ISSN 2319-3670

Journal of Rice Research

Volume 12, No. 1 June 2019

Society for Advancement of Rice Research

Society For Advancement of Rice Research

(Regd. No.2175/2004 of A.P. Society Regn. Act. 2001)

The Society for Advancement of Rice Research is a registered society started with main objective of providing a platform for exchange of information and knowledge related to latest developments in rice research.

Aims and Objectives

- 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.
- To cooperate with other organizations having similar aims and objectives.
- To promote any other scientific/professional activities conducive for the advancement of science of rice and rice improvement.

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Journal of Rice Research is an official publication of Society for Advancement of Rice Research (SARR), ICAR- Indian Institute of Rice Research, Hyderabad - 500030 and published twice a year i.e., June and December. All correspondence related to SARR should be addressed to secretary, Dr R M Kumar (secretary@sarr.co.in).

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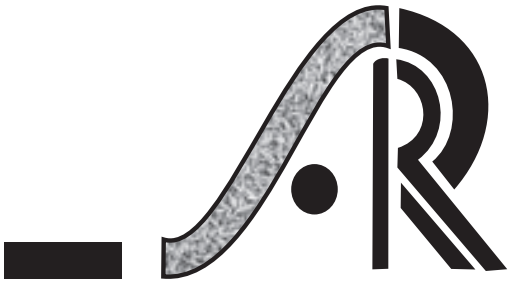
ISSN 2319-3670

Journal of Rice Research

Volume 12, No. 1 June 2019



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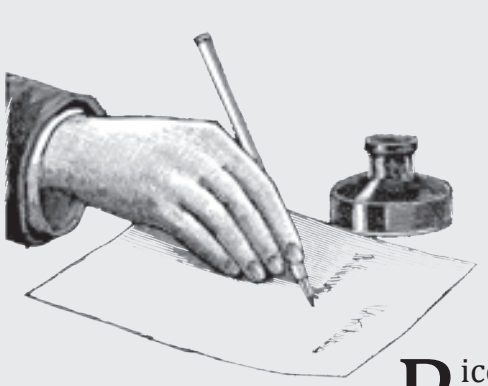

From the SARR President's desk....

Rice is one of the most important cereal food crops in Asia including India, meeting the caloric value of half of the World population. Today, rice cultivation is being faced by many challenges like reducing water resources, depleting soil health, emerging new insect pests and diseases, increasing temperatures due to climate change, market price instability leading to the vulnerability of rice farmers' livelihoods and food security. With the advent of emerging technologies, rice research and communication of research findings needs a paradigm shift to reach various stakeholders. Journal of Rice Research is emerging as one of the means for quick science communication with respect to rice research and development.

I am happy to note that the new editorial board has made efforts to bring out the Journal of Rice Research in a renewed format. I hope that the new editorial board will strive hard to improve the NAAS rating of the Journal with the support from the India's largest network of rice researchers, All India Co-ordinated Rice Improvement Program (AICRIP) and other rice research organizations who are working for the cause of rice research and development.

(Dr SR Voleti)

I am happy to inform you that, for the first time, plagiarism check using iThenticate software was introduced to further improve the quality and authenticity of the original research articles published in this Journal. I acknowledge iThenticate services rendered by NAARM.

- Chief Editor

Journal of Rice Research

Volume 12 : Issue No. 1 June 2019

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Molecular screening and diversity analysis of rice (*Oryza sativa* L.) genotypes for biotic and abiotic stresses using SSR markers

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Received: 16th March 2019; Accepted: 26th May 2019

Abstract

In this study, rice germplasms were screened for biotic (blast, bacterial blight, brown planthopper, gall midge) and abiotic stress tolerance (drought, salinity) in 50 rice genotypes using 19 trait linked SSR markers.Among the genotypes; B 40 showed amplification of the resistance/tolerance specific alleles for multiple traits, *viz.*, blast, bacterial blight, gall midge, saltol and IRBB 57 showed presence of resistance/tolerance specific alleles for blast, bacterial blight, gall midge and drought. Moreover the genotypes, IR 11 A 546, IR 11 A 581, IR 11 N 169, IRBB 2, IRBB 4, IRBB 5, IRBB 13, IRBB 64, IRBB 12 DS-GMEI-22, IR 64, IRBB 55, IRBB 62, IRBB 14, IRBB 50, IR 11 N 223 showed presence of resistance specific alleles for blast, bacterial blight, gall midge, brown planthopper and drought, Saltol by combination of any of the above three traits. The rice line IRBB 10 showed presence of resistance linked alleles for two gall midge resistance genes,*Gm4* (270bp) and *Gm8* (170bp). Genotypes IRBB 61 and IRBB 62 showed presence of resistance linked alleles for two of the *Saltol* linked loci. The rice genotypes displaying amplification of resistance/tolerance linked alleles for multiple traits can be used as donors for these traits after their validation through phenotypic screening. All the nineteen SSR primers used in this study amplified and showed the polymorphism among the rice genotypes. With an average of 5.42 alleles per locus, a total of 103 alleles were detected. The polymorphism information content (PIC) values ranged from 0.40 to 0.90 with an average PIC value of 0.65 per primer. Rice genotypes were grouped into two main clusters by UPGMA, which were further divided into two sub-clusters. Through Marker Assisted Selection (MAS), this study will assist in selection of parental lines and also for development of new breeding population that will be tolerant to specific biotic and abiotic stresses.

Keywords: SSR, molecular screening, MAS, diversity, biotic and abiotic stress

Introduction

Rice (*Oryza sativa* L.) is one of the most widely cultivated (118 countries) crops in the world. Rice ranks second after wheat in terms of area harvested but in terms of importance as a food crop, rice provides more calories per hectare than any other cereal crop. It's a staple food for more than three billion people in the world (Ma *et al*., 2007).

Exposure of rice crop to various environmental stresses such as abiotic (salinity, heat, drought, cold, submergence, radiation, and heavy metals) and biotic factors (pathogens and herbivores) cause a rigorous yield loss (Gomez, 2013). The emergence of new diseases and insect pests and the changing climate are the major issues that address the requirement for sustainable crop development and resistance to biotic and abiotic stresses. For precise genetic manipulation of complex quantitative traits like yield, tolerance against biotic/abiotic stresses, quality etc., understanding the genetic/molecular basis of target traits needs to be investigated thoroughly.

In the recent times, due to technological innovations and development of DNA based molecular markers it has become possible for the transfer of genes that confer resistance to biotic stresses (bacterial blight, blast, gall midge etc.) and abiotic stresses (submergence, drought, salinity etc.). With the improvements made in the area of molecular markers, the tracking of the genes for resistance has become easier by following the path of markers that are linked/ tagged to each gene for resistance, thus making the identification of plants with two and more genes possible.

Traditional breeding approaches are effective but delay production of climate-resilient variety as they rely on extensive phenotypic screening methods and also are not suitable for making rapid improvement in tolerance to multiple stresses. Hence, molecular breeding can be

preferred as it offers an opportunity to increase the speed and efficiency of plant breeding (Whitford *et al*., 2010). Molecular markers are promising and effective tools for measuring genetic diversity in germplasm collection and elucidating their evolutionary relationships. Using molecular marker technology, it is now feasible to analyze the quantitative traits and identify the chromosomal regions associated with such characters known as quantitative trait loci (QTLs) (Choudhary *et al*., 2008). Identifying such regions will help to increase the selection efficiency in the breeding program.

Among various PCR based markers, SSR markers are more popular in rice because they are highly informative, mostly monolocus, codominant, easily analysed, highly reproducible and cost effective (Gracia *et al*., 2004). Microsatellites or SSR markers are sequences of a few repeated and adjacent base pairs and are abundant throughout the eukaryotic genome (Powell *et al*., 1996). Variations in the number of repeats can be detected by polymerase chain reaction (PCR) with the development of primers (20–30 basepairs) specifically built for amplification and complementary to conserved sequences flanking the microsatellite. SSR markers are able to detect high level of allelic diversity and they have been extensively used to identify genetic variation among rice subspecies (Ni *et al*., 2002). SSR markers are efficient in detecting genetic polymorphisms and discriminating among genotypes from germplasm of various sources, even they can detect finer level of variation among closely related breeding lines within a same variety (Lapitan *et al*., 2007).

Different rice varieties of distinct genetic background are a good promise for the future of rice crop improvement programmes as genetic diversity helps in estimating and establishing of genetic relationship in germplasm collection, identifying diverse parental combinations to create segregating progenies with maximum genetic variability and superior recombinations for further selection and introgressing desirable genes from diverse germplasm (Thompson *et al*., 1998 and Islam *et al*., 2012).

The major objective of this study was to screen the genotypes for biotic and abiotic stresses using SSR markers linked with tolerance traits or QTLs and to identify the best genotype to be used as donor for tolerance to multiple stresses in breeding program in the future for development of new rice varieties that are equally beneficial for farmers and the scientific community. In the investigation reported here, 19 SSR markers were used to fingerprint 50 rice accessions. The SSR data was used to evaluate the level of genetic diversity within the rice genotypes and to assess genetic relationship among the accessions.

Materials and Methods

The present study was conducted at the Plant Biotechnology Centre, Dapoli, Ratnagiri district, India in 2017-2018. Fifty rice genotypes obtained from Regional Agricultural Research Station, Karjat, District-Raigad, Maharashtra, India were used for the study (Table 1).

Table 1.Genotypes used in this study

Sr. No.	Genotypes	Sr. No.	Genotypes	Sr. No.	Genotypes
1	IR 11 A 546	18	IRBB 5	35	IRBB 56
2	IR 11 A 581	19	IRBB 7	36	IRBB 57
3	IR 11 N 121	20	IRBB 8	37	IRBB 58
4	IR 11 N 137	21	IRBB 10	38	IRBB 59
5	IR 11 N 169	22	IRBB 11	39	IRBB 60
6	IR 11 N 239	23	IRBB 13	40	IRRI 123
7	IR 11 N 313	24	IRBB 14	41	IRBB 61
8	IR 12 L 201	25	IRBB 21	42	IRBB 62
9	SAKHA 105	26	IRBB 50	43	IRBB 63
10	B 40	27	IRBB 51	44	IRBB 64
11	IR 552	28	IRBB 52	45	IRBB 65
12	IR 11 A 106	29	IRBB 53	46	IRBB 66
13	IRBB 1	30	IR 11 N 223	47	12 DS-GMEI-22
14	IRBB 2	31	IRBB 54	48	HHZ5-DT20-DT3-Y2
15	IRBB 3	32	IR 09 L 226	49	IR 05 A 272
16	IR 12 L 125	33	IRBB 55	50	IR 64
17	IRBB 4	34	IR 11 N 400		

The DNA was isolated by following the protocol of Edwards *et al*., (1991). For DNA isolation, 10 days old leaves were collected and sterilized with 70% ethanol to avoid the contamination. Leaf tissue (100 mg) was collected and kept in 1.5 ml eppendorf tube which leads to ensure uniform size of sample. Collected tissue was macerated by micro pestle at room temperature without buffer for 15 sec. Extraction buffer (500 µl) was added and leaf tissue macerated gently for few seconds and kept in hot water bath for 45 minutes at 65°C. The sample was cooled down to room temperature and centrifuged at 10000 rpm for 10 minutes. Aqueous layer was transferred to fresh eppendorf tube and 200 µl of chloroform: iso-amyl

alcohol (24:1) was added and mixed by gentle inversion for 5-6 times. The contents were then centrifuged at 8000 rpm for 10 minutes. Supernatant was mixed with double volume of chilled Iso-propanol and incubated at –20°C for overnight. On the next day the solution was centrifuged at 8000 rpm for 10 minutes and pellet was collected. Pellet was washed with 100 µl of 70 per cent ethanol followed by centrifugation at 8000 rpm for 10 minutes. Pellet was dried and re-suspended in 50 µl of 1x TE buffer and incubated at 37°C in a water bath for 30 minutes and stored at -20°C till further use. Quality of the isolated DNA was confirmed by Agarose gel electrophoresis method.

Simple sequence repeat (SSR; *i.e.* microsatellite) marker analysis: For the molecular screening of rice germplasm, nineteen different trait specific SSR markers well distributed on all the 12 chromosomes of rice were used (Table 2). These SSR markers were chosen based on their physical position on the 12 chromosomes of rice genome according to the 'Gramene' database (http//:www.gramene.org) and also based on their linkage to different genes conferring resistance to bacterial blight blast, gall midge and tolerance to salinity and drought. PCR reactions were carried out in Thermal cycler with the total reaction volume of 20µl containing, 10ng of genomic DNA, 10X assay buffer, 10mM of dNTPs, 25mM $MgCl_2$, 10pmol of forward and reverse primers and 3 U Taq polymerase enzyme and Nano pure water. The PCR cycles were programmed as 95°C for 5 min, 94°C for 20 sec, 55°C for 30 sec, 72°C for 45 sec for 35 cycles and an additional temperature of 72°C for 7 min for final extension. The amplified products were separated on 2% agarose gel prepared in 1X TAE buffer and stained with Ethidium bromide.The gel was run in 1X TAE buffer at constant voltage of 80 V for a period of 100 minutes.

Scoring and data analysis: Marker alleles were scored as present (+/1) or absent (-/0). The data was used for similarity based analysis using the programme MVSP-A (Multivariate Statistical Package_5785, Version 3.1).

Table 2. List of SSR primers with their sequences and linked gene

Sr. No	Primer	Sequence Forward primer	Sequence Reverse Primer	Chromosome No.	Linked gene	Reference
1.	RM 140	TGCCTCTTCCCTGGCTCCCCTG	GGCATGCCGAATGAAATGCATG	1	*Saltol*	Karmarkar *et al*.,2012
2.	RM 1287	CCATTTGCAGTATGAACCATGC	ATCATGCAATAGCCGGTAGAGG	1	*Saltol*	Ganie *et al*.,2016
3.	RM 562	GGAAAGGAAGAATCAGACACA-GAGC	GTACCGTTCCTTTCGTCACTTCC	1	*Saltol*	Ganie *et al*.,2016
4.	RM 3412	AAAGCAGGTTTTCCTCCTCC	CCCATGTGCAATGTGTCTTC	1	*Saltol*	Islam *et al*.,2015
5.	RM 6775	AATTGATGCAGGTTCAGCAAGC	GGAAATGTGGTTGAGAGTTGAGAGC	6	*Bph25*	Myint *et al*.,2012
6.	RM 309	CACGCACCTTTCTGGCTTTCAGC	AGCAACCTCCGACGGGAGAAGG	12	*Bph26*	Myint *et al*.,2012
7.	RM 5479	CTCACCATAGCAATCTCCTGTGC	ACTTCGTTCACTTGCATCATGG	12	*Bph26*	Myint *et al*.,2012
8.	RM 5926	ATATACTGTAGGTCCATCCA	AGATAGTATAGCGTAGCAGC	11	*Pi1*	Thippeswamy *et al*.,2015
9.	RM 8225	GCGTGTTCAGAAATTAGGATACGG	GATCTCGCCACGTAATTGTTGC	6	*Pi-z*	Ashkani *et al*.,2011
10.	RM 206	ATCGATCCGTATGGGTTCTAGC	GTCCATGTAGCCAATCTTATGTGG	11	*Pi-kh*	Kumar *et al*.,2013
11.	RM 212	AAGGTCAAGGAAACAGGGACTGG	AGCCACGAATTCCACTTTCAGC	1	*Dr*	Ashfaq *et al*.,2014
12.	RM 302	TGCAGGTAGAAACTTGAAGC	AGTGGATGTTAGGTGTAACAGG	1	*Dr*	Ashfaq *et al*.,2014
13.	RM 3825	CCACTAGCAGATGATCACAGACG	GAGCACCTCATAAGGGTTTCAGC	1	*Dr*	Kanagraj *et al*.,2010
14.	RM 201	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA	9	*Dr*	Kanagraj *et al*.,2010
15.	RM 1233	ATGGGCACGTGTAATTCATTCG	ATCCTCGAAAGTAGGAGTAG-GAAAG	11	*Pi-1*	Ramadevi *et al*.,2015
16.	pTA248	AGACGCGGAAGGGTGGTTCCCGGA	AGACCGGGTAAT CGAAAGATGAAA	11	*Xa21*	Sabar *et al*.,2016
17.	RM 122	GAGTCGATGTAATGTCATCAGTGC	GAAGGAGGTATCGCTTTGTTGGAC	5	*Xa5*	Sabar *et al*.,2016
18.	RM 22709	CGCGTGGGCGAGACTAATCG	CCTTGACTCCGAGGATTCATTGTCC	8	*Gm8*	Mohapatra *et al*.,2016
19.	RM 547	TTGTCAAGATCATCCTCGTAGC	GTCATTCTGCAACCTGAGATCC	8	*Gm4*	Kalpana *et al*.,2016

Similarity coefficients were used to construct UPGMA (Unweighted Pair Group Method with Average) to generate dendrogram. Distance matrix and dendrogram was constructed based on diversity coefficient generated from pooled data by using UPGMA, a computer programme for distance estimation. The polymorphism percentage of the obtained bands was calculated by using following formula,

$$\text{Percent Polymorphism} \frac{\text{Total number of Polymorphic alleles}}{\text{Total number of alleles}} \text{ x100}$$

Polymorphism Information Content: Polymorphism Information Content (PIC) value were calculated as per formula developed by Powell *et al.*,(1996).

$$PIC= 1-\sum P_{ij}^{2}$$

Where, P_{ij} is the frequency of i^{th} and j^{th} locus, summed across the entire locus over all lines. PIC values range from 0 (monomorphic) to 1 (very highly discriminative, with many alleles each in equal and low frequency) were estimated for each profile generated across 50 rice genotypes.

Results and Discussions

Biotic stresses

Blast: Four primers *i.e.* RM8225, RM206, RM5926 and RM1233 were used to identify blast resistance linked alleles in 50 rice genotypes. Marker RM8225 specific to gene *Pi-z* indicated presence of resistance linked alleles (221bp) in the genotypes, IRBB 53, IR 11 N 223, IRBB 57, IRBB 58. Fjellstrom *et al*., (2004), Askani *et al*., (2011) reported resistance linked alleles specific to gene *Pi-z* by using marker RM8225. Marker RM5926 specific to gene *Pi-1* indicated presence of resistance linked alleles (at 176bp) in the genotypes, B 40, IRBB 13, IRBB 65, IRBB 66, 12 DS-GMEI-22, IR 64 (Plate No.1). Thippeswamy*et al*., (2006) and Thippeswamy *et al*., (2015) also evaluated rice genotypes for presence of resistance linked alleles specific to gene *Pi1* by marker RM5926.

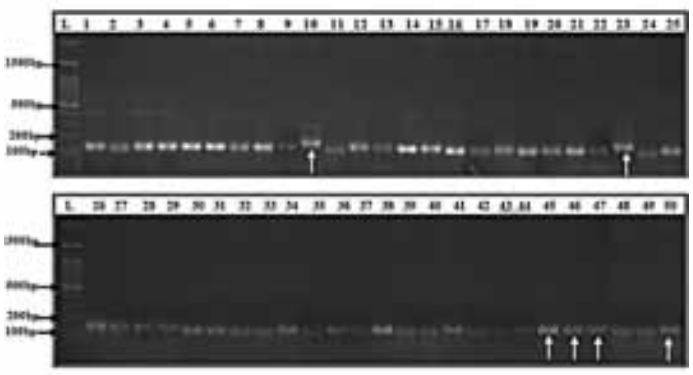

Plate No. 1: Amplified DNA bands of 50 rice genotypes using SSR marker RM5926 linked to Blast resistance trait. L- 100bp ladder. (Arrow indicates presence of the resistance linked allele).

The genotypes were screened for the presence of resistance linked allele for gene *Pi1* by visualization of amplicons of 170 bp fragments using SSR marker, RM1233. The results showed that all the genotypes indicated absence of resistance linked alleles for gene *Pi1* specific marker RM1233. Earlier studies (Ashkani *et al*., 2011; Ramadevi *et al*., 2015; Yadav *et al*., 2017) reported the use of marker RM1233 specific to gene *Pi1* for screening blast resistance in rice genotypes. The genotypes were verified for the presence of blast resistance gene, *Pi-kh* by using the gene specific primer, RM206 which is expected to amplify a 140bp fragment in the genotypes containing the resistance linked allele. No genotype was observed to possess the resistance linked allele for the blast resistance gene, *Pi-kh* for the primer RM206. Kumar *et al*., (2013) screened rice genotypes for blast resistance linked alleles specific to gene *pi-kh* by marker RM206.

Bacterial Blight: In this study, 50 rice accessions were screened to determine resistance status for BLB-resistance genes *viz., Xa5* and *Xa21* by using PCR based microsatellite markers RM122 and pTA248, respectively. Screening for the *Xa5* resistance gene by the amplification of the microsatellite marker RM122, which was employed to track the resistant amplicons of 240-250bp revealed resistance linked alleles in almost all the genotypes in this study. Studies performed by Islam *et al*., (2015), Ullah*et al*., (2012) and Sabar*et al*., (2016) also revealed presence of resistance linked alleles at 240-250bp for *Xa5* gene specific marker RM122. This indicates that bacterial blight gene *Xa5* specific marker RM122 is effective in detecting presence of resistance linked alleles in all the genotypes used in this study. No amplicons (1040bp) specific to resistance linked alleles for *Xa21* gene were detected by marker pTA 248. This indicates that resistance linked alleles for gene *Xa21* were absent in all the genotypes.

Brown Planthopper: The 50 germplasms of rice were evaluated for brown planthopper (BPH) resistance using the SSR markers *viz.,* RM5479, RM6775 and RM309. Among these, RM6775 was most effective in identification of the resistant linked alleles in the genotypes. Marker RM6775 specific to gene *Bph25* indicated presence of resistance linked alleles (192bp) in the genotypes, IRBB 13, IRBB 14, IRBB 64, 12 DS-GMEI-22, HHZ5-DT20-DT3-Y2, IR 05 A 272, IR 64. Marker RM309 and RM5479 specific to gene *Bph26* (at 152bp) indicated absence of resistance linked alleles in all the genotypes. These genotypes may

either have resistance linked alleles for other *Bph* genes which can be detected by using corresponding gene specific markers or these genotypes are devoid of resistance linked alleles for any of the BPH genes. Myint *et al*., (2012) also screened rice genotypes for *Bph25* and *Bph26* genes by the primers RM6775, RM309 and RM5479. Several other workers (Rahman *et al*., 2009; Harini *et al*., 2013; Shabanimofrad *et al*., 2015a and 2015b and Bhogadhi *et al*., 2015) also reported similar marker study for *Bph* genes.

Gall midge: The SSR markers RM547 and RM22709 used in this study reported the presence of resistance linked alleles for biotypes *Gm4* (270bp) and *Gm8* (160 to 170bp), respectively. RM547 at 270bp showed resistance linked alleles in eight genotypes, IR 11 N 169, IR 11 N 239, B 40, IRBB 2, IRBB 4, IRBB 5, IRBB 10, IRBB 50 and marker RM22709 specific to gene Gm8 (at 170bp) indicated presence of resistance linked alleles in the genotypes, IR 11 A 546, IR 11 A 581, IRBB 8, IRBB 10, IRBB 14, IR 11 N 223. Both the markers used in this study were effective in detecting resistance linked alleles. The genotypes showing presence of resistance linked alleles can further be used in various breeding programs. Using flanking SSR markers Sama *et al*., (2012) detected *Gm8* gene in nine rice genotypes. Various previous reports (Kalpana *et al*., 2016 and Mohapatra *et al*., 2016) revealed the strong association of locus specific makers which are in agreement with this study.

Abiotic stresses

Salt Tolerance: Salt tolerance linked markers used in this study were RM140, RM1287, RM562 and RM3412. Saltol QTL detected by the marker RM140 (at 260bp) was observed in the genotypes, IR 11 A 546, IR 11 A 581, IR 11 N 121, IR 11 N 137, IR 11 N 169, B 40, IRBB 7, IRBB 50, IRBB 61, IRBB 62. Saltol QTL detected by the marker RM1287 (at 160bp) was observed in the genotypes, IR 552, IRBB 1, IRBB 2, IRBB 3. Saltol QTL detected by the marker RM3412 (at 211bp) was observed in the genotypes, IR 11 N 137, IR 11 N 169, B 40, IRBB 4, IRBB 5, IRBB 50, IRBB 51, IRBB 53, IRBB 54, IRBB 55, IRBB 56, IRBB 57, IRBB 60, IRBB 61, IRBB 62 (Plate No. 2). Marker RM562 (at 243bp) indicated absence of resistance linked alleles in all the genotypes. Markers used in this study were effective in detecting Saltol QTL in several genotypes screened. Further study may confirm that some of these genotypes might have Saltol QTL and can be used as alternative donors in salt tolerant rice breeding programmes. All the four Saltol linked SSRs used in this study amplified polymorphic bands in the 50 genotypes. Studies performed by Zeng *et al*., (2004), Karmakar *et al*., (2012) Islam *et al*., (2012), Iqbal *et al*., (2015) and Ganie *et al*., (2016) also reported that these markers are highly polymorphic in nature.

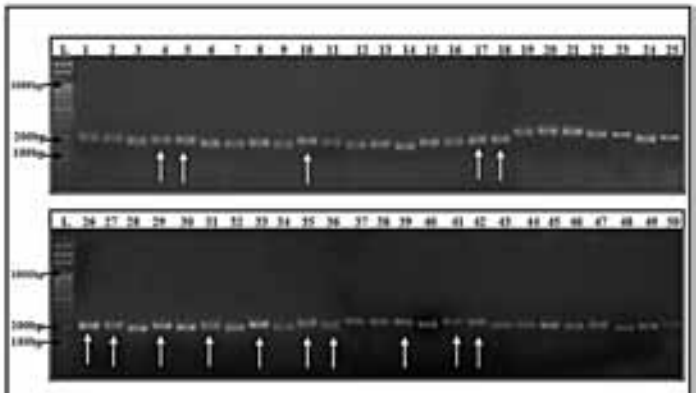

Plate No. 2: Amplified DNA bands of 50 rice genotypes using SSR marker RM3412 linked to salt resistance trait. L= 100bp ladder. (Arrow indicates presence of the resistance linked allele).

Drought Tolerance: Molecular markers linked to drought tolerance in rice are an important tool for screening and selection of drought tolerant genotypes for use in future breeding programs. Drought resistance linked markers used in this study includes RM212, RM302, RM3825 and RM201. Resistance linked alleles Dr (135 bp) detected by the marker RM212 were present in the genotypes, IRBB 55, IR 11 N 400, IRBB 57, IRBB 62, IRBB 63. Markers RM302 (at 140bp), RM3825 (at 147bp) and RM201 (at 220bp) indicated absence of resistance linked alleles in all the genotypes. As these genotypes showed no amplification for these primers, we can suggest the use of other drought related marker combinations to screen the genotypes for drought resistance.

Studies performed by Ashfaq *et al*.,(2014) stated that the markers RM315, RM212 and RM302 on chromosome 1 may be useful for evaluation of diverse germplasm and on the basis of these molecular markers some genotypes of rice were identified as drought tolerant genotypes. This was also linked with the root traits, *i.e.* root length of the genotypes. It is also evident from results given by Kanagaraj *et al*., (2010) that the genomic region RM212–RM302–RM3825 on chromosome 1 is linked to drought resistance traits and may be useful in marker assisted breeding for drought resistance in rice. Various similar reports were observed in the studies done by Kanagaraj *et al*., (2010), Ashfaq *et al*., (2014), Ramadan *et al*., (2015), Freeg *et al*., (2016) and Sindhumole *et al*., (2017).

Genetic Diversity: The polymorphism percentage for each primer was calculated by the ratio of number of

polymorphic bands obtained over the total number of bands produced across the 50 rice germplasms. All the 50 rice accessions were genotyped with 19 trait linked microsatellite markers and were selected for their ability to produce amplified product and detect polymorphism level among the varieties and consistency of the pattern. Total 103 alleles were scored from 19 markers and 100 per cent were found to be polymorphic (Table 4). The overall size of amplified products ranged from 100bp to 1050bp.

Table 3: Genotypes showing amplification of resistance/tolerance specific alleles for multiple resistance / tolerance for different traits

Sr. No	Genotypes	Blast	Bacterial Blight	Brown Plant hopper	Gall Midge	Saltol	Drought
1.	IR 11 A 546	–	+	–	+	+	–
2.	IR 11 A 581	–	+	–	+	+	–
3.	IR 11 N 121	–	+	–	–	+	–
4.	IR 11 N 137	–	+	–	–	+	–
5.	IR 11 N 169	–	+	–	+	+	–
6.	IR 11 N 239	–	+	–	+	–	–
7.	IR 11 N 313	–	+	–	–	–	–
8.	IR 12 L 201	–	+	–	–	–	–
9.	SAKHA 105	–	+	–	–	–	–
10.	B 40	+	+	–	+	+	–
11.	IR 552	–	+	–	–	+	–
12.	IR 11 A 106	–	+	–	–	–	–
13.	IRBB 1	–	+	–	–	+	–
14.	IRBB 2	–	+	–	+	+	–
15.	IRBB 3	–	+	–	–	+	–
16.	IR 12 L 125	–	+	–	–	+	–
17.	IRBB 4	–	+	–	+	+	–
18.	IRBB 5	–	+	–	+	+	–
19.	IRBB 7	–	+	–	–	+	–
20.	IRBB 8	–	+	–	+	–	–
21.	IRBB 10	–	+	–	+	–	–
22.	IRBB 11	–	+	–	–	–	–
23.	IRBB 13	+	+	+	–	–	–
24.	IRBB 14	–	+	+	+	–	–
25.	IRBB 21	–	+	–	–	–	–
26.	IRBB 50	–	+	–	+	+	–
27.	IRBB 51	–	+	–	–	+	–
28.	IRBB 52	–	+	–	–	–	–
29.	IRBB 53	+	+	–	–	+	–
30.	IR 11 N 223	+	+	–	+	–	–
31.	IRBB 54	–	+	–	–	+	–
32.	IR 09 L 226	–	+	–	–	–	–
33.	IRBB 55	–	+	–	–	+	+
34.	IR 11 N 400	–	+	–	–	–	+
35.	IRBB 56	–	+	–	–	+	–
36.	IRBB 57	+	+	–	–	+	+

Sr. No	Genotypes	Blast	Bacterial Blight	Brown Plant hopper	Gall Midge	Saltol	Drought
37.	IRBB 58	+	+	–	–	–	–
38.	IRBB 59	–	+	–	–	–	–
39.	IRBB 60	–	+	–	–	+	–
40.	IRRI 123	–	+	–	–	–	–
41.	IRBB 61	–	+	–	–	+	–
42.	IRBB 62	–	+	–	–	+	+
43.	IRBB 63	–	+	–	–	–	+
44.	IRBB 64	+	+	+	–	–	–
45.	IRBB 65	–	+	–	–	–	–
46.	IRBB 66	+	+	–	–	–	–
47.	12 DS-GMEI-22	+	+	+	–	–	–
48.	HHZ5-DT20-DT3-Y2	–	+	+	–	–	–
49.	IR 05 A 272	–	+	+	–	–	–
50.	IR 64	+	+	+	–	–	–

("+" indicates presence of the resistance linked allele; "-"indicates absence of the resistance linked allele for the marker).

The PIC values were calculated to find out the effectiveness of primers in distinguishing individual accessions (Table 4). The PIC values ranged from 0.40 to 0.90 with an average of 0.65 per primer. Total of 103 alleles were detected with an average of 5.42 alleles per locus. The marker pTA-248 generated a maximum number of alleles (16) while the marker RM5479 produced minimum number of alleles (2). The SSR marker, pTA 248 revealed highest (0.90) PIC value whereas the marker RM22709 revealed the lowest (0.40) PIC value. The higher the PIC value, the more informative is the SSR marker. Hence, primers pTA 248, RM547 and RM302 were found to be highly informative (Table 4).

In this study, a total of 103 alleles were detected with an average number of alleles of 5.42 per locus (ranged from 3 to 16 per locus). It was observed that marker detecting the lower number of alleles showed lower gene diversity than those which detected higher number of alleles with higher gene diversity. The average number of alleles (5.42) obtained in this study is higher than the values reported by earlier studies (Islam *et al*., 2012; Gholizadeh *et al*., 2015; Freeg *et al*., 2016; Krupa *et al*., 2017) on smaller germplasm sets and comparable to values reported by Hoque *et al*., (2014). However, lower than the values reported by Jain *et al*., (2004), Giarrocco *et al*., (2007); Thomson *et al*., (2007) and Roy *et al*., (2015) with large germplasms (Table 5). These inconsistencies might be due to the genotypes used and selection of SSR markers.

The markers showed an average PIC value of 0.65 which indicated that SSR markers used in this study were highly informative because only PIC values higher than 0.5 indicate high polymorphism. Markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at a specific locus (Dewoody *et al*., 1995). The overall genetic diversity (PIC=0.65) of the 50 rice germplasm accessions included in this study was comparable to the value reported in previous studies (Jain *et al.,* 2004; Giarrocco *et al*., 2007; Thomson *et al*., 2007 and Roy *et al*., 2015) and higher than the values reported by Hoque *et al*., (2014), Gholizadeh *et al*., (2015), Freeg *et al*., (2016) and Krupa *et al*., (2017) who reported average PIC value equivalent to 0.54, 0.45, 0.52 and 0.49 and smaller than the values reported by Nguyen *et al*., 2012 and Nachimuthu *et al*., (2015) who reported average PIC value of 0.73 and 0.75, respectively. PIC shows how the marker can indicate the population polymorphism depending on the number and frequency of the alleles (Botstein *et al*., 1980). So the PIC reflects a discriminating ability of the marker and, in fact, depends on the number of known alleles and their frequency distribution, thus being equal to genetic diversity.

Genetic distance values between germplasm accessions: On the basis of analysis of SSR scoring, the alleles were converted to binary score based on their presence (1) or absence (0). This data was used for similarity based

analysis using the programme Multivariate Statistical Package(MVSP) to determine the Jaccard's coefficient matrices *i.e.* estimate of similarity among the fifty genotypes.The genetic distances ranged from 0.308 to 1 with an average of 0.77 among these 50 promising genotypes of rice.The lowest GD value (0.308) was found between the genotypes IRBB 59 vs. IRBB 58, IRBB 60 vs. IRBB 59 whereas highest genetic distance value (1) was found between the genotypes IRBB 5 vs. IR 64.Further, the average genetic distance values per genotype from rest of the genotypes based on Jaccard's similarity index of all germplasm lines in rice were also analysed separately.It was revealed that an average genetic distance among the 50 rice accessions ranged from 0.711 (IR 11 N 169) to 0.819 (IR 64)from MVSP analysis (Table 5).

Clustering analysis based on SSR marker analysis: The UPGMA based dendrogram of 50 rice genotypes was generated with Multivariate Statistical Package (MVSP). Clustering pattern of dendrogram generated by using the pooled molecular data of 19 primers of 50 genotypes produced two main clusters namely I and II (Table 6). The major cluster-I comprised of 17 accessions and was further found to be divided into two sub clusters (IA and IB). The major cluster-II comprised of 33 accessions and was further found to be divided into two sub clusters (IIA and IIB). The dendrogram revealed that the genotypes that are derivatives of genetically similar type clustered together (Figure 1).

Table 4: Molecular polymorphism, PIC Values, No. of alleles and size of loci by SSR Primers in rice genotypes

Sr. No.	Primer	Total no. of polymorphic Band	Average No. of bands/ genotype	% Polymorphism	No. of alleles	PIC	Range of amplified products
1	RM 8225	50	1.0	100	6	0.73	180-330
2	RM 206	58	1.16	100	8	0.80	320-530
3	RM 5926	56	1.12	100	5	0.65	120-190
4	RM 122	50	1.0	100	2	0.43	230-270
5	RM 1233	50	1.0	100	5	0.65	150-250
6	pTA248	50	1.0	100	16	0.90	520-1050
7	RM 5479	50	1.0	100	2	0.50	230-340
8	RM 6775	69	1.38	100	7	0.78	120-190
9	RM 309	50	1.0	100	3	0.44	150-220
10	RM22709	52	1.04	100	3	0.40	100-180
11	RM 547	78	1.56	100	9	0.86	190-370
12	RM 140	50	1.0	100	5	0.69	200-320
13	RM 1287	50	1.0	100	5	0.66	150-230
14	RM 562	50	1.0	100	4	0.70	100-200
15	RM 3412	50	1.0	100	4	0.60	180-250
16	RM 212	50	1.0	100	4	0.64	140-220
17	RM 302	83	1.66	100	8	0.83	170-360
18	RM 3825	50	1	100	4	0.61	160-240
19	RM 201	50	1	100	3	0.62	110-160
	Total	**1046**	**20.92**	**-**	**103**		**-**
	Average	**55.05**	**1.10**	**100.00**	**5.42**	**0.65**	

Table 5: Average genetic distance estimates by SSR marker analysis based on Jaccard's dissimilarity coefficient

Sr.No.	Accession	Avg. GD	Sr.No.	Accession	Avg. GD
1.	IR 11 A 546	0.738	26.	IRBB 50	0.786
2.	IR 11 A 581	0.738	27.	IRBB 51	0.765
3.	IR 11 N 121	0.745	28.	IRBB 52	0.751
4.	IR 11 N 137	0.722	29.	IRBB 53	0.740
5.	IR 11 N 169	0.711	30.	IR 11 N 223	0.750
6.	IR 11 N 239	0.715	31.	IRBB 54	0.768
7.	IR 11 N 313	0.762	32.	IR 09 L 226	0.769
8.	IR 12 L 201	0.712	33.	IRBB 55	0.791
9.	SAKHA 105	0.745	34.	IR 11 N 400	0.771
10.	B 40	0.767	35.	IRBB 56	0.754
11.	IR 552	0.766	36.	IRBB 57	0.781
12.	IR 11 A 106	0.794	37.	IRBB 58	0.777
13.	IRBB 1	0.763	38.	IRBB 59	0.773
14.	IRBB 2	0.826	39.	IRBB 60	0.772
15.	IRBB 3	0.778	40.	IRRI 123	0.768
16.	IR 12 L 125	0.773	41.	IRBB 61	0.758
17.	IRBB 4	0.778	42.	IRBB 62	0.825
18.	IRBB 5	0.798	43.	IRBB 63	0.809
19.	IRBB 7	0.786	44.	IRBB 64	0.768
20.	IRBB 8	0.763	45.	IRBB 65	0.767
21.	IRBB 10	0.760	46.	IRBB 66	0.774
22.	IRBB 11	0.764	47.	12 DS-GMEI-22	0.800
23.	IRBB 13	0.767	48.	HHZ5-DT20-DT3-Y2	0.811
24.	IRBB 14	0.779	49.	IR 05 A 272	0.783
25.	IRBB 21	0.815	50.	IR 64	0.819
AVERAGE GENETIC DISTANCE= 0.77					

Table 6: Distribution of 50 rice accessions into different clusters based on SSR analysis

Cluster	Sub cluster	Sub-sub cluster	Number of genotypes	Genotypes
I	IA		2	IRBB 63, IRBB 62.
	IB	IB (i)	5	IRBB 58, IRBB 59, IRBB 60, IRRI 123, IRBB 61.
		IB (ii)	10	IRBB 51, IRBB 53, IR 11 N 223, IRBB 52, IR 09 L 226, IRBB 54, IRBB 55, IR 11 N 400, IRBB 56, IRBB 57.
II	IIA	IIA (i)	3	HHZ5-DT20-DT3-Y2, IR 05 A 272, IR 64.
		IIA (ii)	4	IRBB 64, IRBB 65, IRBB 66, 12 DS-GMEI-22.
	IIB	IIB (i)	12	IR 11 N 313, IR 11 A 106, SAKHA 105, IRBB 1, IR 552, IRBB 3, IR 12 L 125, IRBB , B 40, IRBB 4, IRBB 5, IRBB 7.
		IIB (ii)	14	IR 11 A 546, IR 11 A 581, IR 11 N 121, IR 11 N 137, IR 11 N 169, IR 11 N 239, IR 12 L 201, IRBB 8, IRBB 10, IRBB 11, IRBB 13, IRBB 14, IRBB 21, IRBB 50.

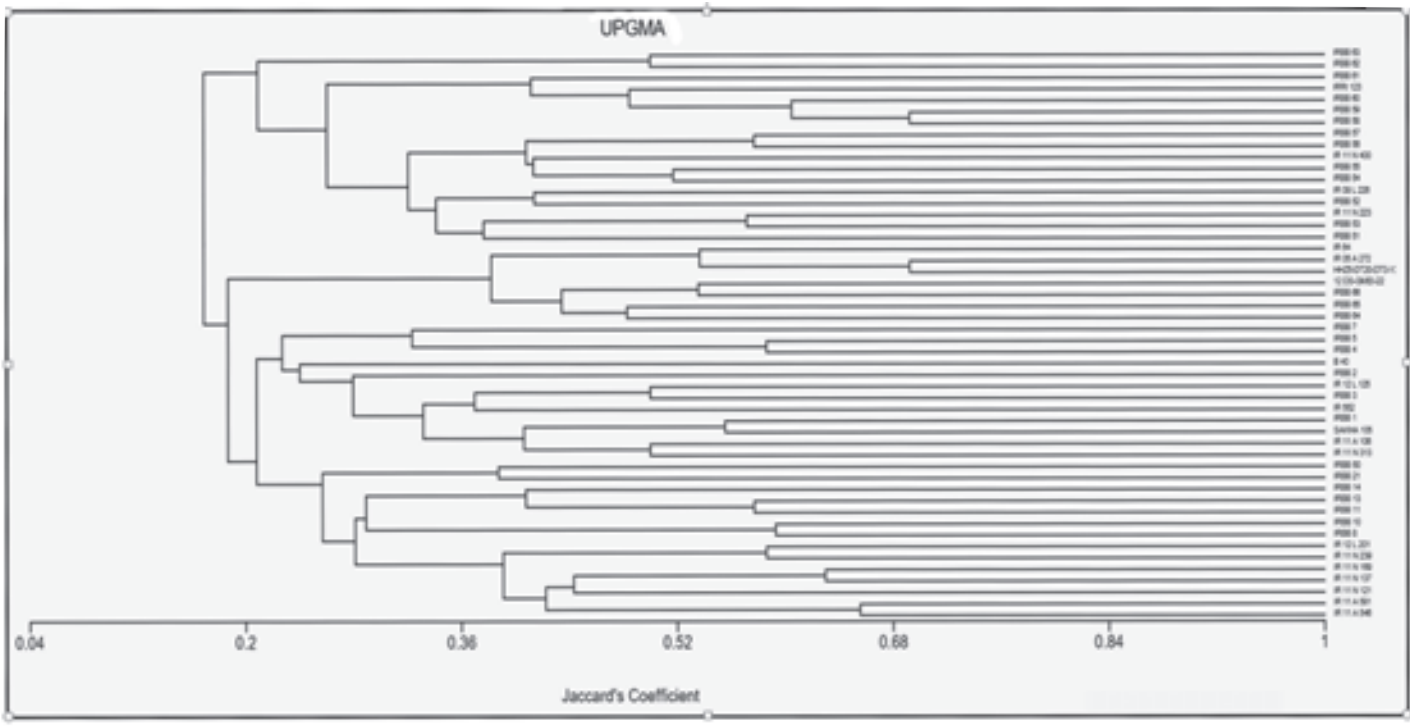

Figure 1: Dendrogram constructed using Jaccard's Similarity Coefficient

Similarly, Choudhary *et al*., (2013) constructed the unweighted neighbour-joining (UNJ) dendrogram on the basis of genetic similarity matrix and grouped 100 genotypes into five clusters *viz.*, landraces, 1970s, 1980s, 1990s, and 2000s. Yadav *et al*., (2013) grouped 88 rice accessions that included landraces, farmer's varieties and popular Basmati lines into two major clusters at the dissimilarity coefficient of 0.55 and further into four clusters at a dissimilarity coefficient of 0.58. Mohiuddin *et al*., (2014) constructed dendrogram based on the Nei's genetic distance calculated from 27 SSR markers generated from the 30 rice accessions. Singh *et al*., (2016) grouped 729 rice varieties into two major clusters, 400 varieties in cluster 1 whereas 329 varieties were grouped into cluster 2. From this study it is revealed that rice varieties are more divergent and the genetic diversity detected using molecular markers in the present investigation indicates the high discrimination capacity of SSR markers precisely due to the multi-allelic nature of SSR markers.

Conclusion

Of all the screened genotypes, B 40 showed presence of multiple resistance traits for the blast, bacterial blight, gall midge and Saltol while IRBB 57 showed presence of multiple resistance traits for blast, bacterial blight, gall midge and drought. Moreover, some genotypes showed presence of three different combinations of resistance traits *i.e.* genotypes IR 11 A 546, IR 11 A 581, IR 11 N 169, IRBB 50, IRBB 2, IRBB 4 and IRBB 5 for bacterial blight, gall midge and Saltol; IRBB 13, IRBB 64, DS-GMEI-22 and IR 64 for blast, bacterial blight and brown planthopper; IRBB 14 for bacterial blight, brown planthopper and gall midge; IRBB 53 for blast, bacterial blight and Saltol; IR 11 N 223 for blast, bacterial blight and gall midge; IRBB 55 and IRBB 62 for blast, Saltol and drought. Many other genotypes also showed amplification of the resistance/ tolerance specific alleles in the present study. These rice genotypes can be exploited for marker-assisted breeding after their validation through phenotype based screening for the target traits. The SSR marker gave more clusters with fewer genotypes in each cluster and therefore, more variation within each cluster. In the present study, the rice varieties are grouped into two major clusters. The genetic diversity and cluster analysis together for stress resistance provides some useful guides for assisting plant breeders in selecting genetically diverse parents for crossing programme and also helps in broadening the genetic base of the rice germplasm.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Estimation of genetic diversity in rice (*Oryza sativa* L.) genotypes for heat tolerance using SSR markers

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Received: 3[4d] Nov. 2018; Accepted: 20[th]April 2019

Abstract

Genetic diversity among a set of 48 rice lines which included 24 heat tolerant genotypes and 24 heat susceptible genotypes was investigated with the help of a set of 16 rice SSR markers. All 16 primers were polymorphic amplifying a total of 127 alleles and mean number of 7.9 alleles per locus. The polymorphism information content (PIC) values ranged from 0.921(RM7075) to 0.995 (RM27289) with an average of 0.965 per locus. The rice lines were grouped into two major clusters and a clear separation of tolerant genotypes and susceptible genotypes into different clusters was observed. The results obtained from the study could be useful for selection of donors for heat tolerance for development of new rice varieties with heat stress tolerance

Keywords: Genetic diversity, heat tolerance, SSR markers, heat susceptible, genotypes

Introduction

Rice (*Oryza sativa L.*) occupies 23% of the total area under cereal production in the world (FAO. 2003) and is the staple food for nearly half of the world's population (Maclean *et al.,* 2002; Wassmann *et al.,* 2009). Most of the world's rice is grown and consumed in Asia where the production levels are estimated to decline by 4% due to climate change (Jagadish *et al.,* 2007). High temperature at anthesis stage in rice crop (which is the most sensitive stage to temperature) is expected to occur more frequently in the future (Jagadish *et al*., 2010). Climate change would affect the growth and developmental aspects of rice significantly. Additionally, yield reduction, head rice recovery (HRR), chalkiness, amylose content and gelatinization temperatures will also get affected (Subba rao *et al*., 2010). Therefore, it is necessary to identify the genotypes with heat tolerance. DNA markers that differentiate genotypes are reliable and convenient also complementing the phenotype process (Zeng *et al.,* 2004). Among various PCR based markers, SSR markers have many advantages due to their multi-allelic and co-dominant nature of amplification, high degree of polymorphic information content, high reproducibility, abundance etc. Hence this work was taken up with an objective to analyze a set of rice lines possessing varied level of tolerance to heat stress using a set of rice SSR markers and identify possible associations between marker amplification pattern and tolerance.

Materials and methods

Plant material: The experimental plant material for genetic diversity assessment of forty eight rice genotypes (detailed in Table 1) were collected from ICAR-IIRR, Hyderabad, India.

DNA extraction and SSR marker analysis: The DNA of 48 selected lines for genotyping was isolated from young leaves harvested after 15 days of sowing using CTAB method as described by Doyle and Doyle (1990). The genomic DNA of these genotypes was subjected to PCR amplification using a set of 16 rice SSR markers (Table 1) as per the procedure described by Chen *et al*., (1997). PCR reactions were carried out in thermal cycler with the total reaction volume of 20µl containing, 2µl of genomic DNA, 10X assay buffer, 10mM of dNTPs, 25mM $MgCl_2$, 5pmol of forward and reverse primer and 3U Taq polymerase enzyme and nano pure water. The PCR cycles were programmed as 95°C for 5 min, 94°C for 20 sec, 55°C for 30 sec, 72°C for 45 sec for 35 cycles and an additional temperature of 72°C for 7 min for final extension. The amplified products were separated on 3.5 per cent agarose gel prepared in 1X TAE buffer. The gel was run in 1X

Table 1: List of forty eight rice genotypes used for diversity analysis

S.No.	Genotype	S.No.	Genotype	S.No.	Genotype	S.No.	Genotype
1	GP:7858	13	GP:8109	25	E-191	37	E-8
2	GP:8001	14	GP:7868	26	E-750	38	GP:8551
3	GP:8067	15	E-849	27	E-224	39	GP:7862
4	GP:7860	16	GP:8058	28	E-186	40	GP:8219
5	GP:8706	17	E-888	29	E-179	41	GP:8205
6	E 147-145	18	GP:8709	30	E-743	42	GP:8178
7	GP:8182	19	GP:8600	31	E-156	43	GP:8170
8	GP:8595	20	GP:8130	32	GP:8447	44	GP:8338
9	E-846	21	GP:8716	33	GP:8331	45	E-321
10	E-437	22	E-851	34	GP:8309	46	GP:8441
11	E-929	23	GP:8142	35	E-283	47	GP:8028
12	E-601	24	GP:7880	36	E-729	48	GP:8762

TAE buffer at constant voltage of 80 V for a period of 100 minutes and stained with ethidium bromide.

Data analysis: Bands were scored as present (+/1) or absent (-/0). The size of each allele was determined by running simultaneously a DNA ladder by using a software (Uvitec, Fire-reader software version 15.12). The data was used for similarity based analysis using the programme NTSySPC (Rohlf, 2000). Similarity coefficients were used to construct UPGMA (unweighted pair group method with average) to generate dendrogram. Distance matrix and dendrogram was constructed based on diversity coefficient generated from pooled data by using Unweighted Pair Group Method of Arithmetic Means (UPGMA), a computer programme for distance estimation. Polymorphism Information Content (PIC) values were determined as per the procedure described by Senior *et al.* (1998)

Results and discussion

Polymorphism of SSR markers

Sixteen microsatellite markers were utilized for the determination of genetic diversity among 48 lines. All were observe to be polymorphic as presented in Table 2. A total of 127 alleles were detected by the 16 SSR markers. The number of alleles per locus varied from 4 to 17 with a mean of 7.9 alleles per locus. The PIC values of the 16 polymorphic SSR primers varied from 0.921 (RM 7075; Figure 1A) to 0.995 (RM 27289; Figure 1B) with an average of 0.969 per locus. Similar results were reported by Madhavi *et al*., (2011).

The SSR markers have significantly superior allelic diversity as compared to other co-dominant markers like RFLPs (McCouch *et al*., 1997). The average number of alleles per locus was 7.9 and this indicates a greater magnitude of variability among the plant materials analyzed in this work. These results agree with those of Olufowote *et al.,* (1997) who reported an average of 7.4 alleles per locus among 71 cultivars of rice. The results obtained in this study indicate that the rice lines selected for the present study have sufficient genetic divergence for possible use in future breeding programmes.

Polymorphism information content (PIC) value reflects the allele diversity and frequency among the genotypes. The informativeness of each SSR marker was established by using PIC value. The usefulness of a marker for different purposes like mapping, molecular bredding and germplasm evaluation is indicated by PIC value. DNA markers with greater PIC values have greater potential to indicate allelic variation. The markers analyzed in this study showed average PIC value of 0.969, indicating that the SSR markers utilized in this work were very informative and are helpful in identifying the polymorphism among rice lines. Higher PIC values reported in this study could be due to diverse origin of the used lines. The higher the mean PIC value of the locus, larger the number of alleles detected. This observation was consistent with report of Yu *et al.,* (2003), wherein SSR markers were noticed to exhibit high PIC valve because of their co-dominant nature and multi-allelic amplification pattern..

Table 2: List of primers and their chromosome location, product size and PIC values of 48 genotypes of rice

S. No	Name of the primer	Chromosome location	Product size (bp)	No. of alleles	PIC content
1	RM 7075	1	376	4	0.921
2	RM 3763	2	194	5	0.948
3	RM 12349	2	297	7	0.967
4	RM 1024	5	190	7	0.966
5	RM 19983	6	176	5	0.953
6	RM 542	7	201	11	0.99
7	RM 22524	8	176	6	0.969
8	RM 22710	8	182	15	0.994
9	RM 24035	9	231	8	0.984
10	RM 1026	9	195	7	0.98
11	RM 27258	11	220	9	0.984
12	RM 27289	11	194	17	0.995
13	RM 206	11	288	7	0.978
14	RM 27973	12	498	6	0.95
15	RM 28157	12	175	5	0.96
16	RM 16216	12	493	8	0.978
	Mean			**7.9**	**0.969**

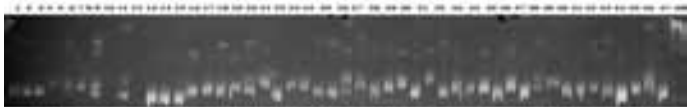

Figure 1A: Amplification pattern of 48 rice genotypes obtained by SSR marker RM7075

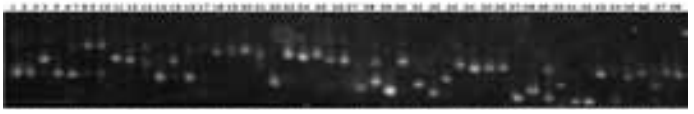

Figure 1B: Amplification pattern of 48 rice genotypes obtained by SSR marker RM27289

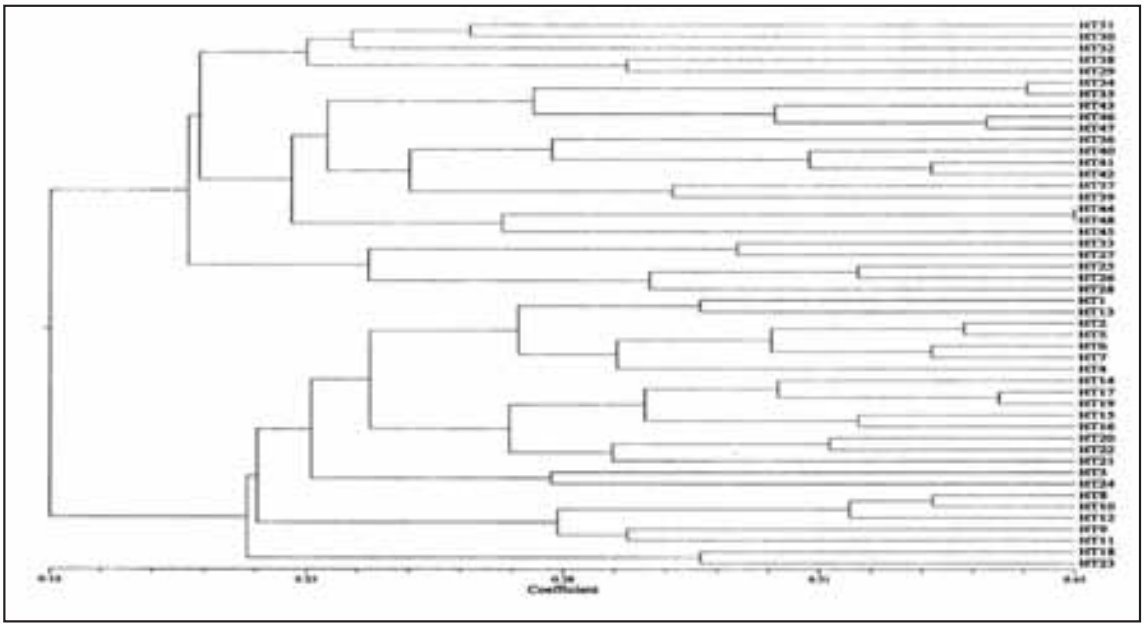

Figure 2. . Dendrogram resulting from UPGMA cluster analysis of 48 genotypes of rice

Genetic diversity Pattern

A dendrogram constructed based on UPGMA cluster analysis grouped the 48 rice lines into two clusters (Figure 2). The Jaccard's similarity coefficient ranged between 0.12 and 0.65. At the genetic similarity of 12%, the lines were grouped into two different clusters. Cluster I included all the 24 genotypes which were heat susceptible and cluster II had 24 heat tolerant genotypes. These findings were consistent with those reported by Saker *et al*., (2005). The results obtained clearly indicate that the genotypes belonging to cluster II can be used for development of heat tolerant varieties. It can also be concluded that the 16 carefully selected hyper-variable SSR markers are able to distinguish among tolerant and susceptible genotypes. Thus, marker-based identification and selection of the diverse genotypes could be helpful for the plant breeders and farmers for the improvement of new high yielding rice varieties.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Correlation and path studies of local germplasm of rice of Himachal Pradesh

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Received: 5th May 2019, Accepted: 21st June 2019

Abstract

The present investigation was carried out to study the correlation and path analysis in forty five genotypes including checks of rice (*Oryza sativa* L.). In general, genotypic correlation coefficient was higher in magnitude than the phenotypic correlation, indicating more genetic association among the various traits. Grain yield per plant exhibited significantly positive association with days to 50% flowering, plant height, panicle length, grains per panicle, spikelets per panicle, 1000-grain weight, grain length, grain breadth and spikelet fertility at both genotypic level and at phenotypic level indicating that simultaneous improvement of all the characters is possible. Path analysis revealed that at genotypic level, grain length had the highest positive direct effect on the grain yield per plant followed by spikelet fertility, effective tillers per plant, spikelets per panicle, panicle length, 1000-grain weight and protein content. Hence, emphasis can be laid out on these traits during selection for further improvement in grain yield in rice.

Keywords: Genotypic correlation, phenotypic correlation, path analysis, direct and indirect effects

Introduction

Rice (*Oryza sativa* L.) is the staple food crop for more than 60 per cent of people across the world. About 60 per cent of the world's rice is grown and consumed in Asia, which is known as "Rice bowl of the World", where it accounts for 50 to 80% of daily calorific intake (Pratap *et al*., 2012). Rice is being planted on approximately 11 per cent of earth's cultivated land area and ranks second in production after wheat (Anis *et al.*, 2016).

Rice is grown under various agro-climatic conditions ranging from foot-hills to an altitude as high as 2,200 m above mean sea level. Based upon genotypic and phenotypic correlation, the breeder would be able to decide the method of breeding that could be used to exploit the desirable associations and apply diverse methods of recombination to break the undesirable associations. Path coefficient analysis specifies the cause and measures the relative importance of each variable. The yield potential of each variety can be exploited if the relative importance of each component is ascertained and is increased to a certain desired degree by suitable management practices.

Materials and Methods

Forty five diverse genotypes of rice along with one check (HPR 2880) from different geographical origin in Himachal Pradesh were transplanted in 3 replications in RBD design at Rice and Wheat Research Centre, Malan, CSK HP Krishi Vishvavidyalaya, Palampur, during *Kharif*, 2018. In each replication, single seedling was transplanted per hill in 4 rows with 20 x 15 cm spacing.

The observations were recorded on five plants taken randomly from each plot for days to 50 per cent flowering, days to 75 per cent maturity, plant height at maturity, total tillers per plant at maturity, effective tillers per plant at maturity, grain yield per plant, spikelets per panicle, grains per panicle, panicle length, 1000-grain weight, spikelet fertility, kernel elongation, grain length (L), grain breadth (B), length breadth ratio (L:B), gel consistency (GC), gelatinization temperature (GT) rating, protein content and amylose content. Phenotypic and genotypic coefficients of correlation were worked out by following the procedure of Al-Jibouri *et al*., (1958) and Dewey and Lu (1959). The path analysis of important component traits and quality traits was done following Dewey and Lu (1959).

Results and Discussion

In general, genotypic correlation coefficient was higher in magnitude than the phenotypic correlation coefficient, indicating more genetic association among the various traits. Grain yield per plant exhibited significantly positive association with days to 50% flowering, plant height, panicle length, grains per panicle, spikelets per panicle, 1000-grain weight, grain length, grain breadth and spikelet

Table 1: Estimates of phenotypic correlation coefficient among various yield, morphological and quality traits in rice

Traits	Days to 75% maturity	Plant height (cm)	Total tillers/ plant	Effective tillers/ plant	Panicle length (cm)	Grains /panicle	Spikelets/ panicle	Grain yield/ plant (g)	1000-grain weight (g)	Grain length (mm)	Grain breath (mm)	L:B ratio	Kernel elongation (mm)	Spikelet fertility (%)	Gel consistency (mm)	GT rating	Amylose (%)	Protein (%)
Days to50% flowering	0.667*	0.214*	-0.385*	-0.386*	0.569*	0.355*	0.451*	0.132	-0.007	0.260*	-0.162	0.314*	0.152	-0.229*	0.051	-0.157	-0.259*	0.413*
Days to75% maturity		0.144	-0.219*	-0.196*	0.416**	0.188*	0.297*	0.021	-0.052	0.294*	-0.207*	0.350*	0.179*	-0.284*	0.251*	-0.087	0.005	0.428*
Plant height (cm)			-0.408*	-0.430*	0.308*	0.359*	0.268*	0.244*	0.242*	-0.133	0.321*	-0.304*	-0.175*	0.267*	-0.089	-0.005	-0.110	0.052
Total tillers/ plant				0.964*	-0.110	-0.489*	-0.485*	0.046	0.063	0.102	-0.047	0.102	0.063	0.010	-0.013	-0.149	0.057	-0.147
Effective tillers/ plant					-0.159	-0.463*	-0.451*	0.050	-0.014	0.062	-0.099	0.109	0.034	-0.010	-0.025	-0.127	0.062	-0.111
Panicle length (cm)						0.210*	0.265*	0.369*	0.393*	0.460*	-0.011	0.343*	0.371*	-0.111	0.024	-0.175*	-0.114	0.304*
Grains /panicle							0.939*	0.395*	-0.215*	-0.312*	-0.092	-0.146	-0.314*	0.252*	0.074	-0.152	-0.021	0.058
Spikelets/panicle								0.322*	-0.256*	-0.224*	-0.192*	-0.024	-0.245*	-0.074	0.128	-0.124	-0.008	0.159
Grain yield/ plant (g)									0.324*	0.121	0.188*	-0.058	0.080	0.298*	-0.128	-0.071	0.025	-0.009
1000 grain weight (g)										0.479*	0.481*	-0.021	0.390*	0.146	-0.170*	0.089	0.021	-0.120
Grain length (mm)											-0.160	0.746*	0.749*	-0.278*	-0.075	-0.024	0.050	0.158
Grain breatdh (mm)												-0.755*	-0.084	0.269*	-0.085	0.189*	-0.103	-0.276*
L:B ratio													0.558*	-0.356*	0.022	-0.180*	0.061	0.331*
Kernel elongation (mm)														-0.218*	-0.005	-0.088	-0.023	0.108
Spikelet fertility (%)															-0.139	-0.154	-0.059	-0.300*
GC (mm)																-0.074	0.025	0.084
GT rating																	0.206*	0.021
Amylose (%)																		0.016

*Significant at 5% level of significance

Table 2: Estimates of genotypic correlation coefficient among various yield, morphological and quality traits in rice

Traits	Days to 75% maturity	Plant height (cm)	Total tillers/ plant	Effective tillers/ plant	Panicle length (cm)	Grains /panicle	Spikelets/ panicle	Grain yield/ plant (g)	1000-grain weight (g)	Grain length (mm)	Grain breadth (mm)	L:B ratio	Kernel elongation (mm)	Spikelet fertility (%)	Gel consistency (mm)	GT rating	Amylose (%)	Protein (%)
Days to 50% flowering	0.811*	0.234*	-0.455*	-0.469*	0.604*	0.379*	0.496*	0.169*	-0.002	0.308*	-0.208*	0.359*	0.200*	-0.324*	0.054	-0.181*	-0.273*	0.425*
Days to75% maturity		0.182*	-0.260*	-0.276*	0.513*	0.266*	0.448*	0.110	-0.064	0.348*	-0.280*	0.416*	0.231*	-0.573*	0.292*	-0.099	0.018	0.518*
Plant height (cm)			-0.493*	-0.548*	0.319*	0.365*	0.281*	0.268*	0.265*	-0.162	0.374*	-0.335*	-0.205*	0.357*	-0.091	-0.007	-0.107	0.054
Total tillers/ plant				0.986*	-0.109	-0.589*	-0.600*	-0.051	0.034	0.219*	-0.057	0.176*	0.152	0.011	-0.013	-0.204*	0.066	-0.183*
Effective tillers/ plant					-0.176*	-0.574*	-0.575*	-0.067	-0.053	0.168	-0.135	0.197*	0.111	-0.008	-0.028	-0.177*	0.086	-0.147
Panicle length (cm)						0.188*	0.252*	0.404*	0.450*	0.550*	-0.006	0.379*	0.449*	-0.161	0.025	-0.197*	-0.125	0.319*
Grains /panicle							0.966*	0.432*	-0.277*	-0.343*	-0.089	-0.158	-0.350*	0.329*	0.079	-0.187*	-0.026	0.069
Spikelets/panicle								0.364*	-0.321*	-0.231*	-0.220*	-0.010	-0.278*	0.074	0.141	-0.151	-0.013	0.182*
Grain yield/ plant (g)									0.385*	0.238*	0.270*	-0.035	0.110	0.395*	-0.159	-0.128	0.021	-0.002
1000 grain weight (g)										0.582*	0.609*	-0.042	0.464*	0.098	-0.193*	0.154	0.018	-0.143
Grain length (mm)											-0.297*	0.784*	0.849*	-0.451*	-0.092	-0.032	0.062	0.204*
Grain breadth (mm)												-0.814*	-0.182*	0.458*	-0.101	0.262*	-0.108	-0.305*
L:B ratio													0.650*	-0.552*	0.020	-0.227*	0.065	0.361*
Kernel elongation (mm)														-0.325*	-0.006	-0.122	-0.017	0.139
Spikelet fertility (%)															-0.196*	-0.229*	-0.100	-0.426*
GC (mm)																-0.081	0.025	0.085
GT rating																	0.225*	0.021
Amylose (%)																		0.016

*Significant at 5% level of significance

Table 3: Estimates of direct and indirect effects at phenotypic level for different traits in rice

Traits	Days to 50% flowering	Days to 75% maturity	Plant height (cm)	Total tillers/ pant	Effective tillers/ plant	Panicle length (cm)	Grains / panicle	Spikelets/ panicle	1000 grain weight (g)	Grain length (mm)	Grain breadth (mm)	L:B ratio	Kernel elongation (mm)	Spikelet fertility (%)	Gel consistency (mm)	GT rating	Amylose (%)	Protein (%)	Grain yield/ plant
Days to50% flowering	**0.037**	-0.067	0.012	0.084	-0.233	0.112	0.002	0.265	-0.001	0.191	0.074	-0.289	0.018	-0.082	-0.003	-0.009	-0.010	0.035	0.132
Days to75% maturity	0.025	**-0.100**	0.008	0.048	-0.118	0.082	0.001	0.175	-0.007	0.217	0.079	-0.319	0.021	-0.102	-0.016	-0.005	0.000	0.036	0.021
Plant height (cm)	0.008	-0.014	**0.055**	0.089	-0.260	0.061	0.002	0.158	0.032	-0.098	-0.136	0.271	-0.020	0.096	0.006	0.000	-0.004	0.004	0.244*
Total tillers/ plant	-0.014	0.022	-0.023	**-0.217**	0.582	-0.022	-0.003	-0.285	0.008	0.078	0.015	-0.094	0.007	0.003	0.001	-0.009	0.002	-0.012	0.046
Effective tillers/plant	-0.014	0.020	-0.024	-0.209	**0.604**	-0.031	-0.003	-0.265	-0.002	0.047	0.036	-0.100	0.004	-0.004	0.002	-0.007	0.002	-0.009	0.050
Panicle length (cm)	0.021	-0.042	0.017	0.024	-0.096	**0.197**	0.001	0.156	0.053	0.338	0.005	-0.316	0.043	-0.040	-0.002	-0.010	-0.004	0.025	0.369*
Grains /panicle	0.013	-0.019	0.020	0.106	-0.280	0.041	**0.006**	0.552	-0.029	-0.230	0.045	0.137	-0.037	0.090	-0.005	-0.009	-0.001	0.005	0.395*
Spikelets/panicle	0.017	-0.030	0.015	0.105	-0.272	0.052	0.005	**0.588**	-0.034	-0.166	0.084	0.027	-0.029	-0.026	-0.008	-0.007	0.000	0.013	0.322*
1000 grain weight (g)	0.000	0.005	0.013	-0.014	-0.008	0.077	-0.001	-0.151	**0.134**	0.350	-0.202	0.013	0.046	0.052	0.011	0.005	0.001	-0.010	0.324*
Grain length (mm)	0.010	-0.030	-0.007	-0.023	0.039	0.091	-0.002	-0.133	0.064	**0.734**	0.065	-0.696	0.088	-0.099	0.005	-0.002	0.002	0.013	0.123
Grain breadth (mm)	-0.007	0.019	0.018	0.008	-0.052	-0.003	-0.001	-0.119	0.065	-0.116	**-0.414**	0.699	-0.010	0.094	0.006	0.012	-0.003	-0.023	0.176*
L:B ratio	0.012	-0.035	-0.016	-0.022	0.066	0.067	-0.001	-0.017	-0.002	0.553	0.313	**-0.924**	0.065	-0.125	-0.001	-0.010	0.002	0.027	-0.048
Kernel elongation (mm)	0.006	-0.018	-0.010	-0.014	0.021	0.073	-0.002	-0.144	0.052	0.551	0.035	-0.516	**0.117**	-0.078	0.000	-0.005	-0.001	0.009	0.080
Spikelet fertility (%)	-0.009	0.029	0.015	-0.002	-0.006	-0.022	0.001	-0.043	0.020	-0.202	-0.108	0.323	-0.025	**0.358**	0.009	-0.009	-0.002	-0.025	0.298*
Gel consistency (mm)	0.002	-0.025	-0.005	0.003	-0.015	0.005	0.000	0.075	-0.023	-0.056	0.040	-0.017	-0.001	-0.050	**-0.065**	-0.004	0.001	0.007	-0.128
GT rating	-0.006	0.009	0.000	0.032	-0.076	-0.034	-0.001	-0.073	0.012	-0.020	-0.085	0.163	-0.010	-0.055	0.005	**0.058**	0.008	0.002	-0.071
Amylose (%)	-0.010	-0.001	-0.006	-0.012	0.037	-0.022	0.000	-0.005	0.003	0.036	0.033	-0.055	-0.003	-0.021	-0.002	0.012	**0.039**	0.001	0.025
Protein (%)	0.015	-0.043	0.003	0.032	-0.067	0.060	0.000	0.093	-0.016	0.115	0.113	-0.299	0.013	-0.107	-0.005	0.001	0.001	**0.084**	-0.009

Residual effect = 0.477 ; *Significant at 5% level of significance; Bold values are direct effects

Table 4: Estimates of direct and indirect effects at genotypic level for different traits in rice

Traits	Days to 50% flower-ing	Days to 75% matu-rity	Plant height (cm)	Total tillers/ plant	Effec-tive tiller/ plan t	Pan-icle length (cm)	Grains / pani-cle	Spike-lets/ pani-cle	1000-grain weight (g)	Grain length (mm)	Grain breath (mm)	L:B ratio	Kernel elon-gation (mm)	Spike-let fertili-ty (%)	GC (mm)	GT rating	Am-ylose (%)	Pro-tein (%)	Grain yield/ plant
Days to50% flowering	**-2.221**	2.851	-0.313	1.503	-1.032	0.818	-1.104	0.778	-0.001	1.134	1.228	-2.631	-0.062	-0.974	-0.029	-0.010	0.164	0.069	0.169*
Days to75% maturity	-1.801	**3.517**	-0.243	0.858	-0.609	0.694	-0.775	0.703	-0.063	1.300	1.461	-3.054	-0.071	-1.721	-0.156	-0.006	-0.010	0.084	0.109
Plant height (cm)	-0.520	0.639	**-1.337**	1.628	-1.208	0.431	-1.064	0.442	0.263	-0.602	-2.047	2.390	0.062	1.071	0.049	0.000	0.064	0.009	0.269*
Total tillers/plant	1.011	-0.914	0.659	**-3.302**	2.172	-0.147	1.717	-0.942	0.034	0.828	0.214	-1.292	-0.046	0.032	0.007	-0.011	-0.039	-0.030	-0.050
Effective tillers/ plant	1.040	-0.971	0.733	-3.255	**2.204**	-0.238	1.673	-0.901	-0.052	0.640	0.623	-1.432	-0.033	-0.026	0.015	-0.010	-0.051	-0.025	-0.067
Panicle length(cm)	-1.340	1.801	-0.426	0.358	-0.387	**1.355**	-0.551	0.396	0.449	2.035	0.046	-2.811	-0.138	-0.484	-0.013	-0.011	0.074	0.052	0.405*
Grains /panicle	-0.841	0.935	-0.489	1.946	-1.266	0.256	**-2.914**	1.515	-0.276	-1.278	0.592	1.182	0.106	0.987	-0.042	-0.011	0.016	0.012	0.432*
Spikelets/panicle	-1.102	1.577	-0.376	1.983	-1.267	0.342	-2.814	**1.569**	-0.319	-0.862	1.260	0.112	0.084	0.223	-0.075	-0.008	0.007	0.030	0.363*
1000-grain weight (g)	0.003	-0.223	-0.354	-0.112	-0.116	0.611	0.808	-0.504	**0.995**	2.141	-3.329	0.243	-0.143	0.289	0.103	0.009	-0.012	-0.023	0.385*
Grain length (mm)	-0.681	1.235	0.217	-0.739	0.381	0.745	1.006	-0.366	0.576	**3.699**	1.574	-5.834	-0.258	-1.364	0.050	-0.002	-0.037	0.033	0.236*
Grain breath (mm)	0.498	-0.938	-0.500	0.129	-0.251	-0.011	0.315	-0.361	0.605	-1.062	**-5.479**	5.907	0.053	1.272	0.058	0.015	0.049	-0.049	0.250*
L:B ratio	-0.792	1.456	0.433	-0.579	0.428	0.516	0.467	-0.024	-0.033	2.926	4.387	**-7.377**	-0.197	-1.631	-0.009	-0.012	-0.038	0.058	-0.021
Kernel elonga-tion (mm)	-0.451	0.822	0.274	-0.500	0.242	0.617	1.017	-0.435	0.468	3.141	0.958	-4.795	**-0.303**	-0.974	0.000	-0.007	0.011	0.023	0.108
Spikelet fertility (%)	0.721	-2.017	-0.477	-0.035	-0.019	-0.218	-0.959	0.117	0.096	-1.681	-2.322	4.009	0.098	**3.001**	0.105	-0.013	0.060	-0.069	0.396*
GC (mm)	-0.119	1.027	0.122	0.042	-0.062	0.032	-0.231	0.221	-0.192	-0.344	0.596	-0.122	0.000	-0.591	**-0.533**	-0.005	-0.014	0.014	-0.159
GT rating	0.401	-0.350	0.009	0.674	-0.389	-0.267	0.546	-0.236	0.154	-0.134	-1.477	1.622	0.037	-0.685	0.043	**0.056**	-0.136	0.004	-0.128
Amylose (%)	0.608	0.062	0.143	-0.215	0.189	-0.168	0.076	-0.019	0.021	0.231	0.452	-0.470	0.005	-0.302	-0.012	0.013	**-0.598**	0.003	0.018
Protein (%)	-0.940	1.808	-0.075	0.615	-0.335	0.432	-0.209	0.290	-0.141	0.753	1.639	-2.626	-0.044	-1.277	-0.044	0.001	-0.010	**0.163**	-0.001

Residual effect =0.210; Bold values are direct effects; *Significant at 5% level of significance

fertility at both genotypic level and at phenotypic level (Table 1 & 2). Days to 50% flowering and grain length was not significantly correlated with grain yield per plant. Thus, the results indicate that improvement in grain yield per plant can be obtained by laying more emphasis on the above characters. Correlation coefficient at the genotypic level also showed similar trends as at the phenotypic correlation level for almost all traits. The results were similar to the findings of Dhanraj *et al*., (1987) for panicle length, Sinha *et al*., (2004) for 1000-grain weight, Abdul *et al*., (2011) for effective tillers per plant, panicle length and spikelet fertility, Haider *et al*., (2012) for spikelets per panicle.

Path analysis revealed that at genotypic level, grain length had the highest positive direct effect on the grain yield per plant followed by 75% days to maturity, spikelet fertility, effective tillers per plant, spikelets per panicle, panicle length, 1000-grain weight and protein content. However, at the phenotypic level, grain length had the highest positive direct effect on the grain yield per plant followed by effective tillers per plant, spikelets per panicle, spikelet fertility, panicle length, 1000-grain weight, kernel elongation, protein content, gelatinization temperature, plant height, amylose content, days to 50% flowering, and grains per panicle (Table 3). Concurrently, at genotypic level, grain breadth showed highest indirect effect on grain yield per plant *via* L:B ratio followed by L:B ratio *via* grain breadth, spikelet fertility *via* L:B ratio, kernel elongation *via* grain length, L:B ratio *via* grain length (Table 4). At phenotypic level, grain breadth had highest indirect effect on grain yield per plant *via* L:B ratio followed by L:B ratio *via* grain length, grains per panicle *via* spikelets per panicle, kernel elongation *via* grain length and panicle length *via* grain breadth. These findings were in agreement with reports of Bhadru *et al*., (2012) for plant height, Garg *et al*., (2010) for days to 75% maturity, Madhavilatha *et al*., (2005) for effective tillers per plant. The overall results obtained from path analysis are more or less in concurrence with earlier findings of Akhi *et al*., (2016) and Sharma and Sharma (2009).

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Development, evaluation and release of biofortified rice varieties

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Received: 10th Feb 2019; Accepted: 22nd May 2019

Abstract

Polished rice is a poor source of micronutrients and was reported to be responsible for malnutrition in the developing countries where rice is the major energy source. Biofortification for zinc (Zn) in polished rice is a promising and cost effective approach for the development of Zn-dense rice to alleviate micronutrient malnutrition. Several donors, especially landraces with >50 ppm in brown rice and >35 ppm in polished rice were identified and used in the development of breeding lines with high Zn and yield. The developed lines were evaluated under ICAR - All India Coordinated Rice Improvement Project for their high yield and Zn across the locations and across the years and promising lines were identified and released as varieties. Five varieties since 2015 were released through Central Varietal Release Committee for high Zn in polished rice in India.

Keywords: Biofortified rice varieties, high zinc, polished grains, development and release

Introduction

Rice (*Oryza sativa* L.) is the staple food crop of 50% of the world and a major energy source especially in India. Polished rice, the most preferred form for consumption, is a poor source of micronutrients and the excess dependence on polished rice was reported to be responsible for malnutrition whose daily caloric intake is mainly confined to rice (Juliano, 1993; Bouis and Welch, 2010). Polished grains of most of the rice varieties have 12-14 ppm of Zn, thus providing only one fifth of daily recommended Zn requirement of ~15 mg (though varies across sex and age) (Promo-u-thai *et al.*, 2010).

Dietary deficiency of Zn is a substantial global public health and nutritional problem with one third of the world population at risk due to low dietary intake of Zn (Krishna swamy, 1998; www.zinc.org/health/). A breeding target of 28 ppm was set in polished rice based on the nutrient needs, daily food intake, retention and bioavailability analyses in order to meet at least 25% of the estimated average Zn requirement for overcoming the most severe Zn deficiency (www.harvestplus.org). Enhancing the Zn content of polished rice has lot of potential to address wide spread Zn deficiency problem responsible for malnutrition in developing countries. Different strategies, such as biofortification, foliar or soil application of Zn fertilizers have been suggested and demonstrated to increase the Zn content of cereals (Nestel *et al.*, 2006; Swamy *et al.*, 2016). Of these, biofortification, an approach of the development of micronutrient-dense staple food crops to alleviate micronutrient malnutrition is targeted, sustainable and cost effective, hence most preferred. Since 2000, several attempts are being made in rice for Zn biofortification through conventional breeding approaches across the world. Using the donors for high Zn content, several breeding lines with high Zn are being developed and evaluated under HarvestPlus, international and national programs. As a proof of concept of combining high Zn with high yield, breeding lines with high Zn in polished rice were developed through conventional breeding with funding received from HarvestPlus, Department of Biotechnology (DBT), and Indian Council of Agricultural Research (ICAR), Government of India. These breeding lines were evaluated in India through national trials on Biofortification in All India Coordinated Rice Improvement Project since 2013 (www.harvestplus.org ; www.irri.org ; http://www.icar-iirr.org/). The entries of the trial constitute the biofortified breeding lines developed from all over the country. The present study summarizes the observations and the lessons learnt during the course of development of varieties with high Zn in polished rice and its release since 2013 in the Indian situation.

Materials and Methods

Identification of donors: Around 100 genotypes comprising landraces, breeding lines and farmer varieties

were evaluated across four locations *viz.*, rice experimental fields at Hyderabad (IIRR), Bengaluru (UAS), Chinsurah (Rice Research Station) and Coimbatore (TNAU) during wet season 2012. The soil properties of the plots of the four locations were given in Table 1.

Development of segregating material and selection of promising lines: The identified promising donors were crossed with rice varieties known for their yield, quality and adoption by the farmers. The favorable recombinants for yield and high Zn in polished rice were selected among the segregants, multiplied and submitted for evaluation.

Evaluation through ICAR - All India Coordinated Rice Improvement Project (AICRIP), ICAR-IIRR, Hyderabad: The nominated promising lines for yield and Zn were evaluated at 10 to 20 locations all over the country with two check varieties (control) *viz*., IR64 and BPT5204 for yield and high Zn *viz*., Kalanamak and Chittimuthyalu in polished rice. The seed samples are being sent to ICAR-IIRR for estimation of Zn in polished rice. The lines screened as Initial varietal trial (IVT), advanced varietal trial 1 (AVT 1) and advanced varietal trial 2 (AVT 2) for three years and the consistent lines were tested for 12 quality parameters for their suitability (http://www.icar-iirr.org/).

The varieties with recommended level of Zn, yield and desirable quality parameters were released as varieties through Central Varietal Release Committee (CVRC). As a case study, the evaluation data of AICRIP- Biofortification 2015-17 was presented.

Estimation of Zn: The seed of each plant was harvested and divided into three parts to be analyzed as three replicates. The seeds were dehusked using JLGJ4.5 testing rice husker (Jingjian Huayuan International Trade Co., Ltd) sponsored by HarvestPlus and polisher (Krishi International India Ltd.) with non-ferrous and non-Zn components. Each sample of brown and polished rice (5 g) was subjected to energy dispersive X-ray fluorescent spectrophotometer (ED-XRF) (OXFORD Instruments X-Supreme 8000) at ICAR-IIRR as per standardized protocols (Sanjeeva Rao *et al.*, 2014).

Results and Discussion

Zn deficiency is a major public health and nutritional issue affecting mostly the developing countries whose staple diet is polished rice (Krishnaswami, 1998; Juliano, 1993). The objective of Zn biofortification is to increase Zn concentration in polished rice and of the various approaches to address Zn deficiency in the human diet, biofortification is the most feasible, sustainable and economical approach. Several biofortified rice varieties have been released in Bangladesh and India and are being evaluated in Philippines and Indonesia (Swamy *et al.*, 2016).

Rice grain Zn concentration is affected by a large number of plant and environmental factors (Welch and Graham, 2002). Zn reported to be poorly available under the irrigated conditions (Fageria, 2013). The Zn content in the grain appears to be significantly affected by the pH, organic matter content and available Zn levels of native soil. However, genotypic variation was observed to be the most significant factor to affect grain Zn content (Chandel *et al.*, 2010). In the present study, the genotypes were evaluated in four locations with differential soil profiles (Table 1). The Zn found to be on higher side in the plots owing to the application of Zn fertilizer as part of the package of practices in rice experimental fields. However, Zn deficiency is reported to be widespread in soils and crops of India and is one of the significantly depleted mineral nutrients under intensively cropping systems (Takkar, 1997). On the basis of the analysis of about 65,000 soil samples, it has been found that about 51.2% of Indian soils are deficient in Zn. The critical levels of DTPA- Zn for cereal crops were found to vary among the soil types and crops (Singh *et al.*, 2015). Thus, farmers need to be sensitized regarding package of practices for growing the biofortified varieties.

Wide genetic variation was observed among the evaluated genotypes for grain Zn content in brown and polished rice. The rice land races or traditional varieties are considered to have high nutritive and therapeutic value (Deb *et al.*, 2015). Extensive genetic variability for grain Zn content has been earlier reported in rice wild accession and landraces (Anandan *et al.*, 2011; Anuradha *et al.*, 2012; Nachimuthu *et al.*, 2014; Huang *et al.*, 2016). Many landraces were identified with >50 ppm in brown rice and >35 ppm in polished rice and have been successfully used or being used in breeding programs (Table 2A and 2B). The screening of landraces for high nutrient content should be a continuous process for identification of new donors for high Zn in polished rice.

Across the locations, the genotypes found highly variable in their grain Zn content and only one genotype was found to be in the top ten genotypes. Surprisingly, the extent of polishing also showed lot of variation across the locations

and genotypes (Table 3). The genotypes with high Zn content in brown rice were found not to necessarily contain high Zn content in polished rice, suggesting the effect of quality parameters and filling of the grain on polishing. The rice grain

Table 1. Soil properties of experimental plots of the four locations during wet season 2012

Trait	Hyderabad (HYD)		Chinsurah (CHN)		Coimbatore (CBT)		Bengaluru (BLR)	
	Before Planting	After Harvesting	Before Planting	After Harvesting	Before Planting	After Harvesting	Before Planting	After Harvesting
pH	8.52	8.47	6.04	7.12	7.82	7.87	6.02	5.99
E.C ($dS\ m^{-1}$)	0.92	0.71	0.14	0.19	0.64	0.47	0.25	0.27
O.C (%)	0.56 (M)	0.70 (M)	0.75 (M)	0.72 (M)	0.73 (M)	0.67 (M)	0.24 (L)	0.23 (L)
N (kg/ha)	230 (L)	187 (L)	164 (L)	183 (L)	195 (L)	227 (L)	181 (L)	183 (L)
P_2O_5 (kg/ha)	81 (H)	107 (H)	39 (M)	41 (M)	100 (H)	84 (H)	9 (L)	14 (L)
K_2O (kg/ha)	616 (H)	641 (H)	458 (H)	410 (H)	957 (H)	926 (H)	359 (H)	336 (H)
Fe (ppm)	3.48 (L)	2.30 (L)	56.50 (H)	31.92 (H)	0.74 (L)	0.94 (L)	28.82 (H)	30.50 (H)
Zn (ppm)	3.66 (H)	3.61 (H)	1.61 (H)	1.28 (H)	3.61 (H)	3.50 (H)	1.55 (H)	1.00 (H)

quality is highly influenced by environmental factors like temperature, soil moisture, crop management and post-harvest practices. Before releasing of a biofortified variety, multi-locational evaluation should be compulsorily made.

Around 20% average loss of Zn is observed in the polished rice samples in comparison to brown rice across the locations, the percentage loss of Zn content on polishing found to be broadly varying from 1.2 to 59.5. Among the genotypes, the range of percentage reduction was too wide, only two genotypes showed <10 % across the locations.

Combining the yield and high Zn in polished rice is a major challenge owing to the dilution effect of the micronutrient content (Impa *et al.*, 2013). Earlier focus of the rice breeding programs was the production of high yielding varieties to ensure the food security, thus breeding for high micronutrient contents was not a priority objective (Graham and Welch, 1996). With growing awareness of importance of biofortification, especially of Zn, breeding for high Zn in polished rice is one of the important objectives in global rice breeding programs.

Table 2A. Zn content in the brown rice of the top ten genotypes in the four locations estimated through ED-XRF

Location	HYD		BLR		CHN		CBT
Name	Zn (ppm)	Name	Zn (ppm)	Name	Zn (ppm)	Name	Zn (ppm)
GMP 22*	51.9	GMP 33	39.5	GMP 23*	39.4	GMP 14*	24.5
GMP 23*	48.5	GMP 45	39.3	GMP 24**	37.9	GMP 24**	22.2
GMP 35*	45.2	GMP 31	38.9	GMP 20*	30.0	GMP 12*	21.0
GMP 24**	45.1	GMP 43*	38.2	GMP 22*	29.9	GMP 22*	21.0
GMP 25	44.8	GMP 24**	35.6	GMP 38	29.6	GMP 11*	19.3
GMP 39*	43.3	GMP 40*	34.3	GMP 36*	28.9	GMP 43*	19.1
GMP 28*	41.8	GMP 28*	34.1	GMP 8	26.3	GMP 44	20.9
GMP 32	41.6	GMP 29	33.9	GMP 39*	26.2	GMP 13	16.8
GMP 40*	40.0	GMP 5	33.8	GMP 11*	25.8	GMP 27	16.4
GMP 14*	39.6	GMP 1	32.7	GMP 35*	25.6	GMP 23*	16.3

* top genotype in more than one location, ** top genotype in four locations

Table 2B. Zn content in the polished rice of the top ten genotypes in the four locations estimated through ED-XRF

Location	HYD		BLR		CHN		CBT
Name	Zn (ppm)	Name	Zn (ppm)	Name	Zn (ppm)	Name	Zn (ppm)
GMP 24*	42.7	GMP 45*	36.1	GMP 20	28.7	GMP 24*	21.3
GMP 23*	42.0	GMP 33	35.1	GMP 24*	27.9	GMP 44*	18.1
GMP 25	38.5	GMP 31	32.9	GMP 23*	27.0	GMP 14	17.3
GMP 28*	38.3	GMP 40	32.2	GMP 39*	24.7	GMP 43*	17.1
GMP 22*	36.6	GMP 43*	32.1	GMP 43*	23.7	GMP 27	14.5
GMP 39*	34.5	GMP 28*	32.0	GMP 45*	23.0	GMP 12	14.1
GMP 44*	34.1	GMP 29	31.7	GMP 35*	22.6	GMP 23*	13.9
GMP 35*	34.1	GMP 1	31.6	GMP 21	22.5	GMP 22*	13.6
GMP 37	33.6	GMP 26	30.8	GMP 41	22.5	GMP 6	13.3

* top genotype in more than one location

Table 3. Percentage reduction of Zn content in polished rice to brown rice

Location	HYD	BLR	CHN	CBT		
Genotype						
Range	3.7 - 39.6	1.2 - 56.5	2.2 – 59.5	2.8 – 43.2	Mean	Range
GMP 1	16.4	3.4	25.0	43.2	22.0	3.4 – 43.2
GMP 2	18.1	11.2	24.0	15.1	17.1	11.2 – 24.2
GMP 3	21.9	3.5	14.3	20.3	15.0	3.5 – 21.9
GMP 4	7.8	14.0	18.8	22.9	15.9	7.8 - 22.9
GMP 5	14.8	15.4	6.9	16.3	13.3	6.9 – 16.3
GMP 6	22.8	6.1	4.8	7.0	10.2	7.0 - 22.8
GMP 7	33.7	3.3	33.8	21.4	22.3	3.3 – 33.8
GMP8	35.2	3.4	23.6	28.7	22.7	3.4 – 35.2
GMP 9	15.3	12.1	22.8	13.8	16.0	12.1 – 22.8
GMP 10	27.7	10.0	8.7	14.1	15.1	8.7 – 27.7
GMP 11	21.0	13.7	17.4	34.2	21.6	13.7 – 34.2
GMP 12	27.4	27.2	24.4	32.9	28.0	24.4 – 32.9
GMP 13	7.2	12.7	31.6	38.7	22.5	7.2 – 38.7
GMP 14	39.6	12.5	21.7	29.4	25.8	12.5 – 39.6
GMP 15	9.7	4.8	7.1	2.8	6.1	2.8 – 9.7
GMP 16	7.3	1.2	14.7	31.3	13.6	1.2 – 31.3
GMP 17	19.3	5.3	5.1	25.2	13.8	5.1 – 25.2
GMP 18	19.2	2.6	4.5	30.4	14.2	2.6 – 30.4
GMP 19	3.7	3.1	29.5	28.4	16.2	3.1 – 29.5
GMP 20	22.4	10.7	4.3	26.4	16.0	4.3 – 26.4
GMP 21	27.5	9.6	2.2	36.7	19.0	2.2 – 36.7
GMP 22	29.5	6.2	49.5	35.2	30.1	6.2 – 49.5
GMP 23	13.4	9.5	31.5	14.7	17.3	9.5 – 31.5
GMP 24	5.3	16.0	26.4	4.1	12.9	4.1 – 26.4
GMP 25	14.1	12.4	18.4	3.2	12.0	3.2 – 18.4
GMP 26	16.1	5.5	26.9	7.0	13.9	5.5 – 26.9
GMP 27	21.6	6.7	28.9	11.6	17.2	6.7 – 28.9
GMP 28	8.4	6.2	9.0	6.7	7.5	6.2 – 9.0

Location	HYD	BLR	CHN	CBT		
Genotype						
Range	3.7 - 39.6	1.2 - 56.5	2.2 – 59.5	2.8 – 43.2	Mean	Range
GMP 29	13.1	6.5	36.4	19.1	18.8	6.5 – 36.4
GMP 30	10.5	11.7	40.7	24.1	21.8	10.5 – 40.7
GMP 31	23.5	15.4	26.7	14.7	20.0	15.4 – 26.7
GMP 32	23.8	1.4	13.6	20.0	14.4	1.4 – 23.8
GMP 33	15.1	11.1	11.5	35.1	18.2	11.1 – 35.1
GMP 34	26.8	4.1	25.6	26.7	20.8	4.1 – 26.8
GMP 35	24.6	56.5	11.7	23.0	28.9	11.7 – 56.5
GMP 36	25.8	11.5	57.8	20.8	29.0	11.5 – 578
GMP 37	14.1	20.8	27.8	18.8	20.4	14.1 – 27.8
GMP 38	15.6	3.8	59.5	6.0	21.2	3.8 – 59.5
Mean	**20.0**	**15.0**	**30.5**	**21.0**	**21.6**	

Several combination of crosses were developed using promising donors for Zn in polished rice and popular rice varieties. In a cross combination of a land race donor and a popular variety, out of the selections made in fixed lines, only one line could show high yield (>20 g single plant yield) and high Zn (> 28 ppm) (Figure 1). Similarly, though a few, promising lines were selected from all cross combinations and are being evaluated.

Figure 1.Common breeding line between the RILs for high yield and high Zn

The evaluation constitutes the comparison of biofortified lines with existing varieties for yield (IR64) and Zn content (landraces with high zinc) as check varieties. Initially, a concentration of 20 ppm was set as threshold value in AICRIP during 2013; however the threshold value has been raised to 24 ppm. The recommended dietary allowance (RDA) of Zn for human population in the age group of 25-50 years is 12-15 mg, respectively (FAO/WHO, 2000). In India, the average daily intake of rice is around 220 g and the polished rice with 28 ppm Zn would give 6.16 ppm Zn which is just 50% of RDA (Sanjeeva Rao *et al.*, 2014).

With funding from HarvestPlus, DBT and ICAR, several institutions have developed breeding lines for high Zn in rice. Based on the need for the evaluation of the breeding lines, a trial was constituted for biofortification during 2013 in ICAR – AICRIP and till date, several lines have been screened at multi-locations. Around 50 % of the nominated lines are advanced to AVT 1 based on their yield and Zn content. Approximately half of the AVT 1 lines were advanced to AVT 2 and based on the stringent yield, Zn and quality parameters, and the varieties are released (Table 5 and Table 6a & 6b).

Table 5. The number of breeding lines nominated under AICRIP

Year	IVT	AVT1	AVT2
2013	29/9		
2014	45/11	6/3	
2015	45/26	30/14	4/2
2016	32/10	12/11	12/3
2017	31/3	12/2	11/1
	182/59	60/30	27/6

Table 6a.An example data set for yield and Zn content

Entry details	2017		2016		2015	
	Mean yield (20)	Zn (ppm)	Mean yield (17)	Zn (ppm)	Mean yield (14)	Zn (ppm)
IET 25477	4791	25.5	4360	28.3	3620	28.4
IET 25475	5102	25.3	4478	27.7	3881	27.6
IET 25443	4728	25.0	4660	26.5	3629	24.4
IR 64	5941	18.5	5044	19.3	3933	17.2
Chittimuthyalu	4597	24	3185	24.8	2804	21.5

Table 6b.An example data set for quality parameters

Entry details	2017			2016		
	HRR	AC	GT	HRR	AC	GC
IET 25477	51.4	24.2	71	52	24.58	59
IET 25475	57	27.57	23	50.8	25.93	53
IET 25443	61.5	25.63	22	56.3	25.96	22
IR 64	na	na	na	52	23.29	55
Chittimuthyalu	65.7	23.52	22	60.8	23.4	22

HRR: head rice recovery, AC: amylose content, GC: Gel consistency

Table 7.Summary of five released varieties with high Zn

Variety	III year		II year		I year	
	Mean yield (20)	Zn (ppm)	Mean yield (17)	Zn (ppm)	Mean yield (14)	Zn (ppm)
IET 25477 (Zinco Rice)	4791	25.5	4360	28.3	3620	28.4
West Bengal, Chattisgarh and Odisha IGKV Raipur						
IET 24760 Surabhi (NSL)	4804	22.84	4693	20.0	4214	24.0
Maharashtra and Gujarat Nuziveedu Seeds Limited						
IET 24555 (DRR Dhan 48)	5008	20.91	4989	19.8	6202	na
Andhra Pradesh, Telangana, Tamil Nadu, Karnataka and Kerala.						
(IET 24557 (DRR Dhan 49)	4562	26.13	5079	20.6	6373	na
Gujarat, Maharashtra and Kerala						
IET 23832 (DRR Dhan 45)	4068	22.9	4222	22.68	4356	20.23
Andhra Pradesh, Tamil Nadu and Karnataka						

Till now, five varieties were released through central release varietal committee for high Zn in polished rice in India through ICAR - All India Coordinated Rice Improvement Project. One of the varieties released was a contribution from private sector, Nuziveedu Seeds Limited (Table 7). Many studies reported a significant negative association between the yield and grain Zn content (Jiang *et al.*, 2008; Norton *et al.*, 2010; Wissuwa *et al.*, 2008). Non-significant correlations were also reported between yield and grain Zn (Gangashetty *et al.*, 2013; Sathisha, 2013) in a set of landraces. However, from our experience in developing the breeding lines for high Zn and yield and from the nominations to AICRP-rice, we suggest the feasibility of combining the yield and high Zn.

Invitro bioavailability studies of a released variety *viz.*, DRR Dhan 45 showed that the Zn content and bioavailability was 50% more than the control IR64 variety. The coupled *in vitro* digestion/Caco-2 cell model studies with extrinsic ^{65}Zn isotopic labeling demonstrated higher absorption of Zn from DRR Dhan 45 in intestinal cells (Neeraja and Voleti, 2019).

Thus, with consorted efforts of various disciplines of plant breeding, soil science, biochemistry and plant physiology, the biofortified rice varieties have been developed. An evaluation system for biofortified varieties has been evolved by ICAR- IIRR through AICRIP for their release. However, the impact of the biofortified rice varieties in addressing malnutrition of the nation appears to be a long way to go, owing to its adoption by the growers and consumers.

Policy recommendations

- Proof of concept for the development of rice biofortified varieties with high yield and Zn
- Combination of genetic and agronomic strategies for biofortified varieties
- Multi-location evaluation is compulsory for stable biofortified varieties
- Clinical trials as proof of concept for inclusion of high Zn rice in public distribution system and mid-day meal schemes.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

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

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Received: 3rd May 2019; Accepted: 2nd June 2019

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.

Acknowledgement

National Professor Project (F.No: Edn/27/4/NP/2012-HRD) of Indian Council of Agricultural Research is gratefully acknowledged.

Contribution No: IIRR/DIR/PD/PMEC/2019-20/Res. paper/355

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

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

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Received: 12th March 2019; Accepted: 10th June 2019

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).

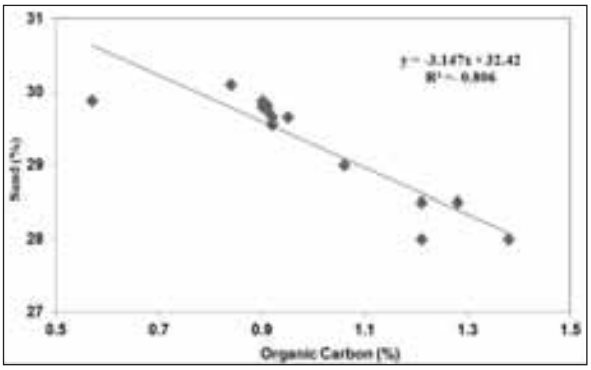

Fig.1. Relationship of organic carbon (%) with sand (%)

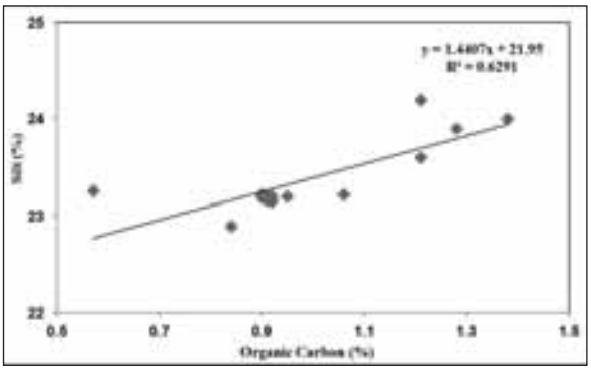

Fig.2. The relationship of organic carbon (%) with silt

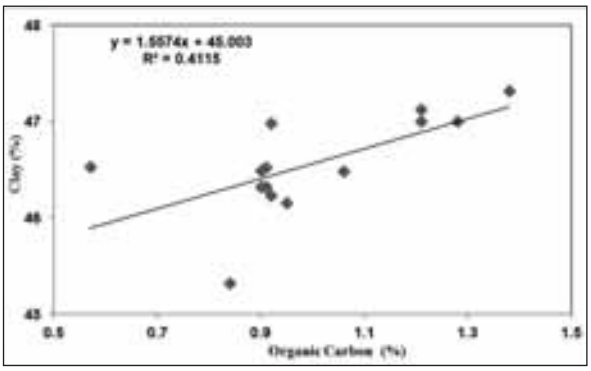

Fig.3. The relationship of organic carbon (%) with clay (%)

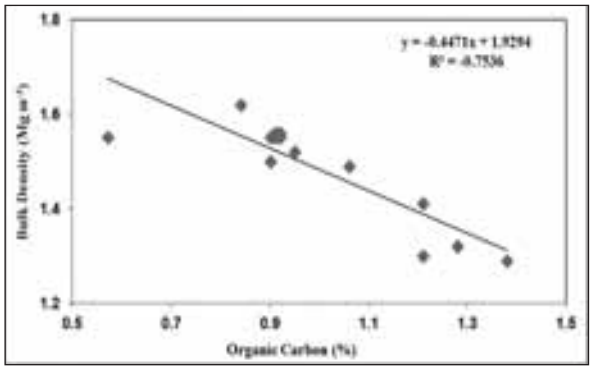

Fig.4. The relationship of organic carbon (%) with Bulk density (Mgm-3)

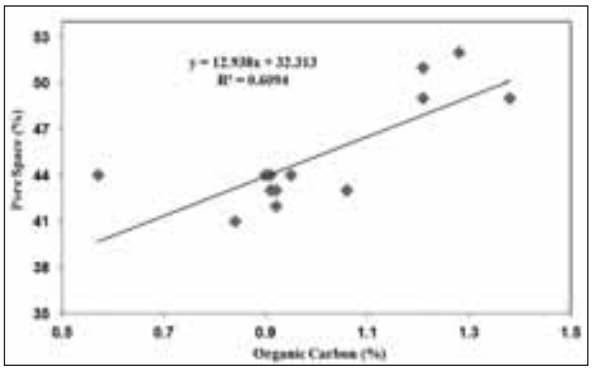

Fig.5. The relationship of organic carbon (%) with pore space (%)

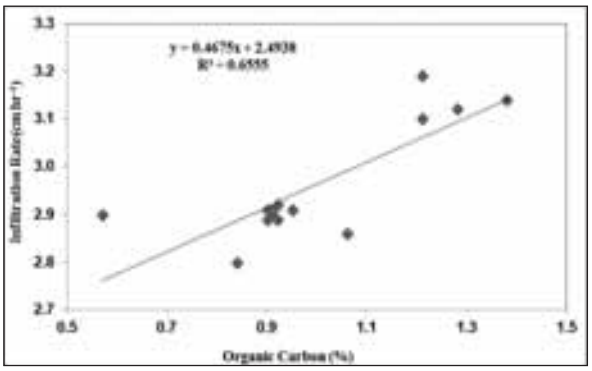

Fig.6. The relationship of organic carbon (%) with infiltration rate (cm hr^{-1})

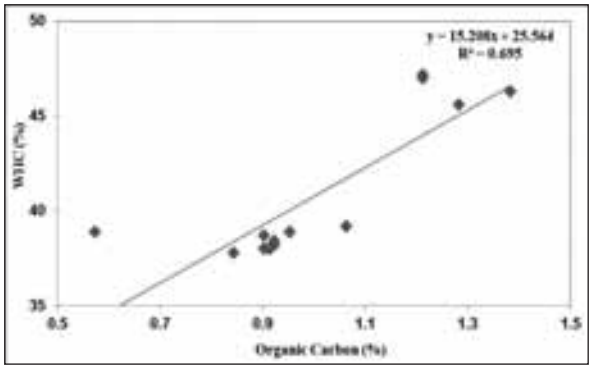

Fig. 7 The relationship of organic carbon (%) with water holding capacity (%)

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.

Acknowledgments

The senior author thanks Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India for providing financial support and the Director, Tamil Nadu Rice Research Institute (TRRI), Aduthurai, Tamil Nadu, India for providing facilities for successful completion of the research work.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Effect of ACC deaminase producing bacteria on germination and seedling growth of rice under heat stress

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Received: 12th May 2019; Accepted: 20th June 2019

Abstract

Ethylene is a gaseous phytohormone regulating plant growth at all stages commencing from seed germination and development and extending to senescence. It is also a stress responsive hormone regulating responses of plants to abiotic and biotic stress conditions. The hormone has been associated with stress-induced senescence in plants and manipulation of ethylene synthesis is known to affect plant stress tolerance. 1-aminocyclopropane-1-carboxylate (ACC) deaminase is a bacterial enzyme that has been known to influence plant ethylene production by degrading the immediate precursor of ethylene biosynthesis specifically ACC, into ketobutyrate and ammonia resulting in reduced ethylene production during stress. In the present study, an ACC deaminase producing bacteria isolated from rhizosphere of rice and identified as *Rhizobium* sp was able to show ACC deaminase activity of 2.52 ± 0.73µM α- ketobutyrate /µg protein/24h. The bacterium was observed to partially offset the negative effects on seedling growth which occurred due to the presence of 3mM ACC in the growth medium. Rice seeds treated with *Rhizobium sp* showed highest germination percentage and vigour index under heat stress at 45 °C, when compared to uninoculated control.

Keywords: ACC, rice, *Rhizobium sp*, heat stress and germination

Introduction

The present day agriculture is plagued by various abiotic (extreme temperatures, drought, salinity, water logging) and biotic stresses (weeds, insect pests, nematodes and pathogens) affecting agricultural productivity (Mariani and Ferrante, 2017; Gimenez *et al*., 2018). Among the various stressors, heat and drought are most important, having a huge impact on growth and productivity of the crops (Fahad *et al.*, 2017). Rice *(Oryza sativa* L.), a major cereal crop and staple food for nearly half of the world population is highly vulnerable to high temperatures. With each 1 ºC rise in day temperature from 28 to 34 °C, a 7-8% reduction in rice yield has been predicted (Baker *et al*., 1992). Although high temperature affects all growth stages of rice from seed germination to seed setting and ripening (Shah *et al*., 2011), it's influence on seed germination and early seedling growth stage is crucial as these stages are important for obtaining a good plant stand and subsequently, high yields (Weitbrecht *et al*., 2011; Hasanuzzaman *et al*., 2013). The optimum temperature for germination of rice seed is 28- 30 °C while the threshold temperature at the seedling stage has been identified as 35 °C (Sarsu, 2018). Seedlings experience a decrease in stomatal conductance and photosynthetic rate due to high temperatures which can lead to poor plant growth (Sanchez-Reinoso *et al*., 2014; Yoshida, 1981)..

In response to stresses, plants protect themselves by modulating the expression of hormones which induce production of stress related proteins and other molecules. One important plant hormone that mediates stress response is ethylene, a gaseous phyto hormone which if accumulated in excess of threshold level, hinders plant growth and development. Manipulation of ethylene biosynthesis or perception has been advocated as a means to create plants with desirable traits like tolerance to various stresses (Stearns and Glick, 2003). Crop engineering targeting plant ethylene signaling as a new strategy for crop improvement, has resulted in crops that show improved growth in the field due optimization of their ethylene responses (Dubois *et al*., 2018).

Plant associated microbes also play a key role in maintaining plant health under various stresses (Kumar and Verma, 2018; Ma *et al*., 2019). The lowering of

ethylene levels is in fact considered as one of the major mechanisms employed by plant growth-promoting bacteria to sustain plant growth under stress. Certain bacteria produce an enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase by which the bacterium cleaves ACC, the precursor of plant ethylene biosynthesis into ketobutyrate and ammonia. Since ethylene production mainly depends on the endogenous levels of plant ACC (Gupta *et al*., 2019), these bacteria when associated with plants acts as a sink for ACC, thereby decreasing internal ACC levels leading to a concomitant reduction in plant ethylene production (Glick, 2014). Inoculation of plants with bacteria expressing ACC deaminase activity has been helpful in allowing plant growth and development under stress conditions by reducing stress-induced ethylene production and significantly decreasing the severity of stresses (Saleem *et al*., 2007; Glick, 2014).

In the present investigation, an ACC deaminase producing plant growth promoting bacteria isolated from rice rhizosphere was evaluated for its ability to attenuate the effect of exogenously supplied ACC and to improve rice seed germination and seedling growth under heat stress.

Materials and Methods

Rhizobium sp. with accession number (KY348774) maintained at ICAR- Indian Institute of Rice Research, Hyderabad was used for the study. Seeds of heat susceptible genotype, *O. sativa* cv. Swarna were also obtained from ICAR-IIRR.

Quantification of ACC deaminase activity

Rhizobium sp. was grown overnight in nutrient broth, centrifuged at 10,000 rpm for 10 min and the cell pellet obtained was washed with modified Dworkin and Foster minimal medium (DF) (containing g/l KH_2PO_4- 4g, Na_2HPO_4 - 6g, $MgSO_4.7H_2O$- 0.2g, glucose - 2g, gluconic acid- 2g, citric acid- 2g, trace elements ($FeSO_4.7H_2O$- 1mg, H_3BO_3- 10mg, $MnSO_4.7H_2O$- 11.19mg, $ZnSO_4.7H_2O$- 124.6mg, $CuSO_4.5H_2O$- 78.22mg, MoO_3- 10mg). After washing, the cell pellets were suspended in DF minimal broth containing 3mM ACC (1- amino cyclopropane carboxylic acid), incubated for 24 h, washed in Tris buffer (pH- 7) and resuspended again in Tris buffer (pH-8.5) and toluene. Toluenized cell suspensions were used for measuring ACC deaminase activity according to the procedure of Dworkin and Foster (1958). The ACC deaminase activity was estimated spectrophotometrically as α-ketobutyrate production at 540 nm using a standard curve of α-ketobutyrate ranging between 1- 10µM concentrations (Honma and Shimomura, 1978). Protein content in the toluenized cell suspension was determined by Lowry's method with bovine serum albumin (200-1000 µg/ml) serving as standard (Lowry *et al*., 1951). ACC deaminase activity was expressed as the amount of α-ketobutyrate liberated in nmol per milligram of cellular protein per 24 h.

PCR amplification of ACC deaminase producing (*acdS)* gene

ACC deaminase (*acdS)* gene was amplified from the DNA extracted from the isolate using ACCf (5´ATGAACCTGAATCGTTTTRAA 3´) as forward primer and ACCr (5´TCAGCCGTTGCGRAACARAACARGAA3´) as reverse primer. PCR was performed in a Thermal Cycler (T-100, Biorad) with the following conditions for 35 cycles, *i.e.,* initial denaturation at 95 °C for 5 min, initial extension 72 °C for 1 min and annealing temperature at 55 °C for 45 sec followed by final denaturation at 95 °C for 1min and final extension 72 °C for 10 min, with expected PCR product being ~996 bp (Farajzadeh *et al.,* 2010).

Seedling survival under exogenous ACC treatment

Three day old seedlings of rice (cv. Swarna) grown under aseptic conditions were transferred to sterile test tubes containing 10 ml of Yoshida medium (containing g/lit: NH_4NO_3- 91.4, NaH_2PO_4. $2H_2O$- 35.6, K_2SO_4- 71.4, $CaCl_2$. $2H_2O$- 117.35, $MgSO_4.7H_2O$- 324 and micronutrients: $MnCl_3.2H_2O$-1.5, $(NH_4)6Mo_7O_{24}4H_2O$-0.074, $ZnS0_4.7H_20$-0.14, H_3BO_3- 0.934, $CuSO_4.5H_2O$- 0.031, $FeCl_3.6\ H_2O$- 7.7 and citric acid- 11.9) and seedlings were grown at 28 ± 30 °C under 16 h/8 h light and dark illumination regime in the presence and absence of 3mM ACC and *Rhizobium sp*. inoculation (1x 10^8 CFU/ml). Seedling growth was evaluated after ten days by measuring the root and shoot growth.

In vitro assay for heat stress tolerance

Seed germination and seedling growth of rice seeds treated with *Rhizobium* sp were evaluated after imposition of heat stress. Surface sterilized Swarna seeds were soaked overnight in bacterial suspension (3 x 10^8 CFU/ml of *Rhizobium sp.*) and allowed to germinate at, i) ambient temperature of 30 °C while a second set of seeds, ii) were exposed to 45 °C heat stress for 24 h (Mastouri *et al*., 2010) and then grown at ambient temperature. Rice seeds soaked in sterile water were used as control. After seven days, the lengths of root and shoots of germinating seeds were

recorded and used for calculation of germination indices (Kandasamy *et al*., 2009)

Germination percentage (%) = (Number of seeds germinated in petri plate/Total no of seeds in the petri plate used for test) X 100

Vigour Index = (Mean of root + shoot length) x Germination percentage

Vigour Index Increment = Vigour index in treatment – Vigour index in control

Statistical analysis

The experiments were conducted in completely randomized design with three replicates and the data are presented as mean ± SD.

Results and Discussion

ACC deaminase production

ACC is taken up by bacterial cells and the ACC deaminase enzyme found inside the cytoplasm of bacterial cells degrades ACC into α-ketobutyrate and ammonia. The bacterial isolate used in this study, identified as *Rhizobium sp.* showed ACC deaminase activity of 2.52 ± 0.73 µM α-ketobutyric acid /µg protein/24h (Table 1).

Table.1 ACC deaminase activity of the bacterial isolate

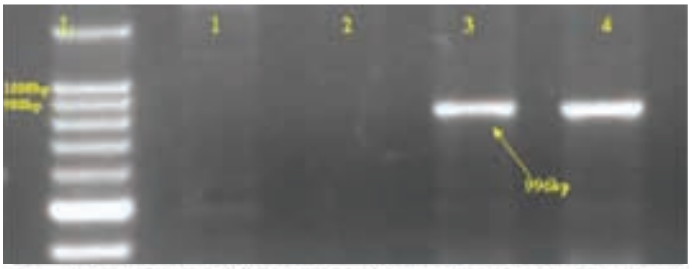
S.No	Name of the isolate	ACCdeaminase activity* µM α – ketobutyrate /µg protein/24h
1	*Rhizobium sp.*	2.52 ± 0.73

*Mean ± SD of three replicates

According to Glick (2005), high ACC deaminase activity is observed in rhizosphere, phyllosphere and endophytic bacterial inhabitants and the bacteria present in these niches can act as a sink for ACC produced by plant as a consequence of stress. Studies by Timmusk (2011) have also revealed that ACC deaminase activity is relatively common in rhizosphere bacteria, especially in soils that are often subjected to stressful conditions. Rice rhizosphere bacteria exhibiting ACC deaminase activity was also observed by Bal *et al*., (2013). ACC deaminase enzyme has been found in both Gram negative and Gram-positive bacteria and also some fungi (Saleem *et al*., 2007). The presence of ACC deaminase activity in *Rhizobium* was also reported by Duan and his colleagues (2009) in a study wherein 27 strains of *Rhizobium* (12%) expressed ACC deaminase during a screening of large number (233) of rhizobial isolates.

ACC deaminase gene amplification

Growth on ACC medium and a detectable deaminase activity in general is not considered confirmative of bacteria with ACC deaminase enzyme production. The reasons attributed are that nitrogen-fixing bacteria without ACC deaminase activity are able to grow on the ACC medium, while certain other bacteria are able to grow on trace amounts of nitrogen present in medium components and in the agar used for isolations (Li *et al*., 2015). Hence, the presence of ACC deaminase structure gene (*acdS*) is important for predicting and for identifying ACC deaminase production by bacteria. PCR amplification of the *acdS* gene using degenerate primers has been widely employed for confirmation of ACC deaminase activity. *Rhizobium* sp used in this investigation showed ACC deaminase gene amplification with the expected product size of 996 base pairs using gene specific primers (Figure1) as described by (Farajzadeh *et al*., 2010; Mahmooda *et al*., 2019) thereby confirming the ACC deaminase activity of the bacterium.



L – 100bp Ladder, 1. Water blank, 2. *Gluconacetobacter diazotrophicus*, 3. *Serratia marcescens* and 4. *Rhizobium* sp.

Figure 1. Amplification of *acds* gene (996 bp) from *Rhizobium sp.*

Effect of exogenous ACC application and bacterial inoculation on seedling growth

Exogenously applied ACC is known to increase ethylene production in plant tissues (Shaharoona *et al*., 2007) including rice. Dark-grown seedlings of rice show a double response in differential root and shoot growth due to ethylene treatment (Ma *et al*., 2013). In this study, rice seedling grown in the presence of ethylene precursor ACC showed comparatively reduced root and shoot growth (5.1 and 9.1 cm respectively) relative to untreated seedlings (3.8 and 5.3 cm respectively). Rice seedlings which were inoculated with the isolated *Rhizobium* strain was found to improve both root and shoot growth irrespective of the presence of ethylene precursor in the growth medium when compared to uninoculated seedlings. Average root growth of 6.7 cm and shoot growth of 12.8 cm were observed

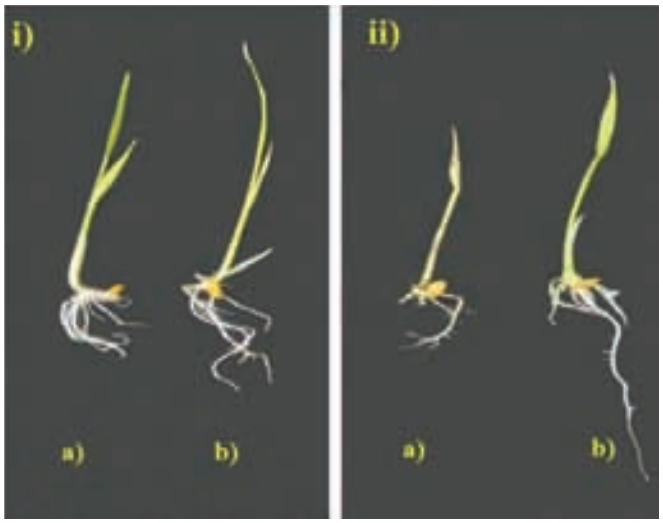
in rice seedlings grown in the presence of the isolated *Rhizobium* strain (Table 2).

Table 2. Rice seedling response to ACC and *Rhizobium sp.* inoculation

S. No	Treatment	Root length (cm)*	Shoot length (cm)*
1	Control	5.1 ± 0.55	9.1 ± 0.55
2	*Rhizobium sp.*	6.7 ± 0.17	11.3 ± 0.2
3	Control + 3mM ACC	3.8 ± 0.7	5.3 ± 1.1
4	*Rhizobium sp.* + 3mM ACC	5.4 ± 0.25	6.7 ± 0.35

*Mean ± SD of three replicates

The reduction in growth due to ACC treatment was partly alleviated by the presence of inoculated bacteria as rice seedlings grown in the presence of both ACC and *Rhizobium* showed better root and shoot growth (5.4 cm and 6.7 cm respectively) compared to seedlings treated with ACC in the absence of bacteria (Figure 2). The dilution of the effect of ACC on plant growth could probably be due deamination of ACC by the isolate into a-ketobutyrate and ammonia (Barnwal *et al*., 2014; Heydarian *et al*., 2016., Ali and Kim, 2018; Saleem *et al*., 2018; Zhang *et al*., 2018).



Seedling growth in i) absence and ii) presence of ACC under a) uninoculated and b) inoculated (*Rhizobium* sp.) conditions

Figure 2. Rice seedling growth in the presence of *Rhizobium sp.* and ACC

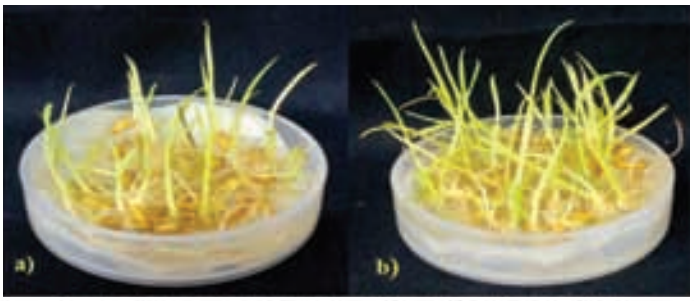
Effect of *Rhizobium* inoculation on germination and seedling growth under heat stress

Seeds inoculated with *Rhizobium* recorded increased germination percentage of 86 and 34% when compared to control (82 and 20%) under ambient and heat stressed conditions. Similarly bacterial treatment also increased vigour index (1074.1 ± 9.49 and 177.32 ± 39.44 under ambient and heat stress respectively), contrary to control uninoculated seedlings with vigour indices of 831.48 + 7.71 and 55.6±21.35 under ambient and heat stress respectively. Vigour index increment due to inoculation was 242.66 ± 23.4 and 121.72 ± 54.78 under ambient and heat stressed conditions (Table 3). Seed inoculation with the isolate was able show improved germination when compared to uninoculated control under ambient and heat stressed conditions (Figure 3).

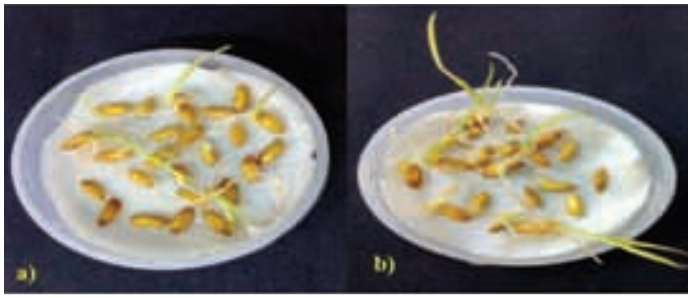
Table 3. Effect of *Rhizobium sp.* inoculation on rice germination indices under heat stress.

S. No	Germination indices	Control		*Rhizobium sp.*	
		Ambient	Temp (45 °C)	Ambient	Temp (45 °C)
1	Germination percentage*	82 ± 19.8	20 ± 10	86 ± 13.4	55 ± 39.43
2	Vigour index*	831.48 ± 7.71	55.6 ± 21.35	1074.1 ± 9.49	177.32 ± 39.44
3	Vigour index increment*	-	-	242.66 ± 23.4	121.72 ± 54.78

*Mean ± SD of three replicates



Germination in ambient conditions: a) control and b) + *Rhizobium sp.*



Germination under heat stress (45°C): a) control and b) + *Rhizobium sp.*

Figure 3. Effect of *Rhizobium sp.* inoculation on germination of rice under heat stress

Fluctuation in temperature leads to hormonal imbalances in plants with changes in ethylene phytohormone in particular leading to growth retardation. Microorganisms have been demonstrated to play a key role in alleviating the stress induced in plants caused due to abiotic and biotic

factors (Grover *et al*., 2011; Glick, 2014; Gamalero, 2015 and Gupta *et al*., 2019). Thermotolerance in sorghum seedlings was observed to be induced by *Pseudomonas* sp. that improved biochemical status of plants in terms of proline, sugar, amino acid and chlorophyll content (Ali *et al*., 2009) thereby leading to improved plant biomass. Seed treatment using another *Pseudomonas aeruginosa* strain 2CpS1 was found to ameliorate the deleterious effects of temperature stress on wheat (Meena *et al*., 2015). In concordance with our results, previous studies have also reported that ACC deaminase producing growth promoting bacterium *Burkholderia phytofirmans* PsJN could protect potato plants in maintaining normal growth under heat stress (Bensalim *et al*., 1998).

Conclusion

Plant-associated bacteria can play an important role in conferring tolerance in crops against abiotic stresses. In this study, a rice rhizospheric bacterium identified as *Rhizobium sp.* was able to impart heat stress tolerance to rice seedlings. Subsequent follow through experiments in glasshouse and field conditions can consequently lead to the use of these beneficial bacteria as a cost effective and environmental friendly approach for ensuring sustainable rice production under heat stress induced by high temperatures.

Acknowledgements

The authors are thankful to National Innovations on Climate Resilient Agriculture (NICRA) project and Director, ICAR-Indian Institute of Rice Research for financial assistance and providing necessary facilities for research.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Efficacy of new insecticide molecules against yellow stem borer and leaf folder in rice

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Received: 22nd Feb 2019; Accepted: 25th April 2019

Abstract

In order to find the efficacy of newer insecticides against rice yellow stem borer, *Scirpophaga incertulas* (Walker) and leaf folder, *Cnaphalocrocis medinalis* (Guenée). Field experiments were conducted during *Kharif* and *Rabi* seasons during 2015-16 at Regional Agricultural Research Station, Pattambi using the Jyothi rice variety. The insecticide molecules tested were DPX-RAB 55@ 237.5ml/ha, flubendiamide + thiacloprid 240SC @ 250 ml/ha, flubendiamide 480SC @ 50 ml/ha, thiacloprid 480 SC @ 250 ml/ha, chlorantraniliprole 18.5% SC @ 150 ml/ha with dinotefuran 20% SG @ 200 g/ha as check insecticide and an untreated control. The pooled results of two crop seasons revealed that chlorantraniliprole 18.5 % SC, flubendiamide 480 SC and flubendiamide + thiacloprid 240 SC were found superior against stem borer (dead hearts and white ears) and leaf folder with significantly lower per cent of leaf damage. Significantly higher grain yield was obtained from chlorantraniliprole 18.5 SC (4196 kg/ha) followed by flubendiamide 480 SC (3885 kg/ha) and flubendiamide + thiacloprid 240 SC (3321 kg/ha) treated plots.

Keywords: New insecticides, rice, yellow stem borer, leaf folder, DPX-RAB 55

Introduction

Rice is a staple food crop of India grown in diverse ecologies leading the world in area and production but lower than the world productivity. Insect pests are major constraints limiting rice productivity besides diseases and weeds (Behura, *et al.,* 2011). Damage occurred during vegetative phase (50 per cent) contributes more to yield reduction than at reproductive (30 per cent) or ripening phase (20 per cent) (Gupta and Raghuraman, 2003). Yield losses in rice due to stem borer, *Scirpophaga incertulas* (Walker) varied between 11.2 – 40.1% and 27.6 – 71.7% due to dead heart and white ear damage, respectively, while it was 65% due to leaf folder, *Cnaphaocrocis medinalis* Guenee (Krishnaiah and Verma, 2010). Chlorantraniliprole 25% SC, a new insecticide found effective against major lepidopteran pests (Sidde Gowda, 2009). In order to find the efficacy of chlorantraniliprole 18.5 % SC molecule against major rice pests, experimental trials were conducted under field condition during *Kharif*' 2015 and *Rabi*'2015-16 seasons.

Materials and Methods

Field experiments were conducted at Regional Agricultural Research Station, Pattambi, Kerala Agricultural University during two cropping seasons *viz., Kharif* 2015 and *Rabi*'2015-16. Twenty five day old seedlings of cultivar Jyothi were transplanted in a plot of size 7 x 4 m with a spacing of 20 x 15 cm at the rate of two seedlings per hill. The experiment included seven treatments with five test insecticides using dinotefuran as check insecticide and an untreated control with four replications. The experiment was conducted in completely randomized block design. The sprays were made at 20, 40 and 60 days after transplanting with a hand operated knapsack sprayer of nine litre capacity. The observations were made a day before spraying and a week after spraying on per cent tiller damage (dead heart) at vegetative stage and white ears at reproductive stage for yellow stem borer and per cent damaged leaves in case of leaf folder. The grain yield was recorded in treatment wise after maturity. Data were subjected to ANOVA and the means were compared for significance using CD at 0.05 % level (Gomez and Gomez, 1984).

Results and Discussion

Effect on Stem Borer

The results of the first crop season (*Kharif*'15) showed flubendiamide 480 SC @50 ml/ha as most effective in reducing the yellow stem borer (dead heart) damage with

0.25, 0.66 per cent dead hearts at 30 and 50 DAT. Next best treatments were chlorantraniliprole 18.5%SC @ 150 ml/ha and flubendiamide + thiacloprid 480 SC treated plots with 0.25, 1.53 and 0.78, 1.73 per cent, respectively at 30 and 50 DAT and was superior to dinotefuran (check) treated plots which recorded 1.27 and 2.93 per cent dead hearts, respectively at 30 and 50 DAT. In case of white ear damage at 80 DAT, chlorantraniliprole 18.5% SC recorded lowest white ear damage of 2.87 per cent followed by thiacloprid treated plots with 5.89 per cent and was superior over check insecticide (6.70 per cent white ears) (Table 1).

Table 1. Effect of different insecticide treatments against rice Yellow stem borer and leaf folder (*Kharif*'2015)

Trt. No	Treatments g /ml / ha	%DH 30DAT	%DH 50DAT	% WE 80DAT	%LF 45 DAT	%LF 60 DAT	Grain Yield (Kg/ha)
T1	DPX-RAB55 @ 237.5	2.30 (0.15)	4.71 (0.21)	8.86 (0.29)	19.73 (0.45)	13.44 (0.37)	3366
T2	Flubendiamide 240%g/L + Thiacloprid 240%g/L@ 250	0 78* (0.06)	1.73 (0.09)	12.38 (0.33)	3.10* (0.18)	3.30* (0.18)	3574
T3	Flubendiamide 480 SC @50	0.25* (0.03)	0.66* (0.06)	8.05 (0.29)	6.20* (0.25)	1.68* (0.13)	3638
T4	Thiacloprid 240 SC @250	2.04 (0.12)	1.61 (0.13)	5.89 (0.24)	17.88 (0.43)	12.95 (0.35)	3372
T5	Chlorantraniliprole18.5 % SC @150	0.28* (0.03)	1.53* (0.12)	2.87* (0.16)	2.95* (0.17)	2.58* (0.15)	3768
T6	Dinotefuran20SG @200	1.27 (0.11)	2.93 (0.16)	6.70 (0.26)	18.72 (0.43)	16.78 (0.42)	3405
T7	Control	6.47 (0.25)	5.87 (0.24)	10.86 (0.33)	10.44 (0.31)	12.55 (0.36)	3100
	CD (0.05%)	0.09	0.11	0.15	0.12	0.13	NS

SB: stem borer, WE: white ear, , LF: leaf folder

* Figures in parentheses are arcsine transformed values

*Figures followed by different letters are significantly different at p=0.05

During the second crop season in the following *Rabi* season, chlorantraniliprole 18.5 SC and flubendiamide + thiacloprid recorded lowest dead heart incidence of 1.65 per cent followed by flubendiamide with 1.68 % and were superior over check insecticide (3.19 per cent dead hearts) at 50 DAT. Similarly, chlorantraniliprole 18.5%SC recorded lowest incidence of white ears of 5.60 per cent followed by flubendiamide 480SC and flubendiamide + thiacloprid 480 SC treated plots with 6.83 and 8.34 per cent, respectively (Table 2).

Table 2. Effect of different insecticide treatments against rice pests (*Rabi*'2015-16)

Trt. No	Treatments g /ml / ha	%DH 30DAT	%DH 50DAT	% WE (80 DAT)	% LF 45 DAT	% LF 65 DAT	Grain Yield (Kg/ha)
T1	DPX-RAB55 @ 237.5	9.18 (0.31)	2.68 (0.13)	14.47 (0.39)	13.66 (0.38)	7.44 (0.27)	3134
T2	Flubendiamide 240%g/L + Thiacloprid 240%g/L@ 250	10 24 (0.32)	1.65* (0.11)	8.34* (0.28)	4.19 * (0.20)	2.63* (0.16)	3067
T3	Flubendiamide 480 SC @50	9.02 (0.29)	1.68* (0.11)	6.83* (0.26)	2.69* (0.16)	2.13* (0.15)	4132*
T4	Thiacloprid 240 SC @250	9.51 (0.31)	2.07 (0.12)	14.07 (0.38)	21.28 (0.47)	9.24 (0.30)	3158
T5	Chlorantraniliprole18.5% SC @150	8.01 (0.28)	1.65* (0.10)	5.60* (0.22)	4.11* (0.19)	2.10* (0.15)	4624*
T6	Dinotefuran20SG @200	5.14 (0.23)	3.19 (0.16)	9.81 (0.32)	19.67 (0.45)	8.44 (0.29)	3184
T7	Control	12.20 (0.35)	11.38 (0.34)	13.97 (0.38)	21.47 (0.48)	9.16 (0.31)	2690
	CD (0.05%)	NS	0.12	0.12	0.12	0.07	530

SB: stem borer, WE: white ear, LF: leaffolder,

* Figures in parentheses are arcsine transformed values

*Figures followed by different letters are significantly different at p=0.05

The pooled analysis of two crop seasons data showed that chlorantraniliprole 18.5%SC and flubendiamide 480 SC were most effective with 4.14 and 4.64 per cent dead hearts at 30 DAT while at 50 days after transplanting flubendiamide 480 SC recorded lowest dead hearts (1.17 per cent) followed by chlorantraniliprole 18.5 SC and flubendiamide + thiacloprid 480 SC with 1.59 and 1.69 per cent dead hearts. With respect to white ears, chlorantraniliprole 18.5% SC

was most effective with lowest white ear damage (4.24 per cent) followed by flubendiamide and flubendiamide + thiacloprid 480 SC (7.44 and 8.26 %, respectively) which is statistically superior over check insecticide treated plots (10.36 %) (Table 3). These results were in confirmation with the earlier study of Karthikeyan and Christy (2014) and Srinivasan *et al.,* (2012) who reported that foliar spraying with chlorantraniliprole 18.5% SC @ 30g a.i./ha reduced stem borer (dead heart and white ear) incidence. Sekh *et al.,* (2007) reported the efficacy of flubendiamide 480 SC against rice stem borer. Vinothkumar *et al.,* (2010) reported the efficacy of flubendiamide + thiacloprid 480 SC against tomato fruit borer larvae, aphids, white fly and leaf hoppers.

Table 3. Effect of different insecticide treatments against rice pests (Pooled analysis)

Trt. No	Treatments g /ml / ha	%DH 30DAT	%DH 50DAT	% WE	% LF 45 DAT	% LF 60 DAT	Grain Yield (Kg/ha)
T1	DPX-RAB 55 @ 237.5	5.74 (0.23)	3.70 (0.17)	11.66 (0.34)	16.70 (0.42)	10.44 (0.32)	3250
T2	Flubendiamide 240%g/L + Thiacloprid 240%g/L@ 250	5 51 (0.19)	1.69* (0.10)	8.26* (0.29)	3.64* (0.19)	2.96* (0.17)	3321*
T3	Flubendiamide 480 SC @50	4.64* (0.16)	1.17* (0.09)	7.44* (0.28)	4.44* (0.21)	1.91* (0.14)	3885*
T4	Thiacloprid 240 SC @250	5.78 (0.22)	1.84 (0.13)	9.98 (0.31)	19.58 (0.45)	11.10 (0.33)	3265
T5	Chlorantraniliprole18.5% SC @150	4.14 * (0.16)	1.59* (0.11)	4.24* (0.19)	3.53* (0.18)	2.34* (0.15)	4196*
T6	Dinotefuran20SG @200	5.20 (0.17)	3.06 (0.16)	10.36 (0.31)	15.20 (0.31)	12.61 (0.36)	3295
T7	Control	9.34 (0.30)	8.62 (0.29)	12.42 (0.36)	16.10 (0.40)	10.90 (0.34)	2895
	CD (0.05%)	0.11	0.11	0.11	0.22	0.13	877

SB: stem borer, WE: white ear, LF: leaffolder,

* Figures in parentheses are arcsine transformed values

*Figures followed by different letters are significantly different at p=0.05

Effect on Leaf f older

During *Kharif* 2015, the incidence of leaf folder in terms of per cent damaged leaves was lowest in chlorantraniliprole 18.5 % SC (2.95 and 2.58 per cent) at 45 and 60 DAT followed by flubendiamide + thiacloprid 480 SC (3.10 and 3.30 per cent) and flubendiamide 480 SC (6.20 and 1.68 per cent) and were superior to all other treatments including the check (18.72 and 16.78 per cent) (Table 1). During the second crop season (*Rabi*' 2015-16), lowest leaf folder damage was observed in flubendiamide 480 SC sprayed plots (2.69 and 2.13 per cent) followed by chlorantraniliprole 18.5% SC (2.69 and 2.13 per cent) and flubendiamide + thiacloprid 240 SC (4.19, 2.63 per cent) at 45 and 60 DAT and were significantly superior over check insecticide (19.67 an 8.44 per cent) (Table 2). The pooled analysis of two crop seasons data showed that chlorantraniliprole 18.5 SC showed the lowest leaf folder damage with 3.53 and 2.43 per cent followed by flubendiamide 480 SC (4.44 and 1.91 per cent) and flubendiamide + thiacloprid 480 SC (3.64 and 2.96 per cent) at 45 and 60 DAT while check insecticide treated plot recorded higher leaf damage (15.20 and 12.61 per cent) (Table 3). These findings were in confirmation with the earlier study of Karthikeyan and Christy (2014) and Srinivasan *et al.,* (2012) who reported the efficacy of chlorantraniliprole 18.5 SC and flubendiamide 480 SC against rice leaf folder (Kulagod *et al.,* 2011 ; Haider *et al.,* 2014). Kumar *et al.,* (2010) reported that flubendiamide + thiacloprid 480 SC was found very effective to cotton boll worms, aphids, white flies and leaf hoppers. Sangamithra *et al.,* (2018) reported that the flubendiamide 24% + thiacloprid 24% SC w/v @ 84 + 84 g a.i / ha had incredible reduction of shoot and fruit borer and sucking pests of brinjal and was safer to natural enemies like spiders and coccinellids.

Grain Yield

During the first crop season (*Kharif*'2015), there were no significant differences among treatments in grain yield (Table 1). During the second season, *Rabi* 2015-16, chlorantraniliprole 18.5 EC treated plots recorded significantly higher yield (4624 kg/ha) followed by flubendiamide 480 SC (4132 kg/ha) (Table 2). The pooled analysis of all the crop seasons also showed that chlorantraniliprole 18.5% SC treated plots recorded significantly higher grain yield of 4196 kg/ha followed by flubendiamide 480 SC (3885 kg/ha) and flubendiamide + thiacloprid 480 SC (3321 kg/ha) sprayed plots and control plots recorded lowest yield of 2895 kg/ha (Table 3). These findings are in agreement with previous reports of Karthikeyan and Christy (2014) and Haider *et al*. (2014) who found the increase in rice yield in chlorantraniliprole 18.5 EC and flubendiamide 480 SC treated plots.

Acknowledgement

The authors express sincere thanks for funding from Indian Institute of Rice Research, Hyderabad under All India Co-ordinated Rice Improvement programme for conducting the trial.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

In vitro studies on *Ustilaginoidea virens*, a rice false smut pathogen

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Received: 11th March 2019; Accepted: 23rd April 2019

Abstract

False smut of rice is caused by *Ustilaginoidea virens* (Cke.) Tak. [teleomorph: *Villosiclava virens* (Nakata) Tanaka & Tanaka] has emerged an important grain disease causing significant yield losses in major rice producing states of India. In the present study, false smut pathogen was successfully isolated from the infected smut balls and pathogenicity of *U. virens* was proved in BPT-5204 by artificial inoculation of conidial suspension (2 x 10^5 conidia) during booting stage. Growth of *Ustilaginoidea virens* (CG-1 isolate) was evaluated in different media, wherein Potato Sucrose Agar (PSA) medium recorded the maximum growth (with a growth rate of around 2.12 mm/day) and minimal growth was recorded in Czapek's Dox Agar medium. Among the natural substrates tested for mass multiplication, rice and barley grains supported maximum growth of the pathogen. The temperature *viz*., 55^{0}C, 60^{0}C and 65^{0}C affected the germination of chlamydospores.

Keywords: *Ustilaginoidea virens*, pathogenicity, chlamydospore, mass multiplication

Introduction

Plant diseases reduces yield and affects the quality and stability of production in addition to their affect on agricultural sustainability. Earlier, false smut disease of rice was considered as farmer's friendly disease and locally known as 'lakshmi disease' as it was found associated with bumper yield. The occurrence of the disease was irregular/ sporadic and the symptoms were mostly restricted to one or two grains per panicle. Use of high fertilizer-responsive varieties and hybrids, heavy application of nitrogenous fertilizer and changes in climatic conditions had paved the way for the outbreak of rice diseases. Thus, false smut disease emerged as one of the important grain diseases of rice in India as well as in the world. Presently, the false smut pathogen can affect huge number of grains in a panicle which can lead to disease epidemic and heavy yield loss. The pathogen affects the young ovary of the individual spikelet and transforms it into large, yellow colour smut balls and symptoms are visible from milky stage onwards. Initially, the smut balls are small in size and remain confined between glumes, white in colour, gradually change into yellow and covered with white colour membrane. Later, the membrane bursts and the colour changes to yellowish orange, olive green and finally greenish black. In India, the disease has been reported to occur in moderate to severe intensity from 2000 onwards (Guo *et al.,* 2012; Singh and Pophaly, 2010; Ladhalakshmi *et al.,* 2012; Laha *et al.,* 2016). The yield losses in different states of the country have been estimated to vary between 0.2% to 49% depending on the disease intensity and rice varieties grown in those areas (Dodan and Singh, 1996). Apart from direct loss, *U. virens* also produces a toxins known as ustiloxins and ustilaginodins (Koiso *et al.,* 1994; Sun *et al.,* 2017). Due to the increased importance of this disease the present work focussed on basic studies on false smut which including isolation and pathogenicity of *U. virens*, identification of suitable media for mass multiplication and also temperature influence on germination of chlamydospores.

Materials and Methods

Isolation, purification of *U. virens*

Isolation was done from the false smut diseased samples collected from different rice growing regions of India. The smut balls collected were thoroughly washed with running tap water and surface sterilized with 1% sodium hypochlorite for one minute and subsequently washed three times with sterile distilled water. Smear of chlamydospores

was streaked onto petri dishes containing Potato Sucrose Agar (PSA) medium using a sterilized inoculation loop. under complete aseptic conditions. To check the bacterial contamination, the medium was added with streptomycin (100 ppm). The petri dishes were incubated in BOD incubator at 25 ± 2° C for one week for obtaining fungal growth (Ladhalakshmi *et al.*, 2012).

Pathogenicity of *U. virens*

Multiplication of *U. virens* isolates

Pure culture of *U. virens* was multiplied by inoculating with an 8 mm mycelial disc of 2 weeks old culture in 100 ml Potato Sucrose Broth (PSB) in 250 ml flask and incubated in a shaking incubator at 120 rpm at 25 ± 2°C for a week.

Preparation of inoculums

The conidia of *U. virens* was harvested 6-7 days after inoculation from inoculated PSB. The culture was filtered using a muslin cloth and centrifuged at 4500 rpm for 20 minutes. The supernatant is discarded and 2-3 ml of sterile distilled water was added to the pellet and the concentration of conidial suspension (2×10^5 conidia ml^{-1}) was adjusted with the help of haemocytometer.

The susceptible rice cultivar *viz.*, BPT-5204 was used and the plants with booting stage were selected and injected with conidial suspension of 2 ml (2×10^5 conidia/ml) using a syringe. The plants were incubated at 24-26°C for 6 days at 95% R.H. in a humidity chamber and then kept under normal room temperature, until appearance of symptoms. The rice panicles injected with sterile distilled water served as control (Ladhalakshmi *et al.*, 2012).

Effect of different media on the radial growth of *U. virens*

Different media were evaluated against the growth of *U. virens.* Studies were made on eight different culture media *i.e.* Potato Sucrose Agar (PSA), Potato Dextrose agar (PDA), Oat meal agar, Corn meal agar, Rice polish agar, Rice polish yeast extract, Malt extract with dextrose and Czapeck-Dox agar.

Evaluation of natural substrate for mass multiplication of *U. virens*

Five natural substrates *i.e.* Rice, Barley, Ragi, Maize and Sorghum grains were selected for the study. Except rice, all the grains were pre-soaked in 2 per cent sucrose solution for 12 hrs. To the rice grain, double the quantity of water with 2 per cent sucrose was added before autoclaving. The flasks containing the substrates were autoclaved twice at 121°C for 15-20 minutes. Three mycelial discs of 8 mm diameter from the 2 week old culture of *U. virens* (C.G-1) were used for inoculation under aseptic conditions. Then the inoculated flasks were incubated at 25 ± 2°C for 30 days. Three replications were maintained for each medium. Flasks were regularly shaken after every 2-3 days for the uniform growth of the fungus (Rani, 2014).

Effect of temperature on the germination of chlamydospore

Different temperatures were evaluated to find out the optimum temperature for the germination of the chlamydospore. Black coloured smut ball (1 No.) from the diseased sample was sterilized with 0.1 % mercuric chloride and washed with sterile distilled water for 2-3 times repeatedly and finally about 3 ml of sterile distilled water was added and mixed thoroughly. From the glass vial about 200 µl was transferred into sterilized eppendorf tubes. Then individual eppendorf tube was subjected to heat at different temperature starting from 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C for ten minutes and the control treatment without heat. Potato Sucrose Agar (PSA) medium was melted and poured @ 20ml into the sterilized petri plates under aseptic conditions. Using a sterilized inoculation loop, a smear of solution was taken from each heat-treated eppendorf tubes and was streaked into the pre-poured petri plates with PSA medium. A control plate was also maintained. Three replications of each were maintained. Observations were recorded after 7 days of inoculation.

Results and Discussion

U. virens culture

Small tiny white colour colony was observed after seven days of incubation. Mycelium is septate in nature, produces primary conidia. The white colour of the fungus gradually changed into yellow and then changed into green colour. Sometimes, pathogen produced small ball like structures or clumps either on the middle or end of mycelia. Later, these balls changed into mass of chlamydospores and bursting of the clump was observed.

Pathogenicity

After 10-15 days of inoculation, the inoculated plants expressed the symptoms of 5-6 smut balls per panicle and the *U. virens* was isolated from these smut balls on PSA medium and Koch's postulate was proved for *U. virens.*

Effect of different solid media on radial growth of *U. virens*

Eight different media were tested and *U. virens* isolate CG-1 used for the study. Colony diameter was recorded at 10 days interval. From the eight-media used, PSA medium supported the maximum growth with a colony diameter of 63.16 mm and Czapeck's-Dox Agar medium supported the minimal growth with a diameter of 19.83 mm. (Table 1). Two of eight media, Rice Polish Agar (RPA) and Rice Polish Yeast Extract Agar (RPYA) media exhibited colony diameter of 54.83 mm and 55.17 mm, respectively and they were on par (Fig 1).

Table 1: Effect of different media on the growth of *U.virens*

Name of the Media	Media Composition	Radial Growth (mm)			Average growth rate per day(mm)	Observation on mycelial growth
		10 DAI	20 DAI	30 DAI		
Potato Sucrose Agar	Potato (from 200 g) extract; Sucrose – 10 g; Agar – 20 g; Water – 1 lt	25.00	55.30	63.16	2.12	White, yellow coloured mycelium
Potato Dextrose Agar	Potato (from 200 g) extract ; dextrose – 10 g; Agar – 20 g; Water – 1 lt	21.33	34.5	49.5	1.65	White, yellow coloured mycelium
Oat meal Agar	Oat meal – 60g; Agar – 12.5 g ; Water – 1 lt	24.33	35.33	45.5	1.50	White coloured mycelium
Corn Meal Agar	Corn meal – 40g; Agar- 15g; Water – 1 lt	18.33	39.16	59	1.96	White, light yellowish mycelium
Rice Polish Agar	Rice polish – 10g; Sucrose – 10g; Agar – 10g	11.33	31.33	54.83	1.81	White coloured mycelium
Rice Polish Yeast Extract Agar	Rice polish – 10g; Yeast extract – 0.5 g; Peptone – 0.5 g; Sucrose – 10g; Agar – 10g	12	29.50	55.17	1.84	White coloured mycelium
Malt Extract with Dextrose Agar	Malt extract – 20g; Peptone – 1 g; Sucrose – 20 g; Agar – 15 g; Water – 1 lt	8.67	15.17	22.5	0.77	Yellowish white coloured mycelium
Czapeck's-Dox Agar	$NaNO_3$- 2g ; K_2HPO_4 – 1 g; $MgSO_4$. 7 H_2O - 0.5 g; KCl – 0.5 g; $FeSO_4$ – 0.01 g; Sucrose – 30 g; Agar – 20g; Water – 1 lt	8.5	15.17	19.83	0.66	Very poor mycelial growth
CD at 0.05%		1.33	2.04	1.808		
CV (%)		4.71	3.67	2.24		

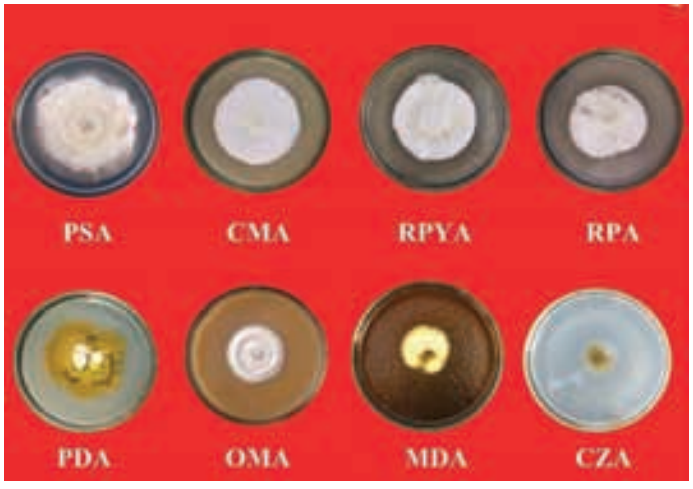

Figure 1. Evaluation of different media for the growth of *U. virens*

PSA - Potato Sucrose Agar; CMA- Corn meal agar; RPYA - Rice polish yeast extract; RPA - Rice polish agar; PDA- Potato Dextrose agar; OMA- Oat meal agar; MDA - Malt extract with dextrose; CZA - Czapeck-Dox agar

The above experimental findings were in accordance with the reports of Rani (2014), Baite *et al.*, (2014) and Fu *et al.,* (2013), who evaluated different carbon sources and different media. Results have shown that sucrose and starch were the best carbon sources and Potato Sucrose Agar medium was the best suitable medium for the growth of *U. virens.*

U. virens growth on different natural substrates

Different natural substrates like rice, barley, ragi, sorghum and maize grains were tested for mass multiplication of *U. virens.* Results indicated that rice and barley grains were shown maximum growth and the minimum growth was observed in maize grains. Colour of the culture was changed from white to yellow and green was observed in rice, barley, ragi; white to yellow in sorghum grains and only white colour in maize grains. In maize grains,

the growth of pathogen was minimum. Change in the colour of the mycelium indicates the formation of the chlamydospores. These results were supported by Rani (2014) who evaluated the different natural substrates for mass multiplication of *U. virens* and reported that the barley grains have shown the maximum growth compared to rice seeds and rice husk.

Effect of different temperatures on the germination of chlamydospore

The results revealed that the germination of the chlamydospores were not affected at temperature *viz*., 30°C, 35°C, 40°C, 45°C and 50°C, and hence colony growth was observed, Whereas temperatures of *viz*., 55°C, 60°C and 65°C affect the germination of the spore and thereby colony growth was not observed. The control treatment has shown the good germination and colony growth. Similar results were reported by Muraleedharan (2004), wherein the germination of chlamydospores was observed up to 40°C. Results of this study would help to have an idea on the survival of chalmydospores during extreme weather conditions.

False smut disease of rice is gaining importance because of its effect on both quantity and quality yield loss under favourable conditions. In the recent years, basic studies on the false smut disease *viz*., pathogen isolation, artificial culturing of the pathogen, pathogenicity and infection process were studied in detail. The results of the present study will assist in the development of the artificial inoculation screening technique for the identification of resistant sources..

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Agribusiness prospects and challenges of black rice produced in North- East India

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Received: 16th March 2019; Accepted: 26th May 2019

Abstract

Black Rice, a variety of rice from the species *Oryza sativa* L. subspecies *indica,* is known for its high nutritional and medicinal values and of high antioxidant properties. It is also known as purple rice, forbidden rice or king's rice. In India it is mostly cultivated in north - eastern region of India. Though, it is reported to be cultivated in small quantity by the villagers, there are also some of the agri-entrepreneurs, who have realized its agribusiness potential, and presently have been engaged in the business of black rice very successfully. Their achievements have been reported in local and national newspapers and websites. The present study is an attempt to trace those successful agri- entrepreneurs, compile, analyse and present the details to a wider audience. The paper also tries to identify various brands of black rice sold through online marketing platform, like Amazon, Flipkart , Indiamart etc. and identify the various companies/ traders involved in it. Finally, the paper also makes a SWOC analysis of black rice production and marketing, so that suitable policies can be formulated. Due to its medicinal properties, with proper consumer awareness and marketing policies along with government support, the farmers of north-east can reap high economic benefits by cultivating black rice.

Keywords: Black rice, agribusiness, online marketing, SWOC

Introduction

Black rice refers to a variety of rice from the species *Oryza sativa* L. Subspecies *indica*. It has high levels of nutrients, high antioxidant property and is glutinous in nature. It is also known as purple rice, heaven rice, imperial rice, king's rice, prize rice and forbidden rice. Recently it was referred as "Super Food" (Saha, 2016). It is cultivated in South - East Asian countries (Kong *et al*., 2008). The specific data for this coloured rice date back to 2003, where Chaudhary (2003) quoted China as the richest country in Black rice resources (62%) followed by Sri Lanka (8.6%), Indonesia (7.2%), India (5.1%), the Philippines (4.3%), Bangladesh (4.1%), and few in Malaysia, Thailand and Myanmar (Chanu, 2015; Sompong *et al*., 2011).

Even though it has a long history of cultivation, only recently Black rice is recognized for its medicinal values (Chanu, 2015). The dark purple colour of Black rice is due to the high anthocyanin content, located in the pericarp layers (Takashi *et al.,* 2001). Anthocyanin pigment which is present in black rice has been documented as health promoting food ingredients because of antioxidant activity (Nam *et al*., 2006; Philpott *et al*., 2006), can reduce the risks of cardiovascular diseases and cancer with anti-inflammatory, antioxidant and chemoprotective properties (Park *et al*., 2008; Hyun and Chung, 2004; Tsuda *et al*., 2003; Tsuda *et al*., 2002). Due to growing demand, there has been increased interest in the alternative sources of anthocyanin, which is inexpensive sources of natural and stable pigments (Hu *et al*., 2003). The Black rice was also reported as good source of fiber, minerals, and several important amino acids (Zhang *et al*., 2005). Black rice also contains higher levels of proteins, vitamins and also relatively richer in the mineral contents such as Fe, Zn, Mn and P as compared to common white rice (Suzuki *et al*., 2004; Qiu *et al*., 1993; Liu *et al*., 1995; Zhang, 2000).

In an era, where value added agriculture became an important strategy to both agricultural entrepreneurship and rural development (Coltrain *et al.,* 2000; Kilkenny and Schluter, 2001; Womach, 2005), the Black rice comes with its own value added properties. It's processing and enhancing value through the focus on identity characteristics (including local and organic destinations), will double its worth, and may play a great role in doubling farmer's income. However, despite rising consumer interest in Black rice, little is known about the variety of value chain relationships that move Black rice from farms

to consumers and consequently the economic and social performance of these Black rice value chains are also not well understood.

In India, though Black rice is not cultivated in large area for commercial purpose as compared to other rice varieties, there are evidences that it has been grown in India for centuries in different states with maximum concentration in North- East region. The present paper is an attempt to compile the reported success stories of people involved in cultivation of Black Rice to have a clear understanding of the agribusiness opportunities it creates. It also compiles the different brands and the rates of Black rice sold to popular online marketing platforms like amazon, flipkart and indiamart among many.

Methodology

This paper is a compilation of various success stories of Black rice cultivation from north- east India reported in different media (both print and electronic). The basic objective is to bring all the reported success stories under single compilation. After compilation of the reported success stories, a SWOC analysis of NE Black Rice is done.

The present study is an attempt to trace those successful agri- entrepreneurs, compile, analyse and present to a wider audience. The paper also tries to identify various brands of black rice sold through online marketing platform, like Amazon, flipkart, indiamart etc. and identify the various companies/traders involved in it. Finally, the paper also makes a SWOC analysis of Black rice production and marketing, so that suitable policies can be formulated.

Case I: Black Rice Success Story of Mr.Upendra Rabha, Assam

The Google search engine finds information about Mr. Upendra Rabha in minimum six websites https://assam.mygov.in,http://www.nezine.com,http://www.drbhupensaikia.com, http://www.ianslive.in,https://www.tribuneindia.comand https://www.newsgram.com. Out of these three websites one is Government of Assam website. Mr. Upendra Rabha, a resident of Amguripara village near Dudhnoi in Goalpara district, was reported by Assam Government website (https://assam.mygov.in) as a progressive farmer who is the pioneer of black rice in Assam. With the guidance of Krishi Vigyan Kendra, Dudhnoi, he started cultivation of black rice. According to an article published in https://www.newsgram.com on 27th May, 2016, in the year 2011, Mr.Upendra planted a sole seedling in the corner of his paddy field, from which he harvested 150 gram seed. In 2012, he harvested about 48 kg of paddy from the 150 gram seeds. In 2013, he harvested 1500 kg of paddy by cultivating black rice in five bighas of land. In 2014, he cultivated black rice with 50 farmers as community farming in about 100 bighas of land and produced 300 kg of rice per bigha of land which gave them total earning of Rs.300,000.00. In 2015, about 100 farmers of Dudhnoi area got into cultivation of black rice in 500 bighas of land with a very good yield. In 2015, around 100 quintals (10,000 kg) of black rice was purchased by a Mumbai based organization for exporting to different European countries. The farmers are now getting queries from different organizations in the US, Japan and Korea who want to buy the black rice from them. In an article published on 6thJan, 2016 in http://www.nezine.com, it was reported for his contribution to innovative technology pertaining to black rice conservation and production, the Meghalaya based Indian Council of Agriculture Research Complex for the NEH Region has recognized him as an outstanding innovative farmer. Having seen the success of Rabha and fellow villagers, farmers from many districts of Assam are now coming to him to purchase the seed (Talukdar, 2016).

Case II: Black Rice Success Story of Mr. Potshangbam Devakanta, Manipur

The Google search engine finds information about Mr Potshangbam Devakanta in minimum seven websites https://www.agricultureinformation.com, https://www.rediff.com, https://farmeruncle.com, https://explorers.zizira.com, https://rochaktathya.wordpress.com, https://www.downtoearth.org.in and http://www.e-pao.net. He was also mentioned by Lambalmayum (2018) in *Journal of Innovation for Inclusive Development*. An article about him was also published in SAARC Agri News in 2015. He was also one of the farmers' representing various Biodiversity Hotspots in '1st International Agro- biodiversity Congress: Science, Technology and Partnership', New Delhi, India, November 6-9, 2016. He also won the PPVFRA (Protection of Plant Varieties and Farmers Rights Act) conservation award in 2012. He cultivates five varieties of the rare and highly nutritious variety of black rice, called 'chakhao poireiton' by following organic agricultural practices. He is currently working with about 200 farmers to increase the cultivation of chakhao poireiton in their farms as well (https://www.rediff.com/business/report/pix-special-manipur-farmer-grows-black-rice-that-cures-cancer/20151028.htm).

Case III: Black Rice Success Story of Mr. Kerli Shylla, Meghalaya

The success story of Mr. Kerli Shyllaas resident of Larnai village in Jaintia hills district was documented Zizira explorers and reported on https://explorers.zizira.com/story-progressive-farmer-turned-tradition-profitable in 2016. He harvested 60-70 bags of black rice in a year with a weight of 100 Kg per bag. He sold it to Wahiajer and Ummulong, closest market of Larnai.

Major constraints of black rice production in North East India

Though, recently black rice is known as "super food", the production of black rice in North East India is less as most of the farmers grow in small plot of land. Regarding consumption of black rice, people of North East India consumed less as compared to white rice due to contained of more fats. It is used as delicacy and consumed only during ceremonial feast, traditional practices and occasionally (Chanu, 2015). Though, black rice is served as high rated dishes for its scented and dark purple color and much demand in domestic market and possibilities of export farmers in North East India neglected to grow it as it has low yield (Asem *et al.,* 2015). In North East India most of the farmers followed traditional way of cultivation which is another constraint that lower the production of black rice.

Online Marketing of Black Rice

In addition to the above success stories, a number of companies/traders across India are in Black rice business. Some of those along with brand and online marketing platform is given below.

Table 1: Black Rice Brands Sold on Online Platforms

Sl No.	Brand	Rate	Sold by	Sold on
1	FOR8 Aromatic Black Rice - Forbidden Rice - 500g	Rs. 260.00	FOR8	https://www.amazon.in
2	FOR8 Aromatic Black Rice - Forbidden Rice - 1000g	Rs. 500.00	FOR8	https://www.amazon.in
3	FOR8 Black Rice Flour- 500g	Rs. 280.00	FOR8	https://www.amazon.in
4	Neotea Black Rice with Forbidden Rice Nutrition (1 Kg)	Rs. 549.00	Neotea	https://www.amazon.in
5	KisaanMaitrey Organic Hand Pounded Black Rice (1 Kg)	Rs. 210.00	Kisaan Maitreya	https://www.amazon.in
6	Green Habit Black Rice 1 KG	Rs. 325.00	Green Habit	https://www.amazon.in
7	Green Habit Wild Black Rice 4.5 kg (aka Forbidden Rice)	Rs. 1450.00	Green Habit	https://www.amazon.in
8	Green Habit Wild Black Rice 3 kg (aka Forbidden Rice)	Rs. 950.00	Green Habit	https://www.amazon.in
9	Green Habit Black Rice 900 gm	Rs. 295.00	Green Habit	https://www.amazon.in
10	Green Habit Black Rice 500gm	Rs. 175.00	Green Habit	https://www.amazon.in
11	Green Habit's Black Rice 1.5KG	Rs. 495.00	Green Habit	https://www.amazon.in
12	Home of Spices Wild Black Rice 1KG	Rs. 375.00	Home of Spices	https://www.amazon.in
13	Mystique Hills Black Rice (Premium Quality)1 Kg	Rs. 440.00	Mystique Hills - Organic Living	https://www.amazon.in
14	Original Indian Table Black Rice, 400g	Rs. 121.60	Original Indian Table	https://www.amazon.in
15	The Forbidden Rice - Black Rice 500 gm	Rs. 260.00	himalaya2home (H2H)	https://www.amazon.in
16	SpiceMart Organically Grown Black/Khasi Rice 200 g	Rs. 84.00	SpiceMart	https://www.amazon.in
17	Sri Sri Ayurveda Black Rice, 1Kg	Rs. 299.00	Sri Sri Ayurveda	https://www.amazon.in
18	Eliva Black Rice (Emperor Rice), 500gm	Rs. 300.00	Eliva	https://www.amazon.in
19	Purvai Black Rice - 500 Gms	Rs. 135.00	Purvai	https://www.amazon.in

Sl No.	Brand	Rate	Sold by	Sold on
20	Paraman The Supernatural Paraman Black Rice 500 Gms	Rs. 175.00	Paraman The Supernatural	https://www.amazon.in
21	Aromatic Black Rice Veg Beneficial For Health 500g	Rs. 299.00	Ayushmaanbharat	https://www.amazon.in
22	Seeds village Paddy OryzaSativa Linn Black Forbidden Rice with Herbal Properties and Nutrient, Fragrant Value	Rs. 700.00	Seeds village	https://www.amazon.in
23	Seeds village Rarest Paddy/Black Rice Seeds -Pack of 0.5 kg	Rs. 1975.00	Seeds village	https://www.amazon.in
24	seeds village Rarest Jasmine Black Rice Paddy Seeds for Growing -250 g	Rs. 1250.00	Seeds village	https://www.amazon.in
25	Seeds village 100 Seeds of Rarest Paddy Black Rice Forbidden Seeds/ Grains for Sowing	Rs. 200.00	Seeds village	https://www.amazon.in
26	"Our Organik Tree" ORGANIC Black Rice	Rs. 335.00	Our Organik Tree	https://www.amazon.in
27	Black Rice - Healthy Alternatives - 500 g	Rs. 285.00	Godrej Natures Basket	https://www.naturesbasket.co.in
28	True Elements Black Rice 1000gm	Rs. 400.00	HW Wellness Solutions Pvt Ltd	https://www.healthyworld.in
29	Chahao 500 gram Black Rice	Rs. 145.00	High Vision Consultants	https://www.indiamart.com
30	B&B Navara Rice 3 Kg Black Navara Rice (Medium Grain, Parboiled) (3000 g)	Rs. 1157	B & B Organics	https://www.flipkart.com
31	BnBkarunkuruvai Black KavuniArisi Rice (Medium Grain, Boiled) (3 kg)	Rs. 665.00	B & B Organics	https://www.flipkart.com
32	Millet Valley Gluten Free Black Rice Black KavuniArisi Rice (Medium Grain, Parboiled) (800 g)	Rs. 245.00	Millet Valley	https://www.flipkart.com
33	El World Organic Aromatic Black Rice (Medium Grain) (500)	Rs. 140.00	ELWORLD	https://www.flipkart.com
34	Fields Of Gold Rice Black Rice (Medium Grain) (1000)	Rs. 190.00	Pristine	https://www.flipkart.com
35	Millet Valley Black Rice (1.8 Kg) Black KavuniArisi Rice (Medium Grain, Unpolished) (1800 g)	Rs. 470.00	Millet Valley	https://www.flipkart.com
36	Chahao Rice Black Raw Rice (Medium Grain) (1 kg)	Rs. 279.00	High Vision Consultants	https://www.flipkart.com
37	Chahao 500gm Black PoreitonChakhau Rice (Raw) (10)	Rs. 145.00	High Vision Consultants	https://www.flipkart.com
38	Truefarm Organic Black Rice (Medium Grain, Unpolished) (750 g)	Rs. 140.00	Truefarm	https://www.flipkart.com
39	Hathmic Wild Black Rice, 500g Black Wild Rice (Small Grain, Raw) (500 g)	Rs. 294.00	Hathmic	https://www.flipkart.com
40	Hathmic Wild Black Rice, 1kg Black Wild Rice (Small Grain, Raw) (1 kg)	Rs. 430.00	Hathmic	https://www.flipkart.com
41	Manipuri Black Rice (CHAK-HAO) - 500g	Rs. 140.00	Assamica Agro	https://www.assamicaagro.in
42	Aromatic Black Rice (ChakHao) - Manipur - 450 g	Rs. 165.00	Giskaa	http://www.giskaa.com
43	Chahao Black Rice; Glutenfree Black Rice; Raw Black Rice	Rs. 279.00	High Vision Consultants	https://www.indiamart.com

Note: Data collected on 08/01/2019 at 8 PM from the respective websites

In addition some companies/traders like High Vision Consultants, Shalimar Bagh, Panchkula; Vedusha Foods, Govindpuri, New Delhi; Kisaan Maitreya, Yerwada Society, Pune; Pathway India, Amalpada, Phulabani; Vishal Trading Co., Vashi, Navi Mumbai; Home Of Spices, Palam Colony, New Delhi; Thiva Exim, Muthuramalingapuram, Madurai; Resource Foyer, Syed Ali Guda, Hyderabad; Prakrati Organic Foods, Indira Nagar, Lucknow; Brightcrop Agro Products Private Limited, Ballygunge, Kolkata; Pradhan Agrico, GopalPura, Agra; The Taj Urban Grains, Govindpuram, Ghaziabad; Forest Products, Vashi, Mumbai; Green India Future, Virugambakkam, Chennai; Crop Connect Enterprises Private Limited, LadoSarai, New Delhi; Amedi Greens Pvt Ltd, Pune; Shree Lakhi Narayan Enterprise, Tinsukia; Pachaa Traders Organic Traditional Natural Wholesalers, Koyambedu Wholesale Market Complex, Chennai; Agro One, Kalyan Nagar, Bengaluru; GVL Traders, Woraiyur, Tiruchirappalli; Sakaria Trade Corporation, Mukund Nagar, Pune; Glorious Enterprise, Dergaon, Golaghat; Pro Organic Farmacy, Near Redhills, Chennai; Green Connection Agro, Parnasree Bazar, Kolkata; Oganic Foods Private Limited, D.L Khan Road, Kolkata; Folkspice LLP, Jorhat; Zikra Green Farm, Haibargaon, Nagaon etc. market black rice through https://www.indiamart.com platform under different brand name and packing sizes and different prices.

The SWOC Analysis of North East Black Rice

The SWOC analysis of NE Black rice is presented in the Figure 1.

Strengths : As people are becoming more conscious on health, research was done on black rice. It was found that black rice is a good source of antioxidant (Park *et al*., 2008) and has medicinal properties. It helps in preventing a risk in diabetics, obesity and heart disease problem which naturally also known as detoxifier. Black rice which is rich in protein and fibre helps the consumer to stay fit and healthy.

Weaknesses : Black rice, though a good source of antioxidant, minerals, fibre having numerous medicinal properties but it is still cultivated in less quantity as compared to white rice.The consumption of black rice is in less quantity as compared to normal white rice. Mostly, people of North East India mainly consume it during festive season and occasionally. Due to this, the demand of black rice is not constant in whole year as compared to other normal rice.

Opportunities : Due to its medicinal property black rice has potential of Agri enterprenuership. As people are more conscious of their health recently, there is a huge demand especially in developed countries which automatically helps to increase the economic status as well as the social status. Due to technology advance it can be traded online giving employment opportunity by easy earning at home.

Challenges : In North East India, most of the farmers are illiterate and reluctant to adapt with the changes. Farmers must be aware about the medicinal property of black rice with the help from government and Agriculture Department. As most of the farmers are day-to-day life earners, incentives from government is needed in order to go for large scale cultivation. They must be aware about the potential of Agribusiness and also the post-harvest processing and value addition.

Strengths	**Weaknesses**
• Rich Source of Antioxidant • Natural Detoxifier • Good Source of Fiber • Prevents Risk of Diabetics • Prevents Risk of Obesity • Rich in Protein • Good for Healthy Heart	• Cultivated in Less Quantity • Can't be Consumed in Large Quantity • No Proper Supply Chain/Value Chain • No Proper Marketing Platform • No Constant Demand
Opportunities	**Challenges**
• Can be used as Natural Medicine • Have Agripreneurship Potential • Huge Export Potential Especially in Developed Countries • Can Double Farmers Income • Can be Traded Online	• Medicinal Properties Need to be Popularised • Cultivators to be Explained about its Agribusiness Potential • Post-harvest Processing and Value Addition Should be Ensured • Government Support is needed for Large Scale Cultivation and Marketing • Black Rice Based Efficient Value Chains should be Developed

Figure 1: The SWOC Analysis of N-E Black Rice

Conclusion

Based on the above success stories, it is concluded that eventhough black rice is not consumed in large quantity as compared to white rice but after knowing the medicinal properties, farmers can motivate fellow farmers to grow the variety in order to produce in large quantity to meet the need of the market and consumers demand. It is not only beneficial to health, but also with value addition, uplifts the economic and social status of the farmer.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

How digital is helping farmers in irrigation: case of auto pump starters using smart phones

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Received: 20th April 2019; Accepted: 20th June 2019

Abstract

This paper deals with one of the digital solutions for farmers (Auto Pump Starters Using Smart Phones) which is simple, yet needs a critical review. In recent past, several organizations started promoting smart solutions to irrigation control through mobile phones. The smart phone based pump starters allow farmers to monitor and operate irrigation pumps for irrigating crops in remote locations. A study was conducted among farmers who have currently installed and are using the auto starters for irrigating their fields during 2017-18. Benefits of Auto pump starters include; relative easiness to operate/ irrigate compared to earlier, time saving, safety and the flexibility of irrigating. More than 74% of the farmers opined that remote accessibility of pump sets using the auto starters was a striking feature. Update about status of irrigation was perceived as an excellent option by 96.3% farmers. Ease in planning irrigation cycles was also found to be an added advantage by 94.5% of farmers. Indirect benefits include reduced water wastage, electricity wastage, labour wastage and fuel wastage (POL). These smart irrigation solutions will help small farmers in a big way. The improvement in irrigation efficiency will also lead to higher yield and productivity in long term.

Keywords: Rice, automated irrigation, auto pump starters, smart phones, farmers perception, digital solution in agriculture

Introduction

Farmers expect much more than access, quality and affordability of knowledge (advisories) and services (financial inclusion, supply chain and marketing) through digital strategies (Shaik N Meera, 2018). The digital models need to take into consideration personalised, exceptional, retail-like experience: time and mission criticalness of extension services. It is about providing these services as how, when and where it is most convenient for them, not extension organizations (Shaik N Meera, 2018). This paper deals with one of such digital solutions (Auto Pump Starters Using Smart Phones) which is simple, yet needs a critical review.

Agricultural industry is the largest employment sector in India; about 64 million hectares of operational holdings have access to irrigation facilities, with over 60 per cent of the irrigated farmland relying on pumped ground water (Sinha, 2016).

At individual farmer's level, a major challenge is effective management of irrigation activities. Significant time and energy is required for frequent trips through hazardous terrain to operate remote water pump systems. This difficult and costly process leads many farmers to neglect daily pump management activities and leave their pumps running, causing substantial waste of water and electricity, erosion and lowering of soil quality.

At systems level, water use efficiency is significant in agricultural sustainability. For example, the world produces approximately 700 million tons of paddy rice each year. This is enough to provide the staple food for more than three billion people, of which some 700 million live in poverty (Bas Bouman, 2018). On average, the world's rice fields use some 1,400 litres of water by evaporation and transpiration to produce 1 kilogram of paddy rice which is on the same order of magnitude as what wheat uses (Bas Bouman, 2009).

At the field level, farmers can adopt a system of alternate wetting and drying, in which fields are not continuously flooded anymore but are allowed to dry out for a few days in between irrigations. This can save up to 30% of the water while maintaining or sometimes even increasing rice yields. Second is the system of "aerobic rice", in which the field is not flooded at all anymore but just "wetted" like a farmer does with irrigated maize or wheat. Suppose

if we can reduce water use in the world's rice fields by a mere 10%, this would free up to 100 cubic kilometers of water (equivalent to water for 40 million Olympic-sized swimming pools) which is enough to provide half to a quarter of the world's population with domestic water annually.

Traditional manual field irrigation systems need lot of labour and material resources and it goes against the development of long-term agricultural production and sustainable utilization of water resources (Khriji *et.al*., 2014).

For other crops as well there is a need to adopt the strategies that reduce the water consumption. One of the innovative approaches is using digital solutions that, among many others, would benefit farmers reducing the water consumption.

Auto Pump Starters

Farmers spend all the time in the field to manage their field efficiently to achieve higher productivity but one cannot be successful because of the unavailability of any information. They do not know exactly how much to irrigate at any time. Along with this, a farmer faces one more problem while irrigation that is supply of electricity, because they irrigate their field only when the electric power is available.

Water use efficiency in crops is much talked about concept keeping in view the sustainability for agricultural systems. Automated irrigation system can be used to improve water management. In recent past several organizations started promoting smart solutions to irrigation control through mobile phones. For example, Indian mobile operator Tata Teleservices together with agro-automation company Ossian is helping farmers monitor and switch on irrigation pumps remotely, using a low-end Nokia phone and mobile modem called 'Nano Ganesh' which is connected to the electric starter of the pump. These innovations are literacy neutral as the advisories can also be listened through audio. The system helps to save time, water, and electricity/fuel.

Some advanced features available with such chips / sensors include; Microcontroller based software Technology, Model to suit every single / three phase range, Start stop motor from home or anywhere in world and Electrical safety from all electrical faults. The smart phone based pump starters allow farmers to monitor and operate irrigation pumps for irrigating crops in remote locations. While the public and private sector stakeholders are convinced about the digital solutions, it is important to assess the perception and acceptability of such initiatives among farmers. User experiences are important when we introduce such digital solutions that involve initial investments on the part of farmers. The assessment of the digital systems in terms of relative advantage over existing irrigation methods would help to further refine and roll out the strategies across the locations. In this context, the present study was carried out in Telangana state of India to critically analyse the grass roots factors that helped use of smart phone based auto starters.

Methodology

The study was conducted among 111 farmers who have currently installed and are using the auto starters for irrigating their fields during 2017-18. The study area is Jagityala district of Telangana, which is purposefully selected due to the fact that a large number of farmers have installed the auto starters recently in this area. The data were collected using survey method and also with focused group discussions. For collecting data, a structured questionnaire was developed with comprehensive indicators. The data was collected during June and July 2018 and the data analysis was done using SPSS.

Results and Discussion

Results of the survey and group discussions are presented in this section. The respondents' age, total cultivated area, automation area and the soil type are presented in Table 1 for general understanding of the context. The respondents are uniformly distributed across different age groups which indicate that farmers, irrespective of their age, have installed the auto starters.

The average cultivated area for the respondents is about 2 ha. It is heartening to note that more than 65 % of the farmers who have cultivated area less than one hectare had installed the auto pump starters compared to only 2.7 % of the farmers having more than 3 ha of land. It is not about the relative large-ness of the landholding that matters in adopting the innovative technologies, rather the relative advantages that are offered by the technology influences the adoption. The proportionate automation area in relation to the total cultivated area was also calculated. Even though few farmers have more cultivated area, not all the area was brought under the auto pump starters. Nearly 85% of the farmers who had installed the auto pump starters tried that in less than 1 ha area. Similarly, 25% of the farmers have used this technology in irrigating 1- 2 hectares area. These pump starters were doing good equally in all types of soils (Heavy, Light and Medium).

Table 1: Frequency of various parameters as perceived by farmers (n=111)

Age in Years	Frequency	%
< 40	35	31.5
41-50	57	51.3
51-62	19	17.2
Total cultivated area (ha)		
a. <1. 0	73	65.7
b. 1.1-2.0	28	25.3
c. 2.1-3.0	7	6.3
d. >3.0	3	2.7
Automation area (ha)		
a. <1. 0	94	84.6
b. 1.1-2.0	13	11.8
c. 2.1-3.0	4	3.6
d. >3.0	0	0
Soil type (Heavy/Medium/Light)		
a. Heavy	30	27.1
b. Light	26	23.4
c. Medium	55	49.5

Table 2 indicates that about 58% of the farmers installed premium units where as 42% installed basic units. Majority of the farmers (30%) got Texmo make pumps while 25% of the respondents have CRI pump installations. The variety of pumpsets among the users of auto starters revealed that these auto starters could be used for any make. Above 93 % of the respondents reported that they would flood their fields, whereas about 7% of the farmers reported drip irrigation. These results indicate, contrary to the popular belief that auto starters are useful only for drip irrigation; they could also be used in flooded conditions.

More than half of the respondents reported that the pumps are used during the day time and 42% reported that there is no specific time period and they may require switching on the irrigation pumps any time. In India, where the electricity supply is erratic, farmers often walk several kilometers to where their irrigation pumps are located, only to find that there is no electricity available. Very few farmers were using these pumps for fertigation. Half of the farmers having drip irrigation facility were using those systems for applying the fertilizers also.

Factors influencing the selection of Pump Starters are presented in Table 3. One of the interesting factors contributing to the installation of auto starters is the influence of peer group or the organizations. The awareness about the auto pump starters and the availability at the optimum cost is very important in adoption of the innovations. The Farmers' Development Centres of eFresh Pvt Ltd., located in the study area played an important role on creating the awareness about the technology and also in making the technology available to the farmers. The awareness was followed by the experience sharing and physical examination of the technology helped farmers in selecting the pump starters. The credibility and reliability of Tata brand, the guarantee offered for the products and eFresh brand contributed to the selection of these starters in this area.

Table 2: Frequency Distribution of Respondents based on Usage of Pump starters (n=111)

Parameters	Frequency	%
Name of the pump starter installed		
Texmo	34	30.63
CRI	28	25.23
Varsha	16	14.41
others	33	29.73
Irrigation system type		
Flooded	103	93
Drip	8	7
Sprinkler	0	0
Make of the pumpset		
TQ Basic	46	41.5
TQ Premium	65	58.5
Water source		
Canal	15	13.51
Well open	61	54.95
Storage tank	0	0.00
Bore well	18	16.22
Others	21	18.92
Main power usage period		
Day	61	54.95
Night	3	2.70
24 Hours	47	42.34
Fertigation Done		
Yes	4	3.6
No	107	96.4

Table 3: Factors influencing the selection of Pump Starters (n=111)

Factors that played role in selection (multiple responses) n=111	Frequency	%
FDC physical center	78	70.27
Word of mouth/fellow farmers	75	67.57
Social media (Facebook, Whatsapp)	41	36.94
Reasons for selection (multiple responses)	**Frequency**	**%**
TATA Brand	101	90.99
Quality standards	85	76.58
Guarantee	85	76.58
eFresh Brand	69	62.16

Interestingly, the high water consumption crops like paddy and sugarcane listed the top (71% and 21% respectively) where these pump starters are being used. This indicates that farmers started perceiving the need for reducing the water usage and wished to look for the alternatives to make best use of available water. By dialing a code number from his mobile phone to a wireless device attached to the pump, farmers can now remotely monitor the electricity supply. This feature could be best harnessed for crops like paddy (Table 4).

Table 4: Crops Cultivated under Automated Pump-starters (n=111)

Crops under automated pump starters	Frequency	%
Paddy	79	71.17
Sugarcane	24	21.62
Banana	2	1.80
Chilli	2	1.80
Cotton	4	3.60
Maize	5	4.50
Malabar Neem	1	0.90
Mango	6	5.41
Turmeric	4	3.60

One of the variables determining the rate of adoption of an idea is the perceived attributes of innovation. The digital technologies may offer a variety of solutions to the farmers, but the adoption of such digital technologies in farmers fields are influenced by the perceived attributes rather than the absolute attributes. An attempt is made to understand the perceived attributes of auto pump starters in terms of direct benefits, indirect benefits and challenges faced by farmers while adopting this technology.

For all the farmers using auto pump starters, the perceived benefits included; relative easiness to operate/ irrigate compared to earlier, time saving, safety (Personal safety, Pump set safety) and the flexibility of irrigating (Table 5).

Accessibility of pump sets during the odd hours has always been challenging, particularly when they are located in difficult terrains. More than 74% of the farmers opined that remote accessibility of pump sets using the auto starters was a striking feature. Another advantage that was reported by 73% of farmers is this could be operated by any family member (unlike only family head has to go in conventional method).

Update about status of irrigation was perceived as an excellent option by 96.3% farmers. Ease in planning irrigation cycles was also seen as an added advantage by 94.5% of farmers. The ability of these pump starters to bypass into manual mode option in case auto starter doesn't work was much appreciated by the users. Update about the status of water in a tank/ well without being physically there – was one of the best benefits of these pump starters (as perceived by 92.7 %).

Table 5: Relative advantages of Auto Pump starters as Perceived by the Farmers (n=111, multiple responses)

Direct benefits	Frequency	%
Easy to operate / irrigate compared to earlier	111	100
Time saving	111	100
Safety (Personal safety, Pump set safety)	111	100
Flexibility of irrigating	111	100
Remote Accessibility of pump sets (vehicle can't get there!)	83	74.7
Can be operated by any family member (unlike only family head has to go)	82	73.8
Gender / Literacy no barrier	104	93.6
Updates about status of irrigation	107	96.3
Ease in planning irrigation cycles	105	94.5
Bypass (Manual mode) option (in case auto starter doesn't work)	111	100
Update about the status of water in a tank/ well	103	92.7
After sales service support	88	79.2
Indirect benefits		
Reduced water wastage	111	100
Reduced electricity wastage	111	100
Reduced labour wastage	111	100
Reduced fuel wastage (POL)	108	97.3
Cost effectiveness	94	84.6
Adding to awareness about resource conservation	109	98.2
Local capacity – business opportunities	98	88.2
Daily / weekly reports – decision making	87	78.3
Increased crop productivity	67	60.3

There are other indirect benefits that were reported by the farmers such as reduced water wastage, electricity wastage, labour wastage and fuel wastage (POL). More than 78% of the farmers reported that auto starters have helped taking timely and effective decisions on irrigation based on the daily / weekly reports. The departments of agriculture and irrigation can develop few strategies based on the data collected from a large number of auto pump starters. This will have far reaching consequences on the release of water in canals or for timely personalised advisories on crop (water) management strategies to the farmers. There is a need to further quantify these benefits using secondary data at macro level.

Table 6: Challenges in large scale adoption of Auto Pump starters as Perceived by the Farmers (n=111, multiple responses)

Difficulties - Negatives	P	F
1. Costly	57.7	64
2. Skills not available to use	24.3	27
3. Skills not available to repair	48.6	54
4. Wiring damage	42.3	47
5. Theft	6.3	7
6. Not working properly	26.1	29
7. Over flooding	27.0	30
8. Poor control	5.4	6
9. Not useful	4.5	5
10. Perceived as excessive sophistication	33.3	37
11. More electricity is consumed	3.6	4
12. Useful only for commercial crops	0.9	1
13. Mobile net working issues (poor signals)	32.4	36

There are few challenges as perceived by the farmers in use of auto pump starters. Some of these challenges could be overcome by interventions by the concerned organizations or even with the group discussions with the other farmers.

About 27% of farmers opined that it required few skills that their family members were lacking in using smart phones for operating. One of the critical challenges faced by farmers was that in the villages there was no man power that could handle trouble shooting and immediately replacing the wiring, if they got damaged. Even 7% of the farmers believed that these sets might get stolen (Table 6).

There are few other challenges observed like excessive sophistication and higher electricity consumption. Increased awareness and long term usage of auto starters can prove this wrong. But there is a need to empirically quantify the benefits at individual farmer's level and at village level, so that effective extension strategies could be chalked out in future.

Conclusion

Several players are engaged in agriculture irrigation automation system that include auto-pump starters, GPRS based drip irrigation automation, IOT applications etc., It is now possible for a farmer to operate his pumpset to irrigate fields with a mobile phone. These smart irrigation solutions will help small farmers in a big way. Besides reducing water wastage, the GSM-based mobile starters were equipped with latest software technology. It will help curtail water and energy wastage as it alerts the farmer in case of faults, overload etc, through a call. The improvement in irrigation efficiency will also lead to higher yield and productivity, in long term.

India has deployed a plethora of digital pilots in the field of agricultural development in last two decades. The Indian government, private industry, civil society organizations are developing and disseminating a series of innovative, networked solutions to increase availability, accessibility and applicability of agriculture services at farm level. In order to exploit the possibilities, digital service have to provide practical solutions that would reduce the cost of cultivation and would bring efficiency in field operations that would eventually increase their productivity and income.

Acknowledgments

This work was carried out as a part of the project "Maximizing the Impact of Rice Technologies through ICT applications" funded by ICAR- Indian Institute of Rice Research, Hyderabad. The support received from ICAR, IIRR, AICRIP co-operators is gratefully acknowledged.

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SHORT COMMUNICATION OPEN ACCESS

Genetic variability for yield and its component traits in upland rice

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Received: 12th Dec. 2018; Accepted: 24th April 2019

Abstract

This study consisted of 33 genotypes including two checks i.e. HPR 1156 and HPR 2656 evaluated in a Randomized Block Design with three replications for various yield traits. Analysis of variance revealed significant differences among the genotypes for all the traits studied and presence of considerable amount of genetic variability for all the traits studied. High heritability coupled with moderate genetic advance and moderate phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) was observed for panicle length, grains/panicle, yield/plant, yield/plot indicated the presence of additive gene action and sufficient variability scope for improvement through selection. On the basis of mean performance for different traits studied *viz*., HPR 2846, HPR 2875, HPR 2839, HPR 2866, HPR 2867 and HPR 2885 were the best genotypes for yield and other yield contributing traits which may be used for the further rice breeding programme.

Keywords: Rice, genetic advance, genetic variability, upland rice, heritability

Introduction

Rice *(Oryza sativa* L.) is one of the major food crops of world especially of the Asian countries like China, India, Pakistan, Bangladesh, Vietnam, and Korea. It is placed on second position in cereal production around the globe and is the staple food of Asia. More than 90% of the world's rice is grown and consumed in Asia, where 60% of the world's population lives. Rice is grown worldwide over an area 160.6 million hectares with total production of 492.2 million tones. Area under rice in India is 42.2 million hectares with production of 104 million tones (Anonymous 2016). It is a staple food for majority of the population of Himachal Pradesh where it is cultivated on an area of about 77 thousand hectares with production of 131.6 thousand metric tons with productivity of 17.05 quintals/hectare (Anonymous 2016). It is grown under various agro-climatic conditions ranging from foot-hills to an altitude as high as 2200 m above mean sea level. As a result of the great diversity of agro-climatic conditions under which rice is cultivated in Himachal Pradesh, there is a great variability in land races present in the crop and a large number of local as well as improved cultivars are available for cultivation Due to scarcity of water and unpredictable rainfall, less availability of agricultural labour and due to hilly terraces in Himachal Pradesh there is less retention of water in rice field. So it is important to develop upland/rainfed rice cultivars for direct sowing. For an effective crop improvement programme, availability of wide range of variability in the genotypes of particular plant species is the basic requirement. It is desirable to understand the nature and magnitude of genetic variability present in a particular material and the methods to make best possible use of this variability.

The investigation was carried out on 31 advance breeding lines at Rice and Wheat Research Centre, Malan, and 2 checks namely, HPR-1156 and HPR-2656. These lines were evaluated in Randomized Block Design with three replications. The observations were recorded on five randomly taken plants from each plot for days to 50% flowering, plant height, panicle length, grains/panicle, spikelets/panicle, yield/plant, 1000-grain weight, grain length, grain breadth, L:B ratio, protein content, aroma, yield/plot (with plot size of 3.50 x,1.20 m), reaction to leaf and neck blast. The data was statistically analyzed as per the procedure given by Panse and Sukhatme (1985). The genotypic, phenotypic and environmental coefficients of variation were estimated following Burton and De Vane (1953). The expected genetic advance (GA) resulting from the selection of 5 per cent superior individuals was calculated as per Burton and De Vane (1953) and Johnson *et al*., (1955).

The analysis of variance revealed significant differences among the genotypes for all the traits studied indicating the presence of wide range of variability in respect of days of 50% flowering, plant height, panicle length, grains/

panicle, spikelets/panicle, yield/plant, 1000-grain weight, grain length, grain breadth, L:B ratio and yield/plot. So the present investigation revealed sufficient variability for yield and morphological character studied. Earlier Parsad *et al.,* (2013) and Singh *et al.,* (2014) reported significant variability for yield and related traits in their studies. An assessment of variability parameters revealed that there was a lot of variation among the genotypes. In general, values of phenotypic coefficient of variation (PCV) were higher than their respective genotypic coefficient of variation (GCV) indicating considerable influence of environment on the performance of genotypes.

In the present study (Table 1), PCV and GCV were lower for days to 50% flowering, plant height, yield/plant, 1000-grain weight, grain length, grain breadth and L:B ratio coupled with high to moderate heritability and low genetic advance indicating that improvement for such traits is very limited. However, these characters can be improved by hybridization. Similar results were reported by Panwar (2005) for low PCV and GCV; Madhavilatha *et al.,* (2005) for low genetic advance and high heritability. PCV and GCV were moderate for panicle length, grains/panicle, spikelets/panicle and yield/plot coupled with higher to moderate heritability and low to moderate genetic advance indicates that these traits can further be improved through selection because of the prevalence of additive gene action. Similar results were recorded by Patil and Sarawagi (2005), who observed moderate PCV and GCV, heritability and genetic advance for grains/panicle.

Table 1: Estimates ofw parameters of variability for different traits in rice genotypes

Traits	Mean	S.E(m)	Range	PCV (%)	GCV (%)	Heritability h^2bs (%)	Expected GA (as percentage of mean)
Days to 50% flowering (No.)	86.780	±0.400	76.00-92.00	6.187	6.136	98.338	12.534
Plant height (cm)	98.102	±1.119	86.70-113.50	6.820	6.527	91.608	12.870
Panicle length (cm)	25.047	±0.334	21.78-28.68	6.166	5.735	86.508	10.988
Grains/panicle (No.)	108.206	±4.610	70.30-150.50	20.242	18.849	86.710	36.157
Spikelets/panicle (No.)	142.133	±8.650	85.20-210.67	25.281	24.071	90.656	47.213
Yield/plant (g)	10.495	±0.440	6.40-14.40	16.813	15.196	81.694	28.294
1000-grain weight(g)	26.568	±0.680	19.67-33.54	9.941	8.899	80.139	16.411
Length of grain (mm)	5.820	±0.110	5.80-7.91	5.809	5.097	77.011	9.215
Breadth of grain (mm)	2.360	±0.070	1.24-2.45	9.011	6.898	58.604	10.879
L:B ratio	3.554	±0.100	3.00-4.23	9.954	9.138	84.272	17.280
Protein content (%)	8.410	±0.100	6.05-12.06	12.643	12.468	97.252	25.328
Yield/plot (Kg)	0.923	±0.017	0.57-1.31	18.109	16.710	85.150	31.765

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Thank you...

Editorial Board of Journal of Rice Research would like to thank the following scientists for reviewing the manuscripts of this June 2019 issue. We thank you for sparing your valuable time and expertise in reviewing each manuscript twice and providing valuable service along with suggestions for improvement.

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We congratulate the outgoing Editorial board headed by Dr. Raman Meenakshi Sundaram and his team for successful completion of the tenure (2014-2016) and thank each one of the members for the services rendered towards the publication of this Journal to advance the cause of rice research and development in the country.

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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. First author or the corresponding author should be member of the Society for Advancement of Rice Research and not in arrears of subscription. All the articles should adhere to the guidelines of the journal.

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Research papers

1. Durvasula V. Seshu. 2017. Networking a Pivotal Strategy for Rice Genetic Improvement. *Journal of Rice Research,* 10(1): 1-8.
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Tables: Tables should be used for reporting extensive statistically analyzed numerical data arranged in an organized manner and 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 103) 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.

Published by :

Dr. S R Voleti, President, Society for Advancement of Rice Research, Hyderabad.

Printed at :

Balaji Scan Pvt. Ltd., Hyderabad - 500 004. Ph: 040-23303424 / 25 E-mail: bsplpress@gmail.com

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