were identified as potential hybrids with respect to characters *viz.,* panicle length, panicle weight, grain yield per plant and per day productivity based on their *perse* performance and heterosis estimates. Marked variation in the expression of heterobeltiosis and standard heterosis for yield and yield components was observed for all cross combinations. These finding are in consonance with those of Saravanan *et al*., (2008), Kumar *et al*., (2012), Singh *et al*., (2013), Sharma *et al*., (2013), Pratap *et al.,* (2013), Bhati *et al*., (2015), Satheesh kumar *et al*., (2016), Yogita *et al.,* (2016), Galal Bakr Anis *et al*., (2017) and Manjunath *et al.*, (2019).

Table 6: Heterobeltiosis and standard heterosis of hybrids for yield and yield contributing traits in rice

Cross	Days to 50 % flowering			Plant height (cm)			Number of productive tillers per plant		
	HB	SHH	SHV	HB	SHH	SHV	HB	SHH	SHV
CMS 23A × RNR 26059	-21.72 **	-22.77 **	-13.50 **	-16.38 **	10.31 **	19.13**	-19.84 **	-5.71	-18.85 **
CMS 23A × RNR26072	-14.81 **	-17.86 **	-8.00 **	-13.23 **	-3.81	3.87	-5.30	11.38	-4.14
CMS 23A × RNR 26074	-16.99 **	-23.66 **	-14.50 **	-13.30 **	-12.33 **	-5.33 *	1.01	18.81 **	2.25
CMS 23A × RNR 26083	-21.56 **	-23.66 **	-14.50 **	5.64 **	10.85 **	19.71 **	-27.78 **	-12.86	-25.00 **
CMS 23A × RNR 26084	-14.16**	-13.39 **	-3	-8.58 **	-1.61	6.25 **	-5.67	10.95	-4.51
CMS 23A × Pusa 1701-10-5-8	-14.29 **	-22.32 **	-13.00 **	9.58 **	-22.20 **	-15.98 **	-30.36 **	-18.10 **	-29.51 **
CMS 23A × PAU 2K10-23-451-2-37-34-0-3	-24.55 **	-25.89 **	-17.00 **	4.94*	-4.66 *	2.95	-15.38 **	-0.48	-14.34 *
CMS 23A × RP 5898-54-21-9-4-2-2	-21.40 **	-19.64 **	-10.00 **	13.64**	-3.59	4.12	-19.84 **	-5.71	-18.85 **
CMS 64A × RNR 26059	-11.76 **	-12.95 **	-2.5	-13.66 **	13.90 **	23.00 **	-27.05 **	-15.24 *	-27.05 **
CMS 64A × RNR26072	-29.63 **	-32.14**	-24.00 **	-11.97 **	-2.42	5.38*	-31.97 **	-20.95 **	-31.97 **
CMS 64A × RNR 26074	0	-6.25 **	5.00*	1.55	2.69	10.90 **	-26.23 **	-14.29 *	-26.23 **
CMS 64A × RNR 26083	1.83	-0.89	11.00 **	8.46 **	13.81 **	22.91 **	-29.76 **	-15.24 *	-27.05 **
CMS 64A × RNR 26084	-18.58 **	-17.86 **	-8.00 **	-1.08	6.46 **	14.96 **	-22.13 **	-9.52	-22.13 **
CMS 64A × Pusa 1701-10-5-8	-7.62 **	-13.39 **	-3	-3.37	-7.44 **	-0.05	-27.87 **	-16.19 *	-27.87 **
CMS 64A × PAU 2K10-23-451-2-37-34-0-3	-5.91 **	-7.59 **	3.5	1.78	-2.51	5.28*	15.74 **	34.48 **	15.74 **
CMS 64A × RP 5898-54-21-9-4-2-2	-5.68 **	-3.57	8.00 **	-3.18	-7.26 **	0.15	-14.75 *	-0.95	-14.75 *
JMS 14A × RNR 26059	-9.95 **	-11.16**	-0.5	-13.12**	14.62 **	23.78 **	-23.26 **	-5.71	-18.85 **
JMS 14A × RNR26072	-12.04 **	-15.18**	-5.00 *	-9.55 **	0.27	8.28 **	-23.26 **	-5.71	-18.85 **
JMS 14A × RNR 26074	-19.42 **	-25.89 **	-17.00 **	12.64**	13.90 **	23.00 **	-6.20	15.24 *	-0.82
JMS 14A × RNR 26083	2.29	-0.45	11.50 **	20.17**	26.10**	36.17**	-11.63 *	8.57	-6.56
JMS 14A × RNR 26084	-23.89 **	-23.21 **	-14.00 **	4.42*	12.38 **	21.36 **	-31.78 **	-16.19 *	-27.87 **
JMS 14A × Pusa 1701-10-5-8	-13.30 **	-21.43 **	-12.00 **	6.87 **	-10.67 **	-3.54	-15.50 **	3.81	-10.66
JMS 14A × PAU 2K10-23-451-2-37-34-0-3	-8.64 **	-10.27 **	0.5	11.85 **	1.61	9.73 **	-17.83 **	0.95	-13.11 *
JMS 14A × RP 5898-54-21-9-4-2-2	-10.04 **	-8.04 **	3	21.04 **	2.69	10.90 **	-23.26 **	-5.71	-18.85 **
JMS 20A × RNR 26059	-26.70 **	-27.68 **	-19.00 **	-17.74 **	8.52 **	17.19**	-4.48	5.71	-9.02
JMS 20A × RNR26072	-20.37 **	-23.21 **	-14.00 **	-9.92 **	-0.15	7.83 **	4.70	14.67 *	-1.31
JMS 20A × RNR 26074	-10.68 **	-17.86 **	-8.00 **	8.98 **	10.20 **	19.01 **	-16.53 **	-3.81	-17.21 **
JMS 20A × RNR 26083	-9.17**	-11.61 **	-1	9.12**	14.50 **	23.65 **	-27.39 **	-12.38	-24.59 **
JMS 20A × RNR 26084	-20.80 **	-20.09 **	-10.50 **	3.5	11.39 **	20.29 **	3.86	15.24 *	-0.82
JMS 20A × Pusa 1701-10-5-8	-17.73 **	-25.45 **	-16.50 **	-20.83 **	-31.66 **	-26.20 **	6.96	17.14 *	0.82
JMS 20A × PAU 2K10-23-451-2-37-34-0-3	-20.45 **	-21.88 **	-12.50 **	7.60 **	-2.24	5.57*	2.61	12.38	-3.28
JMS 20A × RP 5898-54-21-9-4-2-2	-17.90 **	-16.07 **	-6.00 *	11.90 **	-3.41	4.31 *	-6.67	6.67	-8.20

* Significant at 0.05% level, **Significant at 0.01% level

Table 6: contd..

Cross	Panicle length (cm)			Panicle weight (g)			1000 gram weight (g)		
	HB	SHH	SHV	HB	SHH	SHV	HB	SHH	SHV
CMS 23A × RNR 26059	5.92	16.48 **	31.60 **	3.85	3.24	0.96	14.09 **	1.05	78.50 **
CMS 23A × RNR26072	-1.71	17.87 **	33.18**	-26.55 **	-27.06 **	-28.67 **	9.50*	0.92	78.27 **
CMS 23A × RNR 26074	11.61*	-4.21	8.23	-12.95	-50.59 **	-51.68 **	-3.53	-12.02 **	55.41 **
CMS 23A × RNR 26083	7.09	9.96*	24.24 **	-44.01 **	-36.27 **	-37.68 **	-2.98	-5.21	67.44 **
CMS 23A × RNR 26084	-4.98	-1.34	11.47*	-30.50 **	-36.57 **	-37.97 **	0.13	-0.31	76.10 **
CMS 23A × Pusa 1701-10-5-8	27.80 **	0.38	13.42 **	59.36 **	-21.57 **	-23.30 **	-10.97 **	-21.15**	39.29 **
CMS 23A × PAU 2K10-23-451-2-37-34-0-3	-9.36 *	-7.28	4.76	16.99*	1.96	-0.29	0.36	-9.37 **	60.09 **
CMS 23A × RP 5898-54-21-9-4-2-2	13.48 **	0	12.99 **	-26.07 *	-52.45 **	-53.50 **	-7.07	-17.69 **	45.40 **
CMS 64A × RNR 26059	5.57	16.09 **	31.17**	22.19**	21.47 **	18.79 **	13.76 **	-3.72	70.07 **
CMS 64A × RNR26072	-10.70 **	7.09	21.00 **	-35.14**	-35.59 **	-37.01 **	-41.52 **	-46.10**	-4.8
CMS 64A × RNR 26074	22.13**	9.96*	24.24 **	10.47	-19.31 **	-21.09 **	3.7	-5.43	67.05 **
CMS 64A × RNR 26083	18.66 **	21.84 **	37.66 **	-23.94 **	-13.43*	-15.34*	-8.09 *	-10.20 **	58.62 **
CMS 64A × RNR 26084	2.95	6.9	20.78 **	-12.35	-20.00 **	-21.76 **	-0.88	-1.31	74.32 **
CMS 64A × Pusa 1701-10-5-8	6.81	-3.83	8.66	-36.24 **	-53.43 **	-54.46 **	-12.67 **	-26.36 **	30.09 **
CMS 64A × PAU 2K10-23-451-2-37-34-0-3	-6.37	-4.21	8.23	7.54	-6.27	-8.34	10.74 **	0	76.64 **
CMS 64A × RP 5898-54-21-9-4-2-2	9.79*	-1.15	11.69*	14.09	-16.67*	-18.50 **	-10.54*	-24.56 **	33.26 **
JMS 14A × RNR 26059	9.76 **	20.69 **	36.36 **	40.73 **	39.90 **	36.82 **	2.79	-13.00 **	53.67 **
JMS 14A × RNR26072	-4.79	14.18**	29.00 **	-53.60 **	-53.92 **	-54.94 **	-8.50 *	-15.67 **	48.96 **
JMS 14A × RNR 26074	26.81 **	14.18**	29.00 **	-1.49	-22.16**	-23.87 **	-13.15**	-20.80 **	39.91 **
JMS 14A × RNR 26083	7.84*	10.73 **	25.11 **	-1.03	12.65	10.16	2.49	0.13	76.88 **
JMS 14A × RNR 26084	2.58	6.51	20.35 **	-15.15*	-22.55 **	-24.26 **	-12.36 **	-12.74 **	54.14**
JMS 14A × Pusa 1701-10-5-8	0.64	-9.39 *	2.38	-34.37 **	-48.14**	-49.28 **	10.70*	-27.32 **	28.38 **
JMS 14A × PAU 2K10-23-451-2-37-34-0-3	-4.49	-2.3	10.39*	2.59	-10.59	-12.56	5.26	-4.95	67.90 **
JMS 14A × RP 5898-54-21-9-4-2-2	10.21 *	-0.77	12.12**	18.24*	-6.57	-8.63	27.64 **	-27.21 **	28.58 **
JMS 20A × RNR 26059	-9.41 *	-0.38	12.55 **	-0.79	-1.37	-3.55	-2.95	-17.86 **	45.09 **
JMS 20A × RNR26072	-4.15	14.94 **	29.87 **	-46.79 **	-47.16**	-48.32 **	-9.05 *	-16.18**	48.07 **
JMS 20A × RNR 26074	27.23 **	9.20*	23.38 **	35.06 **	-23.33 **	-25.02 **	-17.47 **	-24.74 **	32.95 **
JMS 20A × RNR 26083	7.09	9.96*	24.24 **	-17.48 **	-6.08	-8.15	-20.64 **	-22.46 **	36.97 **
JMS 20A × RNR 26084	5.9	9.96*	24.24 **	-14.5	-21.96 **	-23.68 **	-26.08 **	-26.40 **	30.01 **
JMS 20A × Pusa 1701-10-5-8	7.94	-11.49 **	0	19.85	-36.67 **	-38.06 **	-2.1	-35.73 **	13.53*
JMS 20A × PAU 2K10-23-451-2-37-34-0-3	0	2.3	15.58 **	-32.73 **	-41.37 **	-42.67 **	-5.21	-14.40 **	51.20 **
JMS 20A × RP 5898-54-21-9-4-2-2	9.57*	-3.45	9.09*	26.22 *	-18.82 **	-20.61 **	6.49	-39.27 **	7.27

* Significant at 0.05% level, **Significant at 0.01% level

Table 6: contd..

Cross	Number of filled grains per panicle			Spikelet fertility (%)			Hulling per cent		
	HB	SHH	SHV	HB	SHH	SHV	HB	SHH	SHV
CMS 23A × RNR 26059	21.12**	-5.34	-40.09 **	-9.50 **	-6.50 **	-8.09 **	1.86	-1.28	4.35*
CMS 23A × RNR26072	-7.26	-28.64 **	-54.84 **	-2.99	0.23	-1.48	7.15**	3.85	0.62
CMS 23A × RNR 26074	-14.24	-37.14**	-60.22 **	-15.47 **	-12.67 **	-14.16**	-0.13	-3.21	-6.21 **
CMS 23A × RNR 26083	-50.41 **	41.26 **	-62.83 **	-12.66 **	-9.77 **	-11.31 **	1.92	1.92	-1.24
CMS 23A × RNR 26084	-12.93	-37.86 **	-60.68 **	-16.50 **	-13.73 **	-15.20 **	2.6	1.28	-1.86
CMS 23A × Pusa 1701-10-5-8	-27.21 **	48.06 **	-67.13**	-18.41 **	-15.71 **	-17.14**	1.86	-1.28	4.35*
CMS 23A × PAU 2K10-23-451-2-37-34-0-3	16.12*	-14.32*	-45.78 **	-16.08 **	-13.31 **	-14.78 **	-1.74	-1.28	4.35*
CMS 23A × RP 5898-54-21-9-4-2-2	13.2	-16.75 **	-47.31 **	-16.87 **	-11.31 **	-12.82 **	0.64	0.64	-2.48
CMS 64A × RNR 26059	-18.97 **	-8.74	-42.24 **	3.35	5.37 **	3.57*	3.16	4.49*	1.24
CMS 64A × RNR26072	44.18**	-37.14**	-60.22 **	-10.97 **	-8.44 **	-10.00 **	-2.53	-1.28	4.35*
CMS 64A × RNR 26074	-33.84 **	-25.49 **	-52.84 **	-6.24 **	-4.57 *	-6.19**	0	1.28	-1.86
CMS 64A × RNR 26083	-22.13**	-7.77	-41.63 **	-9.63 **	-7.46 **	-9.04 **	0	1.28	-1.86
CMS 64A × RNR 26084	-29.31 **	-20.39 **	-49.62 **	-0.26	1.05	-0.67	-1.27	0	-3.11
CMS 64A × Pusa 1701-10-5-8	47.41 **	40.78 **	-62.52 **	-5.16**	-3.34	-4.99 **	-0.58	0.69	-2.43
CMS 64A × PAU 2K10-23-451-2-37-34-0-3	-31.47 **	-22.82 **	-51.15**	1.19	2.51	0.77	4.43*	5.77 **	2.48
CMS 64A × RP 5898-54-21-9-4-2-2	2.16	15.05*	-27.19**	-7.08 **	-0.87	-2.56	-2.53	-1.28	4.35*
JMS 14A × RNR 26059	-14.32 **	0.24	-36.56 **	4.08*	6.11**	4.30*	-3.75	-1.28	4.35*
JMS 14A × RNR26072	44.61 **	-35.19**	-58.99 **	-10.27 **	-7.72 **	-9.29 **	-2.97	-0.48	-3.57
JMS 14A × RNR 26074	-12.86*	1.94	-35.48 **	-4.58 **	-2.87	-4.53 **	-17.50 **	-15.38 **	-18.01 **
JMS 14A × RNR 26083	-2.05	16.02 **	-26.57 **	-2.34	0.01	-1.69	1.25	3.85	0.62
JMS 14A × RNR 26084	-22.61 **	-9.47	-42.70 **	5.82 **	6.19**	4.38*	-1.25	1.28	-1.86
JMS 14A × Pusa 1701-10-5-8	-36.51 **	-25.73 **	-53.00 **	3.51*	5.49 **	3.70*	-3.84	-1.38	4.44*
JMS 14A × PAU 2K10-23-451-2-37-34-0-3	-8.09	7.52	-31.95 **	-0.2	0.14	-1.56	-1.25	1.28	-1.86
JMS 14A × RP 5898-54-21-9-4-2-2	-1.66	15.05*	-27.19**	-5.61 **	0.7	-1.01	0	2.56	-0.62
JMS 20A × RNR 26059	35.88 **	12.14*	-29.03 **	1.26	5.27 **	3.47*	-3.14	-1.28	4.35*
JMS 20A × RNR26072	-14.12*	-29.13**	-55.15**	2.26	6.31 **	4.50*	1.89	3.85	0.62
JMS 20A × RNR 26074	-17.06*	-31.55 **	-56.68 **	-11.39 **	-7.88 **	-9.45 **	0.4	2.33	-0.85
JMS 20A × RNR 26083	48.77 **	-39.32 **	-61.60 **	0.35	4.32*	2.55	-0.63	1.28	-1.86
JMS 20A × RNR 26084	-11.76	-27.18**	-53.92 **	-8.58 **	-4.96 **	-6.58 **	-1.54	0.35	-2.76
JMS 20A × Pusa 1701-10-5-8	-0.59	-17.96 **	-48.08 **	-3.27	0.56	-1.15	0.63	2.56	-0.62
JMS 20A × PAU 2K10-23-451-2-37-34-0-3	-13.82	-28.88 **	-54.99 **	-10.93 **	-7.40 **	-8.98 **	-5.66 **	-3.85	-6.83 **
JMS 20A × RP 5898-54-21-9-4-2-2	37.65 **	13.59*	-28.11 **	-5.62 **	0.69	-1.03	-23.90 **	-22.44 **	-24.84 **

* Significant at 0.05% level, **Significant at 0.01% level

Table 6: contd.

Cross	Milling per cent			Head rice recovery (%)			Grain yield per plant (g)			Per day Productivity (kg/ha/day)		
	HB	SHH	SHV	HB	SHH	SHV	HB	SHH	SHV	HB	SHH	SHV
CMS 23A × RNR 26059	3.22	-7.33 **	-7.33 **	6.12**	0.09	-4.23*	12.5	-3.74	8.67	22.04 *	14.86	16.64
CMS 23A × RNR26072	6.19**	-4.67 **	-4.67 **	4.65*	1.5	-2.88	27.16**	9.17	23.24 **	41.71 **	31.16**	33.18**
CMS 23A × RNR 26074	-0.71	-7.33 **	-7.33 **	0.78	-2.26	-6.47 **	-15.96*	-32.49 **	-23.78 **	19.55	10.65	12.36
CMS 23A × RNR 26083	0.71	-6.00 **	-6.00 **	4.43*	-1.5	-5.76 **	-11.91	-32.15**	-23.41 **	-14.04	-18.81	-17.56
CMS 23A × RNR 26084	-0.71	-6.67 **	-6.67 **	2.84	-3.01	-7.19**	-19.47*	-32.11 **	-23.35 **	-15.65	-21.93*	-20.72 *
CMS 23A × Pusa 1701-10-5-8	2.47	-8.00 **	-8.00 **	-3.54	-9.02 **	-12.95 **	-11	-31.46 **	-22.62 **	-16.84	-23.03 *	-21.85*
CMS 23A × PAU 2K10-23-451-2-37-34-0-3	-2.27	-8.00 **	-8.00 **	-10.20 **	-6.77 **	-10.79 **	-10.55	-22.49 **	-12.5	14.24	5.73	7.36
CMS 23A × RP 5898-54-21-9-4-2-2	1.44	-6.00 **	-6.00 **	3.97	-1.5	-5.76 **	-15.75*	-30.33 **	-21.35 **	-7.19	-14.1	-12.78
CMS 64A × RNR 26059	3.57*	-3.33 *	-3.33 *	-5.63 **	-2.79	-6.99 **	26.28 **	12.90*	27.45 **	30.28 **	23.92 *	25.83 *
CMS 64A × RNR26072	1.43	-5.33 **	-5.33 **	-5.11*	-2.26	-6.47 **	4.97	-6.15	5.95	34.52 **	27.95 **	29.92 **
CMS 64A × RNR 26074	1.43	-5.33 **	-5.33 **	-5.11*	-2.26	-6.47 **	4.28	-6.77	5.25	6.03	0.85	2.4
CMS 64A × RNR 26083	2.14	-4.67 **	-4.67 **	-5.84 **	-3.01	-7.19**	-33.61 **	-40.64 **	-32.99 **	-35.61 **	-38.76 **	-37.81 **
CMS 64A × RNR 26084	0.71	-5.33 **	-5.33 **	-5.11*	-2.26	-6.47 **	-13.48	-22.65 **	-12.68	-8.14	-12.63	-11.28
CMS 64A × Pusa 1701-10-5-8	2.14	-4.67 **	-4.67 **	-8.02 **	-5.26 *	-9.35 **	-19.38 **	-27.92 **	-18.62*	-11.9	-16.2	-14.91
CMS 64A × PAU 2K10-23-451-2-37-34-0-3	5.52 **	-0.67	-0.67	-2.96	0.75	-3.6	21.72 **	8.82	22.85 **	24.92 *	18.81	20.65 *
CMS 64A × RP 5898-54-21-9-4-2-2	0.71	-6.00 **	-6.00 **	-5.84 **	-3.01	-7.19**	-22.30 **	-30.54 **	-21.58 **	-26.53 *	-30.12**	-29.04 **
JMS 14A × RNR 26059	-6.21 **	-9.33 **	-9.33 **	12.17**	-3.01	-7.19**	23.22 **	9.34	23.44 **	30.73 **	26.77 *	28.73 **

Table 6: contd.

Cross	Milling per cent			Head rice recovery (%)			Grain yield per plant (g)			Per day Productivity (kg/ha/day)		
	HB	SHH	SHV	HB	SHH	SHV	HB	SHH	SHV	HB	SHH	SHV
JMS 14A × RNR2 6072	-4.83 **	-8.00 **	-8.00 **	-3.1	-6.02 **	-10.07 **	6.19	-5.77	6.38	8.54	5.26	6.89
JMS 14A × RNR 26074	-18.62 **	-21.33 **	-21.33 **	-17.83 **	-20.30 **	-23.74 **	0.59	-10.74	0.77	28.51 **	24.63 *	26.55 *
JMS 14A × RNR 26083	0	-3.33 *	-3.33 *	7.20 **	0.75	-3.6	47.10**	30.54 **	47.37 **	38.91 **	34.71 **	36.78 **
JMS 14A × RNR 26084	-2.07	-5.33 **	-5.33 **	4	-2.26	-6.47 **	26.03 **	11.83	26.25 **	42.20 **	37.90 **	40.02 **
JMS 14A × Pusa 1701-10-5-8	-3.90 *	-7.11 **	-7.11 **	0.8	-5.26 *	-9.35 **	-9.01	-19.26 **	-8.85	8.81	5.52	7.15
JMS 14A × PAU 2K10-23-451-2-37-34-0-3	-2.07	-5.33 **	-5.33 **	-5.86 **	-2.26	-6.47 **	-5.61	-16.24*	-5.44	-11.24	-13.92	-12.59
JMS 14A × RP 5898-54-21-9-4-2-2	-4.83 **	-8.00 **	-8.00 **	-6.35 **	-11.28 **	-15.11 **	1.84	-9.63	2.02	-6.69	-9.51	-8.11
JMS 20A × RNR 26059	-4.83 **	-8.00 **	-8.00 **	9.57 **	-5.26 *	-9.35 **	-9.67	-20.91 **	-10.71	3.19	3.64	5.24
JMS 20A × RNR26072	-1.38	-4.67 **	-4.67 **	0.78	-2.26	-6.47 **	-15.36*	-25.89 **	-16.33*	-4.07	-3.65	-2.17
JMS 20A × RNR 26074	-0.37	-3.69 *	-3.69 *	1.2	-1.84	-6.08 **	1.09	-11.48	-0.07	17.19	17.7	19.52
JMS 20A × RNR 26083	-4.06*	-7.26 **	-7.26 **	1.6	-4.51 *	-8.63 **	-0.36	-12.75	-1.5	-3.82	-3.4	-1.91
JMS 20A × RNR 26084	-2.76	-6.00 **	-6.00 **	-2.4	-8.27 **	-12.23 **	-5.4	-17.16*	-6.48	-6.8	-6.4	-4.95
JMS 20A × Pusa 1701-10-5-8	0.69	-2.67	-2.67	4.80*	-1.5	-5.76 **	-12.32	-23.22 **	-13.32	14.24	14.73	16.5
JMS 20A × PAU 2K10-23-451-2-37-34-0-3	-4.83 **	-8.00 **	-8.00 **	-9.48 **	-6.02 **	-10.07 **	-7.68	-19.16**	-8.74	1.07	1.51	3.07
JMS 20A × RP 5898-54-21-9-4-2-2	-26.90 **	-29.33 **	-29.33 **	-28.03 **	-31.82 **	-34.76 **	0.69	-11.83	-0.47	-4.35	-3.94	-2.45

* Significant at 0.05% level, **Significant at 0.01% level ; HB = Heterobeltiosis; SHH = Standard Heterosis over hybrid (PA 6444); SHV = Standard Heterosis over variety(RNR 15048).

Table 7: Top ranking hybrids based on mean, heterosis over varietal and hybrid check and *sca* effect.

S. No.	Hybrids	Grain yield/ plant (g)	Heterosis over varietal check (%)	Heterosis over hybrid check (%)	*sca* Effect
1	JMS 14A × RNR 26083	41.15	47.37 **	30.54 **	10.00 **
2	CMS 64A × RNR26059	35.59	27.45 **	12.90*	4.36 **

Conclusions

Present investigation showed that superior performance for all the characters was not expressed in any single hybrid combination. However, different cross combinations were found to be superior for various characters. The *gca* effects revealed that among the lines JMS 14A and among the testers, RNR 26059 and RNR 26072 had significant *gca* effects in the desired direction for several traits including grain yield. Hence, these lines and testers could be considered as potential donors in improving grain yield per plant and associated components in future breeding programme.The degree of dominance was more than unity for the traits *viz.*, days to 50 per cent flowering (1.05), number of productive tillers per plant (1.59), kernel length (1.00), kernel breadth (1.05), kernel length breath ratio (1.32), hulling per cent (2.08), milling per cent (1.70), head rice recovery (1.57), grain yield per plant (1.25) and per day productivity (1.35) indicating predominance of non-additive gene action, while plant height (0.53), panicle length (0.83), panicle weight (0.94), 1000 grain weight (0.83), number of grains per panicle (0.73) and spikelet fertility (0.79) exhibited additive gene action. Among 32 hybrids, JMS 14A × RNR 26083 and CMS 64A × RNR 26059 (**Table 7**) were identified as promising hybrids based on *per se* grain yield per plant, heterosis and specific combining ability which would be further tested in bigger plot for advancement.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Production of doubled haploids from rice hybrid KRH-2 through anther culture and their evaluation for agro-morphological traits

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Abstract

The present study was aimed to optimize conditions of anther culture and production of doubled haploids of a popular rice hybrid, KRH-2. Immature panicles collected from field grown donor plants were pre-treated at 8°C for eight days and plated on four different basic media for callus induction with different hormonal concentrations and supplements. N6 media with maltose as the carbon source, supplemented with 2 mg L^{-1} of 2,4-dichlorophenoxyacetic acid (2,4-D), 0.5 mg L^{-1} of kinetin and 10 mg L^{-1} of silver nitrate (designated as N6-4 medium) was found to give the highest callus induction (22.4%). The compact calli, which were transferred to MS regeneration medium supplemented with 2.5 mg L^{-1} of benzyl amino purine (BAP), 0.5 mg L^{-1} of kinetin and 1.5 mg L^{-1} of naphthalene acetic acid (NAA) recorded a good regeneration (25.4%). A total of 125 stable, doubled haploid lines (DHLs) were produced through spontaneous doubling of KRH-2 microspores. The lines showed significant variability with respect to key agro-morphological traits.

Keywords-Rice androgenesis; Callus induction; Green plant regeneration; KRH-2; morpho-agronomic evaluation

Introduction

Among the various alternatives available for increasing rice productivity, adoption of hybrid rice technology is one of the most feasible options (Yuan 1994). Since early 1990s hybrid rice has been cultivated in many Asian countries including India. Presently, China has the maximum area under hybrid rice and the technology is one of the key components of food security in China. Despite, the tremendous potential of hybrid rice technology in improving rice production and productivity, the adoption of hybrid rice technology has not been satisfactory in India. Presently, it is estimated that out of 44.5 Mha of cultivated rice, only 3 Mha is under hybrid rice. Higher seed cost, poor seed-set, low to moderate levels of heterosis and poor grain and cooking quality of rice are the major reasons for limited adoption of the technology (Mishra *et al*., 2013). Among these, in particular, the moderate levels of heterosis is one of the main reasons for its lower adoption in the intensive rice cultivation areas of India and a need has been felt to enhance the genetic diversity among the parental lines to increase the heterosis levels.

In recent years, rapidly changing climatic conditions coupled with increased incidence of biotic and abiotic stresses have posed severe challenges for increasing rice production which has already witnessed a scenario of yield stagnation. One of the feasible options available to enhance the rice production in the short and medium term is through adoption of hybrid rice, which has been proved to have considerable yield advantage over the inbred lines (Yuan, 1994).

Among the various methods available for breeding and improving the parental lines used for hybrid development, conventional breeding approach is the most commonly deployed one. However, it involves several cycles of breeding-selection and is cost, resource intensive. This necessitates development of other viable options that could surpass these breeding limitations. Through conventional breeding, it takes a minimum of 6-8 years for development of desirable lines and there is a need to shorten the process of development of homozygous lines (Snape 1989; Raina & Zapata 1997; Baenziger *et al*., 2006). One such viable and feasible option for developing homozygous lines in a single generation is through adoption of doubled haploid (DH) breeding strategy (Hu. 1985; Baenziger *et al*., 1989; Wu *et al*., 2012).

Different fixed (i.e. non-segregating) populations namely the recombinant inbred lines (RIL), doubled haploid lines (DHLs), near isogenic lines (NILs) etc., are considered as valuable tools for use in tagging and mapping of novel traits/genes and also as a population improvement strategy, besides being useful in functional genomics studies (Paterson 1996; Collard *et al*., 2005; Forster & Thomas 2005). Among these populations, DHLs are unique in the sense that their development and fixation takes the shortest possible time (i.e. within one-two breeding seasons). DHLs are considered as valuable tools for identifying gene blocks associated with important quantitative traits such as yield, biotic and abiotic stress tolerance and heterosis (Tinker *et al*., 1996; Wang *et al*., 2001; Senadhira *et al*., 2002; Suriyan *et al*., 2009). Even though there are several reports of development and utilization of DHLs for varietal development/ improvement (Han and Huang 1987), their utilization in molecular mapping of heterotic loci/QTL is limited.

Even though protocols for anther culture and doubled haploid production have proved to be quite simple in japonica rice, success rate in indica rice has been limited (Zapata and Arias, 2003; Bagheri and Jelodar, 2008). Though many attempts were made to produce the fertile DH plants by manipulating the factors that govern androgenesis in indica rice, the success of optimized anther culture conditions have been fairly unsatisfactory (Ratheika and Silva, 2007). The reasons identified for the limited success of indica rice is principally due to the recalcitrant nature as a result of early anther necrosis, inherently diminished competence to form callus, higher frequency of albino plant regeneration and lower frequency of green plant regeneration (Balachandran *et al*., 1999; Grewal *et al*., 2009). As limited reports are available on the production of DH lines of indica rice (Mishra *et al*., 2015; Naik *et al*., 2016) this study outlines the optimized culture conditions for an elite Indian rice hybrid, KRH-2 and evaluation of the DH lines.

Materials and Methods

Plant material: A medium duration rice hybrid, Karnataka Rice Hybrid-2 (KRH-2 derived from the cross IR58025A × KMR-3R), with long and bold grain type, developed by Regional Agricultural Research Station (RARS), Mandya, Karnataka was used as the experimental material, along with the parents, IR58025B (recurrent parent) and KMR-3R (donor parent). The parental seed material along with the seeds of varietal checks was obtained from the Hybrid Rice Section, ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad, India. For evaluation of the developed DH population, Akshayadhan (AKD) and Varadhan (VRD) were used as varietal checks.

Heterozygosity assessment of KRH-2 hybrid: Purity assessment of parents and F_1 seeds of the hybrid KRH-2 was done before every batch of KRH-2 hybrid anthers were plated on callus induction medium. This was conducted with a simple sequence repeat (SSR) marker RM19660 located on chromosome 6 that exhibited amplification of unique alleles in KRH-2 as per the procedure described in Yashitola *et al*., (2002) and Sundaram *et al*., (2008).

Anther culture and double haploid production in KRH-2: Genetically pure seeds of parents and

the elite rice hybrid KRH-2 (derived by crossing IR58025A with KMR-3R) obtained from the Hybrid Rice Section, ICAR-Indian Rice Research Institute, Rajendranagar, Hyderabad, India, were grown during *Kharif* 2015 and the recommended package of practices were adopted for raising the crop. Immature panicles of KRH-2 were collected from the field grown plants at booting stage in the morning between 8-9 AM as described by Balachandran *et al*., (1999). The panicles were packed in wet cloth wrapped in aluminum foil and subjected to a cold shock pre-treatment at 8°C for a period of eight days in a cold chamber. After cold shock treatment, spikelets which were in late uni-nucleate to early bi-nucleate stage (determined through microscopy, described by Jahne and Lorz, 1995) were chosen for plating of the anthers. For checking the pollen fertility, excised spikelets were fixed in formaldehyde, acetic acid and 70% alcohol solution (1:1:18). They were then gently macerated on a slide with an autoclaved surgical blade and stained with 1% potassium iodide solution (1% IKI) for observation under bright field microscope for pollen fertility estimation (Mishra *et al*., 2015). Fertile anthers were then aseptically plated on four callus induction medium *viz*., N6 (Chu *et al*., 1975), B5 (Gamborg *et al*., 1968), MS (Murashige and Skoog 1962) and He2 (Huang *et al*., 1978) following the protocol standardized by Balachandran *et al*., (1999) in 25 batches but with slight modifications. The plated anthers were incubated in dark for 45 days at 25°±1°C. The cultures were examined periodically for contaminations and the development of callus formation was closely monitored.

Compact, pale yellow colored calli measuring 2-3 mm in diameter were transferred to MS regeneration medium (Cho and Zapata, 1988; Balachandran *et al*., 1999) and incubated under illuminated conditions with 16/8 hours' light and dark regime. The regenerated green shoots were transferred to hormone free ½ MS medium (rooting medium) and were maintained under illumination. Well-developed plants with profuse roots were transferred to Yoshida's solution for hardening for two weeks, later they were transferred to green house and maintained till maturity. The response of the anthers for callus induction and green plant regeneration was recorded 40 days after plating.

Optimization studies for callus induction were carried out with four different media namely, N6, B5, MS and He2 with 3% maltose as the carbon source supplemented with additives 0.5 g L^{-1} of casein hydrolysate, 0.1 g L^{-1} of myo-Inositol, 10 mg L^{-1} of silver nitrate and five different hormonal concentrations *viz.*, 0.5 mg L^{-1}, 1.0 mg L^{-1}, 1.5 mg L^{-1}, 2.0 mg L^{-1}, 2.5 mg L^{-1} of 2,4-D (2, 4-Dichloro phenoxy acetic acid) + 0.5 mg L^{-1} of Kinetin) and plated in 0.4 % phytagel. Similarly, for green plant regeneration phase, the optimization studies were carried out using the MS medium, 3% sucrose as carbon source supplemented with five different hormonal concentrations *viz*., 0.5 mg L^{-1} of 6-Benzyl Amino Purine (BAP), 1.0 mg L^{-1}, 1.5 mg L^{-1}, 2.0 mg L^{-1} and 2.5 mg L^{-1}; 0.5 mg L^{-1} of Kinetin and 1.5 mg L^{-1} of Napthalene Acetic Acid (NAA) **(Table 1).** , The *in-vitro* androgenesis of the hybrid, KRH-2 and its parents was carried out during both *Kharif* 2015 and *Rabi* (2015-2016).

The regenerated plants at the end of the *Kharif* 2015 (D_0) were grown under greenhouse conditions in earthen pots and upon acclimatization, were maintained till maturity in net-house conditions. These plants were agro-morphologically assessed for the determination of ploidy levels. Total number of regenerated plants, total number of survived plants, total number of sterile (n), diploid (2n) and polyploid plants were recorded. The seeds (D_0 generation) of those plants which were observed to be putative fertile doubled haploids (DHLs) were carefully collected. Only the confirmed and true DHLs were advanced to further generations. Similarly, plants obtained during the *Rabi* (2015-2016), were established and ploidy determination and true-fertile DHL advancement was followed.

Duncan's Multiple Range Test (DMRT) was done using SAS version 9.2 (SAS Institute Inc., Cary,

NC, USA) to assess the effect of different media compositions on callus induction and green plant regeneration. Microsoft excel package was used for the analysis of standard error (SE), standard deviation (SD), coefficient of variation in percentage (CV%) and mean.

Identification of true doubled haploids using hyper-variable SSR marker: At the end of *Kharif* 2015 and *Rabi* (2015-2016), in order to determine the origin of putative fertile doubled haploid (2n) anther culture derived plants (across two seasons) from microspores and to ensure that they have not originated from the somatic anther wall tissue, genomic DNA was extracted from fresh and healthy leaves of 10-20 randomly selected putative doubled haploid (2n) plants by following the protocol of Dellaporta *et al*., (1983) and amplified using set of eight highly polymorphic SSR markers namely RM15326 (chr. 3), RM15679 (chr. 3), RM19410 (chr. 6), RM21539 (chr. 7), RM22554 (chr. 8), RM22837 (chr. 8), RM6925 (chr. 8), RM23958 (chr. 9) and as described by Yashitola *et al*., (2002), Sundaram *et al*., (2008) and Jaikishan *et al*., (2009) .

Agro-morphological evaluation of developed DHLs: A total of 125 regenerated true DHLs (D_0) were advanced from D_1 to D_3 generation were grown for three consecutive seasons (*Rabi* (2016-2017), D_1 generation; *Kharif* 2017, D_2 generation; *Rabi* (2017-2018), D_3 generation) for analyzing their stability, uniformity and for their agro-morphological performance. Seven most crucial yield related agro-morphological characters were recorded from five plants per each entry at suitable stage of the rice plant as per the standard evaluation system recommended by IRRI (IRRI, 2002) in the main field of IIRR, Rajendranagar, Hyderabad, India. For phenotyping of the DHL population, randomized complete block design (RCBD), in two replications, was used. In each replication, five middle plants of each DHL entry were considered for phenotyping. At appropriate growth stage of experimental material, seven traits namely, days to fifty per cent flowering (DFF), total grain yield per plant (YLD), total number of grains per panicle (GP), test (1,000) grain weight (TGW), number of productive tillers (PT) and biomass (BM) were recorded and the collected mean data were considered for further analysis.

Results

The true F_1s obtained after crossing IR58025A with its restorer line, KMR-3R, were identified with the help of a parental polymorphic SSR marker RM19660. PCR analysis showed amplification of 250 bp specific to IR58025A and 400 bp band specific to KMR-3R among most of the F_1s (**Figure 1**) and the true F_1s were then subjected for anther culture to produce doubled haploids.

Figure 1: KRH-2 (F_1) purity assessment with RM 19660 (SSR marker); L-100 bp ladder, A-IR58025A (Parent 1), R-KMR-3R (Parent 2), 1-15 KRH-2; F_1 samples

Anther culture of KRH-2

Only those panicles in which the microspores were in late uni-nucleate (**Figure 2a**) or early bi-nucleate stage (**Figure 2b**) were chosen for anther plating as microspores in these two stages are known to be highly responsive for callus induction. The highest androgenic response was observed in the hybrid when panicles were pre-treated at 8°C for a period of eight days (**Table 5**).

Among the various media used for callus induction, N6 medium was identified to be the best **(Table 1).** Irrespective of media, among the five different combinations of hormones used for callus induction, a combination of 2 mg L^{-1} 2,4-D and 0.5 mg L^{-1} kinetin elicited the highest callus induction frequency (16.7%-22.4%). Based on the results obtained, N6-4 medium (with 2 mg L^{-1} of 2,4-D and

0.5 mg L^{-1} of kinetin) was identified to be the best for callus induction in KRH-2 and its parents, followed by B5-4, MS-4 and He2-4 **(Table 1, Figure 3a, Figure 3b).** The highest per cent of albino plants (25.9%) was observed in parent IR58025B whose calli were induced on He2-4 medium **(Table 1).**

Based on the results obtained, N6-4 medium (with 2 mg L^{-1} of 2,4-D and 0.5 mg L^{-1} of kinetin) was identified to be the best for callus induction in KRH-2 and its parents, followed by B5-4, MS-4 and He2-4 (**Table 1, Figure 3a, Figure 3b**). Similar to callus induction, the highest percentage of green plant regeneration (25.4%) on optimized MS medium was

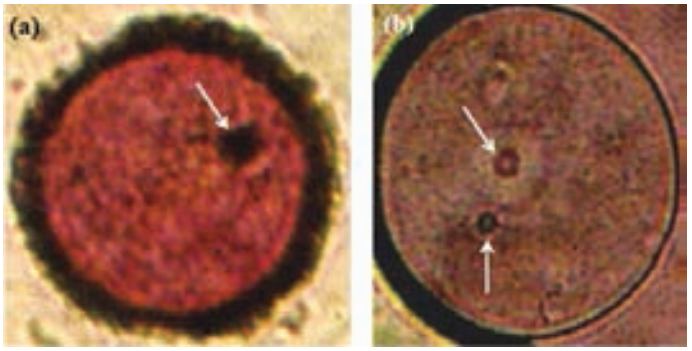

Figure 2: Anthers in late uni-nucleate/early bi-nucleate stage used for callus induction (nucleus indicated with arrows) (a) Microspore in late uni-nucleate stage after staining with 2% acetocarmine solution (b) Microspore in early bi-nucleate stage showing generative nucleus (small) and vegetative nucleus (big) also after staining with 2% acetocarmine solution

Table 1: Response of different media & hormone concentrations on callus induction* and green plant regeneration# in KRH-2 (hybrid) and its parents

Culture Medium	Genotype	No. of anthers plated	No. of calli induced	% callus induction ±SE (%)[a]	No. of calli inoculated	No. of calli producing plants	%Green plant regeneration± SE[a]	% Albino plant regeneration±SE[a]
N6-4	IR58025B	7,500	1,260	16.7±0.56a	223	43	19.1± 0.54b	21.2± 0.68c
	KMR-3R	6,852	829	12.1±0.58b	184	29	15.6± 0.51c	17.8± 0.81b
	KRH-2	7,236	1,534	22.4±0.64c	280	71	25.4 ± 0.43a	15.4± 0.38a
B5-4	IR58025B	6,500	902	13.8± 0.11b	160	26	16.1± 0.17b	20.7± 0.47b
	KMR-3R	6,899	698	10.1±0.66c	123	16	13 ± 0.23c	19.4± 0.68b
	KRH-2	7,023	1,336	18.9± 0.82a	177	40	22.4 ± 0.62a	16.4± 0.74a
MS-4	IR58025B	5,678	656	11.5± 0.22b	144	18	12.4± 0.38b	24.8± 0.77c
	KMR-3R	6,235	575	9.2±0.12c	85	9	10.5± 0.62c	20.8± 0.33a
	KRH-2	7,214	1,090	15.1±0.85a	169	34	20.3 ± 0.67a	18.7± 0.41b
He2-4	IR58025B	7,811	721	9.2 ±0.15b	121	10	8.6± 0.14b	25.9± 0.21c
	KMR-3R	6,778	489	7.2 ±0.36c	85	8	9.2± 0.11b	24.6± 0.23a
	KRH-2	5,789	644	11.1±0.49a	138	22	16.2 ± 0.46a	21.6± 0.54a

Means with the same letter in a column were not significantly different in Duncan's multiple comparison range test ($p < 0.05$); [a]25 replicates per treatment, repeated thrice

*Callus induction media: N6-1, B5-1, MS-1, He2-1 (0.5 mg/L of 2,4-D+ 0.5 mg/L of Kinetin) ; N6-2, B5-2, MS-2, He2-2 (1.0 mg/L of 2,4-D+ 0.5 mg/L of Kinetin) ; N6-3, B5-3, MS-3, He2-3 (1.5 mg/L of 2,4-D+ 0.5 mg/L of Kinetin); N6-4, B5-4, MS-4, He2-4 (2.0 mg/L of 2,4-D+ 0.5 mg/L of Kinetin); N6-5, B5-5, MS-5, He2-5 (2.5 mg/L of 2,4-D+ 0.5 mg/L of Kinetin); 0.1 g/l of myo-Inositol , 10 mg/L of silver nitrate; 30 g/L of maltose

#Green plant regeneration medium: MS-1 (0.5 mg/L of BAP+ 0.5 mg/L of kinetin +1.5 mg/L of NAA), MS-2 (1.0 mg/L of BAP+ 0.5 mg/L of kinetin +1.5 mg/L of NAA), MS-3 (1.5 mg/L of BAP+ 0.5 mg/L of kinetin +1.5 mg/L of NAA), MS-4 (2.0 mg/L of BAP+ 0.5 mg/L of kinetin +1.5 mg/L of NAA), MS-5(2.5 mg/L of BAP+ 0.5 mg/L of kinetin +1.5 mg/L of NAA); 30 g/L of sucrose

observed in those calli which were induced from anthers pre-treated at 8°C for 8 days (**Table 5**). Highest frequency of green plant regeneration (15.6-25.4%) was observed in those calli that were induced on N6-4 medium (**Figure 3c-3d**) followed by B5-4, MS-4 and He2-4 media. MS-5 medium (concentrations of BAP, kinetin and NAA being 2.5 mg L^{-1}, 0.5 mg L^{-1} and 1.5 mg L^{-1}, respectively) was identifed to be the best medium for green plant regeneration (**Table 1, Figure 3c-Figure 3d**). Besides, green plants, a substantial percentage of albino plants in the range of 15.4% to 25.9% was observed. The least per cent of albino plants was observed in the hybrid KRH-2 whose calli were induced on N6-4 medium. The highest per cent of albino plants (25.9%) was observed in parent IR58025B whose calli were induced on He2-4 medium (**Table 1**).

Regenerated shoots with primary roots were transferred to hormone-free ½ MS medium for the development of more roots (**Figure 3e-3f**). The plants were then grown in Yoshida's solution for hardening (**Figure 3g-3h**). Later, the hardened plants were transferred to earthen pots maintained in biosafety screen house (**Figure 3i**) and were maintained till maturity in field (**Figure 3j-3l**).

The details of number of regenerated plants from anther culture of the three genotypes *viz.,* KRH-2, IR58025B and KMR-3R across two seasons are presented in **Table 2**. Regenerated plants constituted fertile doubled haploids, sterile haploid and polyploid plants [**Figure 3k i-iv**]. In *Kharif* 2015, 270 out of 381 regenerated plants of the KRH-2 hybrid were observed to survive. Among them, 58 were fertile double haploids (2n), 140 were sterile haploids (n) and 72 were sterile polyploid plants. Similarly, in *Rabi* (2015-2016), among the 193 plants survived, 67 plants were observed to be fertile diploids (2n), 56 plants were sterile haploids (n) and 70 were sterile polyploids. Similar trend was noticed in case of the other two genotypes, IR58025B and KMR-3R as well.

Table 2: Details on the number of regenerated plants along with their ploidy status across two seasons of androgenesis in KRH-2 and in its parents

Genotype	No. of regenerated Plants		No. of survived Plants		No. of fertile plants (2n)		No. of sterile plants (n)		No. of sterile polyploid plants	
Season	*Kharif* 2015	*Rabi* 2015-16	*Kharif* 2015	*Rabi* 2015-16	*Kharif* 2015	*Rabi* 2015-16	*Kharif* 2015	*Rabi* 2015-16	*Kharif* 2015	*Rabi* 2015-16
KRH-2	381	247	270	193	58 (21.5)	67 (34.7)	140 (51.9)	56 (29.0)	72 (26.6)	70 (36.3)
IR58025B	266	138	242	114	79 (32.7)	23 (20.2)	116 (47.9)	67 (58.8)	47 (19.4)	24 (21.0)
KMR-3R	333	154	266	138	77 (28.9)	48 (34.8)	96 (36.1)	51 (37.0)	93 (35.0)	39 (28.2)

The percentage value of fertile doubled haploid, sterile haploid and sterile polyploid plants is indicated within the parenthesis.

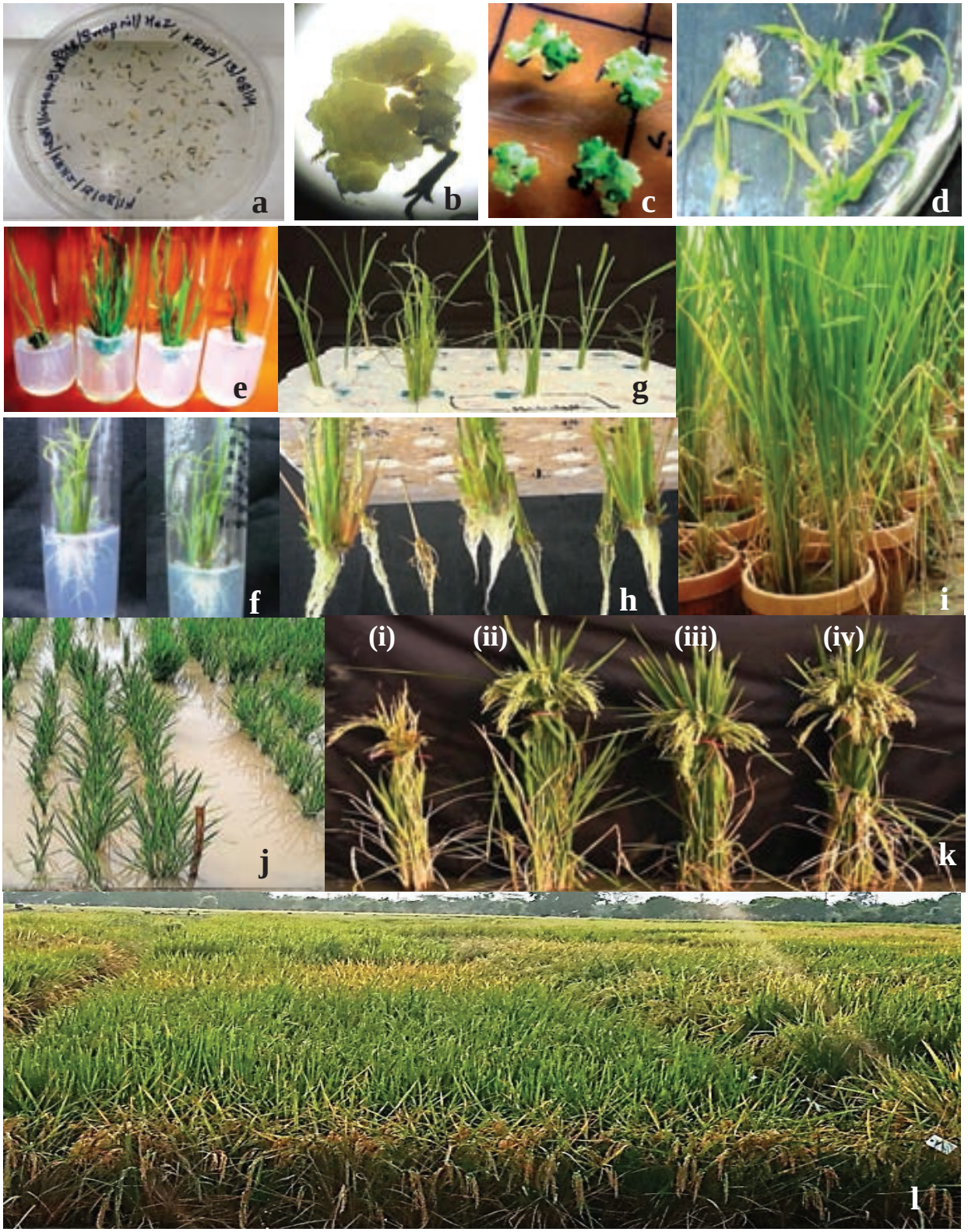

Figure 3: Various stages of androgenesis in KRH-2

(a) Plated KRH-2 anthers on callus induction medium (b) Compact calli emerging from anthers of the hybrid 40 days after anther plating. (c-d) Emergence of green and albino shoots from compact calli on MS regeneration medium (RM) after 10 days of transfer of calli on MS RM (e) Regeneration of roots from regenerated shoots in ½ MS medium (hormone free medium) after 10 days of its transfer (f) Root development in half (½) MS medium; (g-h) Plants hardening in Yoshida's nutrient solution (i) Acclimatization of hardened plants in green house (k) Morphological evaluation of anther culture derived plants for ploidy determination at maturity (i) putative haploid sterile plant (ii) sterile polyploid plant (iii)-(iv) fertile doubled haploid plants (j,l) Field establishment of true KRH-2 derived doubled haploid (DH) population (D_4 generation) (a) Seedling stage (b) Matured DHLs

Molecular analysis of hybrids and doubled haploids

The PCR analysis of regenerated fertile doubled haploid plantlets with parental polymorphic hyper-variable SSR markers namely RM15326, RM15679, RM19410, RM21539, RM22554, RM22837, RM6925 and RM23958 (**Table 3, Figure 4**) showed amplification identical to either of two parents IR58025A or KMR-3R. Also, 12.67% of induced calli were somatic in origin whereas 68.25% were elicited from microspores. Most of the true and fertile DHLs showed amplification identical to parent KMR-3R.

Table 3: Details of SSR markers used in the study

S. No	SSR/EST-marker name	Chr No.	Physical position (Mb)	Forward Primer's Sequence	Reverse Primer's Sequence
1	RM15326	3	20.6	TGAATCTACCGCTC-TACTTGTGG	AAACAGTG-CATCCTTCTTGTGG
2	JGT03-26.8	3	26.80	GAGCGTTTGTAGTA-AGTTTCATGGAC	GGCCCAACCCAAACA-CAAAT
3	RM15679	3	26.87	TAGATGTATGAGTCGGAAT-GGAGTCG	CAGACGCAGTGTGTGTAT-GAAGTTCC
4	RM21539	7	16.44	GCCCAACTACTTCGA-CAGCTTCC	CAATGACCTGAGTAGCATC-CAAGG
5	RM6925	8	0.64	GAATGAGAGGACGCTT-GAAGAGG	GCATTCAGTCCCAGCTTG-TATCG
6	RM22554	8	5.58	TTGTCAAGATCATCCTCG-TAGC	GTCATTCTGCAACCT-GAGATCC
7	RM22837	8	12.36	ACCTGGGTCAGATGTCT-GTTTGG	GGTAGAGCTCCATCCATCT-TAGTGC
8	RM23958	9	7.94	CTACCACTGTTTCATTGT-GTCTCG	GAATTGAAGGAGAAGCAG-GAAGC

Morpho-agronomic performance evaluation in doubled haploids

Doubled haploids produced in different seasons were evaluated for the following yield and yield related traits: i) days to 50% flowering (DFF), ii) total grain yield per plant (YLD), iii) total number of grains per panicle (GP), iv) test (1,000) grain weight (TGW), v) number of productive tillers (PT) and vi) biomass (BM). Varieties, Akshayadhan (AKD), Varadhan (VRD) and the hybrid KRH-2 were used as checks for comparing the performance of DH population **(Table 4).**

Flowering duration ranged between 91 days (SMB-7) and 129 days (Akshayadhan, AKD). The mean value of DFF for parents, varietal-hybrid checks along with the DHLs was observed to be 101 days. All better performing DHLs were observed to be early flowering compared with varietal checks and KMR-3R. The total number of grains (GP) varied between 211 (SMB-10) and 364 (SMB-1 (RP6301-189-17-2) with mean value of 310 **(Figure 5)**. Only one DHL, namely, SMB-1, was observed to have more number of grains than the varietal-hybrid checks. The range of TGW trait value was observed to be between 14.6 g

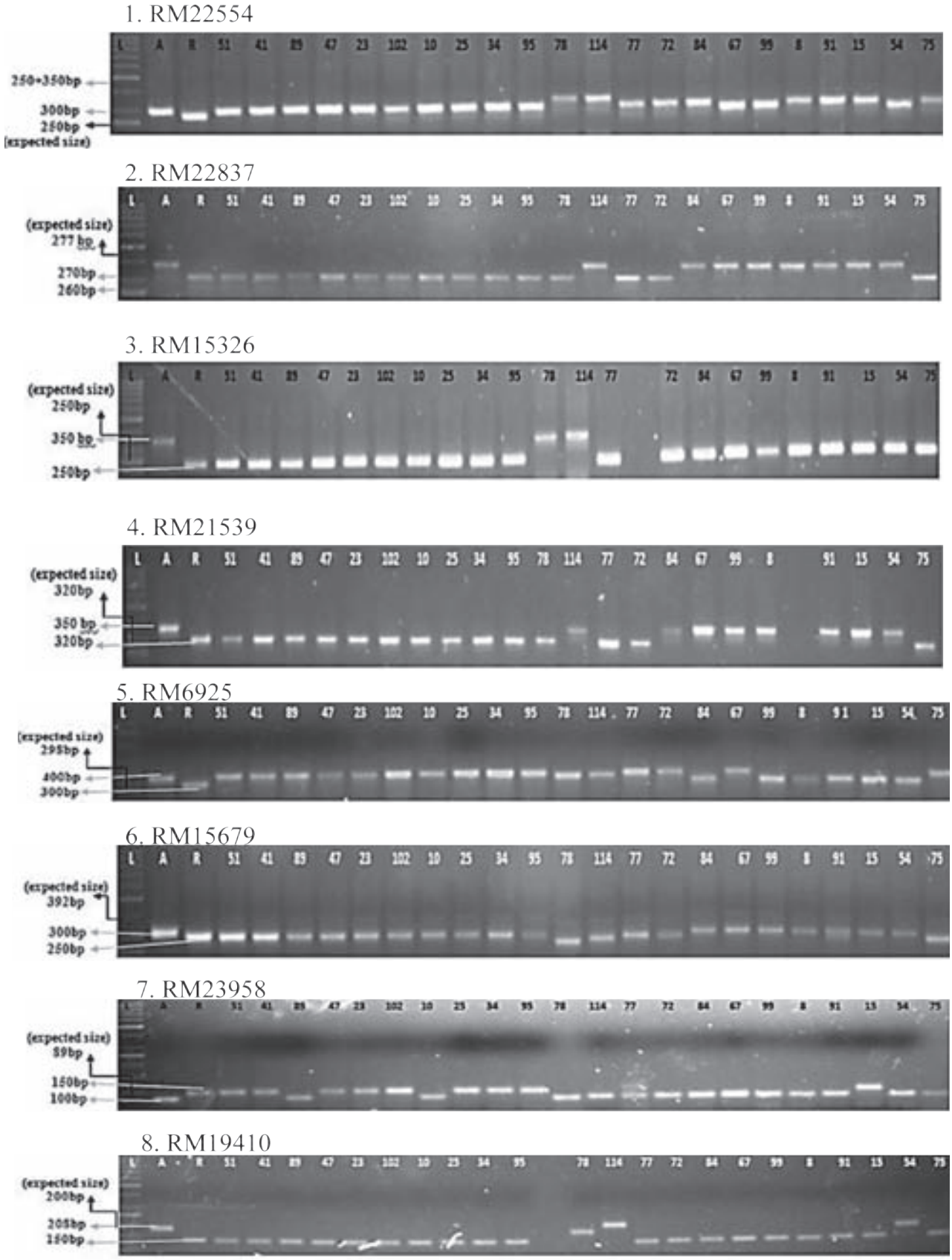

Figure 4: Determination of true doubled haploid lines (DHLs) with hyper variable SSR markers; L-100 bp ladder, A-IR58025A (Parent 1), R-KMR-3R (Parent 2), Sample no. 51 to Sample no. 75 are the randomly selected DHLs chosen for confirmation.

(IR58025B) and 23.68 g (SMB-1) with a mean value of 18.79 g. Number of productive tillers ranged from 8 (SMB-8) to 14 (KMR-3R) with a mean value of 10. No DHL was observed to have more number of productive tillers than KRH-2. For the trait biomass (BM), the range varied from 47.98 g (IR58025B) to 59.98 g (SMB-1) with a mean value of 55.07 g. Among all the DHLs, SMB-1 showed the highest biomass. For grain yield (YLD), the range of values were observed between 18.79 g (IR58025B) and 33.42 g (SMB-1) with a mean value of 24.45 g. Three DHLs namely, SMB-1, SMB-2 (RP6300-188-23-5) and SMB-3 (RP6301-189-27-3-2) were observed to be higher yielding than KRH-2.

Table 4: Performance of doubled haploid (DH) lines derived from the hybrid IR58025A × KMR-3R

	DFF±SE	GP±SE	TGW±SE	PT±SE	BM±SE	YLD±SE
IR58025B	102±0.23	289±0.96	14.6±0.98	10±0.36	47.98±0.13	18.79±0.11
KMR-3R	110±0.15	320±0.34	18.01±0.77	14±0.37	52.36±0.11	24.23±0.74
KRH-2	106±0.36	358±0.85	22.15±0.32	9±0.33	58.68±0.59	27.94±0.31
AKD	129±0.12	335±0.30	20.05±0.15	11±0.25	51.23±0.45	20.08±0.42
VRD	112±0.15	327±0.12	22.03±0.11	12±0.45	53.69±0.23	22.03±0.13
SMB-1	***98±1.20***	***364±0.36***	***23.68±0.33***	***12±0.67***	***59.98±0.71***	***33.42±0.26***
SMB-2	***92±0.88***	***350±0.18***	***20.78±0.29***	***9±0.88***	***58.99±0.69***	***30.84±0.43***
SMB-3	***104±0.88***	***322±0.08***	***20.14±0.33***	***11±0.58***	***57.66±0.62***	***29.97±0.33***
SMB-4	93±0.33	286±0.61	19.21±0.29	9±0.88	56.55±0.50	25.77±0.43
SMB-5	97±0.88	294±0.12	19.07±0.58	10±1.20	50.38±0.58	25.38±0.35
SMB-6	92±0.58	322±0.41	18.04±0.58	10±0.76	55.45±0.62	23.44±0.58
SMB-7	91±0.88	310±0.21	16.22±0.67	11±0.88	56.45±0.51	22.78±0.29
SMB-8	99±1.20	289±0.70	17.44±0.58	8±0.45	58.44±0.28	21.48±0.21
SMB-9	93±1.20	277±0.52	15.11±0.33	9±0.89	54.77±0.39	19.44±0.08
SMB-10	101±0.67	211±0.52	15.33±0.58	9±0.84	53.44±0.32	21.22±0.41
Mean	101	310	18.79	10	55.07	24.45
SD	10.14	38.48	2.75	1.58	3.52	4.41
SE	2.62	9.93	0.71	0.41	0.91	1.14
CV(%)	10.01	12.40	14.64	15.39	6.40	18.02

DFF-Days to 50% per cent flowering; GP-Total grains/panicle; TGW-Test (1,000) grain weight (g); PH-Plant height (cm); PT-Number of productive tillers; BM-Biomass (g); YLD-Total grain yield/plant(g); SD-Standard Deviation, SE-Standard Error, CV%-Coefficient of Variation (CV) in percentage; The best performing three DHLs shown in italics and bold, AKD-Akshayadhan, VRD-Varadhan.

Table 5: Two seasons mean data of cold shock pre-treatment regimes on callus induction on N6 medium and on green plant regeneration percentage on MS medium of parents IR58025B, KMR-3R and hybrid KRH-2 in *Kharif* 2015 and *Rabi* (2015-2016)

Temperature-4°C				
S.No	**Genotype**	**Duration of cold shock (days)**	**Callus induction (%) on N6 medium (Mean±SE)**	**Green plant regeneration (%) (Mean±SE)**
1	IR58025B	2	2.17±0.40	4.73±0.94
		4	4.96±0.57	6.04±0.59
		6	7.13±0.71	7.12±0.13
		8	8.56±0.54	9.97±0.64
		10	8.15±0.59	9.92±0.84
2	KMR-3R	2	1.96±0.43	2.7±0.52
		4	4.21±0.78	3.8±0.81
		6	5.96±0.43	4.53±0.32
		8	7.86±0.61	5.78±0.50
		10	5.69±0.59	8.74±0.26
3	KRH-2	2	2.91±0.55	12.26±0.46
		4	5.09±0.73	13.76±0.69
		6	7.62±0.85	19.41±0.06
		8	10.51±1.01	25.67±0.63
		10	5.89±0.76	15.96±0.13
Temperature-8°C				
1	IR58025B	2	3.79±0.68	5.73±0.23
		4	5.80±0.59	7.30±0.07
		6	6.45±0.47	8.72±0.47
		8	8.60±0.86	10.05±0.80
		10	6.99±0.84	14.53±0.37
2	KMR-3R	2	2.87±0.70	4.18±0.32
		4	4.87±0.55	6.72±0.93
		6	6.16±0.68	7.43±0.60
		8	6.88±0.79	8.63±0.66
		10	5.15±0.69	7.60±0.53
3	KRH-2	2	12.77±0.18	12.35±0.21
		4	14.17±0.25	14.81±0.37
		6	14.82±0.22	18.72±0.22
		8	**17.59±0.23**	**26.37±0.02**
		10	12.25±0.02	16.61±0.27

Table 5: continued....				
S.No	**Genotype**	**Duration of cold shock (days)**	**Callus induction (%) on N6 medium (Mean±SE)**	**Green plant regeneration (%) (Mean±SE)**
Temperature-12°C				
1	IR58025B	2	4.27±0.87	5.90±0.89
		4	5.36±0.45	8.10±0.25
		6	7.89±1.86	9.63±0.14
		8	9.05±0.83	11.07±0.35
		10	7.89±0.70	9.35±0.38
2	KMR-3R	2	3.26±0.73	4.17±0.17
		4	4.78±0.61	9.82±0.27
		6	5.87±0.79	8.56±0.61
		8	6.17±0.86	9.45±0.68
		10	5.24±0.47	6.78±0.17
3	KRH-2	2	8.57±0.94	14.05±0.18
		4	10.10±0.74	17.19±0.25
		6	11.57±1.22	14.82±0.22
		8	14.90±1.02	17.59±0.23
		10	10.88±0.85	12.25±0.02

The highest response for both the stages is indicated in bold

Figure 5: **The best performing DH line (SMB-1) showing larger panicles and more number of productive tillers and of highly desirable medium slender (MS) grain type.**

Discussion

The purpose of the study was to develop the doubled haploid (DH) lines from an elite rice hybrid through which trait fixation *viz*., yield/plant (YLD) could be possible. The stable, fixed and homozygous DHLs were utilized for QTL mapping of yield and its allied traits. KRH-2 is one of the elite, stable and widely adaptable rice hybrid across the country with higher yields and with desirable grain type. Long term goal of the study was to develop DHLs from a high yielding rice hybrid, KRH-2 and to utilize the developed DHLs as pre-breeding material for producing positively heterotic novel hybrids than KRH-2. Moreover, the successful development of doubled haploids has been routinely undertaken from some of the elite hybrids in the recent past for improving the yield/plant, grain quality and grain type. A similar kind of study was done by Mishra *et al*., (2015) where promising DHLs were produced through anther culture of two elite rice hybrids.

The KRH-2 hybrid was produced in every season by undertaking crosses between its parents *viz*., IR58025A and KMR-3R at IIRR. In order to rule out the KRH-2 hybrid's seed production from out-crossing, the heterozygosity assessment (purity assessment) of the KRH-2 hybrid along with its parents was undertaken with a hyper-variable SSR marker, which amplified specific alleles for both the parents. As fresh KRH-2 hybrid seeds were produced in every season and which were employed for DHL production, therefore, commercial hybrid was not used in the study. Checking the pollen fertility of the newly developed test-cross derived hybrids is one of the routinely followed procedures for checking the fertility restoration in novel hybrid. In this study, the pollen of freshly produced KRH-2 hybrid is expected to be completely fertile as KMR-3R (its donor parent) is an elite restorer. To validate the success of crosses made in every season and to validate complete fertility restoration in fresh KRH-2 hybrid, the pollen fertility test was done. We did not have any mechanism to avoid the plating of sterile anthers as most of the anthers were fully fertile.

As opined by Balachandran *et al*., (1999), the androgenic response was genotype specific, so there was a necessity to optimize the anther culture conditions for every genotype. Therefore, as a part of optimization of the anther culture conditions for the hybrid KRH-2 and its parents, many media-hormonal combinations were tried. Out of many anther culture protocols published by this group, the highest androgenic response was observed when protocol enlisted in Balachandran *et al*., (1999), was followed.

Several factors are reported to influence the anther culture response in crops. The process of androgenesis is genotype specific (Kaushal *et al*., 2015). Other factor that significantly influences the successful regeneration of fertile plants through androgenesis is the nutrient medium employed for callus induction and plant regeneration. Our study clearly demonstrated that the anther culture response is significantly dependent on the interaction between the genotype and media and not a single factor is responsible for bringing out the best anther culture response. Similar observations have been made in previous studies (Chen, 1991; Khalequzzaman *et al*., 2005; Bagheri and Jelodhar, 2008). Through the present study, we optimized a few components of anther culture media and established that N6 medium (Chu *et al*., 1975) was the most suitable for callus induction from anthers of KRH-2. This is in congruence with few earlier reports (Mishra *et al*., 2011; Mishra *et al*., 2015; Naik *et al*., 2016).

The androgenesis of the parents was undertaken in order to assess the difference between the parents and the hybrid for androgenic response. Further, the magnitude of difference between the parents was used to gauge KRH-2 hybrid's anther culture response. As opined by Kaushal *et al*., (2014), the utility of parents for anther culture along with the hybrid is useful in knowing which of the two parent's gene (s) contribute for hybrid's higher androgenic response. Among the two parents, IR58025B showed higher callus induction percentage (16.7%) than KMR-3R (12.1%), while the callus induction in the hybrid, KRH-2, was observed to be 22.4%. The highest green plant regeneration percentage in the optimized MS medium (MS-5) was observed in IR58025B (19.1%), followed by KMR-3R (15.6%) while the hybrid showed 25.4% **(Table 1).** The F_1 hybrid recorded a higher callus induction and green plant regeneration response than both the parents. Similar results were reported by Herath and Bandara (2011).

Plant growth regulators have been categorically proved to significantly affect the callus induction and green plant regeneration phases of anther culture (Ball *et al*., 1993). We demonstrated that an optimum concentration of 2 mg L^{-1} of 2,4-D and 0.5 mg L^{-1} of kinetin promoted callus induction efficiency as shown earlier by Mishra *et al*., (2015) and Naik *et al*., (2016). The same concentration of 2,4-D and kinetin was used by Hooghvrost *et al*., (2018) for the development of DHLs.

In addition to callus induction, regeneration of green plants is another vital step in anther culture. In this study, the green plant regeneration was carried out in MS medium supplemented with BAP, kinetin and NAA. Optimum concentration of BAP, kinetin and NAA for successful shoot regeneration in MS-5 medium was 2.5, 0.5 and 1.5 mg L^{-1}. As observed by Naik *et al*., (2016), the increase in the concentration of BAP from 1.5 to 2 mg L^{-1} decreased the rate of shoot regeneration from anther calli by 1.5 times. Similar observation was reported by Kaushal *et al*., (2014), where higher concentration of BAP (2.5 mg L^{-1}) decreased the frequency of shoot regeneration. In the present study, we observed an increment in rate of shoot regeneration by 23% when BAP concentration was increased from 2 to 2.5 mg L^{-1}. Similarly, the rate of primary root formation in regenerated shoots was observed to increase by 17% when the concentration of NAA was increased from 1 to 1.5 mg L^{-1} in our study against the earlier reports (Kaushal *et al*., 2014; Mishra *et al*., 2015). Thus, based on the results of the present study it is clear that N6 medium is the most suitable for successful callus induction at optimized concentrations of 2,4-D (2 mg L^{-1}), kinetin (0.5 mg L^{-1}), maltose (30 g L^{-1}) and silver nitrate (10 mg L^{-1}) and for plant regeneration MS medium with 2.5 mg L^{-1}, BAP, 0.5 mg L^{-1} kinetin and 1.5 mg L^{-1} NAA, was confirmed to be optimum. The Duncan's multiple range test (DMRT) showed the significant differences among the genotypes under study which was due to effect of different media-hormonal compositions on callus induction and green plant regeneration phases.

The initial determination of the ploidy of the regenerated plants was determined based on plant morphology and its fertility/sterility status. Later, the doubled haploidy of the fertile plants was confirmed using a set of hyper-variable SSR markers. A thorough analysis of the DHLs for their ploidy status was done by employing hyper-variable SSR markers (co-dominant in nature, therefore suitable for the analysis). Three different categories of the doubled haploids (DH) are reported to be regenerated from microspores. Those diploid plants which are produced as a result of chromosome doubling (normal reduced gametes), the second being the somatic cell, particularly anther wall induced diploids and the third, are produced from unreduced gametes (Chani *et al*., 2000). In 12.67% of the calli that were induced, it was observed that they showed amplification identical to both the parents IR58025A and KMR-3R when assessed with a set of hyper-variable SSRs indicating their origin from the somatic (2n) anther cell wall whereas 68.25% of the induced calli showed allelic amplification of either of the two parents (as shown in Figure 4) indicating their origin from microspores (n). Further, the utility of the SSR markers for the identification of true doubled haploid has been well-documented in rice and in other crop systems (Tang *et al*., 2006; Shahid *et al*., 2013; Wu *et al*., 2015). The analysis of the DHLs with carefully selected hyper-variable SSR markers *viz*., RM15326 (chr.3), RM15679 (chr.3), RM19410 (chr.6), RM21539 (chr.7), RM22554 (chr.8), RM22837 (chr.8), RM6925 (chr.8), RM23958 (chr.9) (www.gramene.org; Jaikishan *et al*., 2009; Rajendrakumar *et al*., 2009) demonstrated that these markers for multiple loci in different DHLs indicating the regenerants to be true DHLs. Moreover, these markers showed clear amplification and true segregation of alleles of both the parents of the hybrids which was sufficient to show that doubled haploids were indeed true and fertile. Our observations are in accordance with Zhang (1989), Olufowote *et al*., (1997) and Tang *et al*., (2006).

Pertaining to the polyploid plants, we observed that these plants were unusually tall and more importantly were completely sterile. Such plants were discarded after their morphological assessment at maturity stage. The utility of the SSR markers was done to identify/differentiate those doubled haploids which were produced as a result of spontaneous chromosome doubling. The other two groups of doubled haploids *viz*., those induced from anther cell wall (somatic in origin) and those from unreduced gametes were of least interest to us. Therefore, as can be inferred

from Figure 4, only those doubled haploids which showed an allelic amplification to either of the two parents were considered to be true DHLs. Therefore, determination of polyploidy using SSR markers was not possible and polyploids were discarded based on their plant morphology and sterility, upon their maturity.

Agro-morphological evaluation of 10 KRH-2 derived DHLs showed a range of variation for prominent agronomic traits such as DFF, YLD, TGW and PT. Negligible variation was observed within the population for successive generations indicating a high level of uniformity/stability. The agro-morphological data of the 10 DHLs along with the parents, hybrid KRH-2 and varietal checks presented in **Table 4** is the three seasons' mean for all the yield related traits when ten plants (replicates) were considered. As the standard error (SE) of the mean data of each DHLs for yield related traits was observed to be below 1, we inferred that there was negligible agro-morphological variation within the individuals (replicates) of each DHL across three consecutive seasons demonstrating the stability of these DHLs.

Iso-cytoplasmic restorer lines are developed as a process of selfing the promising hybrids. The process of androgenesis or doubled haploidy is akin to selfing as the haploid (n) genome of microspores of a hybrid undergoes endo-reduplication to produce diploid (2n) plants on proper stimulation. The fertile DH plants possess genomes from either of parents but not from both. Iso-cytoplasmic restorer lines carry male sterile cytoplasm which is similar to cytoplasmic male sterile (CMS) lines, thus reducing the cyto-nuclear conflict (Kumar *et al*., 2017). Moreover, similar type of cytoplasm that iso-cytoplasmic lines and CMS lines have rules out the necessity of test crosses for assessment of restoration potential. The genetic relationship among most of the popular Indian hybrids is termed as half-sib, having 50 per cent in commonality, as IR58025A being the common CMS line. Therefore, the iso-cytoplasmic restorer lines serve as a better tool for restorer line diversification. We did not observe any recombinants among the regenerated DHLs as no heterozygous loci were amplified with a set of eight hyper-variable SSRs indicating that the DHLs were regenerated as a result of chromosomal doubling of normal reduced gametes.

Evaluation of DHL population for three consecutive seasons indicated that the lines, SMB-1, SMB-2 and SMB-3 showed significantly higher yield levels compared to the hybrid and the parental lines. SMB-1 showed yield increment of 19.6%, followed by SMB-2 (10.4%) and SMB-3 (7.3%) as compared to KRH-2. As the number of productive tillers and biomass in these three high yielding DHLs were slightly more than KRH-2, the yield increase may be justified. Also, slightly more number of GP and higher TGW of SMB-1 (RP6301-189-17-2) than KRH-2 might have contributed to the latter's higher percentage of YLD heterosis. All the hybrid and varietal checks used in this study are of medium duration, high-yielding with wide cultivation across the country. These commercially released hybrids showed a mean grain yield of 5-7 t /ha with an average yield increment of 15-20% over the checks of the same duration. Our results demonstrated that SMB-1, SMB-2 and SMB-3 showed a positive standard heterosis with respect to KRH-2 which is a high yielding and one of the popular hybrids in India. Further, SMB-1 showed the highest percentage of standard heterosis of 19.61% over KRH-2 which may be considered for commercialization.

Conclusions

The present study analyzed the effect of four different callus induction media and percentage of callus induction in the elite rice hybrid, KRH-2 and established that N6 medium was the most efficient providing for higher green plant regeneration (when plant regeneration was carried out in MS regeneration medium) and lowest albino frequency. Using this optimized protocol, a total of 125 DHLs were developed in this study. Three promising DHLs (SMB-1 (RP6301-189-17-2), SMB-2 (RP6300-188-23-5) and SMB-3 (RP RP6301-189-27-3-2) have been identified which recorded equivalent or superior yield and agro-morphological parameters as compared to KRH-2.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Yield maximization through different sources of nutrients in summer rice

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Abstract

A field experiment was conducted during dry (*boro*) season of 2015-16 and 2016-17 at Rice Research Station, Chinsurah, Hooghly, West Bengal to evaluate the performance of improved nutrient sources in maximizing the productivity of summer rice. The results revealed that maximum grain yield (5.66 t/ha) was achieved through application of recommended dose of fertilizer (RDF) at 130-65-65 kg N-P_2O_5-K_2O/ha in combination with Tabsil at 5.0 kg/ha in two equal splits at 25 and 50 days after transplanting (DAT), on par with RDF in combination with Vigore as basal application (625 g/ha) and also as foliar spray (1.25 g/L) at panicle initiation (5.47 t/ha). Different combinations of RDF with improved sources of nutrient supplements (Vigore and Tabsil) exhibited economic yield advantages to the extent of 2.93-10.55, 3.94-11.64 and 54.09-65.50% over RDF, farmers' fertilizer practice and absolute control, respectively. Supply of nutrients in required quantities through the combinations of these nutrient sources facilitated balanced crop nutrition as well as improved nutrient use efficiency resulting in maximum grain yields due to higher values of growth and yield attributes in summer rice.

Key words: Nano fertilizer, Nutrient use efficiency, Productivity, Summer rice, Tabsil, Vigore, Yield advantage.

Introduction

Rice is an important staple food of more than half of the global population. Although India is the second largest producer of rice after China and contributes about one-fifth of global rice production, rice productivity is still constrained by several factors including climate change, acute water and labour shortage at the time of critical farm operations, escalating input costs, minimal or nominal profit margin *etc*. Besides all these, yield stagnation due to soil health problem coupled with inappropriate supply of nutrients or crop growth supplements is one of the major constraints to sustain rice production in the country. Although conjunctive use of organic and inorganic sources of nutrients is essential to sustain soil health as well as crop productivity, the availability of organic manures is becoming scarce as a result of urbanization and reduction in animal wealth (Subramanian and Tarafdar, 2011). Improved sources of nutrients *viz*. Vigore and Tabsil may be explored for improving crop health and maximizing rice productivity (ICAR-IIRR, 2018; Bhowmick *et al*., 2019a and 2019b). Vigore is a nano-technological product developed from naturally occurring substances in plants, minerals or other materials by using infinite decimal doses and with the process of denomination and potentiating which increases the product effectiveness and helps in removing toxicity (Kumari *et al*., 2016; Ramachandra and Sowmyalatha, 2020). Tabsil is a silica effervescent tablet fertilizer with 100% water

soluble silicon nutrient that can be used as foliar spray and/or soil application for improving crop health and minimizing infestation of insect pests and diseases (ICAR-IIRR, 2018). To address the sustainability of rice-based cropping systems, precision farming has been a long-desired goal toward maximizing crop yields while minimizing the use of chemical inputs through monitoring environmental variables and applying targeted actions (Manjunatha *et al.*, 2016). Keeping these perspectives in view, the present study was undertaken to evaluate the performance of different improved nutrient supplements in enhancing the grain yield of summer rice and to compare yield performance of field-specific fertilizer materials with the existing blanket recommendation for rice crop nutrition.

Materials and Methods

Experimental site and season

A field experiment was conducted during dry (*boro*) season of 2015-16 and 2016-17 at the Rice Research Station, Chinsurah, Hooghly, West Bengal, located at $22^{0}52'$ N latitude and $88^{0}24'$ E longitude with an altitude of 8.62 m above mean sea level. The experimental soil was clay loam having pH 6.90, EC 0.5 dS/m, organic carbon 1.18%, available N 357 kg/ha, available P_2O_5 132 kg/ha and available K_2O 410 kg/ha.

Experimental design and treatment details

The experiment comprising of eight levels of nutrient management practices was laid out in a randomized complete block design with three replications. The treatments included recommended dose of fertilizer (RDF, 130-65-65 kg N-P_2O_5-K_2O/ha) alone, and in combination with Vigore (625 g/ha only as basal), Vigore (625 g/ha as basal + 1.25 g/L as foliar spray at panicle initiation), Tabsil (2.5 kg/ha) at 25 days after transplanting (DAT), Tabsil (5.0 kg/ha at 50 DAT) and Tabsil (2.5 kg/ha, each at 25 and 50 DAT), besides farmers' fertilizer practice (FFP, 120-60-60 kg N-P_2O_5-K_2O/ha) and control (no fertilizer). In the treated plots (5 m × 4 m in size), full doses of P_2O_5 and K_2O along with one-fourth of total N were applied as basal at the time of transplanting whereas the remaining half and one-fourth of total N were applied at active tillering (AT) and panicle initiation (PI) stages, respectively.

Crop establishment

Rice variety '*Triguna*' (IET 12875) was sown in third week of January and last week of December in 2016 and transplanted at the seedling age of 29-34 days in third and first week of February during 2015-16 and 2016-17, respectively **(Table 1).** Young seedlings at 2-3 leaf stage were carefully transplanted at a spacing of 20 cm × 15 cm. The crop was raised with other recommended package of practices (Bhowmick *et al*., 2012 and 2013) and harvested in last and third week of May during 2015-16 and 2016-17, respectively (**Table 1**).

Table 1: Calendar of major field operations in summer rice during dry season of 2015-16 and 2016-17

Date	2015-16	2016-17
Sowing	January 20, 2016	December 30, 2016
Transplanting	February 18, 2016	February 02, 2017
Harvesting	May 30, 2016	May 19, 2017

Data collection and statistical analyses

Twelve hills were randomly sampled from each plot for determining growth attributes *viz.* plant height and tiller number/m^2 at 30 DAT, 60 DAT and harvest, and yield attributes (panicle number and weight) at harvest. Plant samples collected at harvest were oven dried at 70° C ± 1° C till a constant weight was achieved. Number of panicles/hill under each treatment was recorded from twelve hills by visual counting and their average was multiplied by the number of hills/m^2. Panicle weight **(g)** was also determined from the same twelve hills used for other parameters. Grains were harvested, dried and weighed, and grain weight was adjusted to a moisture content of 0.14 g H_2O/g fresh weight. Grain and straw yields were recorded for each plot separately at harvest and converted

into t/ha. Collected data were subjected to statistical analyses as per the procedures outlined by Gomez and Gomez (1984).

Results and Discussion

Effect of treatments on crop growth

Year-wise as well as pooled data on plant height at 30 DAT revealed that the plants grown under RDF + Vigore (either as basal application only or as basal and foliar application both) and RDF + Tabsil (25 DAT) were significantly superior to those established with only RDF or FFP as well as control plots (**Table 2)**. With the advancement of crop growth at 60 DAT, application of RDF in combination with Tabsil (25 and 50 DAT) exhibited maximum plant height although it remained at par with RDF in conjunction with either Vigore (either basal only or basal and foliar both) or Tabsil (25 or 50 DAT) as revealed from second year data and pooled data. Application of RDF in combination with Vigore or Tabsil superseded the other treatments in respect of plant height at harvest, when the plants grown under RDF + Tabsil (25 and 50 DAT) recorded maximum plant height, followed by RDF + Vigore (basal and foliar both) and RDF + Vigore (basal only) according to the results of two years and their pooled data. Being a nano-technology product, Vigore possibly helped in boosting absorption and transportation of micro and macro nutrients, thereby maintaining hormonal balance in plants (ICAR-IIRR, 2018) and registering more plant height. As reported, Vigore also might provide the entire natural mineral required for the fast development of roots that could grow deeper and wider into the soil (ICAR-IIRR, 2018). Subramanian and Tarafdar (2011) and Rai and Rawat (2014) suggested using nano-fertilizers as a strategy to regulate the smart release of nutrients that commensurate with crop requirement, thereby increasing nutrient use efficiencies and preventing environmental hazards. .

Table 2: Effect of treatments on plant height of summer rice during dry season of 2015-16 and 2016-17

Treatment	Plant height (cm)								
	30 DAT			60 DAT			Harvest		
	2015-16	2016-17	Pooled	2015-16	2016-17	Pooled	2015-16	2016-17	Pooled
RDF	40.66	44.60	42.63	65.90	71.50	68.70	92.62	97.00	95.31
RDF + Vigore (basal)	45.30	52.10	48.70	70.37	74.55	72.46	96.06	100.00	98.03
RDF + Vigore (basal + foliar)	45.50	51.30	48.40	72.00	75.11	73.56	96.40	100.20	98.30
RDF + Tabsil (25 DAT)	41.68	49.60	45.64	69.70	72.70	71.20	94.60	97.24	95.92
RDF + Tabsil (50 DAT)	40.41	45.40	42.91	68.82	73.90	71.36	94.45	97.75	96.10
RDF + Tabsil (25+50 DAT)	41.62	51.00	46.31	74.65	76.50	75.58	96.80	100.54	98.67
FFP	39.00	42.90	40.95	64.70	67.94	66.32	92.10	93.43	92.77
Control	33.08	40.20	36.64	61.74	64.60	63.17	87.00	89.40	88.20
LSD (P=0.05)	4.36	4.88	5.25	5.70	6.13	5.68	3.86	5.30	4.10
C.V. (%)	7.49	6.34	6.90	8.61	9.87	9.35	8.29	8.15	7.46

DAT: Days after transplanting; FFP: Farmers' fertilizer practice; RDF: Recommended dose of fertilizer.

Application of RDF along with either Vigore (as basal only or basal and foliar both) or Tabsil (25 or 25 +50 DAT) initially recorded more number of tillers/m^2 at 30 DAT than those with other treatments in both the years (**Table 3**). Significantly more number of tillers/m^2 was recorded under RDF + Tabsil (25+50 DAT) and RDF + Vigore (basal + foliar) as reflected from year-wise data and their pooled values at 60 DAT and harvest **(Table 3).** Kumar *et al*., (2019) also reported higher values of growth attributes with the application of 100% RDF through inorganic fertilizers + Vigore at 625g/ha in wet season. With the content of a wide range of natural minerals, enzymes, vitamins, nutrients and antioxidant in nano form, Vigore helped to supplement the nutrients required for complete growth and healthy development of rice plants (Kumari *et al*., 2016), besides activating enzymatic activities inside the plants (Manjunatha *et al.,* 2016). As reported, Tabsil might help in developing healthy growth of stems and leaves through enhanced uptake of potassium and phosphorus, and thereby reducing the chances of lodging (ICAR-IIRR, 2018). Application of silicate effervescent tablet was reported to activate the enzymatic activity within the plants, and thereby promoting more number of tillers/m^2 (ICAR-IIRR, 2018). Savant *et al*., (1997) detailed the definitive need of silicon (Si) management for sustainable rice production. Compared with data at 30 and 60 DAT, tiller number/m^2 was, however, found to decrease at harvest, irrespective of treatments. Poor tillering was recorded in control plots, which might be due to an impaired transpiration in Si-deficient rice plants as earlier reported by Bergmann (1992).

Table 3: Effect of treatments on tillering profile of summer rice during dry season of 2015-16 and 2016-17

Treatment	Number of tillers/m^2								
	30 DAT			60 DAT			Harvest		
	2015-16	2016-17	Pooled	2015-16	2016-17	Pooled	2015-16	2016-17	Pooled
RDF	242	252	247	412	434	423	391	429	410
RDF + Vigore (basal)	255	274	265	427	457	442	418	438	428
RDF + Vigore (basal + foliar)	256	271	264	429	470	450	425	445	435
RDF + Tabsil (25 DAT)	249	262	256	415	449	432	413	426	420
RDF + Tabsil (50 DAT)	238	256	247	414	451	433	408	433	421
RDF + Tabsil (25+50 DAT)	246	265	256	435	479	457	429	451	440
FFP	236	250	243	409	430	420	387	403	395
Control	216	226	221	369	381	375	340	362	351
LSD (P=0.05)	10.26	10.79	12.30	11.75	10.98	12.61	13.38	11.83	12.77
C.V. (%)	13.18	12.31	13.50	12.90	12.70	12.36	12.35	14.60	12.81

DAT: Days after transplanting; FFP: Farmers' fertilizer practice; RDF: Recommended dose of fertilizer.

Applied at low dosage, Tabsil with the highest percentage (12%) of orthosilicic acid (H_4SiO_4) could increase the P utilization capacity and help in minimizing water loss as well as transpiration since it could easily penetrate the leaves and form a thick silicate layer on the leaf surface, thereby preventing lodging, pest and disease infestation, and enhancing crop growth and yield. Being not very mobile within rice plant, a continued supply of Si would be required during practically all growth stages for healthy and productive development of the plant. Since the active Si absorption by rice was expected to start after tillering or stem elongation stage (Savant *et al*., 1997), the most crucial time of Si application through Tabsil for yield maximization might be during reproductive stage although continued supply of Si element since beginning of crop establishment would be more advantageous. Hence, Tabsil application at 25 and 50 DAT was found to be more effective than that applied only at 25 or 50 DAT in addition to the RDF. Because of high Si requirement, rice crop responded well to Tabsil application. Ma *et al.,* (1989) reported the most remarkable effect of Si supply on the growth and development of rice crop plants at their reproductive stage.

Effect of treatments on yield attributes

Application of RDF + Tabsil (25+50 DAT) significantly registered more number of panicles/m^2 with more panicle weight, on par with RDF plus Vigore or Tabsil during both the years (**Table 4)**. Ramachandra and Sowmyalatha (2020) also reported better results with the application of RDF along with Tabsil 100% Silicate Tabs (2.5 kg/ha at 25 DAT and another 2.5 kg/ha at 50 DAT). Higher values of yield attributes under these treatments were ascribed to improved crop growth owing to better nutrient utilization and abalanced nutrition. Savant *et al*., (1997) reported more (several-fold greater) absorption of Si by rice plants from soil than those of macronutrients (N, P, K, S, Ca and Mg). Even Si was reported to interact with other native or applied nutrients, thereby inducing resistance or tolerance in rice plants to different biotic and abiotic stresses including lodging (Savant *et al*., 1997). Control and FFP plots remained inferior to these treatment combinations in terms of major yield attributes. Pooled data also reflected significantly the highest panicle number and panicle weight with RDF + Tabsil at 25 and 50 DAT (350/m^2 and 3.39 g), followed by RDF + Vigore as basal and foliar (338/m^2 and 3.26 g) and RDF + Vigore as basal (334/m^2

Table 4 Effect of treatments on major yield attributes of summer rice during dry season of 2015-16 and 2016-17

Treatment	Panicles/m^2			Panicle weight (g)		
	2015-16	2016-17	Pooled	2015-16	2016-17	Pooled
RDF	309	319	314	3.18	3.08	3.13
RDF + Vigore (basal)	333	334	334	3.24	3.24	3.24
RDF + Vigore (basal + foliar)	340	336	338	3.26	3.25	3.26
RDF + Tabsil (25 DAT)	329	326	328	3.23	3.20	3.22
RDF + Tabsil (50 DAT)	325	332	329	3.20	3.21	3.21
RDF + Tabsil (25+50 DAT)	348	352	350	3.40	3.37	3.39
FFP	299	303	301	3.15	3.20	3.18
Control	230	236	233	2.96	2.98	2.97
LSD (P=0.05)	40.40	44.43	39.25	0.21	0.24	0.23
C.V. (%)	7.34	8.00	7.60	6.25	4.27	6.12

DAT: Days after transplanting; FFP: Farmers' fertilizer practice; RDF: Recommended dose of fertilizer.

and 3.24 g) and RDF + Tabsil at 25 or 50 DAT (328-329/m^2 and 3.21-3.22 g), compared to RDF (314/m^2 and 3.13 g), FFP (301/m^2 and 3.18 g) and control (233/m^2 and 2.97 g), respectively. More number of effective tillers/m^2 in RDF + Tabsil was obviously due to Si fertilization through Tabsil, compared with RDF alone, conforming to the report of Liang *et al.*, (1994). Even Si imposed a synergistic effect through positive interaction with applied N, P and K fertilizers (Savant *et al*., 1997).

Effect of treatments on crop productivity

Application of RDF in combination with Tabsil (2.5 kg/ha, each at 25 and 50 DAT) maximized the productivity of both grain and straw (5.66 and 7.02 t/ha), and was found equally effective as RDF (**Table 5**) in combination with Vigore as basal application + foliar spray (5.47 and 6.80 t/ha) or as basal application only (5.37 and 6.69 t/ha). Similar trend was observed in both the years of study. As with the combinations of different nutrient sources, economic yield advantages were to the extent of 2.93-10.55, 3.94-11.64 and 54.09-65.50% over RDF, FFP and absolute control, respectively. Supply of nutrients in required quantities through the combinations of nutrient sources facilitated balanced nutrition of rice crop, which resulted in enhanced grain yields due to higher values of yield attributes. Use of nanofertilizer was reported to increase nutrient use efficiency by three times, reduce soil toxicity, impart stress tolerance to crop plants, improve soil aggregation and minimize the potential negative effect associated with injudicious use of chemical inputs (Manjunatha *et al*., 2016). Being a superior organic yield enhancer, Vigore possibly helped in boosting absorption and transportation of micro and macro nutrients, besides maintaining hormonal balance in plants (Bhowmick *et al*., 2019a). Mukhopadhyay (2014) reported substantial improvement in input use efficiency by applying nanomaterials which did not remain confined as a point source application, but spread throughout the field. Being eco-friendly and non-toxic for human, animal, soil and plant, Vigore might be advocated as an important nutrient supplement in addition to RDF (Kumari *et al.*, 2016).

Table 5: Effect of treatments on crop productivity of summer rice during dry season of 2015-16 and 2016-17

Treatment	Grain yield (t/ha)			Straw yield (t/ha)		
	2015-16	2016-17	Pooled	2015-16	2016-17	Pooled
RDF	4.99	5.25	5.12	6.29	6.67	6.48
RDF + Vigore (basal)	5.33	5.41	5.37	6.72	6.66	6.69
RDF + Vigore (basal + foliar)	5.43	5.50	5.47	6.84	6.76	6.80
RDF + Tabsil (25 DAT)	5.28	5.31	5.30	6.65	6.58	6.62
RDF + Tabsil (50 DAT)	5.21	5.33	5.27	6.56	6.61	6.59
RDF + Tabsil (25+50 DAT)	5.69	5.63	5.66	7.17	6.87	7.02
FFP	4.84	5.30	5.07	6.10	6.62	6.36
Control	3.53	3.31	3.42	4.44	4.17	4.31
LSD (P=0.05)	0.39	0.28	0.31	0.51	0.44	0.43
C.V. (%)	9.69	8.31	10.50	11.42	8.11	10.63

DAT: Days after transplanting; FFP: Farmers' fertilizer practice; RDF: Recommended dose of fertilizer.

Kumar *et al*., (2015) reported an improvement in growth and yield attributes along with higher grain yields under 100% RDF + Vigore, which enhanced grain filling percentage by increasing leaf nutrient concentration and photosynthetic rate of flag leaves, and by delaying leaf senescence due to continuous supply of nutrients in sufficient quantity throughout crop growth stages. In anticipation of crop lodging on account of uncertain cyclonic storm at harvesting time, Si application would be an effective strategy for enabling rice plants to acquire necessary mechanical strength against lodging. Comparatively lower levels of grain and straw yields in the FFP plots might be attributed to abysmal utilization of fertilizer nutrients in absence of improved nutrient sources.

Conclusions

The present study clearly showed that use of nano-technological products or improved sources of nutrient supplements in judicious combination with the RDF would be an effective recommendation for enhancing nutrient use efficiency as well as maximizing summer rice productivity.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Response of wet direct-seeded rice to methods of zinc application

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Abstract

A field experiment was conducted at the Research Farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, West Bengal during the wet season of 2018 and 2019 to study the response of direct-seeded rice to methods of Zn application. Results revealed that, growth attributes like plant height, number of tillers m^{-2} and dry matter accumulation were significantly influenced by methods of zinc application. At maturity, higher number of tillers m^{-2} and dry matter accumulation was recorded with split soil application of $ZnSO_4$ which was closely followed by soil application of $ZnSO_4$ + foliar spray of Zn-EDTA. Significantly higher grain yields of 4796 and 4756 kg ha^{-1} were recorded with split soil application of $ZnSO_4$ ha^{-1} compared to 4145 and 4093 kg ha^{-1} in no Zn application during 2018 and 2019, respectively. Improvement in net return was found to the tune of about 29% in split soil application of $ZnSO_4$; and 23% in soil application of $ZnSO_4$ + foliar spray of Zn-EDTA during both the years.

Keywords: zinc, direct-seeded rice (DSR), Zn-EDTA, foliar spray, split application

Introduction

Rice is the staple food of more than half of the world population and meets 15% of protein and 21% of the energy requirement of human population worldwide (Depar *et al*., 2011; McLean *et al*., 2002). For uprooting, carrying and transplanting the seedlings into the field a substantial amount of human workforce is required in conventional transplanting of rice. Availability of human work force in agricultural sector is getting scarce day by day associated with the hike in wage rate due to urbanization and migration of rural daily paid workers. As a suitable alternative, direct-seeded rice (dry and wet) is becoming popular in different parts of India as well as in the world. DSR reduces input requirements and saves time by timely sowing and shortens the crop duration by 7-14 days than transplanted rice and when managed properly, DSR provides grain yield equivalent to transplanting (Gill *et al*., 2014; Saha *et al*., 2020). Dry-direct seeding in rice is strongly advocated when water tables are high or, soils are fine-textured where puddling is not required to slow down water infiltration. Further in conditions where irrigation water availability is not a problem and light soils predominate, wet-DSR is a suitable option to combat labour scarcity (Weerakoon *et al*., 2011).

Rice is generally very sensitive to Zn deficiency particularly in the wet puddle cultivation system (Wissuwa *et al*., 2008). Zinc has important roles in many fundamental biochemical processes in crop plants which include enzyme activation, protein synthesis, starch, auxin and nucleic acid metabolism, and development of pollen (Marschner, 1995). Soils with low availability of Zn not only reduce crop productivity but also impair the nutritional quality of the produce. Worldwide, the most frequent and

widespread micronutrient deficiency problem in crop plants is the deficiency of zinc (Alloway, 2008). Continuous removal of Zn from soils of cereal based cropping systems without adequate Zn supplementation has depleted soils resulting in 49% of Indian soils being Zn deficient (Behera *et al*., 2009). Soil testing by different agencies all over India also indicates the increasing trend of Zn deficient areas and it is estimated that, zinc deficiency in Indian soils is likely to increase up to 63% by the year 2025 (Singh, 2008). The general recommendation to address Zn deficiency in rice crop is the basal application of zinc sulphate in the soil. Foliar sprays of Zn, the combined application of soil+foliar application, seed coating and priming of seeds may also be effective alternatives to combat Zn deficiency in rice, particularly in Zn deficient soils. Since, comparative study on different methods of Zn application comparing soil, foliage and seed treatments of Zn in rice particularly in direct seeding of rice is lacking, an experiment was conducted to study the effect of Zn application methods in direct-seeded rice.

Materials and Methods

The field experiment was conducted during wet (*kharif*) season of 2018 and 2019 at Uttar Banga Krishi Viswavidyalaya, Pundibari, West Bengal, India (26° 24′ 03″ N and 89° 23′ 13″ E, 43 m above the mean sea-level). The amount of rainfall received during the crop growing season (July to November) was 1414 mm and 1911 mm in the year 2018 and 2019, respectively. The soil was sandy loam in texture and acidic in nature with 58.2 % sand, 29.2% silt and 12.6% clay, low in available N (107.8 kg ha^{-1}), high in available P (34.2 kg ha^{-1}), medium in available K (127.6 kg ha^{-1}), pH of 6.14 and had 0.62 mg kg^{-1} of DTPA (diethylene triamine penta acetic acid)-extractable Zn in soil.

The experiment was conducted under puddle soil, with direct sowing of 'MTU 1010' as test variety and laid out in randomized block design with three replications and eight methods of zinc application i.e. T1- no zinc, T2- soil application of 5 kg Zn ha^{-1} as basal through $ZnSO_4.7H_2O$, T3- split soil-application of 2.5 kg Zn ha^{-1} each at basal and at booting stage through $ZnSO_4.7H_2O$, T4- seed coating (1% Zn), T5- seed coating (2% Zn), T6- seed coating (3% Zn), T7- soil application of 2.5 kg Zn ha^{-1} as basal through $ZnSO_4.7H_2O$ + foliar spray of Zn-EDTA at booting stage; and T8- foliar spray of Zn-EDTA at maximum tillering and at booting stage.

Main field for direct seeded rice (DSR) was prepared by deep ploughing once followed by harrowing. A fine puddled structure was achieved by two operations of tractor drawn rotavator with planking after applying an ample amount of irrigation. Two-three seeds were dibbled manually at 20 cm×15 cm spacing in a puddled field with no or negligible standing water on the surface. The recommended dose of 60-30-30 kg $N\text{-}P_2O_5\text{-}K_2O$ ha^{-1} was applied. Zinc fertilization was implemented treatment wise. Zinc sulphate hepta-hydrate and Zn-EDTA were applied in soil and foliar application, respectively. Diseases and insects were effectively managed by integrated measures. Weed control was achieved by a combination of post - emergence herbicides and hand-weeding.

Observations were recorded on growth and yield attributes. The plant height of rice was measured from the base of the plant to the tip of the tallest leaf. Five hills were selected before the harvest of the crop for recording total dry-matter accumulation by the rice crop. Plant samples were air-dried and further dried in a hot air oven at 60±2°C till constant weight was achieved. The number of tillers was noted by counting from the sampling unit at harvest. After threshing of the produce obtained from one square meter (or net plot area) of harvested area, the grain was cleaned, dried and weighed. The grain yield was finally adjusted at 14% moisture and expressed in kg ha^{-1}. Then weight of the straw was also recorded and was adjusted to oven dry weight. Gross and net returns

were calculated based on the grain and straw yield, the minimum support price of grain and prevailing market prices of rice straw in the respective year. B:C ratio or returns per rupee invested was calculated by dividing the gross return by cost of cultivation.

The data obtained from the study were analyzed statistically using the F-test, as per the procedure given by Gomez and Gomez (1984). Critical difference (CD) at P=0.05 was used to determine the significance of the difference between treatment means.

Results and Discussion

Growth and yield attributes

At maturity, two foliar applications with Zn-EDTA at maximum tillering (MT) and booting, and split soil application of $ZnSO_4$ showed greater plant height followed by soil application of $ZnSO_4$ + foliar spray of Zn-EDTA (**Table 1**). Maximum number of tillers m^{-2} was recorded with split soil application (370 and 367) followed by soil application of $ZnSO_4$ + foliar spray of Zn-EDTA (366 and 362) closely followed by two Zn-EDTA sprays at MT and booting (366 and 357). , Split soil application of $ZnSO_4$, soil application of $ZnSO_4$ + foliar spray of Zn-EDTA and foliar application of Zn-EDTA at MT and booting showed better dry matter accumulation (DMA) compared to other methods. The improvement in growth attributes might be due to presence of the zinc as it has an important role in many enzyme systems in rice. These results may also be attributed to the adequate supply Zn through different methods of Zn application and due to higher availability and translocation of nutrients during stages of growth to accelerate the enzymatic activity and auxin metabolism (Alloway, 2008; Sudha and Stalin, 2015).

The number of panicles m^{-2} was significantly influenced by methods of zinc application. A higher number of panicles m^{-2} (293 and 286 in 2018 and 2019, respectively) were observed with split soil application of $ZnSO_4$ followed by $ZnSO_4$ application at basal+ Zn-EDTA spray at booting (287 and 284) and two foliar applications of Zn-EDTA (284 and 281). Higher number of filled grains per panicle was also recorded with split soil application of $ZnSO_4$ and soil application of $ZnSO_4$ + foliar Zn-EDTA

Table 1: Effect of methods of Zn application on growth attributes of rice at maturity

Treatment	Plant height (cm)		No. of tillers m^{-2}		DMA (g m^{-2})	
	2018	2019	2018	2019	2018	2019
T1-No zinc	103.6	100.5	307.4	303.0	999.8	981.1
T2-$ZnSO_4$ application as basal	116.2	113.7	353.0	344.4	1151.2	1111.9
T3-Split application at basal and booting	119.1	115.7	370.4	366.7	1211.8	1179.5
T4-Seed coating 1% Zn	112.5	109.5	337.0	325.7	1090.6	1049.5
T5-Seed coating 2% Zn	114.2	110.6	344.4	334.1	1118.0	1090.8
T6-Seed coating 3% Zn	116.5	112.2	351.9	344.1	1132.0	1100.2
T7-$ZnSO_4$ as basal+ Zn-EDTA at booting	118.5	117.6	366.2	361.7	1200.0	1168.4
T8-Zn-EDTA spray at MT & booting	119.3	117.2	365.6	357.4	1187.2	1162.8
SEm±	2.8	2.7	11.1	12.0	36.3	36.2
CD (P=0.05)	8.5	8.3	33.5	36.5	110.0	109.9

DMA = dry matter accumulation

spray during 2018 and 2019 (**Table 2**), respectively. Different Zn management methods increased per cent filled grain per panicle compared to no Zn application and the highest fertility percentage was recorded with the application of 2.5 kg Zn through $ZnSO_4.7H_2O$ as basal soil application + foliar sprays of Zn-EDTA at booting. Improvement in different yield attributes of rice with the application of Zn might be due to sufficient supply of zinc that might have increased the uptake and availability of other essential nutrients. Higher uptake of Zn as a result of Zn application which resulted in higher biomass accumulation ultimately showed improved yield attributing characters (Shivay *et al*., 2008)

Yield and harvest index

The split soil application of $ZnSO_4$ was found statistically at par in terms of grain yield with $ZnSO_4$ application at basal+ Zn-EDTA spray at booting, foliar application of Zn-EDTA at MT and booting, and basal application of 5 kg Zn ha^{-1}. Seed coating 2% (4421 and 4397 kg ha^{-1}) and 3% (4561 and 4512 kg ha^{-1}) with Zn resulted in significantly higher grain yield than no Zn application during both the years (**Table 3**). The highest straw yield (6352 and 6257 kg ha^{-1}) was registered with split soil application of $ZnSO_4$. Data revealed no significant variation of harvest index during both the years of study.

The positive influence of applied Zn on grain and straw yield might be due to its stimulatory effect on many of the metabolic processes of plants (Mandal *et al*., 2009). Significant improvement in the grain yield of rice due to coating of rice seeds with $ZnSO_4$ was recorded by Mondal *et al*., (2020). Increased grain and straw yield recorded in soil + foliar application of Zn as compared to single basal soil application might be due to better absorption of Zn through stomata of leaves in later phase of the crop and initially with soil application which led to higher photosynthesis. These results have close conformity with the findings of Naik and Das (2008).

Table 2: Effect of methods of Zn application on yield attributes of rice

Treatment	Number of panicles m^{-2}		1000-grain weight (g)		No. of filled grains $panicle^{-1}$		% filled grains $panicle^{-1}$	
	2018	2019	2018	2019	2018	2019	2018	2019
T1-No zinc	249.1	241.2	22.25	22.34	74.3	73.2	67.3	70.1
T2-$ZnSO_4$ application as basal	277.2	278.3	22.46	22.50	79.6	78.2	74.5	74.7
T3-Split application at basal and booting	293.1	286.1	22.32	22.32	83.0	78.8	75.2	76.6
T4-Seed coating 1% Zn	267.3	265.8	22.51	22.26	77.1	75.1	70.5	72.4
T5-Seed coating 2% Zn	272.0	272.8	22.33	22.38	77.8	76.1	72.2	73.6
T6-Seed coating 3% Zn	278.5	278.8	22.33	22.30	79.1	76.7	73.4	74.1
T7-$ZnSO_4$ as basal+ Zn-EDTA at booting	287.4	284.0	22.47	22.53	82.7	80.3	76.2	76.6
T8-Zn-EDTA spray at MT & booting	284.3	280.9	22.37	22.45	80.6	78.2	75.0	75.8
SEm±	8.1	8.0	0.67	0.67	1.6	1.3	1.3	1.3
CD (P=0.05)	24.7	24.3	NS	NS	4.7	4.1	4.0	4.0

Table 3: Effect of methods of Zn application on yield and harvest index of rice

Treatment	Grain yield (kg ha^{-1})		Straw yield (kg ha^{-1})		Harvest index (%)	
	2018	2019	2018	2019	2018	2019
T1-No zinc	4145	4093	5642	5589	42.33	42.27
T2-$ZnSO_4$ application as basal	4587	4548	6248	6136	42.34	42.56
T3-Split application at basal and booting	4796	4756	6352	6257	43.02	43.17
T4-Seed coating 1% Zn	4358	4344	5969	5812	42.21	42.77
T5-Seed coating 2% Zn	4421	4397	6011	5943	42.39	42.50
T6-Seed coating 3% Zn	4561	4512	6121	5990	42.71	42.99
T7-$ZnSO_4$ as basal+ Zn-EDTA at booting	4699	4657	6349	6254	42.54	42.69
T8-Zn-EDTA spray at MT & booting	4665	4592	6310	6172	42.50	42.65
SEm±	80	79	127	118	0.74	0.36
CD (P=0.05)	243	241	386	359	NS	NS

Economics

The highest cost of cultivation was incurred in the treatment with two foliar applications of Zn-EDTA at maximum tillering and at the booting stage (**Table 4**). Higher cost of cultivation of Zn-EDTA treatment in comparison to $ZnSO_4.7H_2O$ was due to the higher price of Zn-EDTA (Ghasal *et al*., 2015). Higher gross and net return was obtained with split soil application of $ZnSO_4$ at basal and at booting stage compared to the rest of the treatments. However, soil (2.5 kg Zn ha^{-1} at basal) +foliar application of Zn-EDTA at booting stage was also found with comparable benefits. Among three seed coating treatments, the coating of rice seeds with 3% Zn was found better than other two and resulted in slightly higher net monetary return

Table 4: Effect of methods of Zn application on economic of rice cultivation

Treatment	Cost of cultivation (Rs. ha^{-1})		Gross return (Rs. ha^{-1})		Net return (Rs. ha^{-1})		B:C (Return per rupee invested)	
	2018	2019	2018	2019	2018	2019	2018	2019
T1-No zinc	43298	43583	78176	80993	34878	37411	1.81	1.86
T2-$ZnSO_4$ application as basal	45298	45583	86515	89911	41217	44328	1.91	1.97
T3-Split application at basal and booting	45298	45583	90287	93822	44989	48239	1.99	2.06
T4-Seed coating 1% Zn	43728	44013	82228	85811	38500	41798	1.88	1.95
T5-Seed coating 2% Zn	43888	44173	83387	86931	39499	42758	1.90	1.97
T6-Seed coating 3% Zn	44048	44333	85946	89075	41898	44742	1.95	2.01
T7-$ZnSO_4$ as basal+ Zn-EDTA at booting	45581	45922	88584	92023	43003	46102	1.94	2.00
T8-Zn-EDTA spray at MT & booting	45863	46260	87954	90754	42091	44494	1.92	1.96

compared to that of 5 kg Zn ha^{-1} applied at basal (soil) through $ZnSO_4.7H_2O$. Farooq *et al*., (2018) observed significant improvement in net benefit and B:C ratio with seed coating and foliar application of Zn in dry-DSR system. Higher net monetary benefit from the split application of Zn might be due to higher yield obtained in that treatment along with no additional cost incurred for application. Mandal *et al*., (2009) obtained higher additional net returns when Zn was applied in splits along with the recommended dose of NPK. Naik and Das (2008) also observed higher net profit and cost-benefit ratio with split application of 10 and 20 kg Zn ha^{-1} as $ZnSO_4$, over the corresponding basal applications.

Based on the experimental findings, it can be concluded that, in wet-direct-seeded rice, split soil application of 2.5 kg Zn ha^{-1} each at basal and at booting stage through $ZnSO_4.7H_2O$ was superior among all Zn management treatments in terms of grain yield and economics. However, coating of rice seeds with 3% Zn was found comparable with a single basal application of Zn in terms of grain yield and net return.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Productivity of direct seeded rice in response to various weed management practices and their residual effect on green gram

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Abstract

Field experiment was conducted during 2015-16 and 2016-17 at Agricultural College farm, Bapatla, Guntur, Andhra Pradesh to study the efficacy of sequential application of herbicides in direct sown rice-green gram cropping system. The experiment was laid out in randomized block design with three replications. Though weed free treatment (T_{13}) resulted in higher gross returns during both the years of study (Rs. 114376 and Rs. 124482 ha^{-1} during 2015-16 and 2016-17, respectively) the net returns and return per rupee investment were markedly higher under pre-emergence application of bensulfuron methyl @ 60 g a.i. ha^{-1} + pretilachlor with safener at 500 g a.i. ha^{-1} followed by post-emergence application of azimsulfuron @ 20 g a.i. ha^{-1} at 25 DAS and post-emergence application of metsulfuron methyl and chlorimuron ethyl @ 4 g a.i. ha^{-1} applied at 45 DAS (T_9) during both the years.

Key words: Direct sown rice, post emergence, herbicides, pre emergence, weed management

Introduction

The rice-pulse cropping system is one of the most important agricultural production systems in the Krishna zone of Andhra Pradesh owing to large acreage and production (Singh *et al*., 2017). Rice-pulse cropping sequence is practically feasible, economic, eco-friendly, water saving technology for sustaining soil fertility and rice productivity. The productivity of rice-green gram system is decreasing due to emergence of multi-nutrient deficiencies, building up of soil pathogens and weed flora.

Weeds are major limiting factor in crop production (Buhler, 1992), causing maximum losses amongst crop pests. They reduce the crop yield and deteriorate the quality of produce and hence reduce the market value of the turn out (Arif *et al*., 2006). Weeds compete for available moisture and nutrients, space and light with crop plants, which result in yield reduction (Khan *et al*., 2004). If left uncontrolled, the weeds in many fields are capable of reducing yields by more than 80 per cent (Karlen *et al*., 2002). Appropriate weed management is considered one of the most important prerequisites in direct sown rice systems to ensure high crop yield. Chemical weed management is the most prominent method to manage weeds in direct sown rice because of its selectivity, cost effectiveness and more labour- and time-saving than other weed management practices (Mazid *et al*., 2003). The use of herbicides in rice for controlling weeds has increased significantly over the last several years (FAO, 2002). Since direct sown rice has complex and diverse weed species, no single herbicide will control all weed species. Therefore, a combination of herbicides applied in sequence is needed for effective control of sedges, broadleaves, and grasses. (Maity and Mukherjee, 2008). Several herbicides, with pre emergence activity, such as oxadiazon

and oxadiargyl, have some limitations *viz*., limited window of application timing and an adequate soil moisture requirement at the time of their application (Singh *et al*., 2006). If optimum conditions are not available, post emergence herbicides may be a better option to manage weeds in direct sown rice systems (Mahajan and Chauhan, 2013). Some herbicides may have negative impact on succeeding crops. In view of this, the present experiment was conducted to study the system productivity and economics of rice-green gram cropping system as influenced by sequential application herbicides in direct sown rice

Materials and Methods

A field experiment was conducted during *Kharif* 2015 and 2016 at the Agricultural College Farm, Bapatla, Guntur, Andhra Pradesh. The soil of the experimental site was sandy loam in texture, slightly alkaline in reaction (pH 8.0 and 7.5), low in organic carbon (0.45 and 0.48%), low in available nitrogen (212 and 230 kg ha^{-1}), medium in available phosphorus (17 and 18 kg ha^{-1}) and medium in available potassium (261 and 285 kg ha^{-1}). There were fourteen treatments, as given here under.

Treatments	Dose (g ha^{-1})	Time (DAS)
$T_{1.}$ Pyrazosulfuron ethyl *fb* Azimsulfuron	25 *fb* 20	Pre *fb* Post
$T_{2.}$ Pyrazosulfuron ethyl *fb* Bispyribac-sodium	25 *fb* 25	Pre *fb* Post
$T_{3.}$ Bensulfuron methyl + Pretilachlor with safener *fb* Azimsulfuron	60 + 500 *fb* 20	Pre *fb* Post
$T_{4.}$ Bensulfuron methyl + Pretilachlor with safener *fb* Bispyribac-sodium	60 + 500 *fb* 25	Pre *fb* Post
$T_{5.}$ Oxadiargyl *fb* Azimsulfuron	75 *fb* 20	Pre *fb* Post
$T_{6.}$ Oxadiargyl *fb* Bispyribac-sodium	75 *fb* 25	Pre *fb* Post
$T_{7.}$ Pyrazosulfuron ethyl *fb* Azimsulfuron *fb* Metsulfuron methyl + Chlorimuron ethyl	25 *fb* 20 *fb* 4	Pre *fb* Post *fb* Post
$T_{8.}$ Pyrazosulfuron ethyl *fb* Bispyribac-sodium *fb* Metsulfuron methyl + Chlorimuron ethyl	25 *fb* 25 *fb* 4	Pre *fb* Post *fb* Post
$T_{9.}$ Bensulfuron methyl + Pretilachlor with safener *fb* Azimsulfuron *fb* Metsulfuron methyl + Chlorimuron ethyl	60 + 500 *fb* 20 *fb* 4	Pre *fb* Post *fb* Post
$T_{10.}$ Bensulfuron methyl + Pretilachlor with safener *fb* Bispyribac-sodium *fb* Metsulfuron methyl + Chlorimuron ethyl	60 + 500 *fb* 25 *fb* 4	Pre *fb* Post *fb* Post
$T_{11.}$ Oxadiargyl *fb* Azimsulfuron *fb* Metsulfuron methyl + Chlorimuron ethyl	75 *fb* 20 *fb* 4	Pre *fb* Post *fb* Post
$T_{12.}$ Oxadiargyl *fb* Bispyribac-sodium *fb* Metsulfuron methyl + Chlorimuron ethyl	75 *fb* 25 *fb* 4	Pre *fb* Post *fb* Post
$T_{13.}$ Weed free	-	-
$T_{14.}$ Weedy check	-	-

Note: Weed free condition maintained by employing manual weeding at regular intervals; *fb* – followed by

Pre and post emergence herbicides were sprayed using a knapsack sprayer fitted with a flat-fan nozzle at a spray volume of 500 l ha^{-1}. A seed rate of 50 kg ha^{-1} was adopted. Seeds were weighed separately for each plot and sown in solid rows in the furrows opened by line markers at 25 cm interval in both the years. Recommended dose of fertilizer (120:60:60 kg NPK ha^{-1}) was applied uniformly, entire dose of phosphorous and potassium was applied as basal dose before last ploughing and nitrogen in three equal splits at basal, active tillering and panicle initiation stages. Irrigation comprised of alternate drying and wetting followed by intermittent irrigation at seven days' interval up to 15 days before harvest. Other agronomic and plant protection measures were adopted as recommended during the crop growth. Grain yield was recorded from net plot and converted to grain yield per hectare.

The gross returns were calculated by multiplying the sale prices of rice and green gram with their respective grain and straw yield. The net returns were calculated by deducting the cost of cultivation from the gross returns. Returns per Rupee Investment (B: C Ratio) was calculated as net returns/ cost of cultivation. Rice equivalent yield was worked out by formula stated by Munda *et al.* (2008).

$$\text{Rice equivalent yield} = \frac{\text{Yield of green gram (kg)} \times \text{Price of green gram kg}^{-1}}{\text{Price of rice kg}^{-1}}$$

Cost of herbicides, fertilizers, rice and green gram seed and labour wages during 2015-16 and 2016-17

Herbicide	**Cost (Rs. ha^{-1})**	**Fertilizers**	**Cost (Rs. ha^{-1})**	**Output price (2015-16)**	
Pyrazosulfuron ethyl:	560	Nitrogen through Urea:	1482	Rice grain (Rs. 14.5 kg^{-1})	Green gram grain (Rs. 48.5. kg^{-1})
bensulfuron methyl + pretilachlor:	2425	Potassium through MOP:	773	Rice straw (Rs. 1.0 kg^{-1})	Green gram haulm (Rs. 0.5 kg^{-1})
Oxadiargyl:	819	Phosphorus through SSP:	3075	Output price (2016-17)	
Azimosulfuron:	1691	Total fertilizer cost :	5330	Rice grain (Rs. 15.1 kg^{-1})	Green gram grain (Rs. 52.3. kg^{-1})
Bispyribac-sodium:	2350	Labour wages :	300 d^{-1}	Rice straw (Rs. 1.0 kg^{-1})	Green gram haulm (Rs. 0.5 kg^{-1})
metasulfuron methyl and chlorimuron ethyl:	3800				

Results and Discussion

Total dry matter production of cropping system as a whole as influenced by weed management practices

Biological production potential of the rice-green gram cropping system as indicated by the total day matter production was significantly influenced by green gram crop in the cropping system as well as different weed management practices in rice (**Table 1**).

For the purpose of evaluating production potential of the cropping system, the total biomass produced was computed by adding the dry matter accrual of individual crop in the respective season. Among the different weed management practices imposed on rice, the treatment weed free (T_{13}) registered the highest dry matter production (13793 and 16847 kg ha^{-1}), which was comparable with the treatments T_9 and T_{10} but was superior to the treatments T_3, T_{14}, T_1, T_2, T_5, T_6 and T_{14}. Weedy check (T_{14}) resulted in the lowest dry matter accumulation of rice-green gram sequence.

Better performance of rice-green gram system under the influence of treatment T_{13} (weed free) was mainly due to higher dry matter accrual of both rice and green gram crops in the system. In the present study the first crop rice followed by green gram in the sequence resulted in elevating the biomass yield of the system. These findings conform to the report of Reddy *et al.,* (2017).

Grain yield of rice (kg ha^{-1})

The highest grain yield (5284 and 5455 kg ha^{-1} during 2015-16 and 2016-17, respectively) was recorded under weed free treatment (T_{13}), which was significantly superior to rest of the treatments except treatment T_9, which was comparable to the treatments T_{10}, T_7, T_{11} and T_8. The lowest grain yield (2159 and 2529 kg ha^{-1}) was obtained in untreated i.e. weedy check (T_{14}) plot, significantly lower than any herbicidal treatment. Appropriate weed management in direct sown rice resulted in lower weed density and weed dry matter and higher dry matter accumulation and nutrient uptake by the crop. These results are in agreement with the findings of Yadav *et. al*., (2009), Singh *et al*., (2010), Naseeruddin and Subramanyam (2013), Hossain and Mondal (2014), Rammu Lodhi, (2016), and and Ajay Singh *et al.,* (2017)**.**

Seed yield of green gram (kg ha^{-1})

The seed yield of succeeding green gram crop after rice was non-significant among the treatments during both the years of study (**Table 2**). This indicates that there was no marked difference among the treatments and the impact of herbicides applied to rice. The applied herbicides which sufficiently got degraded in the soil had no residual effect on the dry matter, number of pods as well as seed and haulm yields of green gram. This indicated that different weed management practices applied to rice had no adverse or favourable effect on growth and yield of succeeding green gram crop. Similar results were also reported by Kumaran *et al.,* (2015) that herbicides applied to rice crop had no residual effect on succeeding crops growth and yields.

System Productivity

Economic yield of system productivity comprising rice-green gram presented as rice grain equivalent yield was not distinctly effected by green gram crop in the cropping system as well as weed management practices to rice during both the years of study (**Table 3**). Various weed management practices to rice in rice-green gram sequence exerted profound influence on the economic yield of the system as a whole. Among the weed management practices weed free (T_{13}) realized the highest economic yield in terms of rice grain equivalent in rice-green gram sequence studied, which was however comparable with treatments T_9 and T_{10}. Weedy check to rice crop has resulted in the lowest economic yield of the system during both the years of study.

For the purpose of judging the economic yield potential of rice-green gram system, the yields of green gram were converted into grain equivalent of rice and to this, the rice yield obtained in *kharif* season in respective treatments was added. Weed free (T_{13})

Table 1: Total dry matter production (kg ha^{-1}) and Grain yield (kg ha^{-1}) of rice-green gram sequence as influenced by weed management practices during *kharif* 2015-16 and 2016-17

Treatments	Dose (g ha^{-1})	Time (DAS)	Total dry matter production		Grain yield (kg ha^{-1})			
					Rice		Green gram	
			2015-16	2016-17	2015	2016	2015	2016
$T_{1.}$ Pyrazosulfuron ethyl *fb* Azimsulfuron	25 *fb** 20	Pre *fb* Post	11407	12010	3844	3619	548	632
$T_{2.}$ Pyrazosulfuron ethyl *fb* Bispyribac-sodium	25 *fb* 25	Pre *fb* Post	11057	12225	3604	3521	532	624
$T_{3.}$ Bensulfuron methyl + Pretilachlor with safener *fb* Azimsulfuron	60 + 500 *fb* 20	Pre *fb* Post	12016	13652	4118	4203	556	652
$T_{4.}$ Bensulfuron methyl + Pretilachlor with safener *fb* Bispyribac-sodium	60 + 500 *fb* 25	Pre *fb* Post	11071	13685	3674	3923	548	548
$T_{5.}$ Oxadiargyl *fb* Azimsulfuron	75 *fb* 20	Pre *fb* Post	11062	11976	3593	3423	537	625
$T_{6.}$ Oxadiargyl *fb* Bispyribac-sodium	75 *fb* 25	Pre *fb* Post	10587	11709	3302	3261	529	617
$T_{7.}$ Pyrazosulfuron ethyl *fb* Azimsulfuron *fb* Metsulfuron methyl + Chlorimuron ethyl	25 *fb* 20 *fb* 4	Pre *fb* Post *fb* Post	13591	14863	4714	4687	559	652
$T_{8.}$ Pyrazosulfuron ethyl *fb* Bispyribac-sodium *fb* Metsulfuron methyl + Chlorimuron ethyl	25 *fb* 25 *fb* 4	Pre *fb* Post *fb* Post	12960	14609	4599	4661	537	655
$T_{9.}$ Bensulfuron methyl + Pretilachlor with safener *fb* Azimsulfuron *fb* Metsulfuron methyl + Chlorimuron ethyl	60 + 500 *fb* 20 *fb* 4	Pre *fb* Post *fb* Post	13764	15833	5107	5313	571	662
$T_{10.}$ Bensulfuron methyl + Pretilachlor with safener *fb* Bispyribac-sodium *fb* Metsulfuron methyl + Chlorimuron ethyl	60 + 500 *fb* 25 *fb* 4	Pre *fb* Post *fb* Post	13365	15618	4828	5014	565	656
$T_{11.}$ Oxadiargyl *fb* Azimsulfuron *fb* Metsulfuron methyl + Chlorimuron ethyl	75 *fb* 20 *fb* 4	Pre *fb* Post *fb* Post	13020	14308	4666	4601	530	649
$T_{12.}$ Oxadiargyl *fb* Bispyribac-sodium *fb* Metsulfuron methyl + Chlorimuron ethyl	75 *fb* 25 *fb* 4	Pre *fb* Post *fb* Post	12670	14549	4371	4437	534	642
$T_{13.}$ Weed free	-	-	14166	16247	5450	5455	585	662
$T_{14.}$ Weedy check	-	-	7971	8802	2159	2529	523	594
SEm ±	-	-	565	490	233	298	19	31
CD (P = 0.05)	-	-	1641	1424	678	865	NS	NS

fb – followed by

Table 2: System Productivity (kg ha^{-1}) of rice-green gram sequence as influenced by different weed management practices in rice-green gram sequence during 2015-16 and 2016-17 *kharif* and *rabi* seasons

Treatments	Dose (g ha^{-1})	Time (DAS)	2015-16			2016-17		
			Rice grain yield (kg ha^{-1})	Rice grain equivalent yield (kg ha^{-1})	System productivity (kg ha^{-1})	Rice grain yield (kg ha^{-1})	Rice grain equivalent yield (kg ha^{-1})	System productivity (kg ha^{-1})
$T_{1.}$ Pyrazosulfuron ethyl *fb* Azimsulfuron	25 *fb** 20	Pre *fb* Post	3844	1821	5664	3619	2188	5806
$T_{2.}$ Pyrazosulfuron ethyl *fb* Bispyribac-sodium	25 *fb* 25	Pre *fb* Post	3604	1767	5371	3521	2158	5679
$T_{3.}$ Bensulfuron methyl + Pretilachlor with safener *fb* Azimsulfuron	60 + 500 *fb* 20	Pre *fb* Post	4118	1848	5966	4203	2255	6458
$T_{4.}$ Bensulfuron methyl + Pretilachlor with safener *fb* Bispyribac-sodium	60 + 500 *fb* 25	Pre *fb* Post	3674	1820	5494	3923	1896	5819
$T_{5.}$ Oxadiargyl *fb* Azimsulfuron	75 *fb* 20	Pre *fb* Post	3593	1783	5376	3423	2163	5585
$T_{6.}$ Oxadiargyl *fb* Bispyribac-sodium	75 *fb* 25	Pre *fb* Post	3302	1757	5059	3261	2136	5397
$T_{7.}$ Pyrazosulfuron ethyl *fb* Azimsulfuron *fb* Metsulfuron methyl + Chlorimuron ethyl	25 *fb* 20 *fb* 4	Pre *fb* Post *fb* Post	4714	1857	6571	4687	2257	6944
$T_{8.}$ Pyrazosulfuron ethyl *fb* Bispyribac-sodium *fb* Metsulfuron methyl + Chlorimuron ethyl	25 *fb* 25 *fb* 4	Pre *fb* Post *fb* Post	4599	1785	6384	4661	2268	6929
$T_{9.}$ Bensulfuron methyl + Pretilachlor with safener *fb* Azimsulfuron *fb* Metsulfuron methyl + Chlorimuron ethyl	60 + 500 *fb* 20 *fb* 4	Pre *fb* Post *fb* Post	5107	1897	7005	5313	2291	7604
$T_{10.}$ Bensulfuron methyl + Pretilachlor with safener *fb* Bispyribac-sodium *fb* Metsulfuron methyl + Chlorimuron ethyl	60 + 500 *fb* 25 *fb* 4	Pre *fb* Post *fb* Post	4828	1877	6706	5014	2271	7284
$T_{11.}$ Oxadiargyl *fb* Azimsulfuron *fb* Metsulfuron methyl + Chlorimuron ethyl	75 *fb* 20 *fb* 4	Pre *fb* Post *fb* Post	4666	1762	6428	4601	2247	6849
$T_{12.}$ Oxadiargyl *fb* Bispyribac-sodium *fb* Metsulfuron methyl + Chlorimuron ethyl	75 *fb* 25 *fb* 4	Pre *fb* Post *fb* Post	4371	1773	6145	4437	2222	6658
$T_{13.}$ Weed free	-	-	5450	1942	7392	5455	2289	7744
$T_{14.}$ Weedy check	-	-	2159	1738	3896	2529	2056	4585

fb – followed by

Table 3: Economics of rice-green gram sequence as influenced by different weed management practices during 2015-16 and 2016-17 *kharif* and *rabi* seasons

Treatments	Dose (g ha^{-1})	Time (DAS)	2015-16			2016-17		
			Gross returns (Rs. ha^{-1})	Net returns (Rs. ha^{-1})	B: C Ratio	Gross returns (Rs. ha^{-1})	Net returns (Rs. ha^{-1})	Return per rupee investment
$T_{1.}$ Pyrazosulfuron ethyl *fb* Azimsulfuron	25 *fb** 20	Pre *fb* Post	88138	38206	1.42	93095	40913	1.58
$T_{2.}$ Pyrazosulfuron ethyl *fb* Bispyribac-sodium	25 *fb* 25	Pre *fb* Post	83704	33114	1.23	91480	38640	1.49
$T_{3.}$ Bensulfuron methyl + Pretilachlor with safener *fb* Azimsulfuron	60 + 500 *fb* 20	Pre *fb* Post	92673	40877	1.47	103790	49743	1.81
$T_{4.}$ Bensulfuron methyl + Pretilachlor with safener *fb* Bispyribac-sodium	60 + 500 *fb* 25	Pre *fb* Post	85495	33040	1.21	94512	39807	1.38
$T_{5.}$ Oxadiargyl *fb* Azimsulfuron	75 *fb* 20	Pre *fb* Post	83702	33511	1.26	90014	37573	1.47
$T_{6.}$ Oxadiargyl *fb* Bispyribac-sodium	75 *fb* 25	Pre *fb* Post	78872	28023	1.07	86969	33870	1.34
$T_{7.}$ Pyrazosulfuron ethyl *fb* Azimsulfuron *fb* Metsulfuron methyl + Chlorimuron ethyl	25 *fb* 20 *fb* 4	Pre *fb* Post *fb* Post	102137	48406	1.63	111866	55885	1.93
$T_{8.}$ Pyrazosulfuron ethyl *fb* Bispyribac-sodium *fb* Metsulfuron methyl + Chlorimuron ethyl	25 *fb* 25 *fb* 4	Pre *fb* Post *fb* Post	99181	44791	1.48	111856	55216	1.90
$T_{9.}$ Bensulfuron methyl + Pretilachlor with safener *fb* Azimsulfuron *fb* Metsulfuron methyl + Chlorimuron ethyl	60 + 500 *fb* 20 *fb* 4	Pre *fb* Post *fb* Post	108647	53050	1.72	122288	64442	2.11
$T_{10.}$ Bensulfuron methyl + Pretilachlor with safener *fb* Bispyribac-sodium *fb* Metsulfuron methyl + Chlorimuron ethyl	60 + 500 *fb* 25 *fb* 4	Pre *fb* Post *fb* Post	104208	47953	1.56	117337	58832	1.94
$T_{11.}$ Oxadiargyl *fb* Azimsulfuron *fb* Metsulfuron methyl + Chlorimuron ethyl	75 *fb* 20 *fb* 4	Pre *fb* Post *fb* Post	100139	46149	1.52	110100	53860	1.87
$T_{12.}$ Oxadiargyl *fb* Bispyribac-sodium *fb* Metsulfuron methyl + Chlorimuron ethyl	75 *fb* 25 *fb* 4	Pre *fb* Post *fb* Post	95825	41176	1.37	107566	50667	1.75
$T_{13.}$ Weed free	-	-	114376	48696	1.41	124482	56552	1.70
$T_{14.}$ Weedy check	-	-	60882	13202	0.63	74005	24075	1.05

fb – followed by

treatment to *kharif* rice realized the highest economic yield in terms of rice grain equivalent yield in the rice-green gram system was however, comparable to other effective treatments (T_9 and T_{10}) owing to the cumulative effect of higher rice yield as well as seed yield of green gram in the system. Reddy *et al.*, (2017) reported similar findings on rice grain equivalent yield with legumes as a component crop.

Economics

Varied weed management practices adopted in the rice rice-green gram system altered the economics of system as a whole during both the years of study (**Table 3**). Though weed free treatment (T_{13}) resulted in higher gross returns during both the years of study (Rs. 114376 and Rs. 124482 ha^{-1} during 2015-16 and 2016-17, respectively) the net returns and return per rupee invested were markedly higher under T_9 and T_{10}, respectively during both the years. Weedy check (T_{14}) registered the lowest gross returns, net returns and return per rupee investment during both the years of study.

The economics of rice-green gram sequence play a vital role in making a recommendation for adoption of technology on farmer's field. In the present investigation the pre-emergence application of bensulfuron methyl @ 60 g a.i. ha^{-1} + pretilachlor with safener at 500 g a.i. ha^{-1} followed by post-emergence application of azimsulfuron @ 20 g a.i. ha^{-1} at 25 DAS, post-emergence application of metsulfuron methyl and chlorimuron ethyl @ 4 g a.i. ha^{-1} applied at 45 DAS (T_9) was the most profitable with the net returns (Rs. 53050 and Rs. 64442 ha^{-1} during 2015-16 and 2016-17, respectively) over the other treatments. The findings are similar to the results in the report of Reddy *et al.*, (2017).

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Effect of liquid inorganic and organic fertilizers on the growth and yield of rice

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Abstract

Two field experiments were conducted with Co-51 variety at Annamalai University in randomised block design comprising ten treatments to determine the effect of foliar application of liquid inorganic and organic fertilizers alone and in combination with 75 % recommended NPK on growth and yield of rice. The results revealed that application of 75 % NPK with alternate spraying of liquid fertilizers - SPIC NPK and Isabion on 10, 20, 30, 40, and 50 DAT increased the plant height (2.3,8.1 cm), leaf area index (1.3,1.9), number of tillers /hill (8.3) and grain yield (530 Kg/ha) compared to 75 % recommended NPK application alone. The same treatment increased the grain yield up to 141 Kg/ha over 100 % recommended NPK.

Keywords: Polyfeed, amino acid, plant height, tillers, LAI, Grain yield

Introduction

Agriculture plays a vital role in the Indian economy and out of the total population 54.6 per cent people are engaged in agriculture and allied activities. Among the Asian countries, China ranks first in production followed by India. Among the South Asian countries, India stands first position in Area (44.16 M ha), Production (116.48 Million metric tonnes), and productivity (3.96 t/ha) (USDA, 2019) which is low compared to world average 0f 4.58 t/ha. In India, rice is grown in 44.16 million hectares and the production level is 116.48 million tonnes, while productivity is 3.96 t/ha. In Tamil Nadu, rice occupied an area of 1.78 M ha with production and productivity of 6 mt and 3.37 t/ha, respectively (Annual report, 2017-18). The applied fertilizers supplying NPK undergo physical, chemical, and biological transformation processes in the soil. The NPK use efficiency is 30-50, 15-20 and 70-80 %, respectively. Several thousand crores of rupees are wasted due to losses of nutrients. Further, leaching and volatilization loss of N pollutes the environment. The N recovery in basal, mid tillering, panicle initiation and flowering stages are in the range of 15-30, 30-50, 45-75 and 35-60 % respectively. Foliar fertilization is one of the plant-based interventions to increase nutrient use efficiency. Water-soluble NPK (19:19:19) fertilizers are designed to provide complete plant nutrition throughout the season by fertigation and through foliar application for field crops, vegetables, fruit trees and flower crops grown in all environments. It is safe to use, free of chloride, sodium, other detrimental elements, with high-quality ingredients, instant uptake and enriched with a high concentration of micronutrients. Foliar applicationimproves the nutrient uptake of plants, nutrient use efficiency and minimizes the losses by leaching. Foliar application of polyfeed @ 1 % along with recommended NPK increased the growth and yield components of ADT-R 47 rice (Kunjammal *et al*., 2016). Isabion is a product of biological activator, composed of free amino acids. Amino acids are important for plant and pollen fertility, necessary to start cell division, regulation of water balance, stimulation of hormone metabolism, readymade building blocks for proteins, stress-resistant and precursor of auxin. It stimulates the basic functions of crops, promotes vegetative

growth, root growth and vigorous development of buds, induces greater flowering, promotes pollination, fruit set, and improves the quantity and quality of the produce. In rice, it helps in overcoming threats due to sudden or extreme heat and cold in young rice plants. Yield components and yield of rice was increased due to isabion application along with recommended NPK (Venkatesh Prasath *et al*., 2017). With a view to enhance the yield of rice, this study was conducted to know the effect of foliar application of Polyfeed, SPIC NPK and Isabion applied individually as well as in combination with 75 per cent of recommended NPK, on growth and yield of rice.

Materials and Materials

Two field experiments were conducted in the experimental farm, Department of Agronomy, Faculty of Agriculture, Annamalai University, during December 2019 and February 2019 with rice variety Co – 51. The rice crops were raised during the Navarai and late Navarai seasons. The experimental farm is situated at 11˚24´ North Latitude and 79 ˚44' East Longitude with an altitude of +5.79 m above mean sea level.

The soil of experimental fields was clayey loam in texture with low in available Nitrogen (240, 220 Kg/ha), medium in available Phosphorus (17.8, 18 Kg/ha), and high in available Potassium (335, 315 Kg/ha). The experiments were conducted in Randomized Block Design, consisting of ten treatments with three replications. The treatments were T_1 - 100 % Recommended NPK, T_2 - 75 % Recommended NPK, T_3 - T_2 + Polyfeed spray 5 times @ 5 g/l at 10, 20, 30, 40, and 50 DAT ,T_4 - T_2 + SPIC NPK spray 5 times @ 5 g/l at 10, 20, 30, 40, and 50 DAT , T_5 - T_2 + Isabion spray 5 times @ 5 ml/l at 10, 20, 30, 40, and 50 DAT, T_6 - T_2 + Polyfeed spray 3 times @ 5 g/l and Isabion spray 2 times @ 5 ml/l at 10, 20, 30, 40, and 50 DAT ,T_7 - T_2 + SPIC NPK spray 3 times @ 5 g /l and Isabion spray 2 times @ 5 ml/l at 10, 20, 30, 40, and 50 DAT,T_8 - T_2 + Alternate spray of Polyfeed @ 5 g/l and Isabion @ 5 ml/l at 10, 20, 30, 40, and 50 DAT ,T_9 - T_2+ Alternate spray of SPIC NPK @ 5 g/l and Isabion @ 5 ml/l at 10, 20, 30, 40, and 50 DAT and T_{10} - T_2 + Urea spray 3 times @ 10g/l and KCl spray 2 times @ 10g/l at 10, 20, 30, 40, and 50 DAT . The spray applications were carried out with the help of water @ 500 l/ha using knapsack sprayer during evening hours. For the experiments 29 and 21 days old seedlings were transplanted @ 2 /hill with a spacing of 15x10 cm respectively. A recommended fertilizer schedule of 120:40:40 was adopted for 100 per cent and from which 75 per cent NPK was calculated and applied to the respective plots as per treatment schedule. The half of N, entire P and half of K were applied basally. The remaining quantities of N and K were top dressed in two equal splits at maximum tillering and flowering stages. Various growth and yield parameters were recorded from five tagged plants in each plot periodically. The grain yield was recorded plot-wise and computed.

Results and Discussion

Data on growth parameters revealed that, among the treatments, T_9 (T_2 + Alternate spraying of SPIC NPK and Isabion 5 times spray at 10, 20, 30, 40 and 50 DAT) resulted in the tallest plants and the heights recorded were 16.9 and 50.8 cm on 25 and 50 DAT, respectively **(Table 1)**. Among the individual foliar sprays along with 75% recommended NPK, T_5 (T_2 + Isabion foliar spray 5 times on 10, 20, 30, 40 and 50 DAT) was the best on par with continuous foliar application of Polyfeed / SPIC NPK, 3 times followed by 2 times Isabion spray. Application of 25 % less than recommended NPK resulted in reduced plant height to the tune of 1.4 and 5.1 cm compared to 100 % NPK application, respectively at both the stages.

The treatment, T_9 (T_2 + Alternate spraying of SPIC NPK and Isabion 5 times spray on 10, 20, 30, 40, and 50 DAT) was significantly superior to remaining treatments in showing the highest number of tillers/hill (23.6). This might be due to the presence of macronutrients in the soil from initial stages of the crop growth and supplemental feeding of water-soluble NPK and amino acid resulting in enhanced metabolic activity of the plants leading to more

number of tillers. The next best treatment was was T8 (T2 + Alternate spraying of Polyfeed and Isabion 5 times spray on 10, 20, 30, 40 and 50 DAT). A comparable effect was observed among the individual foliar sprays along with 75 % recommended NPK. Continuous application of Polyfeed/SPIC NPK 3 times along with Isabion 2 times on 10, 20, 30, 40 and 50 DAT showed similar effect. Application of 25 % less than recommended NPK reduced the number of tillers/hill to the tune of 4.9 compared to 100% recommended NPK.

The treatment, T9 also resulted in the highest leaf area index of 2.98 and 4.89 on 25 and 50 DAT, respectively. Increased leaf area is a base for better photosynthesis. Increased LAI recorded in this treatment might be due to the availability and absorption of macronutrients from initial stages of the crop growth from the soil or through foliar feeding favouring the crop growth, plant height, tiller number, leaf length and width. This result is in conforms to the finding of Kunjammal *et al*., (2016). T8 (- T2 + Alternate spraying of Polyfeed and Isabion 5 times spray on 10, 20, 30, 40, and 50 DAT) was the next best treatment, while among the individual foliar applications, T5 (T2 + Isabion foliar spray 5 times on 10, 20, 30, 40, and 50 DAT) resulted in LAI of 2.23 and 3.72 on 25 and 50 DAT higher than T2 treatment (– 75% recommended NPK). Continuous foliar application of SPIC NPK 3 times along with 2 times spray of Isabion was superior to polyfeed and isabion application at both

Table 1: Effect of inorganic and organic liquid fertilizers on growth and yield of Rice (Pooled data)

Treatments	Plant height (cm) DAT		Number of tillers/ hill 50 DAT	LAI DAT		Grain yield Kg/ha
	25	50		25	50	
T1 – 100 % Recommended NPK	16.0	47.8	20.2	2.57	4.41	4486
T2 – 75% Recommended NPK	14.6	42.7	15.3	1.68	2.98	4097
T3 - T2 + Polyfeed NPK spray 5 times on 10, 20, 30, 40 and 50 DAT	15.1	44.1	17.0	1.97	3.34	4206
T4 - T2 + SPIC NPK foliar spray 5 times on 10, 20, 30, 40 and 50 DAT	15.4	44.6	17.1	2.09	3.54	4237
T5 - T2 + Isabion foliar spray 5 times on 10, 20, 30, 40 and 50 DAT	15.5	45.2	18.2	2.23	3.72	4265
T6 -T2 +Polyfeed 3 times foliar spray +Isabion 2 times foliar spray on 10, 20, 30, 40 and 50 DAT	15.6	45.9	18.5	2.36	3.87	4381
T7 - T2 + SPIC NPK 3 times spray + 2 times Isabion spray on 10, 20, 30, 40 and 50 DAT	15.8	46.7	19.4	2.45	4.09	4439
T8 - T2 + Alternate spraying of Polyfeed and Isabion (totally 5 times spray) on 10, 20, 30, 40 and 50 DAT	16.1	49.4	21.4	2.71	4.64	4588
T9 - T2 + Alternate spraying of SPIC NPK and Isabion (totally 5 times spray) on 10, 20, 30, 40 and 50 DAT	16.9	50.8	23.6	2.98	4.89	4627
T10 - T2 + Urea spray 3 times and KCl spray 2 times on 10, 20, 30, 40 and 50 DAT	14.6	43.25	16.4	1.79	3.16	4147
S. Ed	0.168	0.631	0.984	0.041	0.053	88.6
C.D (P = 0.05)	0.3524	1.323	2.0625	0.0859	0.01112	185.87

the stages of observation. Application of 25 % less than recommended NPK reduced LAI compared to 100 % NPK with values of 1.68, 2.98 on 25 and 50 DAT, respectively.

Among all the the treatments T9 (-T2 + Alternate spraying of SPIC NPK and Isabion totally 5 times spray on 10, 20, 30, 40, and 50 DAT) resulted in the highest grain yield of 4627 kg/ha which was 12.94% and 3.14% higher than T2 (75% recommended NPK) and T1 (100% recommended NPK), respectively and on par with T8 (- T2 + Alternate spraying of Polyfeed and Isabion 5 times spray on 10, 20, 30, 40, and 50 DAT).

The grain yield increase was due to increased uptake of nutrients from the soil, increased N assimilation and vigour inducing character of growth-promoting organic amino acid fractions. All these influenced the physiological and morphological characters of the plant in terms of tallest plants, higher tillers, LAI and finally the grain yield. Similar result was obtained by Sorour *et al*., (2015). Individual foliar application five times on 10, 20, 30, 40, and 50 DAT increased the grain yield by 109 to 168 kg/ha compared to T2 (75% recommended NPK). Among the continuous foliar application of Polyfeed/SPIC NPK 3 times followed by Isabion 2 times spray, T7 (T2 + SPIC NPK 3 times foliar spray followed by Isabion 2 times foliar spray on 10, 20, 30, 40, and 50 DAT) had an edge over T6 (– T2 + Polyfeed 3 times foliar spray +Isabion 2 times foliar spray on 10, 20, 30, 40, and 50 DAT). Application of 25 % less than recommended NPK resulted in reduced yield to the tune of 389 Kg/ha compared to T1 (100 % recommended NPK). Foliar application of urea 3 times followed by KCl 2 times increased the grain yield up to 50 kg/ha over T2 (75% recommended NPK).

Overall, application of 75 % recommended NPK + alternate spraying of SPIC NPK and Isabion on 10, 20, 30, 40, and 50 DAT was the best treatment and is recommended to get higher grain yields.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

A web-based radiation use efficiency calculator for rice genotypes

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Abstract

Radiation Use Efficiency (RUE) is one of the key parameters in measuring the crop biomass and plays a major role in assessing the yield performance of genotypes. RUE can be derived from radiation interception and utilization. Manual process of computing Photosynthetically Active Radiation (PAR) and RUE is tedious and only limited models are available exclusively for computing radiation use efficiency of rice crop. Hence a web-based radiation use efficiency calculator was developed for rice genotypes and successfully evaluated with data collected from field trials conducted under All India Coordinated Rice Improvement Programme (AICRIP). Computed RUE values ranged from 1.01-2.76 g/MJ^{-1} at panicle initiation stage and 0.17-0.26 g/MJ^{-1} at maturity stage for the experiment conducted at IIRR, Hyderabad. This software got registered with the copyright no. SW/13541/2020 and presently available to all users in the IIRR website (www.icar-iirr.org) for facilitating computation of RUE at different phenological stages of rice crop.

Key words: Radiation, rice, web based calculator, genotype, software

Introduction

Rice is the most important staple food crop in India feeding more than half of the population. Rice yields need to increase further to meet the substantial increase in demand of the rapidly increasing population. It is well known that accumulated intercepted radiation and Radiation Use Efficiency (RUE) are key indicators for determining crop biomass (Monteith *et al.*, 1977). Many crop growth models use radiation use efficiency parameter to simulate photosynthesis *i.e* converting light energy and CO_2 into biomass. RUE is a key quantifier of crop production in relation to photosynthesis. It combines both the amount of solar radiation captured by the crop and the efficiency of the crop to produce dry matter. It helps in understanding and modelling the relationship between plant growth and the physical environment. Huang *et al*., (2019) indicated that higher RUE rather than accumulated intercepted radiation contributed more to yield improvement. More importantly, the relative contribution of RUE to yield improvement is growth stage dependent because RUE is not consistent during the whole crop growth period (Yonghui Pan *et al.*, 2019). Hence it is necessary to elucidate the crucial growth stage related with rice yield and to understand the relative contribution of RUE to yield improvement. The process of computing daily total radiation, photosynthetically active radiation (PAR) and measuring the fraction of crop intercepted radiation and calculating RUE at different stages of rice crop is cumbersome and time taking. In view of this, efforts were made to develop a web based radiation use efficiency calculator to facilitate easy computation of RUE at different stages of rice crop.

Materials and Methods

Crop growth can be described as the product of the incident Photosynthetically Active Radiation (PAR); the fraction (f) of PAR intercepted by green leaf (*f*); and the 'efficiency' with which the PAR is used as radiation use efficiency. The PAR intensity depends

on the location and time of the year while seasonal *fraction (f)* is affected by the duration and the area of the canopy. Radiation Use Efficiency (RUE) is defined as the ratio of dry matter produced to absorbed photosynthetically active radiation (APAR Mega Joules-MJ/m^2). It is usually measured in grams of total dry matter per megajoule (g TDM MJ^{-1}). The formulae used for computing Radiation Use Efficiency (Bouman *et al*., 2001) at different stages of rice crop are presented in the **Table 1.**

Table 1: Formulae used to compute Radiation Use Effciency (RUE)

Variable	**Formula**
RUE at Maturity (RUE_{Mat} g/ MJ^{-1})	TDM_{Mat} / ∑APAR from sowing day to maturity TDM: Total Dry Matter
RUE from Panicle Initiation to Maturity ($RUE_{PI\ to\ Mat}$ g/ MJ^{-1})	TDM_{PI-Mat} / ∑APAR from PI to maturity
RUE at Panicle Initiation	TDM_{PI} / ∑APAR from sowing day to maturity
Absorbed Photosynthetically Active Radiation (APAR MJ/m^2)	PAR*40%
Photosynthetically Active Radiation (PAR MJ/m^2)	Shortwave radiation(TMPR1)*fraction of PAR (0.5)
Short wave radiation (TMPR1)	RDD*SINB*(1.0+0.4*SINB)/DSINBE SinB : Actual effective sine of solar inclination
Daily integral of sine of solar angle (DSINB)	2.*3600.*(DAYL*0.5*SINLD-12.*COSLD*ZZCOS/PI)
Daily integral of effective SINB (DSINBE)	2.*3600.*(DAYL*(0.5*SINLD+0.2*SIN-LD**2+0.1*COSLD**2) - & (12.*COSLD*ZZ-COS+9.6*SINLD*COSLD*ZZCOS+ & 2.4*COSLD**2*ZZCOS*ZZSIN)/PI)
Solar Constant (SOLCON)	1370.*(1.+0.033*COS (2.*PI*DOY/365.))
Daily total extraterrestrial radiation (ANGOT- J m^{-2})	SOLCON*DSINB
Declination of Sun (DEC)	-ASIN (SIN (23.45*DEGTRAD)*COS (2.*PI*(DOY+10.)/365.)) Constants: PI=3.1415927, DEGTRAD=0.017453292
Intermediate Variables	SINLD = SIN (DEGTRAD*LAT)*SIN (DEC)
	COSLD = COS (DEGTRAD*LAT)*COS (DEC)
	AOB = SINLD/COSLD
Day Length (DAYL - h)	DAYL = 12.*(1.+2.*ASIN (AOB)/PI)
Intermediate Variable(ZZA)	ZZA = PI*(12+DAYL)/24
Cosine of ZZA(ZZCOS)	ZZCOS = COS (ZZA)
Sine of ZZA(ZZSIN)	ZZSIN = SIN (ZZA)
Daily Total Radiation(RDD kJ m^{-2} d^{-1})	S_0*(a_A+b_A*(sh/day length) Sh: daily sunshine hours S_0 is the theoretical amount of global radiation without an atmosphere (kJ m^{-2} d^{-1}) a_A and b_A are an empirical constants Angstrom A & B parameters

These calculations involve some empirical relationships that calculate the day length and the integral of the sine of the solar angle from the day number and latitude (Goudriaan and van Laar 1994). First, the declination (DEC) is calculated from the day number. Then, the intermediate variables SINLD, COSLD, AOB, ZZA, ZZCOS and ZZSIN are calculated to make the other equations simpler. Appropriate corrections to the daylength (DAYL) and intermediate sine and cosine variables are made by checking the geographic latitude (LAT) within or below the polar circles through AOB. After this, two versions of the integral of the sine of the solar elevation are calculated: the first (DSINB; s d−1) is the straightforward integral of the sine of the solar angle used to calculate daily total extraterrestrial radiation (ANGOT; J m−2 d−1); the second one (DSINBE; s d−1) is a modified integral for radiation at Earth's surface, which takes into account the effect of the daily course in atmospheric transmission. DSINBE is used to calculate the actual radiation at a specific time of the day. The solar constant (SOLCON; W m−2) is calculated as a function of the day number because the distance between Earth and the sun is not constant over the year.

In the present study, daily sunshine hours (sh) were used to compute daily total radiation (RDD kJ m^{-2} d^{-1}). Shortwave radiation was calculated by the product of daily total radiation with the ratio of actual effective sine of solar inclination (SinB) over the integral of effective SINB (DSINBE). Fraction of PAR was calculated from the fraction of diffused radiation which is derived from the atmospheric transmission. This radiation flux at Earth's surface (assuming 100% atmospheric transmission) was calculated from the solar constant, which is the radiation flux perpendicular to the sun rays, multiplied by the sine of the solar inclination (SinB), which changes during the day. APAR (MJ/m^2) and RUE (g/ MJ^{-1}) at different phenological stages of rice crop *i.e* Panicle Initiation (PI), PI to maturity and maturity period were computed using the formulae **(Table 1).** As there are many intermediate calculations to compute RUE, a software was developed to computer variety-wise and replication-wise RUE at PI, PI to maturity and maturity stages of rice crop.

This software was developed using Microsoft Structured Query Language (MS SQL) as backend and .NET as front end. Three input and one output interfaces were designed. Input interfaces are location and sowing details interface, replication wise grid interface and weather data interface. One output interface was designed to display the variety-wise computed values of RUE and APAR.

Results and Discussion

RUE main interface (**Figure 1**) prompts for location, year and date of sowing, sowing level (Early, Medium and late sowings) and number of replications and number of varieties in each replication of the trial.

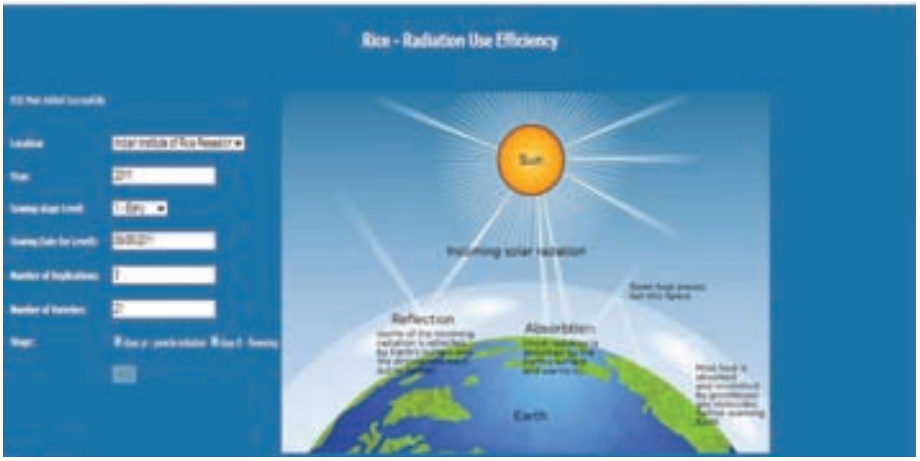


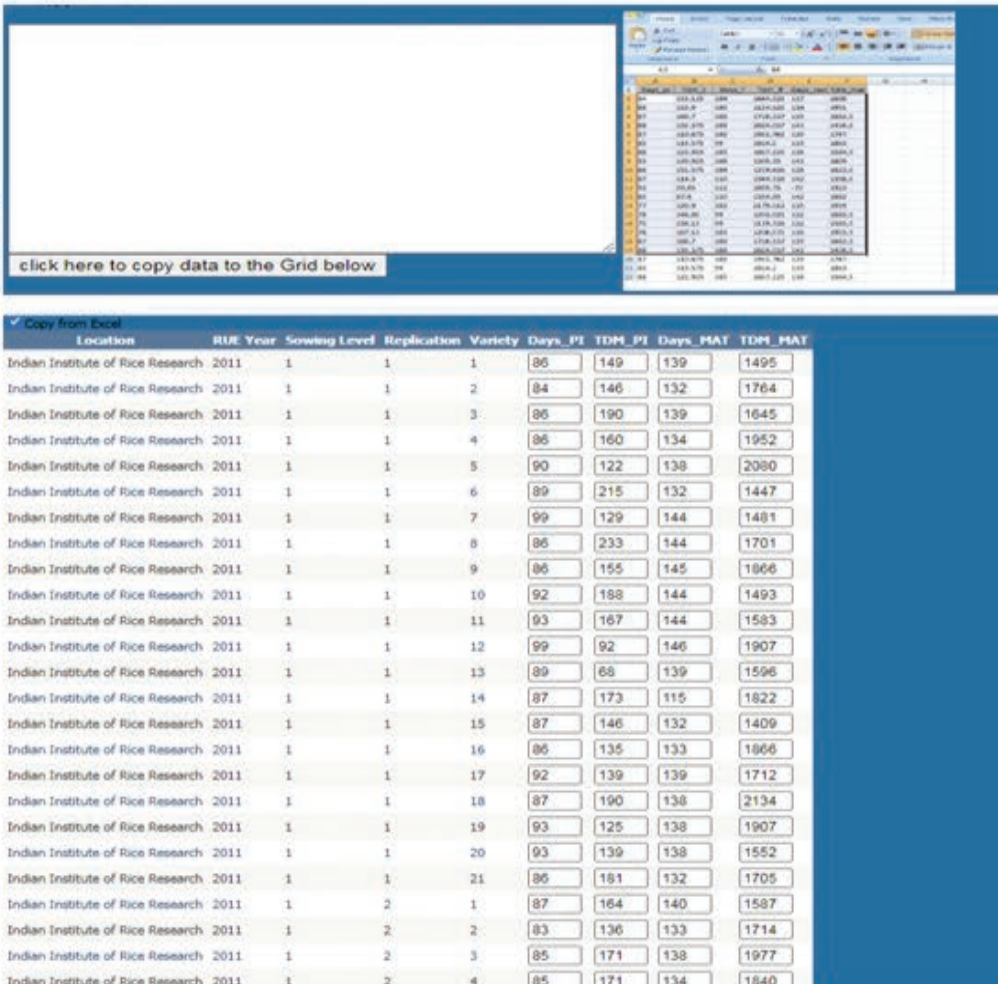
Figure 1: Location and Sowing details interface of Web based RUE

There are two options for stages of crop like panicle initiation and flowering. If user check the boxes then only next screen prompts for the data pertaining to the particular stage, otherwise it will prompt for data at maturity. Immediately after this screen prompts for number of days to maturity stage and total drymatter weight (TDM) to compute radiation use efficency along with opted details for opted crop stages.

Following this, replication-wise grid interface will be displayed to enter the replication data (**Figure 2**). Another provision is also provided to paste the data using '***copy from excel***' check box. Using the '***Add RUE Details'*** button, the data will be saved in the RUE database.

In sequence with this, weather data interface prompts for day-wise sunshine hours from sowing to maturity (**Figure 3**). Here also '***copy from excel***' provision is available to paste the data from excel. By using ***ADD Weather data***, the data will be inserted in the weather table of RUE database.

Then by using the ***Calculate Result*** command button**,** APAR and RUE at different stages will be computed and displayed in the grid (**Figure 4).**



Location	RUE Year	Sowing Level	Replication	Variety	Days_PI	TDM_PI	Days_MAT	TDM_MAT
Indian Institute of Rice Research	2011	1	1	1	86	149	139	1495
Indian Institute of Rice Research	2011	1	1	2	84	146	132	1764
Indian Institute of Rice Research	2011	1	1	3	86	190	139	1645
Indian Institute of Rice Research	2011	1	1	4	86	160	134	1952
Indian Institute of Rice Research	2011	1	1	5	90	122	138	2080
Indian Institute of Rice Research	2011	1	1	6	89	215	132	1447
Indian Institute of Rice Research	2011	1	1	7	99	129	144	1481
Indian Institute of Rice Research	2011	1	1	8	86	233	144	1701
Indian Institute of Rice Research	2011	1	1	9	86	155	145	1866
Indian Institute of Rice Research	2011	1	1	10	92	188	144	1493
Indian Institute of Rice Research	2011	1	1	11	93	167	144	1583
Indian Institute of Rice Research	2011	1	1	12	99	92	146	1907
Indian Institute of Rice Research	2011	1	1	13	89	68	139	1596
Indian Institute of Rice Research	2011	1	1	14	87	173	115	1822
Indian Institute of Rice Research	2011	1	1	15	87	146	132	1409
Indian Institute of Rice Research	2011	1	1	16	86	135	133	1866
Indian Institute of Rice Research	2011	1	1	17	92	139	139	1712
Indian Institute of Rice Research	2011	1	1	18	87	190	138	2134
Indian Institute of Rice Research	2011	1	1	19	93	125	138	1907
Indian Institute of Rice Research	2011	1	1	20	93	139	138	1552
Indian Institute of Rice Research	2011	1	1	21	86	181	132	1705
Indian Institute of Rice Research	2011	1	2	1	87	164	140	1587
Indian Institute of Rice Research	2011	1	2	2	83	136	133	1714
Indian Institute of Rice Research	2011	1	2	3	85	171	138	1977
Indian Institute of Rice Research	2011	1	2	4	85	171	134	1840

Figure 2: Replication wise grid interface of web based RUE

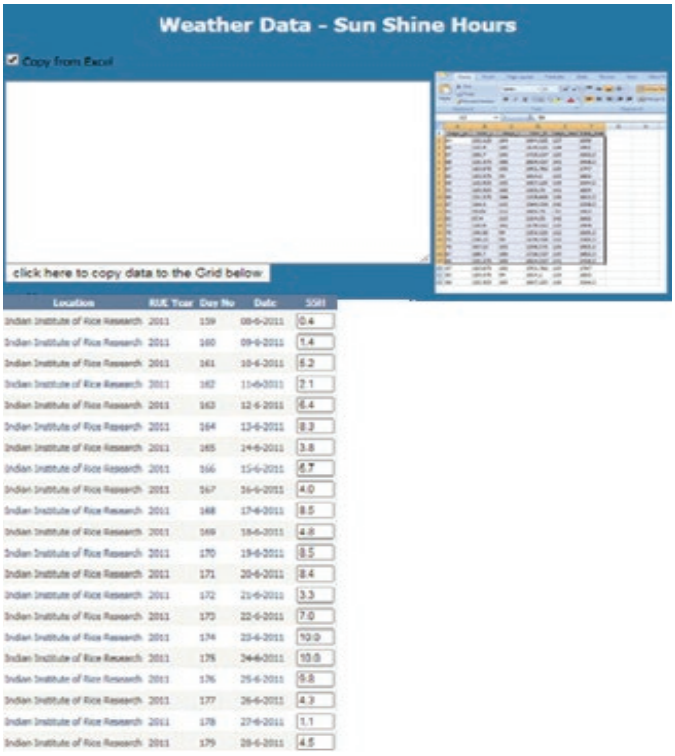


Figure 3: Weather data interface of Web based RUE

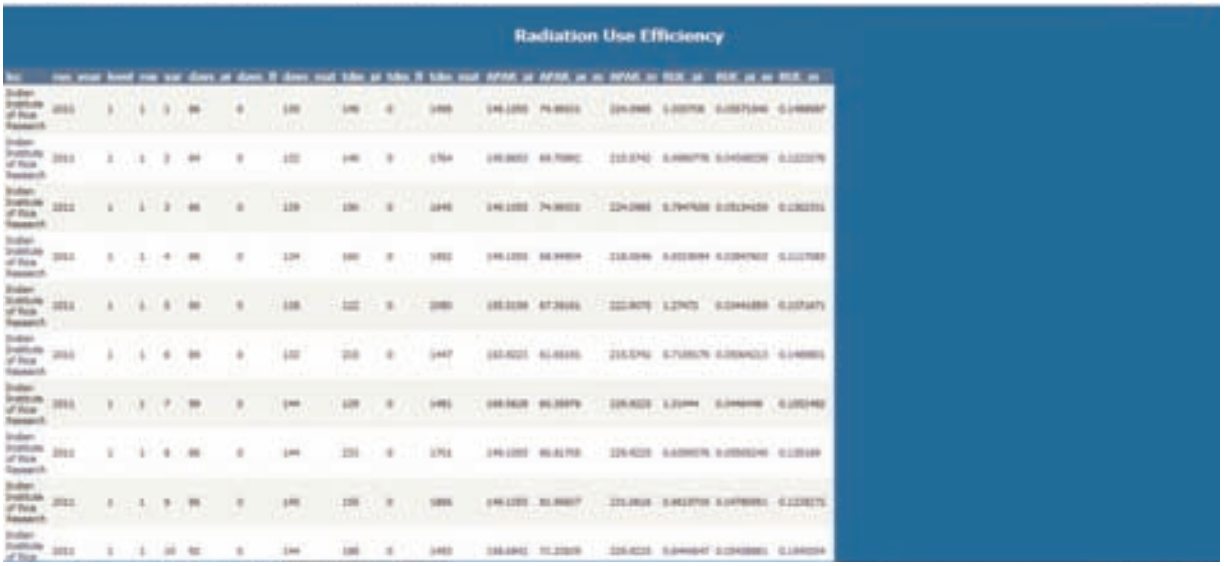


Figure 4: Interface of Web based RUE to display computed values of RUE and APAR

The software was specifically designed to compute rice genotype-wise radiation use efficiency at different phenological stages of rice crop for different locations spread across India.

It was evaluated with six years' data of the radiation use efficiency experiment conducted under All India Coordinated Rice Improvement Programme (AICRIP). The input dataset of RUE experiment under AICRIP during 2011 conducted at IIRR, Hyderabad and computed values of RUE using this software. It was observed that computed RUE values ranged from 1.01-2.76 g/MJ^{-1} at PI stage and 0.17-0.26 g/MJ^{-1} at maturity stage and RUE values were high at PI stage compared to maturity stage (**Table 2**).

Table 2: Computed values of RUE using web based RUE calculator

S. No	Varieties	Input				Output					
		Days_ pi	Days_ mat	TDM_t (g/m^2)	TDM_ mat (g/m^2)	APAR_pi (MJ/m^2)	APAR_ pi_m (MJ/m^2)	APAR_m (MJ/m^2)	RUE_pi (g/MJ^{-1})	RUE_ pi_m (g/MJ^{-1})	RUE_m (g/MJ^{-1})
1	IET 20524	84	138	120.23	1733.17	242.08	146.67	388.75	2.13	0.09	0.23
2	IET 20556	85	133	154.26	1877.17	244.96	130.39	375.35	1.63	0.08	0.20
3	IET 20924	86	139	174.63	1935.83	245.69	145.67	391.35	1.44	0.08	0.21
4	IET 20935	88	141	145.11	1541.50	250.09	147.29	397.38	1.73	0.11	0.26
5	IET 21519	87	139	92.68	1880.17	248.53	141.99	390.53	2.74	0.08	0.21
6	IET 21528	86	133	114.13	1912.17	245.61	128.81	374.41	2.43	0.07	0.20
7	IET 21542	87	137	134.48	1639.00	249.26	137.71	386.97	1.91	0.09	0.24
8	IET 22202	93	141	103.11	1587.33	264.32	132.50	396.82	2.76	0.09	0.25
9	IET 22218	86	138	146.02	1688.67	246.33	142.42	388.75	1.74	0.09	0.23
10	IET 22225	88	143	129.94	1590.67	250.09	149.75	399.85	2.07	0.10	0.25
11	IET 22228	93	73	78.06	1726.17	176.11	91.90	268.01	2.03	0.06	0.17
12	IET 22237	85	143	126.62	1751.17	244.32	155.36	399.68	2.05	0.10	0.23
13	IET 22251	77	136	107.03	1766.00	227.05	155.95	383.00	2.22	0.09	0.22
14	KRH-2	79	132	174.03	1790.33	231.96	140.47	372.43	1.35	0.09	0.21
15	PA-6129	75	132	222.45	1678.33	221.88	149.57	371.44	1.01	0.10	0.22
16	PA-6201	78	135	143.88	1885.17	229.98	151.26	381.24	2.18	0.09	0.20
17	PA-6444	77	135	177.99	1978.67	227.05	154.19	381.24	1.40	0.09	0.19
18	PHB-71	80	134	182.93	1805.00	234.61	142.67	377.28	1.59	0.09	0.21
19	AK.DHAN	86	139	186.03	1852.67	245.61	145.75	391.35	1.37	0.09	0.21
20	NDR-359	86	139	188.92	1947.50	245.61	145.75	391.35	1.43	0.08	0.20
21	VARADHAN	76	133	161.22	1564.00	224.37	150.01	374.39	1.46	0.11	0.24
	Min	**75**	**73**	**78.06**	**1541.50**	**176.11**	**91.90**	**268.01**	**1.01**	**0.06**	**0.17**
	Max	**93**	**143**	**222.45**	**1978.67**	**264.32**	**155.95**	**399.85**	**2.76**	**0.11**	**0.26**

RUE declined as LAI increased, and it decreased significantly after anthesis (Colin *et al*., 2001). Raghuveer Rao *et al.*, (2012) reported that RUE values at PI stage ranged from 0.5-2.36 g/MJ^{-1} and 0.24-0.53 g/MJ^{-1} at maturity stage at IIRR, Hyderabad location. RUE values computed using this model were well in agreement with reported values in the literature (Zhang *et al*., 2009). However, this model needs to be calibrated and validated to use for other crops like wheat, maize etc. This software got registered with the copy right no**. SW/13541/2020.**

Rice genotypes can be assessed easily for efficient RUE and yield at different stages of rice crop using this software. The software prompts for minimum input parameters and facilitates the computation of RUE across locations at different stages of rice crop. The data generated by this software can be easily copied to excel and can be used for further statistical analysis with other datasets. This software is easily understandable and user friendly.

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SHORT COMMUNICATION OPEN ACCESS

Potential yield assessment of red rice landraces under north-eastern agro climatic zone of Tamil Nadu

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Abstract

Among varied types of varieties in rice, red rice is found to be a rich in mineral source. It is the need of hour to conduct agronomical research on red rice landraces to study the increase of production, per unit area in sustained manner. *Kullakar* is a short duration landrace which is naturally hardy and pest resistant. *Kuzhiyadichan* is ideal for lactating mothers since it increases the milk flow and also referred as 'Kulikulichan'. *Kattuyanam* is a very tall red rice landrace which grows up to eight feet tall. TKM 9 is a red rice variety released at Rice Research Station, Tirur from Tamil Nadu Agricultural University, is found suitable for both rainfed and transplanted rice cultivation. Hence, a field study was carried out to identify the best performing red rice landrace for its potential yield. The study revealed that, the yield potential of 3.42 t/ha recorded in *Kuzhiyadichan* red rice landrace, was higher than that of *Kullakar* and *Kattuyanam* rice landraces.

Keywords: Red rice, landraces, yield performance

Introduction

The Rice varieties with a red bran layer are called red rice. Though, the red colour is confined to the bran layer, a tinge of red remains even after a high degree of milling. The colour of the bran ranges from light to dark red colour. The bran layer contains polyphones and anthocyanin, possessing antioxidant properties (Itani and Ogawa, 2004). Red rices are found to possess rich mineral source. In addition to being nutritive and having medicinal value, red rice has many other special features. Red rice varieties are suitable for both rainfed and irrigated rice cultivation under various agro climatic conditions and even in adverse situations (Lindsay Falvey, 2011). These genotypes are commonly cultivated in localized regions. From Agronomical or cultivation point of view, such red rice landraces possess resistance to drought, flood, submergence, alkalinity, salinity, resistance to pests and diseases. Due to the introduction of high yielding varieties and hybrids, red rice landraces cultivation area has declined. In the recent years, consumer preference has also changed over to white polished rice and this trend has lead to the unavailability of red rice landraces in the open market. Conserving biological diversity within food crops such as rice is crucial for sustaining agricultural systems and for maintaining global food security. In this context, a field study was conducted to evaluate the potential yield of red rice landraces.

The research trial was carried out in Rice Research Station, Tirur situated in North Eastern Agro Climatic Zone of Tamil Nadu to evaluate the performance and yield potential of red rice landraces and variety. The geographical co-ordinates of the location were 13˚7'N latitude and 79˚58'E longitude. The altitude of the research station is 39.47m MSL. The soil type of the experimental site is sandy clay, non-calcareous and light brown in colour. The performance of red rice landraces - *Kullakar* (V_1), *Kuzhiyadichan* (V_2) and *Kattuyanam* (V_3) were evaluated along with TKM 9 (V_4) red rice as check variety.

Observations were recorded on growth attributes *viz.*, plant height, tillers, leaf width, leaf length during flowering stage and at harvest stage, the grain and straw yields. At harvest stage, the crop duration was found to be 100 days in *Kullakar,* 110 days in *Kuzhiyadichan*, 110 days in TKM 9 red rice variety and 150 days in *Kattuyanam* land race. Among the landraces, *Kattuyanam* followed by *kuzhiyadichan* recorded comparatively higher values with regard to plant height, leaf length and leaf width. *Kuzhiyadichan* and *TKM 9* were found to have more tillering capacity than the other landraces.

The harvest of the rice crop was done at the physiological maturity stage of each individual variety. The TKM 9 red rice variety exhibited maximum grain yield of 4.28 t ha^{-1} followed by *Kuzhiyadichan* which recorded 3.42 t ha^{-1} while *Kattuyanam* landrace yielded the lowest with 2.25 t ha^{-1}. The straw yield was higher in *Kuzhiyadichan* landrace (12.77 t ha^{-1}) followed by *Kattuyanam* (9.11 t ha^{-1}) whereas lower straw yield was observed in TKM 9 variety (6.12 t ha^{-1}). Maximum harvest index was recorded in TKM 9 red rice variety with 0.41 HI value (**Table 1**).

Table 1: Field performance of red rice landraces in comparison with TKM 9

Treatments/ Varieties	Plant height at flowering (cm)	No. of tillers at flowering	Leaf length at flowering (cm)	Leaf width at flowering (cm)	Grain yield (t/ha)	Straw yield (t/ha)	Harvest Index (HI)
Kullakar (V1)	104.07	11	37.67	0.93	2.59	9.08	0.22
Kuzhiyadichan (V2)	118.27	12	42.33	1.07	3.42	12.77	0.21
Kattuyanam (V3)	173.67	10	59.67	1.29	2.25	9.11	0.19
TKM 9 (V4)	75.32	12	29.90	1.00	4.28	6.12	0.41
SEd					0.35	1.02	
CD (P=0.05)					1.03	2.95	

At present, conserving biological diversity within the food crops, particularly rice crop is crucial for sustaining agricultural systems and for maintaining global food security (Rhodes, 2008). Therefore encouraging the farmers to take up the cultivation of red rice landraces is in the need of hour and this technology would fetch them good returns with less usage of inputs and also the cultivation of landraces stands as the best option for the present scenario of climate resilience (Rao *et al*., 2018). The performance of red carotene rice landraces and variety tested under the north eastern agro climatic zone of Tamil Nadu State in India revealed that TKM 9 rice variety was found to be best performing red rice followed by the *kuzhiyadichan* red rice landrace in recording higher potential grain and straw yield per unit area.

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SHORT COMMUNICATION OPEN ACCESS

The performance of *Kavuni* rice in the western zone of Tamil Nadu

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Abstract

Field experiments were carried out at wetland farms of Tamil Nadu Agricultural University, Coimbatore during *Navarai* and *Samba* seasons with *Red Kavuni* and *Black Kavuni* rice which is indigenous and medicinal rice type to find the feasibility of cultivation of *Kavuni* rice under irrigated rice ecosystem in the western zone of Tamil Nadu. *Red Kavuni* and *Black Kavuni* recorded grain yield of 3,171 and 2,576 kg ha^{-1} respectively. *Red Kavuni* recorded higher straw yield of 7,470 kg ha^{-1}, but was on par with *Black Kavuni* showing7,030 kg ha^{-1}. *Black Kavuni* recorded higher amylose content of 20.8 per cent, total phenol content of 11.83 mg/100g and protein of 6.82 per cent and β-carotene of 420.37 µg/100g. The two *Kavuni* rice types, *viz.*, *Red Kavuni* and *Black Kavuni* can be suggested for cultivation in the western zone of Tamil Nadu and also in rice belt districts of Tamil Nadu and it would be more profitable with the small and marginal farmers.

Key words: Kavuni landrace, rice, performance, western zone

Introduction

In Asia, more than two billion people are getting 60-70 per cent of their energy requirement from rice and its derived products. (FAO, 2013). Many rice varieties with medicinal value are cultivated and used in certain pockets in states of Karnataka, Madhya Pradesh, Kerala, Tamil Nadu, Uttar Pradesh, Himachal Pradesh and Western Ghats. These rice types, being the local landraces is exploited by the rural folks to treat skin disease, blood pressure, fever, rheumatism, lactation and used also as a health tonic. Some of the rice landrace types are used in *sidda* and *ayurvedic* medicine preparations. Research on exploring the nutritional value of traditional rice varieties with its inherent medicinal values needs encouragement and proper documentation.

Kavuni is a rice variety type native to Tamil Nadu, cultivated in certain pockets of Thanjavur, Thirunelveli, Kanniyakumari districts and is basically of two types' *viz.*, *Black Kavuni* and *Red Kavuni* (Valarmathi *et al*., 2015). These rice landraces are highly nutritive and are rich in minerals like potassium, sodium, calcium, micronutrients like iron and zinc. They also contain higher proteins, carbohydrates and vitamins like thiamine, riboflavin and niacin. Though there is no scientific data on the medicinal properties, they are being used in Ayurveda for treating diseases like arthritis, skin diseases and neurological problems. Hence, the present study was planned to find the feasibility of cultivation of *Kavuni* rice under irrigated rice ecosystem in western zone of Tamil Nadu.

The field experiments were carried out at wetland farms of Tamil Nadu Agricultural University, Coimbatore during *Navarai* (Dec-Jan) and *Samba* (Aug-Sep) seasons, the major rice cultivation seasons of Tamil Nadu state. The location of the trial area is situated at 11° N latitude, 77° E longitude and at an altitude of 426.7m above mean sea level. All other package of practices was carried out as per the recommendation of CPG (2012). Five sample hills (plants) were selected randomly in the net plot area, and tagged for recording biometric observations. The experiment was conducted with *Red kavuni* and *Black Kavuni* rice variety which is indigenous and medicinal rice variety in Tamil Nadu. Observations were recorded on growth parameters like plant height, tillers, dry matter production and Leaf Area Index (LAI).

The yield attributes *viz;* productive tillers, spikelet number, filled grains, grain yield, and straw yield were recorded at the time of harvest. The data were subjected to statistical analysis by following standard statistical methods (Gomez and Gomez, 1984). The bio-chemical parameters like amylose per cent, total phenol content, total protein and β - carotenes were recorded on samples taken from another field trial with kavuni rice conducted for four consecutive years (2013-17).

The growth parameters like plant height, tillers and dry matter production were found higher in *Red Kavuni* compared to *Black Kavuni*. *Red Kavuni* recorded more plant height (127.45 cm), tillers per m^2 (538), leaf area index (5.87) at flowering stage and dry matter production of 11.31 t/ha **(Table 1)**. Among the yield attributes, *Red Kavuni* recorded the maximum number of productive tillers m^{-2} (404), total spikelets $panicle^{-1}$ (119) and filled grains $panicle^{-1}$(94).

Table 1: Bio -metric and yield parameters of *Kavuni* rice

***Kavuni* Rice (Type)**	***Black Kavuni***	***Red Kavuni***
Growth parameters		
Plant height (cm)	118.8	127.5
Tillers per m^2	443	538
Leaf Area Index (flowering stage)	4.31	5.87
Dry matter production (t/ha)	11.00	11.31
Yield attributes		
productive tillers m^{-2}	350	404
total spikelet $panicle^{-1}$	114	119
filled grains $panicle^{-1}$	86	94
Yield Parameters		
Grain yield (kg/ha)	2,576	3,171
Straw yield (kg/ha)	7,030	7,470
	Grain yield	**Straw yield**
SEd	204.80	117.81
CD (P=0.05)	614.03	458.71

These results were similar to the findings of Chowdhury *et al*., (1993) who stated that effective tillers $hill^{-1}$ varied with the landrace rice variety. In the present study, *Red Kavuni* and *Black Kavuni* recorded grain yield of 3,171 and 2,576 kg ha^{-1}, respectively. However, the average yield at farmers' fields have been reported to range from 700 to 1200 kg/ha in the traditional rice growing tracts of Tamil Nadu (Ashraf and Subbalakshmi, 2017). *Red Kavuni* recorded higher grain yield (3171 kg/ha) compared to *Black Kavuni*. *Red Kavuni* also recorded higher straw yield of 7,470 kg ha^{-1} but was on par with *Black Kavuni* (7,030 kg ha^{-1}). *Black Kavuni* recorded higher amylose content of 20.8 per cent, total phenol content of 11.83 mg/100g and protein of 6.82 per cent and β-carotene of 420.37 µg/100g (**Table 2**).

Table 2: Bio-chemical parameters of *Kavuni* rice

Parameter	***Black Kavuni***	***Red Kavuni***
Amylose content (%)	20.2	19.9
Total phenol content (mg/100g)	14.79	12.69
Total protein content (%)	6.87	5.83
The β - Carotene (µg /100g)	288.91	208.59

Thus, *Red Kavuni* rice recorded enhanced growth characters and yield attributes compared to *Black Kavuni* rice. *Black Kavuni* has been reported to show higher amylose per cent, total phenol content, total protein content and β - carotene (Savitha and Usha, 2016). These two varieties can be suggested for cultivation in the western zone of Tamil Nadu and also in districts of rice belts of Tamil Nadu. The study also revealed that the traditional rice varieties recorded high grain quality characteristics with medicinal and nutritional properties which needs to be examined and validated along with the popularization of these traditional rice types**.**

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NOTES

NOTES

Journal of Rice Research - Authors Guidelines

Scope: Journal of Rice Research is a channel for publication of full length papers covering results of original research, invited critical reviews or interpretative articles related to all areas of rice science, rice based crop systems and rice crop management. The journal also publishes short communications, book reviews and letters to the editor.

Articles reporting experimentation or research in any field involving rice or rice based cropping systems will be accepted as original articles while critical reviews are generally invited. Short articles concerned with experimental techniques or observation of unique nature will be accepted as short communication. Letters to the editor concerning previous articles are welcome and are published subject to review and approval by the editorial board. The original authors will be invited to reply to the points raised in these letters for their response which are also published together.

General Requirement:

Submission to the journal must be reports of original research of at least two crop seasons and must not be previously published or simultaneously submitted to any other scientific or technical journal. At least one of the authors (in case of joint authorship) should be member of the Society for Advancement of Rice Research (SARR) and not in arrears of subscription. Authors of invited articles are exempted from this.

Submission of Manuscript:

Manuscripts should be sent by email to the chief editor (chintalapatipadmavathi68@gmail.com) as an attachment. All the enclosed figures (as ppt/jpg files), graphs (as MS Excel worksheet with original data) and photographs (as jpg or ppt files with high resolution) may be submitted as separate files. Avoid using more than one font. The manuscript should be typed in double spaced times new roman font with margins of at least 2.5 cm. On the first page give the title, a byline with the names of authors, their affiliation and corresponding author's e-mail ID. Abstract should be followed by a list of key words. The usual order of sections to be included after title and abstract pages are: Introduction which includes literature review; materials and methods; results and discussion; conclusion (optional), acknowledgements and references followed by figures and tables.

Title should give a clear idea what the articles is about. It should be brief and informative (12-15 words).

Materials and Methods should include experimental design, treatment details, replications and techniques/ methods employed.

Results and Discussion should be supported by sound scientifically analysed data along with explanatory text with relevant tables and figures.

References should be quoted in author-year notation system only. All the references should be arranged alphabetically by author. All single author entries precede multiple author entries for the same first authors. Use chronological order within entries with identical authorship and add a low case letter a, b, c, etc., to year for same year entries of the same author. References should be presented in the format given below:

Research papers

1. Durvasula V. Seshu**.** 2017. Networking a Pivotal Strategy for Rice Genetic Improvement. *Journal of Rice Research*, 10(1): 1-8.
2. Kemparaju KB, MS Ramesha, K Sruti, AS Hari Prasad, RM Sundaram, P Senguttuvel and P Revathi**.** 2018. Breeding strategy for improvement of rice maintainer lines through composite population for short term diversity. *Journal of Rice Research*, 11(2): 27-30
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Book chapter

Scott JM 1984. Catabolism of folates. P. 307-327. In R.L. Blackley and S.J. Benkovic (ed.) Folates and Pterims Vol.1. John Wiley & Sons, New York

Book

Subba Rao LV, Shobha Rani N, Chiranjeevi M, Chaitanya U, Sudharshan I, Suneetha K, Jyothi Badri and Dipal R Choudhary 2013 DUS Characterization of Rice Varieties. Directorate of Rice Research, Rajendranagar, Hyderabad-500 030, AP, India. 524 pp

Figures: Photographs and drawings for graphs and charts should be prepared with good contrast of dark and light. Figure caption should be brief specifying the crop or soil, major variables presented and year. Give careful attention to the width of lines and size, and clarity of type and symbols.

Tables: Tables are used for reporting extensive numerical data in an organized manner and statistically analyzed. They should be self explanatory. Prepare tables with the word-processing tables feature and tabs or graphics boxes should not be used. Table head should be brief but complete and self contained. Define all variables and spell out all the abbreviations. An exponential expression (eg. x 10^3) in the unit's line is often needed to keep length of the data reasonably short, and referenced with an explanatory note. Unless otherwise required, two decimal place values are suggested.

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