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Flag leaf dimensions and pigments in backcross progeny of MTU1010/*Oryza rufipogon*

Venkateswara Rao Y[1], Krishnam Raju A[1], Divya B[2], Malathi S[1], Sukumar M[1], Vishnu Kiran T[1], Subrahmanyam D[1], Sarla N[2] and Voleti S R[1]*

ICAR - Indian Institute of Rice Research, Rajendranagar, Hyderabad.
ICAR- National Professor Project, IIRR, Hyderabad, Indian Institute of Rice Research (IIRR)
*Corresponding author email: voletisr58@rediffmail.com

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Abstract

Flag leaf photosynthesis related traits like chlorophyll a, b, total chlorophyll and carotenoids were evaluated in a set of 30 BC_2F_1 plants derived from MTU1010/ *Oryza rufipogon* IC309814. Significant differences were observed in chlorophyll a, b, total chlorophyll and carotenoid content between parents. Chlorophyll a, b and total chlorophyll showed highly significant positive correlation with chlorophyll/carotenoid in the introgression lines (ILs). IL198-15 showed higher chlorophyll a, b, total chlorophyll, chlorophyll/carotenoid, flag leaf width and area than both parents. IL-198-16 and IL-198-29 showed higher chlorophyll a, b, total chlorophyll, carotenoids, chlorophyll a/b ratio, chlorophyll/carotenoid, flag leaf length, width and area than MTU1010.

Keywords: Chlorophyll, carotenoids, flag leaf, pigments, introgression lines

Introduction

Global rice grain production has to be increased for securing food supply in coming years. However, marginal genetic gains have been observed during past 20-30 years in the yield potential of irrigated rice (Dingkuhn *et al.,* 2015). Thus, new methods are required to enhance the productivity, profitability and sustainability of rice yields with limited resources. One of them is developing new varieties with desired traits of yield enhancement. Photosynthesis is one of the crucial mechanisms that can help in improvement of biomass and yield in rice, thus increased photosynthetic pigments and photosynthetic efficiency can also improve the yield (Zhu *et al*., 2010, Ali *et al.,* 2017), and several other morphological traits such as stomatal conductance, transpiration rate and chlorophyll concentration. In our previous study, Swarna /*O. nivara* backcross inbred lines (BILs) were evaluated for photosynthesis and chlorophyll related traits and significant variations were observed for chlorophyll concentration among the BC_2F_8 BILs (Rao *et al.,* 2018a).

Flag leaf is the major source of photosynthesis in relation to grain yield. Wild rice, though agronomically poor has been reported as rich source to enhance photosynthesis and thereby yield (Kiran *et al.,* 2013). Several yield enhancing alleles have been reported from wild species of rice (Swamy and Sarla, 2008; Tripathy *et al.,* 2018; Samal *et al.,* 2018). The aim of this study was to characterize backcross introgression lines derived from MTU1010/ *Oryza rufipogon* for photosynthesis related traits.

Materials and methods

As part of program to develop chromosomal segment substitution lines (CSSLs) in MTU1010 using related wild species *O. rufipogon* IC309814, backcross introgression lines were generated using MTU1010 (Cottondora Sannalu) as recurrent parent and *O rufipogon* as donor parent as this accession showed high photosynthesis efficiency in our previous study (Kiran *et al.,* 2013). True F_1 plants were backcrossed with female parent MTU1010 and BC_1F_1 plants were again backcrossed with MTU1010 to generate BC_2F_1 plants (Rao *et al.,* 2018b). BC_2F_1s were raised in normal irrigated field condition at ICAR - IIRR farm during *Rabi* 2015. Only 30 plants out of 238 BC2F1 plants were used for flag leaf dimensions and pigment studies.

Leaf photosynthetic pigments were extracted in cold 80% acetone. Leaf sample of 50 mg was ground in mortar and pestle and the extract was centrifuged at 4000 rpm for 5 min. The supernatant was used for estimation of chlorophyll concentration. Chlorophyll and carotenoid concentration were determined spectrophotometrically (Spectrascan UV 2600, Toshniwal Instruments Pvt. Ltd., India) by measuring the absorbance at 663.2 nm (Chl a), 646.8 (Chl b), and 470 nm (Carotenoid). The pigment concentration was calculated according to Lichtenthaler and Wellburn (1983). The flag leaf traits were measured 10 days after flowering stage in the main stem panicle. Flag leaf length (FLL) was measured from base of ligula to tip of leaf (cm), Flag leaf width (FLW) was measured in the widest part of flag leaf (cm) and Flag leaf area (FLA) was calculated based on length, width and the factor 0.750 (Bhan *et al.*, 1966).

One-way ANOVA was performed using Statistix 8.1 (Analytical Software Inc. USA) software and the statistical significance of the parameter means were determined using HSD test. MS Excel 2007 was used for correlation analysis.

Results and Discussion

Significant differences were observed in chlorophyll a, b, total chlorophyll and carotenoids concentration between the parents (Table 1). Donor parent has significantly higher chlorophyll and carotenoid concentration than recurrent parent. However, the differences were not significant for chlorophyll a/b ratio and chlorophyll/carotenoid ratios. The mean chlorophyll a and total chlorophyll concentration in introgression lines (ILs) varied between 1.88 and 2.33 mg g^{-1}(FM). The highest concentration chlorophyll a and total chlorophyll was found in IL198-15 and least in IL198-51. Chlorophyll b concentration in BC_2F_1 ILs varied from 0.33 (IL 198-49) to 0.70 (IL 198-15) with a mean of 0.44 mg g^{-1}(FM). Sixteen BC_2F_1 ILs showed higher chlorophyll b content than recurrent parent and four BC_2F_1 ILs showed higher values than donor parent. Haritha *et al.*, (2017) and Rao *et al.*, (2018a) reported increased chlorophyll concentration in introgression lines of Swarna/ *O.nivara* compared to parental lines.

The mean chlorophyll a/b ratio was higher in recurrent parent (4.46) compared to donor parent (4.31). Among the BC_2F_1 ILs, chlorophyll a/b ratio varied from 3.82 (IL198-15) to (IL198-53) 4.57 mg g^{-1}(FM). Flag leaf length in ILs varied from 11.20cm – 27.85cm with a mean of 20.71 cm.

Table 1 Variation in leaf chlorophyll content and flag leaf traits in backcross introgression lines

Line no.	Chl a	Chl b	Total	Carotenoids	Chl a/b
198-1	1.72±0.2 defg	0.42±0.0 cdefghi	2.14±0.2 defgh	1.06±0.1 defgh	4.13±0.0 de
198-2	1.77±0.1 cdefg	0.42±0.0 cdefghi	2.19±0.1 cdefgh	1.09±0.1 cdefgh	4.27±0.0 bcde
198-3	1.81±0.0 bcdefg	0.41±0.0 cdefghi	2.23±0.0 bcdefgh	1.10±0.0 cdefgh	4.41±0.0 abcd
198-4	2.03±0.0 bcde	0.47±0.0 bcdef	2.50±0.0 bcde	1.25±0.0 abcde	4.30±0.0 abcde
198-5	2.13±0.0 bcd	0.50±0.0 bcd	2.63±0.0 bcd	1.31±0.0 abc	4.26±0.0 bcde
198-15	2.69±0.0 a	0.70±0.0 a	3.39±0.0 a	0.75±0.0 i	3.82±0.2 f
198-16	1.92±0.1 bcdef	0.46±0.0 bcdefg	2.38±0.1 bcdefg	1.19±0.1 abcdef	4.17±0.0 cde
198-21	1.80±0.1 bcdefg	0.43±0.0 bcdefghi	2.22±0.1 bcdefgh	1.15±0.0 bcdefg	4.22±0.0 bcde
198-22	1.61±0.1 fg	0.37±0.0 fghi	1.99±0.1 fgh	1.03±0.0 efgh	4.30±0.0 abcde
198-26	2.00±0.0 bcdef	0.48±0.0 bcdef	2.48±0.1 bcdef	1.23±0.0 abcde	4.22±0.1 bcde
198-27	1.81±0.0 bcdefg	0.43±0.0 bcdefghi	2.24±0.0 bcdefgh	1.12±0.0 cdefgh	4.26±0.0 bcde
198-28	1.81±0.1 bcdefg	0.41±0.0 cdefghi	2.21±0.1 cdefgh	1.13±0.0 cdefg	4.46±0.1 ab
198-29	1.95±0.1 bcdef	0.44±0.0 bcdefgh	2.39±0.1 bcdefg	1.20±0.0 abcdef	4.47±0.0 ab
198-33	1.93±0.1 bcdef	0.46±0.0 bcdefg	2.39±0.2 bcdefg	1.21±0.1 abcdef	4.23±0.1 bcde
198-35	1.74±0.0 cdefg	0.39±0.0 efghi	2.13±0.0 defgh	1.08±0.0 cdefgh	4.49±0.0 ab
198-36	1.60±0.0 fg	0.36±0.0 ghi	1.96±0.0 gh	0.97±0.0 fghi	4.49±0.0 ab
198-39	2.15±0.1 bc	0.51±0.0 bc	2.66±0.2 bc	1.38±0.1 ab	4.21±0.0 bcde
198-41	2.00±0.1 bcdef	0.49±0.0 bcde	2.49±0.1 bcdef	1.42±0.0 a	4.05±0.0 ef
198-44	1.96±0.0 bcdef	0.46±0.0 bcdefg	2.42±0.0 bcdefg	1.25±0.0 abcde	4.24±0.0 bcde
198-46	1.99±0.1 bcdef	0.46±0.0 bcdefg	2.45±0.1 bcdefg	1.24±0.0 abcde	4.35±0.1 abcd
198-47	1.97±0.0 bcdef	0.47±0.0 bcdef	2.43±0.0 bcdefg	1.20±0.0 abcdef	4.23±0.1 bcde

Line no.	Chl a	Chl b	Total	Carotenoids	Chl a/b
198-49	1.47±0.1 g	0.33±0.0 i	1.80±0.1 h	0.92±0.1 ghi	4.44±0.0 abc
198-50	2.20±0.0 b	0.53±0.0 b	2.72±0.0 b	1.29±0.0 abcd	4.15±0.0 cde
198-51	1.45±0.0 g	0.34±0.0 hi	1.79±0.0 h	0.88±0.0 hi	4.29±0.0 abcde
198-52	1.62±0.1 efg	0.40±0.0 defghi	2.02±0.1 efgh	1.02±0.1 efgh	4.05±0.1 ef
198-53	1.81±0.0 bcdefg	0.40±0.0 defghi	2.21±0.0 cdefgh	1.11±0.0 cdefgh	4.57±0.0 a
198-54	1.96±0.0 bcdef	0.45±0.0 bcdefg	2.41±0.1 bcdefg	1.20±0.0 abcdef	4.32±0.0 abcde
198-55	2.01±0.1 bcdef	0.48±0.0 bcdef	2.48±0.1 bcdef	1.23±0.1 abcde	4.21±0.0 bcde
198-56	1.81±0.0 bcdefg	0.41±0.0 cdefghi	2.22±0.0 bcdefgh	1.10±0.0 cdefgh	4.42±0.0 abc
198-57	1.76±0.0 cdefg	0.39±0.0 efghi	2.16±0.0 cdefgh	1.07±0.0 cdefgh	4.48±0.0 ab
MTU1010	1.91±0.0 bcdef	0.43±0.0 bcdefghi	2.33±0.0 bcdefg	1.12±0.0 cdefg	4.46±0.0 ab
O. rufipogon	2.12±0.0 bcd	0.49±0.0 bcdef	2.61±0.0 bcd	1.31±0.0 abc	4.31±0.0 abcde
Mean	1.89	0.44	2.33	1.14	4.29
HSD	0.41	0.10	0.51	0.24	0.29
CV	6.76	7.31	6.75	6.50	2.08

Line no.	Chl / Car	FLL	FLW	FLA
198-1	2.02±0.0 bcd	20.9±0.3 cdefghijk	1.27±0.1 bcde	19.9±1.3 cdefgh
198-2	2.01±0.0 bcd	25.5±0.8 ab	1.40±0.0 ab	26.8±0.8 a
198-3	2.02±0.0 bcd	23.0±0.2 bcdefg	1.27±0.0 bcde	21.6±0.3 bcde
198-4	2.00±0.0 bcd	17.7±1.6 hijk	0.77±0.0 j	10.0±1.3 mn
198-5	2.01±0.0 bcd	20.6±0.4 cdefghijk	1.10±0.0 efgh	17.0±0.3 efghijkl
198-15	4.53±0.0 a	17.1±0.4 jk	1.47±0.0 a	18.6±0.0 cdefghij
198-16	2.01±0.0 bcd	23.1±0.5 bcdefg	1.30±0.1 abcd	22.6±1.5 abcd
198-21	1.94±0.0 d	20.7±0.4 cdefghijk	1.30±0.0 abcd	20.2±0.4 cdefg
198-22	1.93±0.0 d	20.9±0.1 cdefghijk	1.37±0.0 abc	21.2±0.4 bcdef
198-26	2.02±0.0 bcd	16.7±0.2 k	1.00±0.0 ghi	12.5±0.1 lm
198-27	2.00±0.0 cd	21.0±0.9 cdefghijk	1.37±0.0 abc	21.3±1.4 bcdef
198-28	1.96±0.0 d	23.4±0.2 bcdef	1.47±0.0 a	25.4±0.3 ab
198-29	1.99±0.0 cd	24.4±0.4 abcd	1.47±0.0 a	26.5±0.9 a
198-33	1.98±0.0 cd	18.6±0.5 hijk	1.10±0.0 efgh	15.4±0.4 ghijkl
198-35	1.98±0.0 cd	20.6±1.0 defghijk	0.93±0.0 hij	14.7±1.2 ijklm
198-36	2.02±0.0 bcd	18.8±0.1 ghijk	1.07±0.0 fgh	14.8±0.3 ijklm
198-39	1.93±0.0 d	17.4±2.3 ijk	1.03±0.0 fghi	13.5±1.7 klm
198-41	1.75±0.1 e	11.2±0.2 l	0.87±0.0 ij	7.10±0.1 n
198-44	1.94±0.0 d	20.9±0.3 cdefghijk	1.07±0.0 fgh	16.5±0.7 fghijkl
198-46	1.98±0.0 cd	24.9±2.0 abc	1.10±0.1 efgh	20.8±2.8 bcdef
198-47	2.02±0.0 bcd	21.5±0.9 bcdefghi	1.10±0.0 efgh	17.7±0.7 defghijk
198-49	1.95±0.0 d	23.5±1.2 abcde	1.10±0.0 efgh	19.4±1.0 cdefghi
198-50	2.11±0.0 b	20.4±0.4 defghijk	1.00±0.0 ghi	15.3±0.3 hijkl
198-51	2.03±0.0 bcd	19.1±0.1 fghijk	1.30±0.0 abcd	18.7±0.1 cdefghij
198-52	1.98±0.0 cd	20.1±0.9 defghijk	1.10±0.1 efgh	16.6±0.9 fghijkl
198-53	1.98±0.0 cd	27.9±0.8 a	1.10±0.0 efgh	23.0±0.6 abc

Line no.	Chl / Car	FLL	FLW	FLA
198-54	2.00±0.0 bcd	19.0±0.3 ghijk	1.00±0.0 ghi	14.3±0.2 jklm
198-55	2.02±0.0 bcd	21.2±1.1 bcdefghij	1.07±0.0 fgh	17.0±1.0 efghijkl
198-56	2.02±0.0 bcd	22.0±0.0 bcdefgh	1.13±0.0 defg	19.0±0.5 cdefghij
198-57	2.01±0.0 bcd	19.0±0.3 ghijk	1.20±0.0 cdef	17.1±0.3 efghijkl
MTU1010	2.08±0.0 bc	19.8±0.7 efghijk	1.13±0.0 defg	17.0±0.2 efghijkl
O. rufipogon	1.99±0.0 cd	20.4±0.5 defghijk	1.20±0.0 cdef	18.4±0.5 cdefghij
Mean	2.07	20.67	1.16	18.11
HSD	0.10	4.35	0.17	4.83
CV	1.56	6.51	4.65	8.25

Each value represents mean of three replications ± SD

Table 2 Relationship among the chlorophyll and flag leaf traits in BC_2F_1 of MTU1010/ *O.rufipogon*.

	Chl a	*Chl b*	*Total chl*	*Caro*	*Chl a/b*	*Chl/Car*	*FLL*	*FLW*	*FLA*
Chl a	1.00								
Chl b	0.97***	1.00							
Total chl	0.98***	0.98***	1.00						
Caro	0.33	0.23	0.31	1.00					
Chl a/b	-0.52**	-0.69***	-0.56**	-0.01	1.00				
Chl/Car	0.61***	0.69***	0.63***	-0.53**	-0.51**	1.00			
FLL	-0.29	-0.37*	-0.31	-0.19	0.50**	-0.16	1.00		
FLW	-0.08	-0.04	-0.07	-0.49**	-0.02	0.33	0.45*	1.00	
FLA	-0.23	-0.25	-0.24	-0.35	0.29	0.05	0.84***	0.85***	1.00

Chl = Chlorophyll, Caro = Carotenoids, FLL = Flag leaf length, FLW = Flag leaf width, FLA = Flaf leaf area. The significance of each correlation is indicated: *P < 0.05; **P < 0.01; ***P < 0.001.

Flag leaf width ranged from 0.75cm (IL198-4) to 1.45cm (IL198-28, IL198-29) with a mean of 1.16 cm. Genetic differences in the cultivars and the different environmental effects influence the growth and development of leaves (Zou *et al.,* 2003). Flag leaf area has one of the key roles in determining photosynthetic capacity (Wu *et al.,* 2017). Leaf area varied from 7.13 cm^2 (IL198-41) to 26.83 cm^2 (IL198-2) with a mean of 18.14 cm^2. In comparison to donor parent, 18 ILs (leaf length), 11 ILs (leaf width) and 15 ILs (leaf area) showed higher values. Among all the ILs, two ILs (IL198-16 and IL198-29) showed higher values for 8 traits (chlorophyll a, b, total chlorophyll, carotenoids, chlorophyll a/b ratio, chlorophyll/carotenoid, flag leaf length, width and area) over recurrent parent and one IL (IL198-15) showed higher values than donor parent for 6 traits (chlorophyll a, b, total chlorophyll, flag leaf length, width and area). In Swarna/ *O.nivara* BILs, total chlorophyll ranged from 1.40 to 2.24 with overall mean value 1.78 (Rao *et al.,* 2018a).

Highly significant correlation was observed between chlorophyll a, chlorophyll b with total chlorophyll and chlorophyll/carotenoid (Table 2). Zhang *et al.,* (2015) reported significant positive correlation between flag leaf length, flag leaf width and flag leaf width was significantly positively correlated with grain yield in rice. In our study, chlorophyll a/b ratio showed significantly negative correlation with chlorophyll a, chlorophyll b and total chlorophyll. Kiran *et al.,* (2013) and Haritha *et al.,* (2017) also reported negative correlation between chlorophyll a/b and chlorophyll a, chlorophyll b and total chlorophyll.

Conclusion

Oryza rufipogon accessions can be used as donors to improve the leaf pigment concentration and flag leaf traits of *O. sativa* which helps to enhance the photosynthetic rate. Three ILs, *viz.*, IL198-15, IL198-16 and IL198-29 showed trait dominance over parents, which could be further dissected to understand their genetic constitution in relation to leaf pigment concentration and flag leaf dimensions.

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References

Ali Y, Aslam Z, Ashraf MY and Tahir GR. 2017. Effect of salinity on chlorophyll concentration, leaf area, yield and yield components of rice genotypes grown under saline environment. *International Journal of Environmental Science & Technology*. 3:221–225.

Bhan VM and Pande HK. 1966. Measurement of leaf area of rice. *Agron. J*. 58:454.

Dingkuhn M, Laza MR, Kumar U, Mendez KS, Collard B, Jagadish K. and Sow A. 2015. Improving yield potential of tropical rice: achieved levels and perspectives through improved ideotypes. *Field Crops Research*. 182:43–59.

Haritha G, Vishnukiran T, Yugandhar P, Sarla N, Subrahmanyam D. 2017. Introgressions from *Oryza rufipogon* increase photosynthetic efficiency of KMR3 rice lines. *Rice Science*. 24(2):85–96.

Kiran TV, Rao YV, Subrahmanyam D, Rani NS, Bhadana VP, Rao PR, Voleti SR. 2013. Variation in leaf photosynthetic characteristics in wild rice species. *Photosynthetica*. 51:350–358.

Lichtenthaler HK, Wellburn AR. 1983. Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans*. 11:591–592.

Rao VY, Divya B, Raju AK, Mesapogu S, Kiran TV, Subrahmanyam D, Sarla N, Voleti SR. 2018a. Characterization of backcross introgression lines derived from *Oryza nivara* accessions for photosynthesis and yield. *Physiology and Molecular Biology of Plants*. 24 (6):1147–1164.

Rao VY, Raju AK, Malathi S, Sukumar M, Kavitha B, Divya B, Sarla N. 2018b. Interspecific hybridization for the development of chromosome segment substitution lines of rice in India. *Oryza*. 55(4): 511–522.

Samal R, Roy PS, Sahoo A, Kar MK, Patra BC, Marndi BC. and Gundimeda JN. 2018. Morphological and molecular dissection of wild rice from eastern India suggests distinct speciation between *Oryza rufipogon* and *Oryza nivara* populations. *Sci Rep*, 8(1): 2773.

Swamy BPM and Sarla N. 2008. Yield- enhancing quantitative trait loci (QTLs) from wild species. *Biotechnology Advances*. 26:106–120.

Tripathy K, Singh B, Singh N, Rai V, Misra G and Singh NK. 2018. A database of wild rice germplasm of *Oryza rufipogon* species complex from different agro-climatic zones of India. *Database*. 1–6.

Wu J, Qi Y, Hu G, Li J, Li Z, Zhang H. 2017. Genetic architecture of flag leaf length and width in rice (*Oryza sativa L.*) revealed by association mapping, *Genes Genom*. 39:341–352.

Zhang B, Ye W, Ren D, Tian P, Peng Y, Gao Y, *et al*. 2015. Genetic analysis of flag leaf size and candidate genes determination of a major QTL for flag leaf width in rice. *Rice*. 8:39.

Zhu XG, Long SP, Ort DR. 2010. Improving photosynthetic efficiency for greater yield. *Ann Rev Plant Biol*. 61:235–261.

Zou JS, Yao KM, Lu CG, Hu XQ. 2003. Study on individual plant type character of Liangyoupeijiu rice. *Acta Agron. Sin*. 29: 652–657.

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Long term effect of fertilization on rice (*Oryza sativa*) yield, nutrient uptake, economics and soil fertility under 25 years old rice-rice cropping system in vertisol of Tamil Nadu

Sharmila Rahale C*

Assistant Professor (SS&AC)
Department of Nano Science & Technology, Tamil Nadu Agricultural University, Coimabtore-3. Tamil Nadu, India
* Corresponding author email: sharmicj@yahoo.com

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Abstract

The continuous use of fertilizers on yield, nutrient uptake, economics and soil fertility was studied under 25 years old rice-rice cropping system in Vertisol of sub tropical India. Significant increase in grain and straw yield and nutrients (N,P and K) uptake in grain and straw was observed in Integrated Nutrient Management (INM) practices. A strong negative correlation occurred between organic carbon and sand and bulk density of soil. Correlation between organic carbon and silt, clay, pore space, infiltration rate and water holding capacity are significantly positive. The INM treatment recorded higher yield resulting in higher economic returns, additional net income and benefit: cost ratio. Application of RDF with organic manures in rice is socially acceptable, economically viable and environmentally sustainable way of nutrient application which helps in sustainable crop production, greater profit and maintaining soil quality.

Keywords: Rice-rice cropping system, integrated nutrient management, soil fertility, net income, partial budgeting

Introduction

Generally in field experiments, when fertilizers are once applied to a particular unit they are not fully utilized on that particular unit. The treatment may leave residual effect on the succeeding crop. Among the nutrients, nitrogen shows a fair response in the same crop season when it is applied while, response of phosphorous and potash is generally visible in the second and third year of experimentation (Liu *et al.,* 2014). The soil gets exhausted due to increase in plant growth inspite of continuous application of nitrogen, phosphorus and potassium. To formulate fertilizer recommendations to crops, it is therefore, essential that the experiment should be repeated over time at the same site, because, the effects of climate, soil , fertilizer, agronomic practices get stabilized only after a period of years and responses to fertilizer treatments also become more stable and reliable (Kidd *et al.,* 2017). Long term field experiments, therefore, form one of the most useful tools for technical advances and are indispensable for framing empirical rules for the conduct of practical agriculture. These experiments can be used for precise monitoring of changes in soil fertility and soil productivity.

Rice is the most important and staple food crop for more than two third of population of India. The slogan "RICE IS LIFE" is most appropriate for India as this crop plays vital role in our national food security and a mean of live hood for millions of rural households. India is the second largest rice producing country in the world after China. Cauvery Delta Zone is the potential tract in the traditional rice cultivated area of Tamil Nadu. Rice-Rice cropping system is the most prevailing system in this zone (Stalin *et al.* 2006). It is being increasingly realized that when crops are grown in system, the fertilizer requirement of the cropping system as a whole is important than individual crop (Sharma and Subehia, 2003). There is a need to revive the age old practice of application of organic manures to maintain soil fertility and also to supplement many essential plant nutrients for crop productivity. The use of inorganic fertilizers in combination with organic manures has been found more advantageous than either of them alone for sustainable agriculture on long term basis (Kumara *et al.,* 2018). However, long term fertilization of inorganic fertilizers with organic fertilizers, farm chemicals and biofertilizers in rice - rice cropping system is very limited. Keeping these points in mind, a Permanent Manurial Experiment (PME) was started to study the continuous use of inorganic fertilizes with organic fertilizers, farm chemicals and biofertilizers on soil fertility and rice productivity in 1992 in Tamil Nadu Rice Research Institute, Aduthuurai, Thanjavur district, Tamil Nadu, India.

Materials and Methods

Experimental Site and Design

Permanent Manurial Experiment with fixed plots has been laid out in a randomized block design with four replications. Thirteen treatments were compared in both *Kharif* and *Rabi* seasons (Table 1). Treatments in the study involving various levels of fertilizers (organics and inorganics), bio-fertilizers, herbicide and soil amendments were compared. A uniform plot size of 22.5 x 3.5 m (78.75 m^2) was adopted.

Crop Management

For treatments of green manure (T_5, T_6 and T_7), *Sesbania rostrata* (3.23 % N, 0.32 % P and 4.30% K dry weight basis) was grown *in situ* and incorporated (35-40th day) at 6.25 t ha^{-1} (dry weight basis) for *kharif* rice while *rabi* rice received farmyard manure (0.67 % N, 0.24 % P and 0.70 % K) at 12.5 t ha^{-1} (dry weight basis) for the same set of treatments. Gypsum was applied at 500 kg ha^{-1} as a source for Ca and S. For the treatment T9, the weedicide Butachlor was applied at 2.5 lit ha^{-1} within eight days of transplanting in both the seasons. In *kharif* crop, Azospirillum at 2 kg ha^{-1} mixed with sand was broadcasted before transplanting. Blue Green Algae (BGA) flakes at 10 kg ha^{-1} were broadcasted 10 days after transplanting in *rabi* season. Composted coirpith at 12.5 t ha^{-1} (1.24% N, 0.06 % P, 1.2 % K, 0.5 % Ca, 0.48 % Mg and 15.8 % Zn) was applied. The N is applied in four splits (25 % each) in both *kharif* and *rabi* seasons (*kharif*: Basal, 15 DAT, 30 DAT, 45 DAT; *Rabi*: Basal, 20DAT, 40 DAT, 60 DAT). In $T_{1,}$ Potassium was skipped in both the seasons. In T_2, Phosphorus was skipped in both the seasons. In T_4, Phosphorus was skipped in *rabi* season alone to study the residual effect of Phosphorus. In T_{11}, 75 % of NPK was applied in *rabi* season. Most popular rice varieties of Cauvery delta region *viz.*, ADT 43 and ADT 45 for short duration (*kharif*) and ADT 38 and ADT 39 for medium duration (*rabi*) were grown in these experiments. Need based plant protection measures were taken to control insect pests and diseases.

Grain yield and straw yield

At maturity, the crop was harvested and the grain and straw yields were recorded from the net area of 5 m^2 in each plot. The grain yield was adjusted to 14 % moisture level.

Soil analysis

At the end of each year (after harvesting of *rabi* crop), representative post harvest soil samples were collected from the surface (0-15 cm). In each plot, the soil was collected from eight points randomly, and mixed into one sample. Then the samples were air dried in shade and ground to pass through 2 mm sieve and used for the estimation of soil chemical properties. Mechanical composition of the soil under various treatments was determined by International Pipette Method (Piper, 1966). Bulk density and particle density and pore space were determined (Blake 1965a & 1965b). Steady state infiltration rate was measured by using double ring infiltrometer. Readings was recorded at 5, 10, 15, 30 minutes and then one hour intervals till constant steady state rate was obtained (Gupta, 1999). Water holding capacity of soil was determined by the Keen-Raczkowski Box Method (Keen and Raczkowski, 1921).

Plant analysis

Plot wise samples of rice grain and straw were dried at 65^{o}C in oven and ground in a Wiley mill for chemical analysis. Total nitrogen was determined after digesting the sample with concentrated H_2SO_4 using digestion mixture of K_2SO_4 and $CuSO_4$ (10:1) followed by steam distillation in a micro-kjeldahl nitrogen distillation unit (Jackson, 1973). For other nutrients, grain and straw samples were digested with di-acid mixture of HNO_3:$HClO_4$ (10:4) and subsequently used for analysis. Total phosphorus content in the acid digest was determined by spectrophotometer after developing vanadomolybdo-phosphate yellow colour complex as described by Jackson (1973). Potassium content in the acid digest was determined by a flame photometer (Jackson, 1973). The nutrient uptake was calculated by multiplying per cent concentration of a particular nutrient with grain and straw yields. The uptake of the nutrients obtained in respect of grain and straw was summed up to compute the amount of total nutrient removed by the crop.

Economics

The simple averages and percentage statistical tools were applied to analyze the data.

Input output ratio = Gross income / Total cost.

Benefit-cost = Net income / Total cost.

Partial Budgeting

Increase in costs and decrease in returns due to adaptation is the total additional cost (A) for that adaptation measure. The benefit (B) is accounted by the decrease in costs and increase in returns due to the adoption of that adaptation measure. The difference (B-A) between the additional returns and additional cost is the net benefit of that adaptation measure (CIMMYT, 1988).

Statistical Analysis

The data for the crop and soil properties were analysed by analysis of variance as outlined by Gomez and Gomez (1984). The significance of the treatment effect was determined using F-test and to determine the significance of the difference between the means of the two treatments, least significant differences (LSD) at 5% probability level. Correlations and regressions were determined using the data analysis tool pack of MS Excel (2003).

Table 1 Treatment details

T. No.	*Kharif* (kg ha^{-1})				*Rabi* (kg ha^{-1})			
	N	P_2O_5	K_2O		N	P_2O_5	K_2O	
1	125	50	0		150	60	0	
2	125	0	50		150	0	60	
3	125	50	50		150	60	60	
4	125	50	50		150	0	60	
5	125	50	50	GM	150	60	60	FYM
6	125	50	50	GM + Azos	150	60	60	FYM+BGA
7	125	50	50	GM+GYP	150	60	60	FYM+GYP
8	125	50	50	$ZnSO_4$	150	60	60	$ZnSO_4$
9	125	50	50	WC	150	60	60	WC
10	125	50	50	GYP	150	60	60	GYP
11	125	50	50		112.5	45	45	
12	125	50	50	CPC	150	60	60	CPC
13	Absolute control				Absolute control			

FYM: Farm yard manure – 12.5 t ha^{-1}; GM:Green manure – 6.25 t ha^{-1}; GYP: Gypsum – 500 kg ha^{-1}; WC: Herbicide (Butachlor 2.5 lit ha^{-1}); CPC : Coirpith compost – 12.5 t ha^{-1}; Azos: Azospirillum – 2 kg ha^{-1}; BGA: Blue Green Algae – 10 kg ha^{-1}; Zn SO_4 @ 25 kg ha^{-1}

For *Kharif* N splits: 4 splits: Basal, 15 DAT, 30 DAT, 45 DAT – 25 % each ; For *Rabi* N splits: 4 splits: Basal, 20 DAT, 40 DAT, 60 DAT – 25 % each

Results and Discussion

Grain and straw yield

All the treatments with fertilizers either alone or in combination with organics/biofertilizer/herbicide/soil amendment under study showed significant increase in yield over control (Table 2). During both the seasons, the application of NPK fertilizer at 125:50:50 kg ha^{-1} along with green manure @ 6.25 t ha^{-1} and gypsum @ 500 kg ha^{-1} (T_7) recorded significantly higher grain yield than other treatments. On an average, the treatment NPK + green manure +gypsum (T_7) was the most productive with yields increasing up to 81% over control (T_{13}), 66% over NPK + Gypsum (T10), 62% over NPK +Green Manure + Azospirillum (T6) and 59% over NPK + Green Manure, respectively. The grain yield data under the study emphasized the need for conjunctive use of organic manures with inorganic NPK fertilizers. Besides application of gypsum might have provided sulphur nutrient for rice and also eliminated ill-effects of toxicity of ferrous and manganese ions due to continuous submergence. Therefore, the combined use of organics, inorganics and specific amendment could sustain the productivity of rice in heavy soil of Cauvery Delta Zone. Organic manures acting as slow release source of N are expected to more closely match with N and supply of other nutrients with demand of rice crop and this could have reduced the N losses and also improved the nutrient use efficiency particularly of nitrogen. Therefore, inorganic fertilizers in combination with organic manures caused the greater translocation of photosynthates from source to sink site that resulted higher grain yield of rice (Naveen kumar *et al.*, 2019).

Table 2 Effect of treatments on grain yield (kg ha^{-1})

Treatments	*Kharif* season		Treatments	*Rabi* season	
	Grain Yield	% increase over control		Grain Yield	% increase over control
NP	4809	47	NP	5932	43
NK	4782	46	NK	5693	37
NPK	4958	51	NPK	5938	43
NPK	4932	50	NK	5341	30
NPK+GM	5216	59	NPK+FYM	6099	47
NPK +GM+Azos	5316	62	NPK +FYM+BGA	6262	51
NPK +GM+GYP	5942	81	NPK +FYM+GYP	6603	59
NPK +$ZnSO_4$	5054	54	NPK +$ZnSO_4$	6149	48
NPK +Herbicide	5092	55	NPK +Herbicide	6089	47
NPK + GYP	5442	66	NPK + GYP	6391	54
NPK	4888	49	NPK -75%	5828	40
NPK + CPC	5180	58	NPK + CPC	6056	46
Absolute Control	3269		Absolute Control	4141	
CD	216*		CD (p=0.05)	225*	

* = Significant at $p \leq 0.05$

FYM :Farm yard manure – 12.5 t ha^{-1}; GM:Green manure – 6.25 t ha^{-1}; GYP: Gypsum – 500 kg ha^{-1}; WC: Herbicide (Butachlor 2.5 lit ha^{-1}); CPC : Coirpith compost – 12.5 t ha^{-1}; Azos: Azospirillum – 2 kg ha^{-1}; BGA: Blue Green Algae – 10 kg ha^{-1}; Zn SO_4 @ 25 kg ha^{-1}

The same trend was observed in straw yield (Table 3) also. Increase in straw yield might be attributed to increase in photosynthetic area and dry matter accumulation. Slow available nitrogen from organic manure is known to enhance the formation of new cells, promotes root and shoot growth. It is also associated with the vital oxidation reduction reactions of various physiological processes determining the supply of photosynthates to proliferating shoots and other parts. Thus, readily available N in organic and inorganic sources of nutrients might have helped in production of large number of shoots and finally their conversion into dry matter accumulation and straw yield per unit area. (Indoria *et al*., 2018; Moe *et al*., 2019).

Table 3 Effect of treatments on straw yield (kg ha^{-1})

Treatments	*Kharif* season		Treatments	*Rabi* season	
	Straw yield	% increase over control		Straw yield	% increase over control
NP	5815	44	NP	6533	24
NK	5859	45	NK	6124	16
NPK	6091	51	NPK	6598	26
NPK	6087	50	NK	6023	15
NPK+GM	6337	57	NPK+FYM	7093	35
NPK +GM+Azos	6450	59	NPK +FYM+BGA	7236	38
NPK +GM+GYP	6906	71	NPK +FYM+GYP	7653	46
NPK +$ZnSO_4$	6398	58	NPK +$ZnSO_4$	7195	37
NPK +Herbicide	6279	55	NPK +Herbicide	6945	32
NPK + GYP	5484	60	NPK + GYP	7212	37
NPK	6054	50	NPK -75%	6523	24
NPK + CPC	6384	58	NPK + CPC	7023	34
Absolute Control	4032		Absolute Control	5236	
CD	312*		CD (p=0.05)	392*	

* = Significant at $p \leq 0.05$

FYM : Farm yard manure – 12.5 t ha^{-1}; GM:Green manure – 6.25 t ha^{-1}; GYP: Gypsum – 500 kg ha^{-1}; WC: Herbicide (Butachlor 2.5 lit ha^{-1}); CPC : Coirpith compost – 12.5 t ha^{-1}; Azos: Azospirillum – 2 kg ha^{-1}; BGA: Blue Green Algae – 10 kg ha^{-1}; Zn SO_4 @ 25 kg ha^{-1}

Nutrient uptake by grain and straw

The data (Table 4) showed that, the treatments were statistically significant in case of grain and straw uptake for all the three (N, P and K) nutrients. The integrated nutrient management treatments had a favourable effect on the uptake of nutrients (N, P and K) than that of inorganic treatments and control. In both the seasons, the absolute control (T_{13}) recorded lower N, P and K uptake values which are again the reflection of the lowest yield recorded in the absolute control plots. The higher NPK uptake may be due to higher yield received in this treatment (Arunkumar *et al*., 2014; Gill and Aulakh, 2018). Generally, application of recommended dose of NPK along with organics increased the N, P and K uptake by rice. Jacqueline *et al.*, (2008) reported that the N uptake by rice grain and straw increased significantly with the combined application of organic manure and chemical fertilizers. Niederberger *et al.*, (2019) reported that organic manures increased labile, moderately stable and stable organic P contents in soil and uptake by plants. Basak *et al*., (2016) reported that application of organic manure and chemical fertilizers significantly increased the K uptake by rice.

Table 4 Effect of treatments on nutrient uptake in grain and straw (kg ha^{-1})

Treatments	*Kharif* season						Treatments	*Rabi* season					
	Grain			Straw				Grain			Straw		
	N	P	k	N	P	k		N	P	k	N	P	k
NP	50.0	9.5	10.5	35.0	6.0	67.8	NP	57.3	11.9	10.9	29.2	8.6	63.7
NK	51.1	9.0	12.5	34.9	5.5	83.6	NK	60.4	11.1	14.8	30.7	6.3	74.4
NPK	57.7	10.7	14.5	42.3	7.3	93.7	NPK	63.3	13.4	15.8	37.0	8.4	78.5
NPK	55.2	9.4	12.6	40.4	6.1	90.3	NK	60.2	11.6	15.3	34.3	7.6	75.0
NPK+GM	61.1	10.5	14.6	45.3	7.1	103.4	NPK+FYM	68.7	14.1	17.5	39.6	8.7	81.5
NPK +GM+ Azos	56.8	10.6	14.5	42.7	6.7	65.9	NPK +FYM+BGA	69.2	13.6	17.5	38.8	9.1	82.4
NPK+GM+GYP	71.0	11.2	17.8	53.7	8.1	115.9	NPK +FYM+GYP	73.9	14.8	19.1	41.1	9.9	90.2
NPK +$ZnSO_4$	61.7	10.5	14.5	41.3	6.8	96.0	NPK +$ZnSO_4$	66.2	13	16.1	34.4	7.8	80.4
NPK +Herbicide	59.7	9.7	13.2	42.1	6.2	89.6	NPK +Herbicide	64.4	12.6	15.6	35.8	8.8	81.7
NPK + GYP	64.2	11.5	15.7	45.5	7.1	104.2	NPK + GYP	64.9	13.5	16.2	37.8	8.3	83.8
NPK	55.0	9.5	13.0	37.2	6.2	87.1	NPK -75%	61.0	11.8	14.2	33.4	8.2	77.2
NPK + CPC	59.1	10.8	14.5	41.5	7.2	96.6	NPK + CPC	63.0	13.0	17.4	36.3	7.6	78.7
Absolute Control	32.1	6.0	7.0	16.7	3.5	37.3	Absolute Control	33.4	7.0	7.7	15.4	4.6	40.4
CD(p=0.05)	7.5*	1.5*	2.3*	4.6*	0.88*	8.1*	CD(p=0.05)	5.3*	1.5*	3.3*	3.6*	1.3*	10.5*

*= Significant at $p \leq 0.05$

FYM: Farm yard manure – 12.5 t ha^{-1}; GM: Green manure – 6.25 t ha^{-1}; GYP: Gypsum – 500 kg ha^{-1}; WC: Herbicide (Butachlor 2.5 lit ha^{-1}); CPC : Coirpith compost – 12.5 t ha^{-1}; Azos: Azospirillum – 2 kg ha^{-1};

BGA: Blue Green Algae – 10 kg ha^{-1}; Zn SO_4 @ 25 kg ha^{-1}

Soil Correlation studies

Simple Correlation Coefficients (r) between soil physical parameters and organic carbon were studied at the end of 25th year. When soil organic carbon increases, sand content decreases. A high degree negative correlation of organic carbon was observed with sand content (r = - 0.806). But when organic carbon increases, the silt and clay content were increased. So a positive correlation of organic carbon was observed with silt (r = 0.629) and clay content (r = 0.411) of soil samples (Fig.1-3). Organic carbon and bulk density of soils showed strong negative correlation between them. Similar results were obtained as strong negative correlation (r = - 0.7536) between organic matter and bulk density (Fig. 4) which is required for the proper growth of the plants. But there was a strong positive correlation between organic carbon and pore space (r = 0.609), infiltration rate (r =0.895) and water holding capacity (r=0.655) (Fig.5-7). The table 5 gives the correlation coefficient between organic carbon and other soil properties in nutshell. Bindhu and Sujata (2017) stated a reverse correlation between organic carbon and bulk density. Similar result was obtained by Sakin (2012).

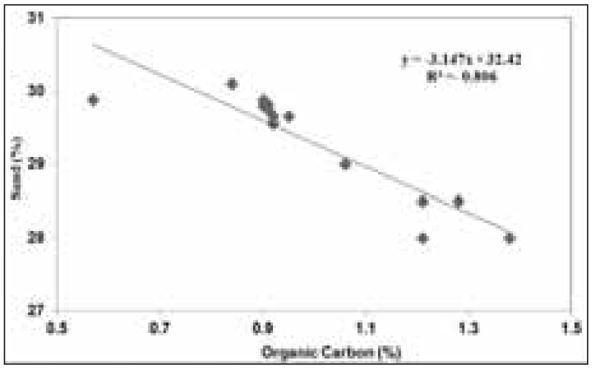

Fig.1. Relationship of organic carbon (%) with sand (%)

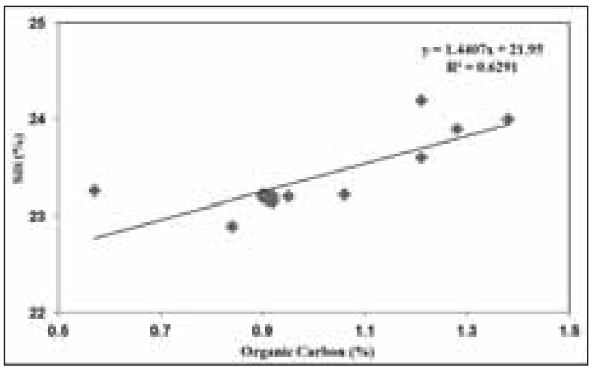

Fig.2. The relationship of organic carbon (%) with silt

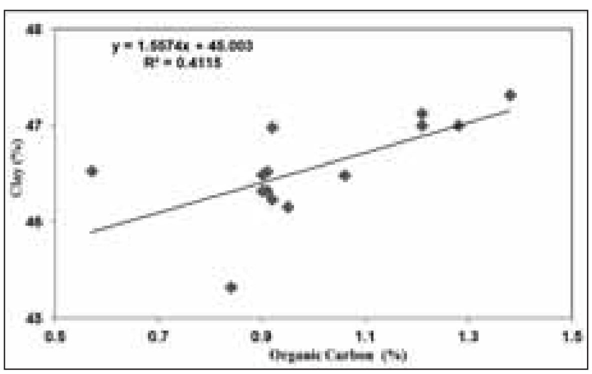

Fig.3. The relationship of organic carbon (%) with clay (%)

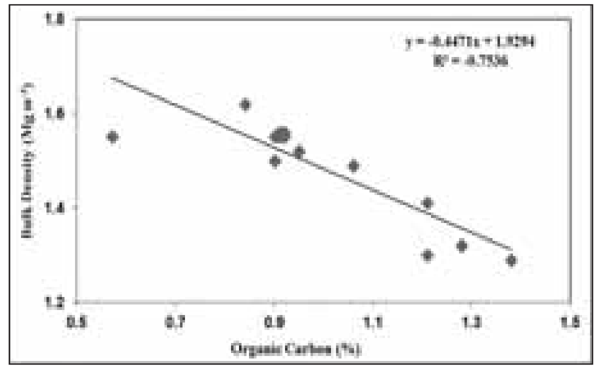

Fig.4. The relationship of organic carbon (%) with Bulk density (Mgm-3)

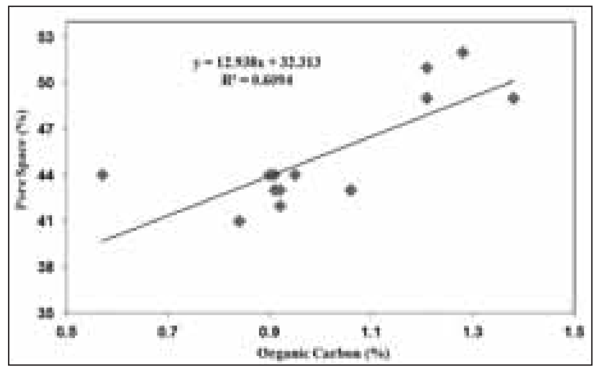

Fig.5. The relationship of organic carbon (%) with pore space (%)

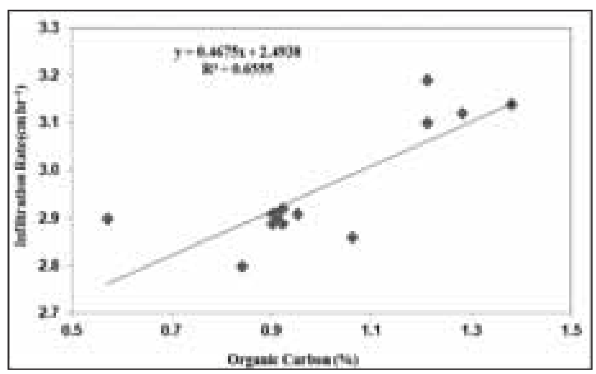

Fig.6. The relationship of organic carbon (%) with infiltration rate (cm hr^{-1})

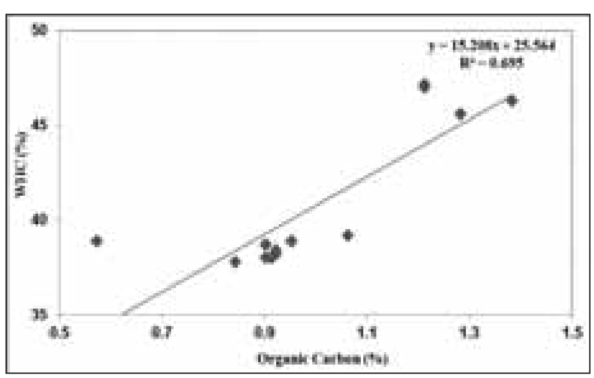

Fig. 7 The relationship of organic carbon (%) with water holding capacity (%)

Table 5 Simple correlation coefficient between soil properties

Related soil parameters	Correlation Coefficient (r)	Level of significance
Organic Carbon – Sand %	- 0.806	Strong negative
Organic Carbon – Silt %	0.629	Significant positive
Organic Carbon – Clay %	0.411	Significant positive
Organic Carbon – BD	- 0.754	Strong negative
Organic Carbon – Pore Space	0.609	Significant positive
Organic Carbon – Infiltration Rate	0.895	Strong positive
Organic Carbon – Water Holding Capacity	0.695	Significant positive

Economics

Net return and B: C ratio was calculated in both the seasons in rice crop fertilized with organic and inorganic fertilizers (Table 6). Gross return was calculated as the total value of grain and straw yield of rice. The highest net return of Rs.60, 230 was obtained in the treatment T_7 during *kharif* season and the same treatment recorded the highest Benefit Cost ratio of 2.96. In *rabi*, the same treatment (T_7) recorded the highest net return of Rs.62, 231 and the Benefit Cost ratio of 2.98. In both seasons, the treatment T_7 was followed by T_6 with respect to maximum net returns and B:C ratio. The minimum net return and benefit cost ratio was obtained in control (T_{13}) in both the seasons. The treatment T_7 recorded the highest additional net income (Rs.31, 808 in *kharif* and Rs.33, 935 in *Rabi*) than other treatments in both the seasons (Table 7). Hence, it can be reasonably concluded that integrating organic manures along with inorganic fertilizers would be the best. Combined use of organic manures with inorganic fertilizers could save part of the money that would have been paid for the greater doses of the chemical fertilizer and is socially acceptable. The higher yield realized under the INM treatment would be the reason for more economic return as against the cost of cultivation with higher net gain, additional net income and benefit: cost ratio. The result was in conformity with the findings of Jat *et al.*, (2018), who also realized higher economic return due to integrated nutrient management practices.

Table 6 Effect of continuous application of fertilizers on net returns (Rs.) and B:C ratio

Treatments	***Kharif* season**		Treatments	***Rabi* season**	
	Net Returns (Rs.)	**B:C ratio**		**Net Returns (Rs.)**	**B:C ratio**
NP	34,890	1.71	NP	35,982	1.71
NK	33,980	1.76	NK	35,432	1.78
NPK	36,262	1.79	NPK	37,122	1.79
NPK	35,170	1.79	NK	36,152	1.79
NPK+GM	38,094	1.96	NPK+FYM	40,194	1.96
NPK +GM+Azos	57,526	2.54	NPK +FYM+BGA	53,556	2.24
NPK +GM+GYP	60,230	2.96	NPK +FYM+GYP	62,231	2.98
NPK +$ZnSO_4$	47,090	1.87	NPK +$ZnSO_4$	49,234	1.88
NPK +Herbicide	43,740	1.80	NPK +Herbicide	42,134	1.80
NPK + GYP	43,990	1.80	NPK + GYP	45,236	1.89
NPK	44,470	1.83	NPK -75%	44,723	1.83
NPK + CPC	41,134	1.70	NPK + CPC	42,351	1.72
Absolute Control	-912	1.01	Absolute Control	-1125	1.02

FYM :Farm yard manure – 12.5 t ha^{-1}; GM:Green manure – 6.25 t ha^{-1}; GYP: Gypsum – 500 kg ha^{-1}; WC: Herbicide (Butachlor 2.5 lit ha^{-1}); CPC : Coirpith compost – 12.5 t ha^{-1}; Azos: Azospirillum – 2 kg ha^{-1}; BGA: Blue Green Algae – 10 kg ha^{-1}; Zn SO_4 @ 25 kg ha^{-1}

Table 7 Effect of continuous application of fertilizers on change in net income (Partial Budgeting)

Kharif season		*Rabi* season	
Treatments	**Change in Net Income (Rs.)**	**Treatments**	**Change in Net Income (Rs.)**
NP	18414	NP	17090
NK	18365	NK	15880
NPK	18375	NPK	17905
NPK	17489	NK	19509
NPK+GM	17372	NPK+FYM	15940
NPK +GM+Azos	20853	NPK +FYM+BGA	22562
NPK +GM+GYP	31808	NPK +FYM+GYP	33935
NPK +$ZnSO_4$	20169	NPK +$ZnSO_4$	22113
NPK +Herbicide	20370	NPK +Herbicide	18341
NPK + GYP	25011	NPK + GYP	23681
NPK	17848	NPK -75%	20239
NPK + CPC	17657	NPK + CPC	22411
Absolute Control	18414	Absolute Control	17090

FYM : Farm yard manure – 12.5 t ha^{-1}; GM:Green manure – 6.25 t ha^{-1}; GYP: Gypsum – 500 kg ha^{-1}; WC: Herbicide (Butachlor 2.5 lit ha^{-1}); CPC : Coirpith compost – 12.5 t ha^{-1}; Azos: Azospirillum – 2 kg ha^{-1}; BGA: Blue Green Algae – 10 kg ha^{-1}; Zn SO_4 @ 25 kg ha^{-1}

Therefore, taking the findings of the present study into consideration, it may be concluded that application of recommended dose of NPK (125:50:50) along with Green Manure @ 6.25 t/ha and gypsum 500 kg/ha in *kharif* and in *rabi* recommended dose of NPK (150:60:60) along with FYM @ 12.5 t/ha and gypsum 500 kg/ha improved rice productivity, maintained soil quality with economic gain in rice cultivation. Application of RDF with organic manures in rice is socially acceptable, economically viable and environmentally sustainable source of nutrient application which helps in sustainable crop production, providing greater profit and maintaining soil quality.

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References

Arun kumar R, Meena N, Lalji Yadav and Gilotia YK. 2014. Effect of organic and inorganic sources of nutrient on yield, yield attributes and nutrient uptake of rice. *The Bioscan* 9: 595-597.

Basak Kapil, Kibria Mohammad, Hossain Mahmud and Hoque Md. 2016. Improvement of rice production through combined use of organic manures and bio-slurries with chemical fertilizers. *Asian Australas. J. Biosci. Biotechnol.* 1 (1): 78-85.

Bindu Sharma and Sujata Bhattacharya. 2017. Soil bulk density as related to soil texture, moisture content, pH, electrical conductivity, organic carbon, organic matter content and available macro nutrients of pandoga sub watershed, Una District of H.P (India). *Int. J. Engg. Res.and Devlop.*, 13: 72-76.

Blake GR. 1965a. Particle density. pp. 371-374, In: Methods of Soil Analysis (Black CA. Ed.), Part I, American. Society of Agronomy. Madison, WI.

Blake GR. 1965b. Bulk density. pp. 374-390, In: Methods of Soil Analysis (Black CA. Ed.), Part I, American. Society of. Agronomy. Madison, WI.

CIMMYT 1988. From agronomic data to farmer recommendations. An economic training manual. Completely Revised Edition. Mexico, pp. 79.

Gomez KA and Gomez AA. 1984. Statistical Procedures for Agricultural Research - An IRRI Book. John Wiley and Sons, New York.

Erdal Sakin. 2012. Organic carbon, organic matter and bulk density relationships in arid-semi arid soils in Southeast Anatolia region. *African J. Biotech.*11: 1373-1377.

Gill PK and Aulakh CS. 2018. Effect of integrated nitrogen management on NPK uptake in basmati rice (*Oryza sativa* L). *J.Applied and Nat. Sc.* 10: 258 - 261.

Gobi R, Stalin P and Sathyamurthi S. 2019. Sustainable agronomic approaches for enhancing growth and yield of rice. *Plant Achieves* 19: 609-612

Gupta PK. 1999. Soil, plant, water and fertilizer analysis. Publication AGRO-BIOS, Jodhpur, India. pp. 24-61.

Indoria AK, Sharma KL, Sammi Reddy K, Srinivasarao CH, Srinivas K, Balloli S, Osman M, Pratibha G and Raju NS. 2018. Alternative sources of soil organic amendments for sustaining soil health and crop productivity in India – impacts, potential availability, constraints and future strategies. *Current Sci.*, 115 December 2018.

Jackson ML.1973. Soil Chemical Analysis. Prentice Hall of India.

Jacqueline A, Prudente A, Gilbert C, Sigua M Manoch K and Alfredo D. 2008. Improving yield and nutrient uptake potentials of Japonica and Indica rice varieties with nitrogen fertilization. *Journal of Agricultural Sciences* 4: 427-434.

Jat N, Yadav R, Kumar Sudhir, Ravisankar, Natesan Shamim, Mohammad. 2018. Evaluation of nutrient management practices under different cropping systems in north western Indo-Gangetic plains of India. *Annals of Pl. and Soil Res.* 20: 409-415.

Jörg Niederberger, Martin Kohler, and Jürgen Bauhus.2019. Distribution of phosphorus fractions with different plant availability in German forest soils and their relationship with common soil properties and foliar P contents. *SOIL*. 5:189–204. https://doi.org/10.5194/soil-5-189-2019.

Keen BA and Raczkowski H. 1921. Relation between the clay content and certain physical properties of a soil. *Journal of Agricultural Sciences.* 11: 441-449.

Kidd J, Manning P, Simkin J, Peacock S, Stockdale E. 2017. Impacts of 120 years of fertilizer addition on a temperate grassland ecosystem. *PLoS ONE* 12(3): e0174632. https://doi.org/10.1371/journal.pone.0174632

Kyi Moe, Aung Zaw Htwe, Thieu Thi Phong Thu, Yoshinori Kajihara and Takeo Yamakawa.2019. Effects on NPK status, growth, dry matter and yield of rice (*Oryza sativa*) by organic fertilizers applied in field condition. *Agriculture* 9:109. doi:10.3390/agriculture 9050109.

Kumara BH, Antil RS, Priya HR and Devraj. 2018. Long-term effects of organic manures and inorganic fertilizers on organic carbon and nutrient contents in soil under pearl millet-wheat cropping sequence. *J of Pharmacognosy and Phytoche.* 1335-1339.

Liu CW, Sung Y, Chen BC, Lai HY. 2014. Effects of nitrogen fertilizers on the growth and nitrate content of lettuce (*Lactuca sativa* L.). *Int. J Environ Res Public Health.* 11:4427–4440.

Piper CS.1966. Soil and Plant Analysis. Hans Publishers, Bombay, India.

Stalin P, Ramanathan S, Nagarajan R. and Natarajan K. 2006. Long term effect of continuous manurial practices on grain yield and some soil chemical properties in rice based cropping system. *Journal of Indian Society of Soil Science.* 54:30-37.

Sharma SP and Subehia SK.2003. Effect of 25 years of fertilizer use on maize-wheat yields and quality of an acidic soil in Western Himalayas. *Experimental Agriculture* 39**:** 55- 64.