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Society for Advancement of Rice Research

Society For Advancement of Rice Research

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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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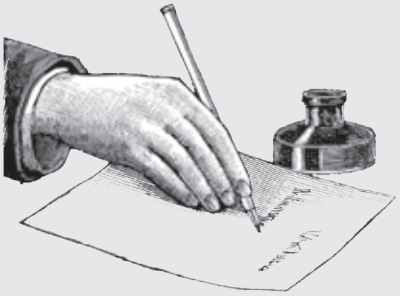

From the SARR President's desk....

Rice is the primary staple food for two thirds of the population in India, contributing to about 30% of the calories consumed. Today, India is one of the leading producer of rice and gained supremacy as leading exporter also. In spite of the pandemic situation prevailing in the last two years, rice production is estimated at 127.93 million tonnes for 2021-2022. Currently, rice cultivation is being faced by many challenges due to shrinking water resources, depleting soil health, emerging new insect pests and diseases, increasing temperatures due to climate change, instability in market prices leading to the vulnerability of rice farmers' livelihoods and food security. To meet the growing demand, the concerted efforts by various rice scientists of AICRIP, SAUs, ICAR institutes and private companies is essential to meet the challenges. With the advent of emerging next generation technologies and renewed focus on researchable issues to increase the yield, there is a need to have a paradigm shift in the rice research and communication 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 executive committee and the editorial board has made significant and appreciable efforts to bring out the Journal of Rice Research in a renewed format and are striving hard to improve the NAAS rating of the Journal with support from the India's largest network of rice researchers, i.e. All India Coordinated Rice Improvement Program (AICRIP) and other rice research organizations who are working for the cause of rice research and development in the country.

(Dr. RM Sundaram)
Director, ICAR-IIRR &
President, SARR

Production Editor

Dr. P Ananda Kumar joined as Production Editor of the Journal of Rice Research

Dr P. Ananda Kumar served as Director, ICAR-National Institute of Plant Biotechnology, New Delhi, India; Director, Institute of Biotechnology, Acharya N.G. Ranga Agricultural University, Hyderabad and Director (A), ICAR-Indian Institute of Rice Research, Hyderabad. Dr P. Ananda Kumar specialized in Plant Molecular Biology and Biotechnology. He obtained M.Sc degree in Botany from Sri Venkateswara University, Tirupati. He joined as a Scientist in the Agricultural Research Service of Indian Council of Agricultural Research (ICAR) in 1978. He obtained Ph.D degree in Plant Physiology from Indian Agricultural Research Institute (IARI), New Delhi. After working as Alexander von Humboldt Fellow in the University of Hannover, he moved to Biotechnology Centre, IARI, in 1993. He specialized in the area of transgenic development for insect resistance utilizing the genes encoding insecticidal proteins of *Bacillus thuringiensis*. Dr Kumar developed fruit borer resistant brinjal and tomato, which were licensed to private companies. In collaboration with Assam Agricultural University (Jorhat), he developed Pod borer-resistant Chickpea. His associates are currently involved in the development of Pod borer resistant Pigeonpea, utilizing the codon-modified Bt genes constructed by him. Dr Kumar published over 150 research articles, books and book chapters. He has three patents on codon-modified and chimeric Bt genes. His other research interests include functional genomics of aerobic rice, brinjal, ragi and cotton.

Honours and Awards

- Mahindra Krishi Samriddhi Award – 2011 (as Director, NRCPB)
- Best Institution Award of ICAR – 2010 (as Director, NRCPB)
- Recognition Award, National Academy of Agricultural Sciences – 2005.
- National Bioscience Award-Department of Biotechnology – 2001
- VASVIK Award for Agriculture and Industry – 2000
- Fellow, National Academy of Agricultural Sciences – 1997
- Fellow, National Academy of Sciences, India-2011
- Fellow, Alexander von Humboldt Stiftung, Germany – 1991
- Young Scientist Award-Indian National Science Academy -1989
- Prof Hiralal Chakravarty Award-Indian Science Congress Association – 1996
- Editor, GM Crops and Food (USA)
- Secretary, Society for Plant Biochemistry and Biotechnology, India.

Patents:

1. Synthetic gene encoding a chimeric δ-endotoxin of *Bacillus thuringiensis.* (Patent No. 237912; 14-1-2010).
2. Synthetic gene encoding Cry1Fa1 δ-endotoxin of *Bacillus thuringiensis.* (Patent application: No. 242768; 9-9-2010)
3. Promoter from *Gossypium hirsutum* L. for enhanced expression of foreign genes in late boll developmental stages of cotton. (Patent No. 382816; 26-11-2021)

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ORIGINAL RESEARCH ARTICLE

Evaluation of improved drought-tolerant parental lines of KMR3R for fertility restoration by molecular analysis

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Abstract

The present investigation was carried out to identify fertility restoration in improved drought tolerant parental lines with ideal agronomic trait performances and their utilization in the hybrid rice breeding program. Backcross derived lines obtained from a cross between a drought-tolerant Vandana NIL (donor, possessing *qDTY12.1* and *qDTY1.1*) and a drought susceptible restorer, KMR3R (recurrent parent, possessing *Rf3* and *Rf4* genes). Based on foreground and background selection, the backcross population was advanced to BC_2F_4 population by stringent Marker-Assisted Backcross Breeding (MABB). Molecular markers were utilized in the marker assisted restorer selection in WA-CMS from large source of nurseries to keep away from regular test cross nursery (TCN) in the hybrid rice breeding technology. The tightly linked/or gene-specific markers *viz.*, RM6100, RMS-PPR-9-1, DRCG-Rf4-14, for *Rf4* on chromosome 10, and DRRM Rf3-10, RM 10313, and RMS-SF21-5 for *Rf3* located on chromosome 1 were used to screen for the presence or absence of specific restorer allele in the population. 71 improved drought-tolerant backcross inbred lines (BILs), including checks, and parents were screened for their fertility restoration. Results were skewed in their frequency distribution by showing 48.48% of *Rf3* and *Rf4* genes (*Rf3Rf3/Rf4Rf4*). These double allelic combination containing genotypes exhibit better fertility restoration than any of single *Rf3Rf3/rf4rf4* or *rf3rf3/Rf4Rf4* individually. Ultimately ten genotypes were identified as complete restorers (RP6340 NPVR1, RP6340 NPVR3, RP6340 NPVR10, RP 6340 NPVR24, RP6340 NPVR27, RP6340 NPVR32, RP6340 NPVR48, RP6340 NPVR52, RP6340 NRR5 and RP6340 NRR11) with drought QTLs for drought-prone lowland ecosystems and can be utilized in the hybrid rice breeding programme under unfavorable drought ecologies.

Keywords: Rice, hybrid, drought tolerance, fertility restoration, molecular markers

Introduction

Rice is an essential food cereal crop for more than half of the world's population and livelihood. The utmost priority of rice production is to satisfy the hunger of the escalating population and to improve food security. The productivity is inadequate to meet the future demand of escalating population in India (Shidenur *et al.*, 2019). Among various genetic approaches available today to enhance the yield potential, hybrid rice technology is the most encouraging, and accepted strategy for improving rice productivity. Though rice is a self pollinated crop, exploitation of heterosis through male sterility system is a prerequisite and distinctly shown standard heterosis of 15–20% over the commercially cultivated indica genotypes/high yielding varieties in a similar growth environment (Virmani *et al.*, 2003; Gramaje *et al.*, 2020). The heterosis proportion depends on the possible course of existing hybrid rice parental lines.

India occupied second place after China in the production of hybrid rice. India covers an area of 2.8 Mha of hybrid rice production (Senguttuvel *et al.*,

2019). However, India reaches only about 5.6% of the total rice area; ten times lower than that of China (Katara *et al.,* 2017). Nevertheless, distinct heterosis and adaptability, the slow spread of hybrid rice technology is attributed to various biotic and abiotic stresses. Hence, the development of genetically potential and suitable hybrids for different ecologies with a high level of heterosis along with desirable grain and cooking quality is imperative to enlarge its cultivated area (Verma *et al.,* 2021).

In India, hybrid rice is primarily developed using a three-line system, which involves cytoplasmic male sterile line (A-line; male-sterile), an isonuclear maintainer line (B line; male fertile) and restorer line (R line; male fertile with fertility restorer gene). Drought is the primary and most important abiotic factor among all abiotic stresses. Exploitation of plant tolerance to drought stress is considered a viable strategy to enhance the potential of rice hybrids under drought-prone ecology. Cytoplasmic male sterility (CMS) is a maternally inherited trait that results in the plant lacking the capacity to produce fertile pollen. Nuclear-encoded genes restore pollen fertility called the fertility restorer (*Rf*) gene. Hence, to develop high-yielding heterotic hybrids, the first and foremost step is to identify restorers (R line) that can efficiently restore the fertility of CMS (A) lines.

The restorer lines employed to develop new rice hybrids remain susceptible to drought stress. Therefore, in the present study, we attempted to fortify the restorer line, KMR-3R (a restorer line), as a recurrent parent carrying major restorer genes (*Rf3* and *Rf4*) to develop hybrids tolerant to drought stress. The donor parent, Vandana NIL (drought tolerant), possessed major qDTYs *viz.,* qDTY12.1 and qDTY1.1. This approach was carried out by marker-assisted backcross breeding (MABB) and 71 (BC_2F_4) backcross inbred lines (BILs) were generated.

Two fertility restorer *Rf3* and *Rf4* genes are essential for viable pollen production in the WA-CMS type of CMS system (Bhati *et al.,* 2018). The use of molecular markers linked to *Rf* genes can enhance the selection efficiency and remove the impediments related to phenotype screening. The analysis of genetic linkage indicated that SSR markers RM6100 (Singh *et al.,* 2005) on the long arm of chromosome 10, is linked with the *Rf4* gene (at 1.2 cM) and RM10313 (Neeraja *et al.,* 2009) on the short arm chromosome 1 is linked with *Rf3* gene (at 4.2 cM). SSR marker RM6100 may facilitate MAS selection of WA-CMS-based restorer lines by avoiding regular testcross in large breeding genotypes in a hybrid rice breeding program (Sheeba *et al*., 2009; Kiani *et al.,* 2015). Other candidate gene markers (Suresh *et al.,* 2012; Pranathi *et al.,* 2016) have been utilized efficiently to screen the BIL population to identify superior drought tolerant restorers The MAS (Marker-assisted selection) program was carried out to introgress the drought-tolerant qDTYs, while maintaining fertility restoration (*Rf3* and *Rf4*) genes into popular restorer lines to develop agronomically superior hybrid rice parental lines for drought-prone lowland ecologies.

Materials and Methods

Plant genetic material

The study material comprises of 71 (BC_2F_4) improved Backcross Inbred Lines (BILs) (including parents and checks) derived from a drought-tolerant donor parent, Vandana NIL (*rf3rf3/rf4rf4*), and an elite restorer recurrent parent KMR3R (*Rf3Rf3/Rf4Rf4*). Along with parents, checks including two maintainers (B) lines, IR79156B and APMS-6B devoid of fertility restoration (*rf3rf3/rf4rf4)* and two restorers (R) lines, RPHR1005 and BK49-72 with complete fertility restoration (*Rf3Rf3/Rf4Rf4)*, were utilized as negative and positive controls respectively, for Rf3 and Rf4 alleles.

Experimental details

All the experiments were conducted during the wet season (*Kharif,* 2018) at Research Farm, ICAR-Indian Institute of Rice Research (IIRR, 17.3200˚ N, 78.3939˚ E), Hyderabad, India. Standard agronomical practices and plant protection measures were followed to ensure healthy crop. 21-day old seedlings were transplanted into the main field using a randomized complete block design (RCBD) with three biological replications. The stringent phenotypic and genotypic screening was employed to identify desired plants with restoring ability and drought tolerance at BC_2F_4

population using marker-assisted backcross breeding (MABB) approach.

Genotyping of parents and BILs

Genomic DNA was extracted from the young leaf tissues of parents collected at active tillering stage and BILs using the CTAB method (Dellaporta *et al.,* 1983). DNA quantification was done using 0.8% of agarose gel. For microsatellite assay, PCR reaction mix was prepared and carried out using 50 ng/l of isolated template DNA, containing 0.5µl of each forward and reverse primer, 1µl (2.5 mM of each) of dNTP, 0.2 µl of Taq DNA polymerase, 10µl of 10X PCR reaction buffer in a total volume of in thermal cycler (Eppendorf, USA). PCR Amplification was programmed by following steps: as 94℃ for 4 min, followed by 35 cycles of 94℃ for 30sec denaturation, 55℃ for 1min of annealing, and 72℃ for 1 min of extension and last step is 5min at 72℃ for the final extension. The amplified PCR products, along with 100 bp molecular marker (Bangalore Genie, India), were electrophoresed on a 3.0% agarose gel (Seakem® LE), stained with ethidium bromide, and the gel was documented using Gel documentation unit (Alpha Innotech). Unambiguous and resolved DNA bands were scored for their presence visually for each reported primer. BILs (BC_2F_4) were screened for the fertility restoration status and positive checks for restorer and maintainer. This was successfully done by closely linked reported SSR (Simple sequence repeat) fertility restoration markers for *Rf4* and *Rf3* locus (**Table 1**).

Results and Discussion

Hybrid rice technology has been introduced successfully in more than 40 countries, and India is the second largest country in the adoption and production of hybrid rice since 1989 (Yuan *et al.,* 2017). India has made substantial progress and released 127 hybrids for commercial cultivation which are mostly suitable to irrigated ecologies (Senguttuvel *et al.,* 2019). Due to the unambiguous specificity of hybrids released so far in India, *viz.,* lack of specific ecosystem, tolerance to several biotic and abiotic stresses (drought, salinity, submergence, etc.), and consumer's preference (Rout *et al.,* 2020) exploitation of genetic diversity of drought tolerant parental lines along with fertility restoration is a convenient way for the development of promising drought tolerant hybrids (Singh *et al.,* 2021). Hence, this study was undertaken to develop the parental lines suitable for drought-prone lowland and upland ecosystems besides the normal irrigated environment.

Screening for fertility restoration genes *Rf3* and *Rf4*

Identifying superior parental lines harboring restorer genes through molecular approach is desirable as phenotyping is a very time-consuming and tedious process, and spikelet sterility in testcross progeny needs to be determined (Ahmadikhah *et al.,* 2007). The success of hybrid rice is largely dependent on high pollen and spikelet fertility due to the high compatible interaction of both *Rf3* and *Rf4* genes and CMS cytoplasm (Shalini *et al.,* 20 15). Reported linked molecular markers may be effectively used in the marker assisted restorer selection in WA-CMS from a large source of nurseries to give a wide berth to usual test cross nursery in the hybrid rice breeding (Singh *et al.,* 2021). In the evaluation of testcross nursery, we conducted the present experiment with the help of reported gene-linked/specific markers. For fertility restoration, markers linked to *Rf3* and *Rf4* were used. The SSR marker RM 6100 (175 bp for restorer line), one candidate gene marker DRCG-RF4-14 for *Rf4* locus, and one SSR marker DRRM-RF3-10 (150 bp for restorer line) for Rf3 reported by Suresh *et al.,* (2012) were used for screening the population. The details of markers are given in **Table 1**. The plants were grouped as B (maintainer indicates allele), R (fertility restorer genes) and also partial maintainer and partial restorers, respectively.

The fertility restorer genes (*Rf3* and *Rf4*) reported to restore male fertility in the WA CMS system was mapped on chromosomes 1 and 10, respectively (Zhang *et al.,* 1997; Alavi *et al.,* 2009). Six markers, RM6100, RMS-PPR-9-1, DRCG-Rf4-14, DRRM Rf3-10, RM 10313, and RMS-SF21-5 were already validated as tightly linked with fertility restoration of WA-based cytoplasm by Rf3 and Rf4 genes in rice (Singh *et al.,* 2005, Neeraja *et al.,* 2009, Balaji *et al.,* 2012, Revathi *et al.,* 2013, Pranathi *et al.,* 2016).

Categorizations of BILs based on fertility restoration were represented in **Table 2.**

A total of 71 drought tolerant BILs (BC_2F_4) were screened without prior information of fertility restoration status and known restorers and maintainers linked to fertility restoration genes, namely *Rf3 & Rf4* located on chromosome 1 and chromosome 10, respectively. Possessing a single Rf4 gene in the genotype will not complete the pollen fertility restoration. So, other fertility restorer genes, such as the *Rf3* gene located on chromosome 1, are necessary for a restorer as they express fully restoring WA-CMS (Suresh *et al.,* 2012).

Table.1 Reported SSR markers linked to *Rf3* & *Rf4*genes

Marker	Linked gene	Chromo-some	Reference
DRRM Rf-3–10	*Rf3*	1	Balaji *et al.,* 2012
RM 10313	*Rf3*	1	Neeraja *et al.,* 2009
RMS-SF21-5	*Rf3*	1	Pranathi *et al.,* 2016
RM 6100	*Rf4*	10	Singh *et al.,* 2005
RMS-PPR-9-1	*Rf4*	10	Pranathi *et al.,* 2016
DRCG-Rf4-14	*Rf4*	10	Balaji *et al.,* 2012

Table 2. Advanced drought-tolerant backcross inbred lines (BILs)

S No	Genotype	*Rf3/Rf4*	Restorer/ maintainer
1.	RP6340-NPVR-1	*Rf3Rf3/Rf4Rf4*	Restorer
2.	RP6340-NPVR-2	*rf3rf3/rf4rf4*	Maintainer
3.	RP6340- NPVR-3	*Rf3Rf3/Rf4Rf4*	Restorer
4.	RP6340- NPVR-4	*Rf3Rf3/rf4rf4*	Partial Maintainer
5.	RP6340- NPVR-5	*Rf3Rf3/rf4rf4*	Partial Maintainer
6.	RP6340- NPVR-6	*rf3rf3/rf4rf4*	Maintainer
7.	RP6340- NPVR-7	*rf3rf3/Rf4Rf4*	Partial Restorer
8.	RP6340- NPVR-8	*Rf3Rf3/Rf4Rf4*	Restorer
9.	RP6340- NPVR-9	*Rf3Rf3/Rf4Rf4*	Restorer
10.	RP6340- NPVR-10	*Rf3Rf3/Rf4Rf4*	Restorer
11.	RP6340- NPVR-11	Rf3Rf3/rf4rf4	Partial Maintainer
12.	RP6340- NPVR-12	Rf3Rf3/Rf4Rf4	Restorer
13.	RP6340- NPVR-13	Rf3Rf3/Rf4Rf4	Restorer
14.	RP6340- NPVR-14	Rf3Rf3/Rf4Rf4	Restorer
15.	RP6340- NPVR-15	rf3rf3/rf4rf4	Maintainer
16.	RP6340- NPVR-16	Rf3Rf3/Rf4Rf4	Restorer
17.	RP6340- NPVR-17	rf3rf3/Rf4Rf4	Partial Restorer
18.	RP6340- NPVR-18	rf3rf3/Rf4Rf4	Partial Restorer
19.	RP6340- NPVR-19	rf3rf3/Rf4Rf4	Partial Restorer
20.	RP6340- NPVR-20	rf3rf3/Rf4Rf4	Partial Restorer
21.	RP6340- NPVR-21	Rf3Rf3/Rf4Rf4	Restorer
22.	RP6340- NPVR-22	rf3rf3/Rf4Rf4	Partial Restorer
23.	RP6340- NPVR-23	Rf3Rf3/Rf4Rf4	Restorer
24.	RP6340- NPVR-24	Rf3Rf3/Rf4Rf4	Restorer
25.	RP6340- NPVR-25	Rf3Rf3/Rf4Rf4	Restorer
26.	RP6340- NPVR-26	Rf3Rf3/Rf4Rf4	Restorer
27.	RP6340- NPVR-27	Rf3Rf3/Rf4Rf4	Restorer
28.	RP6340- NPVR-28	rf3rf3/Rf4Rf4	Partial Restorer
29.	RP6340- NPVR-29	Rf3Rf3/rf4rf4	Partial Maintainer
30.	RP6340- NPVR-30	rf3rf3/Rf4Rf4	Partial Restorer
31.	RP6340- NPVR-31	Rf3Rf3/Rf4Rf4	Restorer
32.	RP6340- NPVR-32	Rf3Rf3/Rf4Rf4	Restorer
33.	RP6340- NPVR-33	rf3rf3/Rf4Rf4	Partial Restorer
34.	RP6340- NPVR-34	Rf3Rf3/Rf4Rf4	Restorer
35.	RP6340- NPVR-35	Rf3Rf3/Rf4Rf4	Restorer
36.	RP6340- NPVR-36	Rf3Rf3/Rf4Rf4	Restorer
37.	RP6340- NPVR-37	rf3rf3/Rf4Rf4	Partial Restorer
38.	RP6340- NPVR-38	rf3rf3/Rf4Rf4	Partial Restorer
39.	RP6340- NPVR-39	Rf3Rf3/rf4rf4	Partial Maintainer
40.	RP6340- NPVR-40	Rf3Rf3/Rf4Rf4	Restorer
41.	RP6340- NPVR-41	Rf3Rf3/rf4rf4	Partial Maintainer
42.	RP6340- NPVR-42	Rf3Rf3/Rf4Rf4	Restorer
43.	RP6340- NPVR-43	rf3rf3/rf4rf4	Maintainer
44.	RP6340- NPVR-44	rf3rf3/rf4rf4	Maintainer
45.	RP6340- NPVR-45	Rf3Rf3/Rf4Rf4	Restorer
46.	RP6340- NPVR-46	rf3rf3/rf4rf4	Maintainer
47.	RP6340- NPVR-47	rf3rf3/Rf4Rf4	Partial Restorer
48.	RP6340- NPVR-48	Rf3Rf3/Rf4Rf4	Restorer
49.	RP6340- NPVR-49	Rf3Rf3/Rf4Rf4	Restorer
50.	RP6340- NPVR-50	Rf3Rf3/Rf4Rf4	Restorer
51.	RP6340- NPVR-51	Rf3Rf3/Rf4Rf4	Restorer
52.	RP6340- NPVR-52	Rf3Rf3/Rf4Rf4	Restorer
53.	RP6340- NPVR-53	rf3rf3/Rf4Rf4	Partial Restorer

S No	Genotype	*Rf3/Rf4*	Restorer/ maintainer
54.	RP6340- NPVR-54	rf3rf3/rf4rf4	Maintainer
55.	RP6340- NPVR-55	rf3rf3/rf4rf4	Maintainer
56.	RP6340- NPVR-56	rf3rf3/Rf4Rf4	Partial Restorer
57.	RP6340- NPVR-57	Rf3Rf3/Rf4Rf4	Restorer
58.	RP6340- NPVR-58	rf3rf3/rf4rf4	Maintainer
59.	RP6340- NPVR-59	Rf3Rf3/rf4rf4	Partial Maintainer
60.	RP6340- NPVR-60	Rf3Rf3/rf4rf4	Partial Maintainer
61.	RP6340- NPVR-61	Rf3Rf3/rf4rf4	Partial Maintainer
62.	RP6340- NPVR-62	rf3rf3/Rf4Rf4	Partial Restorer
63.	RP6340- NPVR-63	Rf3Rf3/Rf4Rf4	Restorer
64.	RP6340- NPVR-64	rf3rf3/rf4rf4	Maintainer
65.	RP6340- NPVR-65	Rf3Rf3/Rf4Rf4	Restorer
66.	RP6340- NPVR-66	Rf3Rf3/Rf4Rf4	Restorer
67.	BK-49-77 (Check)	Rf3Rf3/Rf4Rf4	Restorer
68.	IR79156B (Check)	rf3rf3/rf4rf4	Maintainer
69.	APMS-6B (Check)	rf3rf3/rf4rf4	Maintainer
70.	RPHR-1005 (Check)	Rf3Rf3/Rf4Rf4	Restorer
71.	KMR-3R	Rf3Rf3/Rf4Rf4	Restorer
72.	Vandana NIL	rf3rf3/rf4rf4	Maintainer

Genotypes were identified as restorers *(Rf3Rf3/ Rf4Rf4*), partial restorers (*rf3rf3/Rf4Rf4)*, partial maintainers (*Rf3Rf3/rf4rf4)*, and maintainers (*rf3rf3/ rf4rf4)* based on the existence of specific allele band. The amplification of *Rf3 & Rf4* gene-specific/linked markers showed a noteworthy difference in their allelic pattern and represented in **Figures 1 & 2.** Of the total, 42 homozygous plants were identified as positive for fertility restoring gene Rf3 by carrying a single dominant gene (Rf3Rf3/rf4rf4), and a total of 48 genotypes were identified as positive for Rf4 single dominant gene (*rf3rf3/Rf4Rf4)* using gene-specific/linked markers represented in **Table 1**. 32 genotypes were identified as positive (restorers) for both *Rf3* and *Rf4* fertility restorer genes by carrying both dominant non-allelic combinations (*Rf3Rf3/ Rf4Rf4)*. The number of genotypes used for fertility restoration using gene-specific/linked markers in BC_2F_4 generation is provided in **Table 2.**

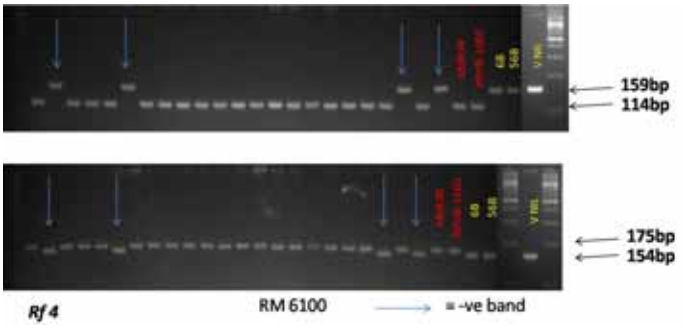

Figure 1: Showing the presence and absence of *Rf4* gene in BILs

Figure 2: Showing the presence and absence of Rf3 gene in BILs

The result out turned in the experiment was skewed by their distribution of allelic frequencies. The presence of *Rf3* (*Rf3Rf3/rf4rf4*) dominant functional gene alone (13.63%) is relatively less than *Rf4* (*rf3rf3/Rf4Rf4*) gene (22.72%). Both fertility restoring genes *Rf3* & *Rf4 (Rf3Rf3/ Rf4Rf4)* were notched with 48.48% with more efficient reported markers. Traditionally, crossing the test genotypes with CMS lines has been reported as a standard protocol to ensure restorer or maintainer lines (Virmani 1996, Singh *et al.,* 2022), which is tedious, laborious, and time-consuming. Hence, molecular analysis of drought-tolerant BILs for the presence of *Rf3* and *Rf4* genes was espoused as a primary selection criterion in this current experiment and can help to reduce number of test crosses for final hybrid development programme.

Revathi *et al.,* (2013) evaluated and reported that 85–92% of efficiency would be there with tightly linked markers of *Rf3* and *Rf4* genes for fertility restoration, and *Rf3Rf3/rf4rf4* genotypes mainly behave as partial maintainers or partial restorers (less than 30% fertility). In the same way, *rf3rf3/Rf4Rf4* genotypes were partial or effective restorers (up to less than 70% fertility). However, double dominant genotypes (*Rf3Rf3/Rf4Rf4*) appeared with greater fertility restoration than the single *Rf3* or *Rf4* genotypes (Katara *et al.,* 2017). Based on the molecular survey for fertility restoration, the results revealed that thirteen genotypes with the absence of *Rf3* and *Rf4* (*rf3rf3/rf4rf4)* allelic combinations were identified

maintainers, and they were categorized in group-1. Nine genotypes with only one *Rf3* dominant allele (*Rf3Rf3/rf4rf4*) were nominated as partial maintainers and categorized as group-2. Of them, 15 genotypes were identified as partial restorers or effective restorers with a single *Rf4* functional dominant allele (*rf3rf3/Rf4Rf4*) and categorized as group-3. Of the total, 35 genotypes were identified as restorers with *Rf3* and *Rf4* dominant fertility restoration allelic combination (*Rf3Rf3/Rf4Rf4*) and were categorized as group-4 (**Figure 3**). Of the identified restorers, ten promising and phenotypically desirable (RP 6340 NPVR1, RP6340 NPVR3, RP6340 NPVR10, RP 6340 NPVR24, RP6340 NPVR27, RP 6340 NPVR32, RP 6340 NPVR48, RP 6340 NPVR52, RP 6340 NRR5 and RP6340 NRR11) restorer lines with qDTY 12.1 and 1.1 in combination or individually were utilized for station trial and on AICRIP trials.

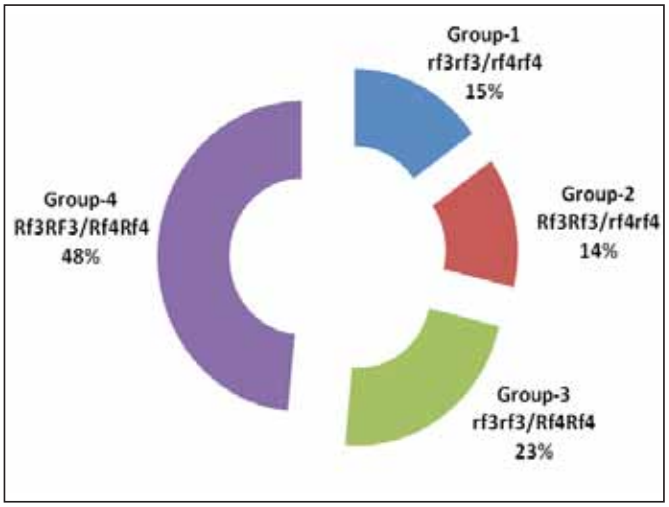

Figure 3: Frequency distribution of Rf3 and Rf4 allelic combination in BILs

Based on a molecular survey undertaken, the identified maintainers can be utilized in the development of a new CMS line for the drought-prone lowland ecology as the genotypes possessing drought-tolerant qDTYs as well as normal irrigated ecology. Besides, identified restorers *viz.*, RP6340 NPVR1 and RP6340 NPVR32 possessing qDTY12.1 and 1.1 with fertility restoration genes (Rf3 and Rf4) may be effectively used as genetic stocks for drought-tolerant parental lines. And also to develop new hybrids in hybrid rice improvement programs for drought-prone lowland environments.

Conclusions

To fulfill the future demand for rice grain, research needs to be intensified in breeding for unfavorable ecologies as an alternative method to substantiate the yield plateauing in rice production. Among all the options available for yield enhancement, exploitation of heterosis through hybrid rice technology is the most feasible one. From this study, a remarkable set of drought tolerant restorers were identified with the help of promising gene-linked/specific markers. This is first of its kind in improvement of restorers and useful in further development of hybrids for drought prone lowland and upland ecologies.

Acknowledgement

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ORIGINAL RESEARCH ARTICLE

Genetics of pollen fertility restoration trait in rice (*Oryza sativa* L.) for wild abortive cytoplasmic male sterility system

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Abstract

Two crosses in F_2 generation *viz.,* APMS 8A × RM 141-91-11-2-1-1 and APMS 6A × RM 71-42-2-1-1 used to study the genetics of fertility restoration, revealed that one major gene governs the fertility restoration, with dominant monogenic gene action. The crosses APMS 8A × RM 141-91-11-2-1-1 and APMS 6A × RM 71-42-2-1-1 segregated in the ratio of 3:1 in F_2 generation, indicating the presence of single independently segregating dominant gene governing the fertility restoration in both the restorers under study.

Keywords: Genetics, Fertility restoration, Gene action, Rice, BSA

Introduction

Rice (*Oryza sativa* L. 2n = 2x = 24, Family: *Gramineae*) is the most important cereal food crop of our country and occupies about 24 per cent of gross cropped area of the country. In India, rice is cultivated over an area of 43.79 m ha with a production of 112.91 m tonnes of milled rice and productivity of 3520 kg ha^{-1}. In Andhra Pradesh area, production and productivity of rice are 22.08 lakh ha, 123.52 lakh tones of milled rice and 5593 kg ha^{-1}, respectively (Statistical Abstracts of AP, 2019). Among the various approaches to improve the yield threshold of rice, exploitation of hybrid vigor is considered to be the most feasible and readily practicable. China pioneered hybrid rice research in 1970's and demonstrated 20-30% yield advantage over conventional varieties. The hybrids grown in India, China, Vietnam, Bangladesh and other countries are based on *indica* rice sources which on an average show a standard heterosis of 15-20% in commercial cultivation. It has been demonstrated very clearly on large scale that hybrids give 15-20% increased yield over the highest yielding varieties under the similar growing conditions by using 'Wild Abortive' (WA) cytoplasmic male sterility (Hossain *et al.,* 2010). High yield potential of CMS derived F_1 hybrids depends upon their high pollen and spikelet fertility which is determined by the number and mode of action of restorer genes present in the restorer parent.

The commercial use of cytoplasmic genetic male sterility (CGMS) is possible only when effective restorers are identified. The identification of restorers for different cytoplasmic male sterile sources will increase cytoplasmic diversification and will help in the development of hybrids with greater adaptability and reduced vulnerability to different pests and diseases. The fertility of CMS system can be restored by nuclear restorer genes, which need to be identified before implementing the production of hybrid rice. Fertility restorer alleles (*Rf*s) are always tightly evolved with cytoplasmic male sterility (CMS) during plant evolution. Fertility restoration is reported to be controlled by two major genes *Rf3* and *Rf4* which are on chromosomes 1 and 10 respectively (Zhang *et al.,* 1997). To study the inheritance of fertility restoration the main three indexes (percentage of fertile pollen, bagged seed setting and opening seed-setting) are often used as the criteria to evaluate fertility restoration (Li *et al.,* 2007). The present investigation was undertaken to understand the genetics of fertility restoration of CMS lines of 'WA' cytoplasm by using two F_2 crosses.

Materials and Methods

Experimental material of the present investigation comprised of two segregating populations in F_2 generation derived from test crosses APMS 8A × RM 141-91-11-2-1-1 and APMS 6A × RM 71-42-2-1-1. The pollen fertility and spikelet fertility of these restorers are 92.41 % & 90.12 % in APMS 8A × RM 141-91-11-2-1-1 and 96.26 % & 91.28 % in APMS 6A × RM 71-42-2-1-1 in F_1 generation. Both the crosses were sown with a spacing of 20 × 15 cm in paired rows during *kharif,* 2016 at Regional Agricultural Research Station, Maruteru, West Godavari district of Andhra Pradesh. All the recommended agronomic practices were followed for a good crop stand.

Fertility restoration in the restorers was identified by pollen sterility study. In both the populations 100 plants were selected in each population and 15 to 20 spikelets from just emerged panicles of each plant were collected in a vial containing 70% ethanol for conducting pollen sterility studies. A glass slide was taken and a drop of 1% iodine potassium iodide (IKI) stain was taken on slide. All the anthers from at least 6 spikelets are taken out with the help of a forceps and placed in the stain. These are gently crushed by using a needle to release the pollen grains. After removing the debris, a cover slip is placed and the slide is observed for the number of fertile and sterile pollen. The entire slide is scanned under microscope and pollen sterility count is taken in 3 random fields.

The pollen grains are classified based on their shape, size, and extent of staining. Fully, dark stained pollen grains were the fertile one whereas unstained pollen grains were sterile. Plants were classified in to different fertility sterility groups as - pollen sterility per cent between 0-20 were grouped as fully fertile, 21-30 per cent as fertile, 31-70 per cent as partially fertile, 71-90 per cent partially sterile, 90-99 per cent as sterile and 100 percent pollen sterility as completely sterile plants (Virmani *et al*, 1997). The goodness of fit for various Mendelian genetic ratios in F_2 generation was tested using the Chi-square Analysis (Pearson, 1901).

Results and Discussion

In the present study, the inheritance of fertility restoration in the crosses APMS 8A × RM 141-91-11-2-1-1 and APMS 6A × RM 71-42-2-1-1, revealed F_2 segregation ratio of 3 (fertile): 1 (sterile), indicating that the restorers *viz.,* RM 141-91-11-2-1-1 and RM 71-42-2-1-1 carry single independently segregating dominant gene for fertility restoration. The Chi square test confirmed the goodness of fit of 3 fertile:1 sterile ratio in both the populations indicating that the fertility restoration in the restorer lines under study was governed by single dominant monogenic gene.

Genetics of fertility restoration by chi square analysis

Population – I (APMS 8A × RM 141-91-11-2-1-1)

Pollen sterility was studied in 100 F_2 plants in the population APMS 8A × RM 141-91-11-2-1-1. Based on the pollen sterility (%), 83 fertile plants and 17 sterile plants were observed which approximately fits in 3 (fertile): 1 (sterile) ratio, which is a typical case of dominant monogenic inheritance **(Table 1).**

Population – II (APMS 6A × RM 71-42-2-1-1)

Pollen sterility was studied in 100 F_2 plants in the population APMS 6A × RM 71-42 2-1-1. Based on the pollen sterility (%), 71 fertile plants and 29 sterile plants were observed which approximately fits in 3 (fertile): 1 (sterile) ratio, which is a typical case of dominant monogenic inheritance **(Table 2).**

Table 1 Chi-square test for goodness of fit in F_2 cross APMS 8A × RM 141-91-11-2-1-1.

S. No	Cross	Phenotype	Frequency	Observed (O)	Expected (E)	D (O-E)	D^2 $(O-E)^2$	$\chi^2=D^2/E$
1	APMS 8A x RM 141-91-11-2-1-1	Fertile	¾	83	75	8	64	0.85
		Sterile	¼	17	25	-8	64	2.56

$\chi^2=3.41$

Table 2 Chi-square test for goodness of fit in F_2 cross APMS 6A × RM 71-42-2-1-1.

S. No.	Cross	Phenotype	Frequency	Observed (O)	Expected (E)	D (O-E)	D^2 $(O-E)^2$	$\chi^2=D^2/E$
1	APMS 6A x RM 71-42-2-1-1	Fertile	¾	71	75	-4	16	0.21
		Sterile	¼	29	25	4	16	0.64

$\chi^2=0.85$

Similar findings were reported by Anandakumar and Subramaniam (1992), Gyan *et al.* (2003), Ahmadikhah *et al.* (2007) and Singh *et al.* (2015). In both the populations the calculated χ^2 value at 0.05 % probability is less than the table value of 3.841 at 1 degrees of freedom and the difference is non-significant between the observed values and expected values for the above cross. The Chi square test confirmed the goodness of fit of 3 fertile:1 sterile ratio in both the populations indicating that the fertility restoration in the restorer lines under study was governed by single dominant monogenic gene.

A total of 21 *Rf3* (10 markers) and *Rf4* (11 markers) gene linked markers were used for parental polymorphism among the parents of both F_2 crosses APMS 8A × RM 141-91-11-2-1-1 and APMS 6A × RM 71-42-2-1-1. Among ten *Rf3* gene linked markers, five markers *viz.*, RM 10287, RM 10305, DRRM *Rf3-5*, DRRM *Rf3-10* and RMS-SF21-1 showed polymorphism among parents in F_2 cross APMS 6A × RM 71-42-2-1-1 (**Figure 1**). Among eleven *Rf4* gene linked markers, one marker *viz.*, RM 6100 showed polymorphism between parents in F_2 cross APMS 8A × RM 141-91-11-2-1-1. Confirmation of markers linked to fertility restoration genes in the populations under study was done by bulk segregant analysis.

Sterile bulks and fertile bulks derived from F_2 population of APMS 8A and RM 141-91-11-2-1-1 did not show any polymorphism for *Rf4* gene linked marker RM 6100. In the population, APMS 6A x RM 71-42-2-1-1 polymorphic markers linked to *Rf3* genes *viz.*, RM 10287, RM 10305, DRRM *Rf3-5*, DRRM *Rf3-10* and RMS-SF21-1 showed no polymorphism between fertile and sterile bulks.

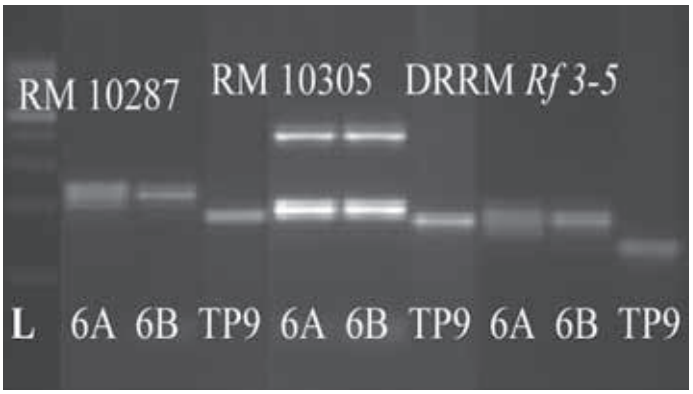

Figure 1: Parental polymorphism using *Rf3* gene linked markers RM 10287, RM 10305 and DRRM *Rf3-5*

(L: 100 bp Ladder, 6A: APMS 6A, 6B: APMS 6B, TP-9: RM 71-42-2-1-1)

The expected marker genotypic segregation ratio within the sterile extremes should be 1 (fertile band):1 (sterile band). But in the current study no such genotypic segregation ratio has been observed. No linkage has been detected between the marker loci and *Rf* gene in these populations. However, chi square analysis of phenotypic data clearly showed the marker loci to be linked strongly with *Rf* genes.

Conclusions

Two F_2 population *viz.*, APMS 8A × RM141-91-11-2-1-1 and APMS 6A × RM71-42-2-1-1 were used in the study of genetics of fertility-restoration. Confirmation of genetic studies in segregating populations was done for pollen sterility (%) in both the populations using Chi square test. Chi square values obtained were χ^2 = 3.41 (APMS 8A × RM141-91-11-2-1-1) and χ^2 = 0.85 (APMS 6A × RM71-42-2-1-1) with 5% level of significance (1 df =3.84) leading to conclusion that both the F_2 populations were segregating in the ratio

of 3 (fertile):1 (sterile), indicating single dominant gene governing the fertility restoration.

Through bulked segregant analysis it was found that no co-segregation of banding pattern of fertile parents and fertile bulks and sterile parents and sterile bulks. It clearly indicated that neither of *Rf3* or *Rf4* genes is contributing for fertility restoration and polymorphic markers used were not tightly linked to *Rf* genes in the two restorers under study. Further screening for presence of fertility restoration genes other than *Rf3* and *Rf4* has to be done to confirm the genes linked to fertility restoration in both the restorers under study.

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ORIGINAL RESEARCH ARTICLE

Grain and cooking quality analysis in heat-tolerant QTL introgressed restorer of hybrid rice

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Abstract

Rice grain yield and quality are affected by the high temperature stress to a large extent. Four promising backcross introgression lines (BC_2F_8) of KMR-3R/N22 carrying qHTSF1.1, qHTSF4.1 and HT Score QTLs were evaluated for high-temperature induced rice grain and cooking quality during *Rabi* 2021. Late-sown staggered planting was adopted to ensure uniform high-temperature stress on the improved restorers along with the parents used, which resulted in significant yield loss and grain quality degradation due to high-temperature stress. For cooking quality metrics, ANOVA showed significant variation among genotypes (G), treatments (T), and G×T. Among the backcross introgression lines (BILs), RP6338-9 possesses qHTSF4.1 noted to be promising for grain quality traits under high temperature stress.

Keywords: Hybrid rice, KMR-3R, high-temperature stress, grain quality and cooking traits.

Introduction

Rice (*Oryza sativa* L) is a staple cereal consumed by more than one half of the world's population and providing 35-80 % of total calorie intake (Wassmann *et al.,* 2009). The episodes of high-temperature and altered precipitation levels affect the global food grain security (IPCC 2014). An increase of 1°C of global mean temperature can reduce the yields of maize (7.4%), wheat (6%), rice (3.2%) and soybean (3.1%) which affords two-thirds of human caloric intake globally (Zhao *et al.,* 2017). By the end of the century, the global mean temperature is anticipated to rise by 2.5°C to 5.8°C, and extreme temperatures may be more common (IPCC 2014). Breeding rice for high-temperature stress is a major priority area of rice research in order to sustain global rice production and meet the expected need of the ever-growing population. In comparison to varieties, hybrid rice technology provides more stability in meeting food grain production goals. Hybrid technology's success is attributed to yield heterosis, and hybrids account for a large portion of global rice yield. Hybrid rice occupies 3-4 million hectares in India and contributes 3–4 million tonnes of rice production (Senguttuvel *et al.,* 2019). Despite succumbing to numerous biotic and abiotic stresses, rice hybrids showed a 10-13 percent yield advantage over popular varieties (Virmani 2003; Serraj *et al.,* 2009; Villa *et al.,* 2012). Madan (2012) observed that the yield advantage of hybrids is being affected by the reduced seed setting rate under high-temperatures (38°C) when compared to normal temperatures (29°C - 35°C). Similarly, Zhou (2009) and Hu (2012) also observed the sensitivity of hybrid rice towards high-temperature stress. Rice hybrid seed production is often followed in India during *Rabi* (Dry) season due to dry weather and absence of precipitation. The high-temperature at the anthesis stage affects the pollen viability (Song *et al.,* 2001; Wassmann *et al.,* 2007) anther dehiscence (Matsui *et al.,* 2000, 2005), pollen tube elongation and stigma receptivity which eventually results in low spikelet fertility rate, reduced grain yield and quality (Satake and Yoshida 1978). To develop heat tolerant quality

rice hybrids, both the parental lines should possess high level of temperature tolerance (Guan-fu *et al.,* 2015) and this strategy showed significant association in the three-line hybrid rice system (Kuang *et al.,* 2002; Gong *et al.,* 2008). Apart from yield, grain quality also plays a major role in the acceptance of the rice variety or hybrid. Based on this background, the restorer line KMR-3R was introgressed with qHTSF1.1 and qHTSF4.1 for heat tolerance through Marker-Assisted Backcross Breeding (MABB) approach and promising backcross introgression lines (BILs) with heat-tolerance were developed (Jaldhani *et al.,* 2021). In this study, the promising heat-tolerant BILs and their parental lines were evaluated for grain quality under ambient and high-temperature stress.

Materials and Methods

Plant material

Four BILs (BC_2F_8) namely RP6338-9 (qHTSF4.1), RP6338-28 (qHTSF1.1), RP6338-48 (qHTSF4.1) and RP6338-66 (HT Score) derived from the KMR-3R/Nagina22 (Jaldhani *et al.,* 2021) were used in this study. These BILs were introgressed with heat-tolerant QTLs and native fertility restoration. KMR-3R is a promising restorer line and Nagina22 (N22) is a potential genetic resource for heat tolerance. N22 is widely employed in heat-tolerance studies (Senguttuvel *et al.,* 2020; Jaldhani *et al.,* 2021).

High-temperature treatment

The study was carried out at the Indian Institute of Rice Research farm, Hyderabad, India (17.53° 19'N latitude and 78.27° 29' E longitude, 542.7 MSL, with a mean temperature of 31.2°C and mean annual precipitation of 988.3 mm) during Dry (*Rabi*) season 2021. The high-temperature stress was imposed by following late sown method in dry season (Senguttuvel *et al.,* 2020). The experiments were replicated thrice in a randomized complete block design (RCBD) with same sets of genotypes in two experiments under normal and late sown method. Standard agronomic practices and integrated pest management was followed throughout the experiment. The weather data recorded throughout the crop duration was indicated in **Figure 1** and **Table 1**.

Grain quality analysis

At physiological maturity, each paddy sample was harvested, thoroughly cleaned from impurities and dried under shade till the moisture content reached to 15%. All the samples were stored under ambient conditions and quality analysis – Milling (M%), Hulling (H%) and Head rice recovery (HRR%), Amylose content (AC), Gel consistency (GC), Kernal length before cooking (KLBC), Kernal breadth before cooking (KBBC), Kernal length after cooking (KLAC), Kernal breadth after cooking (KBAC) were performed at the end of three months aging (Juliano, 1971; Cagampang, 1973).

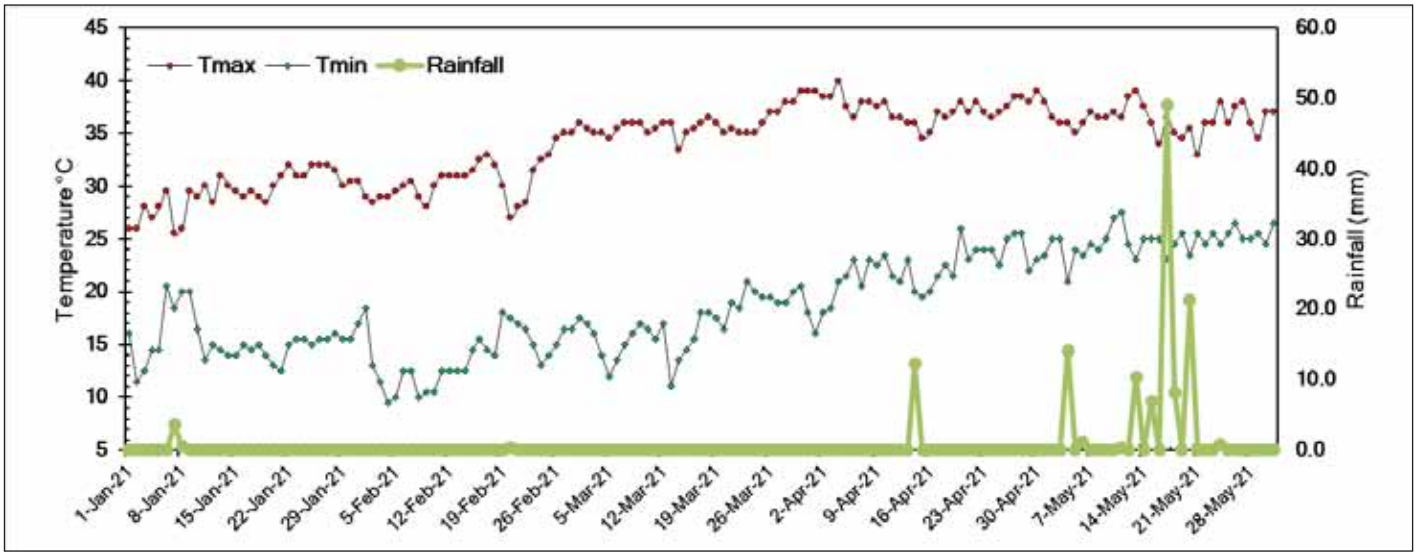

Figure 1: Maximum and minimum temperature (°C), rainfall (mm) during the crop growing period during *Rabi* 2021.

Table 1. Monthly meteorological data recorded during *Rabi* 2021.

Season / Month	Temperature (°C)		Relative Humidity (%)		Rainfall (mm)	Rainy Days	Sun Shine (hours)	Wind Speed (Km h^{-1})
	MAX	MIN	I	II				
January	29.5	15.3	95.0	76.0	4.2	2	7.1	3.0
February	30.7	13.8	88.1	41	0.4	1	8.5	3.5
March	35.9	17.1	80.6	30.0	0.0	0	8.0	3.6
April	37.4	22.1	81.1	47.0	12.2	2	7.5	4.4
May	39.6	24.8	89.1	51.1	112.6	6	8.3	6.1
Mean	34.6	18.6	86.78	49.02	-	-	7.88	4.12
Total	-	-	-	-	129.4	11	-	-

Statistical analysis

The data collected on grain quality was analyzed statistically using two-way analysis of variance (ANOVA) (Gomez and Gomez, 2010) in software *Statistix 8.1* (Analytical software, 2003). The derived data from the ANOVA, represented with standard errors of mean (SE) and Tukey's honest significant difference (HSD) (P = 0.05) between treatments and genotypes.

Results and Discussion

Heat tolerant QTL introgressed lines were evaluated in the present study to understand the effect of high temperature on grain quality. As grain quality plays a major role in the acceptance of rice variety, it is imperative to analyze the influence of high temperature on grain quality in comparison with the same variety cultivated under normal conditions (ambient/control). The results of analysis of variance (ANOVA) indicates significant variation among genotypes (G), treatment (T) and G × T for cooking quality parameters (**Table 2** and **Table 3**). Marginal variation (<0.5%) in AC % was observed between RP6338-9 & RP6338-66, whereas AC increased in other samples at high temperature stress except RP6338-28.

Table 2. Mean squares for AC, GC, GLBC, GBBC, GLAC and GBAC in ambient and high temperature methods.

Source	df	AC	GC	GLBC	GBBC	GLAC	GBAC
Rep	2	1.102	39.69	0.004	0.009	0.05	0.003
Genotype (G)	5	46.82***	3591.04***	0.107***	0.027***	0.78***	0.41***
Treatment (T)	1	14.85***	1877.78***	0.001	0.08***	0.20*	0.071***
G × T	5	12.53***	279.04***	0.015*	0.014 **	0.25 ***	0.022 ***
Error	22	0.75	25.39	0.004	0.003	0.03	0.003

df-Degrees of freedom; *P < 0.05; **P < 0.01; ***P<0.001.

Table 3. Effect of high-temperature stress on AC, GC, GLBC, GBBC, GLAC and GBAC.

Trait	Mean		Difference	Grand Mean	CV (%)	Tukey HSD (P<0.05)		
	Control	HT				Genotype (G)	Treatment (T)	G × T
AC	27.79	29.07	1.3	28.43	3.04	1.55	0.60	2.57
GC	57.44	43	-14.4	50.22	10.03	9.06	3.49	14.96
GLBC	4.68	4.67	0.0	4.67	1.39	0.12	0.04	0.19
GBBC	2.14	2.05	-0.1	2.10	2.68	0.10	0.04	0.17
GLAC	7.78	7.63	-0.2	7.70	2.20	0.30	0.12	0.50
GBAC	2.62	2.71	0.1	2.67	1.99	0.10	0.04	0.16

Change in amylose content from intermediate under controlled conditions to high under elevated temperature was observed in the parents (N22 & KMR 3) and vice-versa in the case of RP6338-28 (**Figure 2**). High-temperature affected amylose content, amylopectin chain length, immature kernels, chalkiness, kernel dimensions, and fissuring in rice (Wassmann *et al.,* 2009). However, amylose and amylopectin contents were similar in high-temperature than in the ambient condition, which indicates that variation in these components at high-temperature varies among the genotypes/varieties. This underlines the need to verify the fitness of every released variety or developed lines at high-temperature (Jaldhani *et al.,* 2022). The GC % was same in both control and treatment for N22 and RP6338-48, however the values decreased significantly for other samples in control and treatment. Before cooking, there were no significant differences in grain length or grain breadth between the samples under high temperature stress and control conditions. The ratio of grain length to grain breadth indicates that the ILs are of medium bold grain type. Similarly, negligible variations were observed under treatment and control conditions for grain breadth after cooking and grain length after cooking except for RP6338-9 which noted around 0.8 mm more under control conditions.

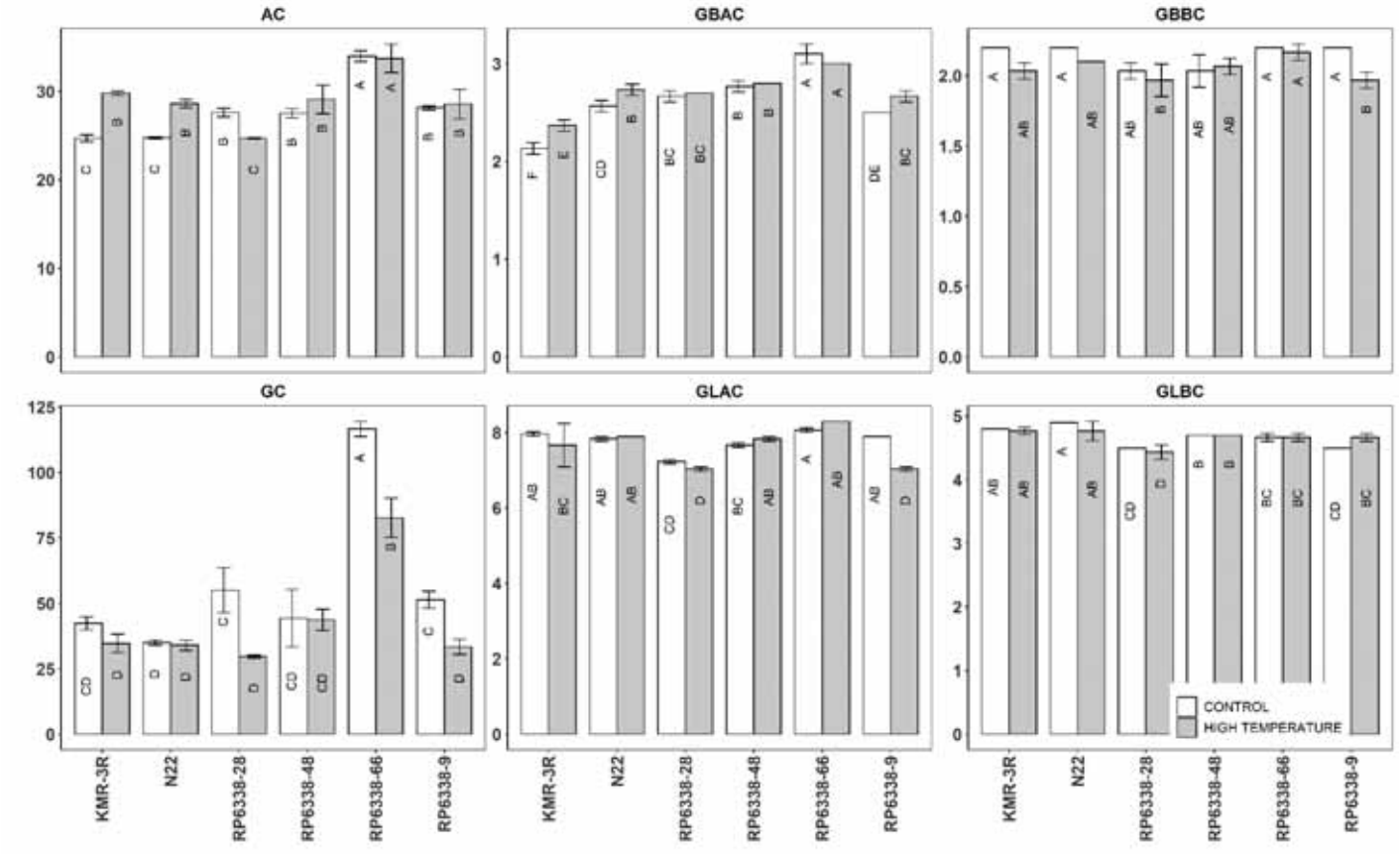

Figure 2: Effect of high-temperature stress on grain quality traits in ILs and parents.

Compared with controlled conditions, marginal variations were observed for Hulling percent and Milling percentage at high-temperature and significant for head rice recovery (**Table 4).** Among the four BILs, only two (RP6338-9 and RP6338-28) noted desired HRR% of ≥60% under controlled conditions. Only one BIL noted (RP6338-9) desired HRR% under high temperature conditions. The reduction in HRR% is largely due to decrease in the density of chalky rice grain that are formed due to high night air temperature. Chalkiness is the opaque region of the brown or polished rice grain. The proportion of chalkiness was high in rice varieties cultivated at high-temperature than the same varieties cultivated

at ambient temperature. Variation in grain appearance itself was noticed among the varieties/genotypes. Variation in individual grain weight was also observed under high-temperature (Jaldhani *et al.,* 2022).

Table 4. Effect of high-temperature stress on H%, M% and HRR

	H%		M%		HRR	
	Control	HT	Control	HT	Control	HT
N22	73.3	75.6	63.5	68.1	59.1	36.3
KMR-3R	75.1	74.5	65.5	66.2	58.6	47.6
RP6338-9	75.1	74.4	66.9	68.1	65.5	59.3
RP6338-28	74.3	74.3	64.3	68.8	62.7	45.4
RP6338-48	72	73.3	63.4	65.5	49.4	48.4
RP6338-66	77.2	76.5	62.4	61.9	39.7	34.5

KMR 3 and N22 had intermediate AC (20-25%) under control conditions, whereas the four ILs exhibited high AC (>25%). Hard GC (35 mm) was observed in N22, soft GC (95 mm) was recorded in RP6338-66, and medium GC was recorded in the other three ILs and KMR 3 (41 to 60 mm). Hybrids with intermediate AC or high AC with soft GC are recommended for release under AICRIP testing, and RP6338-66 with high AC and soft GC is a potential heat tolerant restorer line with desired grain cooking quality. Overall, RP6338-9 possesses qHTSF4.1 and noted to be promising for grain quality under high temperature stress.

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ORIGINAL RESEARCH ARTICLE

Genetic diversity analysis for yield and yield components in rice

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Abstract

The present investigation was carried out to explore the extent of divergence in 55 rice germplasm accessions for twelve characters during *Kharif*, 2019. In D^2 analysis, the 55 genotypes were grouped into fourteen clusters. The clustering pattern indicated that there was no parallelism between genetic diversity and geographical origin as the genotypes of the same origin were included in different clusters and *vice versa*. The maximum inter cluster distance was found between cluster IX and cluster XIII (1313.049) followed by cluster X and cluster XIII (1152.033), cluster XII and cluster XIII (1039.108), cluster III and cluster XIII (1015.310) and cluster I and cluster XIII (978.369). Genetically distant parents from these clusters could be able to produce higher heterosis in progenies on hybridization. Single plant yield (43.29%) followed by grain length (12.99%), 100 grain weight (12.12%) grain width (11.85%) and days to 50% flowering (11.85%) were the major contributors towards the total genetic divergence among the genotypes studied. Selection could be made based on grain yield per plant, hundred grain weight and days to 50% flowering for the progenies identified.

Keywords: Rice, germplasm, genetic diversity, yield & yield components

Introduction

Rice is cultivated in an area of 43.66 million ha with a production of 118.87 million tonnes and a productivity of 2.7 tonnes per hectare (Indiastat, 2020). It contributes to about 40% of the total food grain production. Rice is a source of livelihood for 120-150 million rural households. So, rice is life for Indians as it plays a vital role in diet, economy, employment, culture and history. Even though India had attained self sufficiency in rice, keeping in view the continuously growing population it is estimated that about 140Mt of rice would be required by 2050 to ensure the food and livelihood security of the people. To achieve this, rice production has to be increased by about 1.5Mt/year and the productivity to 3.25t/ha by 2050 (Pathak *et al.,* 2018). It is a challenging task for the rice breeders as yield in rice has attained a plateau. To break the yield barrier in rice, it is imperative to broaden the genetic base of rice cultivars for which existence of genetic variability and diversity in a population is a prerequisite (Allard, 1960). Genetically diverse population would aid in the selection of diverse parents which could be utilized for the development of high yielding rice varieties superior to existing varieties. The germplasm with inherent genetic value and wide variability and diversity would be a potent source for the genetic improvement of rice. Hence, the information on genetic diversity in germplasm collections would immensely help the plant breeder in selection of diverse parents for crossing programmes so as to obtain highly heterotic F_1 and broad spectrum of variability in subsequent segregating generations (Vivekanandan and Subramanian, 1993). Multivariate analysis like Mahalanobis D^2 statistic provides useful tool for measuring the genetic diversity in a given population with respect to different characters considered together. Hence, the present study was undertaken to assess the nature and magnitude of genetic divergence present in rice germplasm for yield, yield attributes and grain characters.

Materials and Methods

The experimental material for the present study comprised of 54 numbers of rice germplasm collection maintained as T numbers at Department of Rice and check CO(R)50. Details of the rice germplasm collections and check used in the present study along with its pedigree details are given in **Table.1** The fifty-five germplasm collections including check were raised in Randomized Block design (RBD) with two replications at Department of Rice, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University and Coimbatore during *Kharif*, 2019. Each genotype was raised in two rows with each row of 3 meters' length. Row to row and plant to plant spacing was maintained at 20 x 20 cm. All the recommended agronomic practices were followed. Data on all yield and yield attributing characters (except days to flowering) *viz*., plant height, leaf length, leaf width, panicle length, number of tillers per plant, number of productive tillers per plant, 100-grain weight, grain length, grain breadth, L/B ratio and single plant yield were recorded in five competitive plants selected at random for each genotype in each replication. Days to 50 percent flowering was recorded on plot basis. Data recorded for twelve quantitative traits were analyzed using GENRES software. Mahalanobis D^2 analysis (1949) was used for working out the genetic distance between the genotypes whereas grouping of varieties into clusters was done using Tochers method suggested by Rao (1952).

Table. 1 Details of rice germplasm accessions used along with pedigree

S.No	Genotypes	Pedigree	Origin
1.	T15	*Chani Brara*	Indigenous landrace of Travancore
2.	T23	*Safeda*	Exotic collection from central province
3.	T26	*Bhangara Bellu Bhatta*	Indigenous landrace of Mysore
4.	T54	*Ratna Chvdi*	Indigenous landrace of Ganjam,Orissa
5.	T65	Chennangi	Indigenous landrace of Salem, Tamil Nadu
6.	T71	*Vellai Gundu Samba*	Indigenous land race of South Arcot, Tamil Nadu
7.	T73	*Godumani Samba*	Indigenous landrace of South Arcot, Tamil Nadu
8.	T75	*Norungan*	Indigenous landrace of Tamil Nadu
9.	T089	*Chintamani*	Indigenous landrace of Tamil Nadu
10.	T146	*Ayyan Samba*	Indigenous landrace of Gobi, Tamil Nadu
11.	T153	*Nyan* 5	An exotic collection from Burma
12.	T181	*Arupatham Vellai*	Indigenous landrace of Tirunelveli
13.	T186	Seevan Samba	Indigenous landrace of Tirunelveli
14.	T209	*Bobbili Ganti*	Indigenous landrace of Vizakapattinam
15.	T227	*Vayan hill paddy*	Indigenous landrace of Malabar
16.	T261	*Mangam Samba*	Indigenous landrace of South Arcot
17.	T268	*Myosan*	Exotic collection from Burma
18.	T271	*Kukka Nyan B.60*	Exotic collection from Burma
19.	T272	*Kukka Nyan 1*	Exotic collection from Burma
20.	T273	*Kunwah B.61*	Exotic collection from Burma
21.	T275	*Naseingale –C.* 27	Exotic collection from Burma
22.	T277	*Matawaka –C.* 89	Exotic collection from Burma

S.No	Genotypes	Pedigree	Origin
23.	T279	*Palak E.19*	Exotic collection from Burma
24.	T307	*Matawaka –C.* 100	Exotic collection from Burma
25.	T309	*Lawangai*	Indigenous collection from north West frontier provinces
26.	T311	Double grain paddy	Indigenous landrace from Pune
27.	T317	*Periavellai Red rice*	Exotic collection from Ceylon
28.	T341	*Poongar*	Indigenous collection from Tamil Nadu
29.	T355	*Nallakonamani*	Indigenous collection from Vizhakapattinam
30.	T356	*Dasaradhinu* an indigenous collection from Ganjam, Orissa	Indigenous collection from Ganjam, Orissa
31.	T374	*Varigarudan Samba*	Indigenous landrace of Tamil Nadu
32.	T378	*Forhina*	Exotic collection from USA
33.	T420	*Vellai Kuruvai*	Indigenous landrace of Tamil Nadu
34.	T422	*Rangoon Samba*	Indigenous landrace of Tamil Nadu
35.	T432	*Thogai Samba*	Indigenous landrace of Tamil Nadu
36.	T433	*Maranel*	Indigenous landrace of Tamil Nadu
37.	T541	*Karthigai Samba*	Indigenous landrace of Tamil Nadu
38.	T542	*Sirumani*	Indigenous landrace of Tamil Nadu
39.	T549	*Alther Samba*	Indigenous landrace of Tamil Nadu
40.	T710	*White Amon*	Indigenous landrace from Assam
41.	T716	*Parichak*	Indigenous landrace from Assam
42.	T722	*Lead rice*	Exotic collection from USA
43.	T730	*Larly-Wreight* an exotic collection from Italy	*E*xotic collection from Italy
44.	T761	*V.Vulgariskom Eat164 F*	Exotic collection from Russia
45.	T777	*Seeraga Samba*	Indigenous landrace of Tamil Nadu
46.	T810	*A*29-16	Exotic collection from Mudon
47.	T812	*Thulasi Vasanai Seeraga Samba*	Indigenous landrace of Tamil Nadu
48.	T854	*Karajano*	Exotic collection from West Africa
49.	T872	*Thengaipoo Samba*	Indigenous landrace of Tamil Nadu
50.	T1001	*Poyongchao*	Exotic collection from China
51.	T1022	*Kothamalli* Samba	Indigenous landrace of Tamil Nadu
52.	T1067	*Thooyamalli*	Indigenous landrace of Tamil Nadu
53.	T1071	*Salem 3*	Indigenous landrace of Tamil Nadu
54.	T1092	*Muthuvellai*	Indigenous landrace of Tamil Nadu
Check			
55.	CO(R)50	CO 43/ADT 38	India

Results and Discussion

The analysis of variance for 12 quantitative characters revealed highly significant differences among the genotypes for all the characters studied (**Table 2).** The results indicated the existence of variation among the genotypes for all the characters under study. Fifty-five genotypes were clustered into 14 clusters based on minimum D^2 values and confirmation of tentative grouping by Tocher's method. Out of fourteen major clusters, cluster XI was the widest with 14 number of genotypes followed by cluster IX (9), clusters XII& XIII (4), cluster XIV (5) and cluster I(3) . Clusters II, Cluster III, Cluster IV, Cluster V, Cluster VI, Cluster VII, VIII and cluster X had the minimum number of 2 genotypes each. Grouping of 55 genotypes in to fourteen clusters itself indicates that the genetic material is divergent enough. The germplasm material used in the present study may serve as a novel source for the selection of the diverse parents for hybridization programmes aimed at isolating desirable segregants for grain yield and other important characters. Presence of substantial amount of genetic divergence in the rice germplasm was earlier reported by Sri Lakshmi *et al.* (2021); Rukmini Devi *et al.*, (2020) and Vishnu Bridha Devi *et al.* (2018).

Distribution of genotypes into various clusters was observed to be at random (**Table 3**). Genotypes originated from same or diverse eco-geographical region were present in different clusters as well as in same cluster. This is in conformity with the report of Kumar *et al.,* (2020). This random grouping may be attributed to the interchange breeding material over locations followed by intensive selection both by natural and human selection for diverse and adaptable gene complexes resulting in genetic drift and consequently increased genetic diversity (Arunachalam and Ram, 1967).

Table. 2 Analysis of variance for grain yield & its component traits and grain characters in rice germplasm

S. No	Source	df	PH	DFF	LL	LW	NT/Plt	ET/Plt	PL	100 GW	GL	GW	L/B ratio	SPY
1.	Repli-cation	1	MSS											
			1.1659	0.4614	2.5943	0.0508	5.1072	5.8069	2.8027	0.0038	0.0005	0.0002	0.0283	4.6715
2.	Geno-type	54	159.6065**	103.0579**	27.5750**	0.1678**	30.6794**	33.3847**	37.0025**	0.2160**	0.237 **	0.0022**	0.5124**	163.5362**
3.	Error	54	12.4773	0.7600	1.0380	0.0113	1.6598	7.8301	2.8855	0.0046	0.0004	0.0001	0.0274	3.4523

PH-Plant height; DFF-Days to 50% flowering; LL-Leaf length; LW-leaf width; NT/Plt Number of tillers per plant; ET/Plt-Number of productive tillers per plant; PL-Panicle length; 100GW-100 grain weight; GL-Grain length; GW-Grain width; L/B ratio-Length/Breadth ratio,100GW-100 grain weight; SPY-Single plant yield *, ** Significant at 1% and 5% levels, respectively

Table. 3 Constitution of D^2 clusters for 55 genotypes of rice

Cluster No.	Number of genotypes	Name of the genotypes
I	3	T15, T422, T590
II	2	T432, T722
III	2	T209, T272
IV	2	T268, T307
V	2	T378, T710
VI	2	T186, T317
VII	2	T181, T273
VIII	2	T341, T1022
IX	9	T23, T26, T54, T65, T71, T75, T73, T374, T854
X	2	T311, T541
XI	14	T146, T153, T227, T261, T271, T275, T279, T309, T355, T356, T420, T433, T542, T1071
XII	4	T1067, T549, T716, T1001
XIII	4	T809, T730, T761, T777
XIV	5	T1092, CO(R)50, T810, T812, T872

Narrow range of genetic variability will be exhibited by the genotypes grouped within a cluster whereas genotypes included in different clusters will show wider variability. The maximum inter cluster distance was found between cluster IX and cluster XIII (1313.049) followed by cluster X and cluster XIII (1152.033), cluster XII and cluster XIII (1039.108), cluster III and cluster XIII (1015.310) and cluster I and cluster XIII (978.369). Greater the genetic distance between two clusters wider the genetic diversity among the genotypes of these clusters. Generally, it is assumed that cross combinations involving the parents belonging to most divergent clusters will manifest maximum amount of heterosis. So, in recombination breeding programme selection of highly divergent superior genotypes from the genetically distant clusters would be of great use in order to get desirable transgressive segregants. Hence, hybridization between the genotypes of cluster IX (T23, T26, T54, T65, T71, T75, T73, T374, T854), cluster X (T311, T541), cluster XII (T1067, T549, T716, T1001), cluster III (T209, T272) with genotypes of cluster XIII (T809, T730, T761, T777) are expected to produce highly heterotic F_1 and broad spectrum of variability in subsequent segregating generations enabling further selection and improvement (Rukmini Devi *et al.,* 2020). The minimum inter cluster distance was observed between cluster IV and VII (67.812) followed by cluster II and cluster VI (82.332), cluster VI and cluster VII (89.089), cluster I and cluster V (110.739), cluster II and cluster VII (111.768) and cluster V and cluster VIII (115.61). So, the genotypes included in them were closely related. The maximum intra cluster distance was exhibited by cluster XIV (852.674) followed by clusters XII (727.193), XI(684.01) , XIII (627.188) and IX (563.403). The minimum intra cluster distance was exhibited by cluster II (35.681) (**Table 4**). To maintain relatively broad genetic base, selection of parents from genetically homogeneous clusters should be strictly avoided. Parallel findings were reported by Nirosha *et al.,* (2016); Mamta Kumari *et al.,* (2016) and Lahari *et al.,* (2017).

Cluster mean analysis revealed wide range of variation for all the traits studied (**Tables 5a&b**). Cluster mean for days to 50% flowering was lowest for cluster X (94.750) and hence germplasm lines in this cluster may be used as a donor for earliness in rice breeding programmes. The highest cluster mean value for leaf length (36.443 cm) and leaf width (1.406cm) was observed in cluster VII and cluster XIV respectively. Hence the genotypes in these clusters would be useful for the improvement of physiological efficiency through hybridization. Regarding number of tillers and productive tillers per plant, cluster III (21.750) had the highest cluster mean value so the germplasm accessions present in this cluster may be used as a donor for the introgression of profuse tillering habit in to the desirable genotypes. Genetic improvement for panicle length could be achieved through hybridization using genotypes possessing long droopy panicles present in clusters IX and XIV due to its highest cluster mean (24.167) for this trait. Genotypes in cluster V (2.320) with high test weight could be excellent donors for the development of bold grain rice varieties. High yielding genotypes can be developed by using elite genotypes present in cluster IV (27.600) as donors in crossing programme. Genotypes with all the desirable traits were not present in any one of the clusters which could be directly selected and utilized. All the minimum and maximum cluster mean values were distributed in relatively distant clusters. Similar kind of results were reported in rice by Muthuramu and Sakthivel (2018); Ranjith *et al., (*2018) and Kavurikalpana *et al.,* (2018). Thus according to the breeding objectives, trait specific lines can be picked out from different clusters and involved in hybridization programs for improvement of the character.

Table 4. Average intra-(in bold) and inter-cluster D^2 distances

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
I	**195.512**	209.165	208.984	535.499	110.739	233.551	411.57	162.197	372.785	127.288	591.790	484.86	978.369	642.725
II		**35.681**	218.546	194.17	217.325	82.332	111.768	134.667	433.217	266.668	366.796	348.730	607.200	425.767
III			**36.85**	283.063	293.746	135.446	262.597	275.542	339.784	152.814	481.279.	540.214	1015.310	528.521
IV				**41.265**	649.751	132.739	67.812	478.449	627.475	568.258	390.953	546.731	713.966	430.703
V					**42.700**	281.895	476.134	115.61	396.471	146.052	644.427	456.66	933.162	706.054
VI						**45.175**	89.089	242.845	379.066	267.818	363.008	373.328	667.009	389.643
VII							**54.017**	341.83	565.555	488.907	362.573	436.227	576.254	391.314
VIII								**57.370**	479.482	174.564	530.560	494.228	701.059	592.232
IX									**563.403**	332.594	762.520	662.177	1313.049	793.155
X										**65.879**	632.169	568.470	1152.033	666.668
XI											**684.01**	711.902	897.179	686.973
XII												**727.193**	1039.108	748.351
XIII													**627.188**	915.583
XIV														**852.674**

Table.5a Cluster mean values of rice genotypes for different yield & yield contributing traits and grain characters

Clusters	Plant height (cm)	Days to 50% flowering (days)	Leaf length (cm)	leaf width (cm)	Number of tillers per plant	Number of productive tillers per plant	Panicle length (cm)
I	93.417	99.167	31.472	1.133	15.528	15.388	21.862
II	95.750	105.000	26.558	1.068	17.625	16.750	22.877
III	90.330	95.500	31.667	1.158	21.750	21.750	22.300
IV	94.583	104.000	31.658	1.260	12.500	12.500	22.458
V	89.585	101.500	29.975	1.217	13.415	12.458	23.000
VI	92.750	102.750	30.458	1.132	16.500	15.250	21.210
VII	92.080	106.500	36.443	1.233	20.417	20.417	24.083
VIII	80.750	101.500	29.608	1.100	13.585	12.417	21.208
IX	88.343	98.167	30.306	1.317	14.463	12.092	24.167
X	83.210	94.750	29.540	1.257	12.500	12.000	21.085
XI	97.738	103.250	32.024	1.297	16.095	15.136	22.607
XII	90.646	107.125	30.171	1.268	15.792	14.416	22.874
XIII	96.250	111.125	36.296	1.335	18.855	17.584	23.667
XIV	89.167	104.000	36.434	1.406	16.667	16.300	24.167

Table.5b Cluster mean values of rice genotypes for different yield & yield contributing traits and grain characters

Clusters	100 grain weight (g)	Grain length (mm)	Grain width (mm)	Length/Breadth ratio	Single plant yield (g)
I	**2.298**	8.12	3.00	2.707	16.200
II	2.243	7.67	3.00	2.557	24.150
III	1.857	6.83	3.00	2.275	18.575
IV	1.773	6.15	3.00	2.055	27.600
V	2.320	8.92	3.00	2.973	12.100
VI	2.090	6.65	2.95	2.287	16.900
VII	1.977	7.00	3.00	2.330	22.075
VIII	2.215	8.57	2.88	2.950	24.175
IX	2.172	7.32	3.11	2.375	14.444
X	2.278	7.58	3.00	2.528	21.150
XI	2.010	7.02	2.94	2.385	24.839
XII	2.280	8.17	3.25	2.557	15.113
XIII	2.079	7.16	2.16	3.474	21.563
XIV	2.078	6.62	2.79	2.673	23.670

Table. 6 Percentage contribution of each character to total genetic divergence

S.No.	Characters	No. of first rank	Percent contribution
1	Single plant yield	643	43.29
2	Grain length	193	12.99
3	100 grain weight	180	12.12
4	Days to 50% flowering	176	11.85
5	Grain width	176	11.85
6	Grain Length/Width ratio	52	3.50
7	Panicle length	41	2.76
8	Plant height	10	0.67
9	Leaf length	7	0.47
10	Leaf width	3	0.20
11	Number of tillers per plant	2	0.13
12	Number of productive tillers per plant	2	0.13
	TOTAL	**1485**	**100**

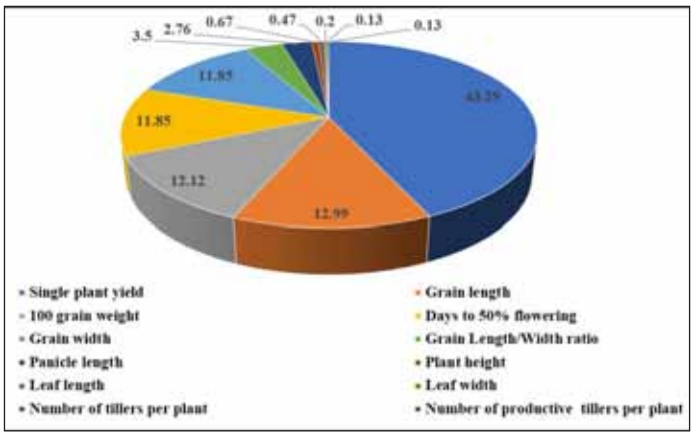

Figure 1: Percentage of contribution of each character to total genetic divergence

From the relative contribution of individual traits towards the divergence, it was found that the maximum contributing trait for divergence was single plant yield (43.29%) followed by grain length (12.99%), 100 grain weight (12.12%) grain width (11.85%) and days to 50% flowering (11.85%). Least contribution (0.13%) towards divergence was by number of tillers and productive tillers per plant (**Table 6**) and (**Figure 1**). These observations were in agreement with report of Kumari *et al.* (2018) and Rashmi *et al.* (2017) for days to 50% flowering; Sowmiya and Venkatesan (2017) for hundred grain weight and grain yield per plant and Vennila *et al.* (2011) for grain length. Hence, during selection of parents for hybridization and selection in the segregating populations traits like grain yield per plant, grain length, grain width, hundred grain weight and days to 50% flowering should be given importance.

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ORIGINAL RESEARCH ARTICLE

Molecular mapping of quantitative trait loci for stigma exsertion trait in rice (*Oryza sativa* L.)

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Abstract

Stigma exsertion is the most important floral trait of female parent/cytoplasmic male sterile (CMS) line for out crossing which increases hybrid seed production at farmer's field. The present study was aimed to map the genomic regions controlling the stigma exsertion in F_2 mapping population derived from IR68897B, a recipient maintainer line of IR68897A with low stigma exsertion (36.78 %) and BF-16B, a donor parent for high stigma exsertion (80.25%). Phenotyping and genotyping of stigma exsertion trait was done for 188 individuals of F_2 population developed from IR68897B X BF16B. Frequency distribution of F_2 population showed continuous variation which indicated polygenic nature of inheritance. Phenotypic evaluation revealed that significant variation for stigma exsertion trait and transgressive segregation was observed. A total of 15 QTLs were identified for total stigma exsertion rate (TSE) which includes single and dual stigma exsertion distributed on chrm 2, 3, 4, 5, 6, 8, 11 and 12 through QTL mapping. The phenotypic variance and LOD value explained by each QTL ranged from 1.19 to 5.83% and 2.54 to 9.29 respectively. In the present study, total 15 QTLs were minor effect QTLs (<10% phenotypic variance), indicating that stigma exsertion is controlled by many small effect QTLs. The QTL *qTSE11-1* on chrm 11 (9.29) and *qTSE3-1* on chrm 3 (6.83) were identified with higher LOD values and *qTSE3-2* on chrm 3 and *qTSE6-2* on chrm 6 explained 5.83 and 5.76 % of phenotypic variance, respectively

Keywords: QTL Mapping, stigma exsertion, maintainer line, phenotyping, grain yield

Introduction

Rice *(Oryza sativa* L.*)* is the second most important cereal crop and occupies a predominant position among major food grains. It is one of the crops responsible for the green revolution in the 1960s and 1970s. Global rice demand estimated to rise from 759.6 million tons in 2018 (FAO RMM, 2018) to 852 million tons in 2035 (Kush, 2013). This demand can be achieved by concentrating breeding efforts to improve rice yield potential with the shrinking natural resource base. With an ever-increasing population, crop scientists continuously work to meet the demands. Different strategies have been suggested to increase the yield potential of rice (Kush, 2005 and 2013). Among various strategies hybrid breeding is one of the strategies to improve rice productivity. Rice hybrids offer an yield advantage of 10-15 % over inbred varieties. Unfortunately, this technology was less adopted by the farming community. The high cost of hybrid seed is one of the major reasons affecting large scale adoption of the technology. This is mainly because of low hybrid seed yields i.e 1-2 t/ha. It is

difficult to reliably produce an acceptable quantity of hybrid rice seeds owing to its self-pollinating nature (Azzini and Rutger, 1982 and Kato and Nimai, 1987). The F_1 seed yield depends on outcrossing potential of female parent of a hybrid. Hybrid rice research should focus on to improve the F_1 seed yields by improving the out crossing potential of CMS lines and by managing seed production practices. This would maintain the hybrid seed cost. Therefore, improvement of hybrid seed production efficiency is an essential factor for large scale commercialization of hybrid rice (Tiwari *et al*., 2011).

In hybrid rice breeding programs, morphological characteristics of florets are of utmost importance in increasing the out crossing rate in hybrid rice seed production. The floral traits which can improve outcrossing potential of CMS lines include days to heading, pollen load, pollen longevity and morphological traits of floret, *viz.*, size of stigma and style, stigmatic receptivity, stigma exsertion, angle of floret opening and duration (Virmani, 1994). Among them, stigma exsertion is emphasized as a significant component in increasing pollination and seed set (Kato and Namai, 1987; Virmani, 1994; Sheeba *et al*., 2006). Stigma receptivity varies from 3-7 days, increasing the chance for pollination (Yan and Li, 1987; Xu and Shen, 1988; Li *et al*., 2004).Yang, (1997) reported that with 1 percent increase in stigma exsertion rate in male sterile lines, hybrid seed yields would increase by 47–68 kg /ha. Therefore stigma exsertion rate is extremely important floral trait for improving seed yields in hybrid seed production plots.

Stigma exsertion includes single, dual stigmas and other stigma traits that play important roles in hybrid seed production and received consistent attention from rice researchers (Virmani and Athwal, 1973; Virmani, 1994; Yan and Li, 1987; Tian, 1993; Li *et al*., 2001; Uga *et al*., 2003; Xu 2003; Yamamota *et al*., 2003; Miyata *et al*., 2007; Sidharthan *et al*., 2007 Yan *et al*., 2009, Huang *et al*., 2012; Dang *et al*., 2016; Zhou *et al*., 2017; Guo *et al*., 2017; Li *et al*., 2014; Liu *et al*., 2015; Sruthi *et al*., 2016; Rahman *et al*., 2017; Zhang *et al*., 2018; Liu *et al*., 2019, Bakti and Tanaka, 2019; Xu *et al*., 2019). Earlier studies have suggested that stigma exsertion is partially dominant (Virmani and Athwal, 1974) or completely dominant (Li *et al*., 1997b), and is a qualitative character controlled by a major gene (Hassan *et al*., 1984). Many studies reported that stigma exsertion is a quantitative trait controlled by polygenes (Li *et al*., 1995; Virmani and Athwal, 1974; Li and Chen, 1985; Wang *et al*., 2008; Xu and Shen, 1987; Sruthi *et al*., 2014). The small size of rice spikelets and the large effect of the environment on flowering in rice add to the difficulty in traditional selection-based breeding. As a result, the development of DNA markers associated with desirable floral traits for breeding programs has received increased attention at the national and international levels. Recently many studies have reported QTLs for stigma exsertion traits. Tan *et al*., (2020) mapped 7 QTLs on 5 chrm. (1, 3, 5, 9 and 10) using single segment substitution lines (SSSLs) derived from *O. glumaepatula*. Xu *et al*., (2019), identified a major QTL for stigma exsertion rate *viz.*, qSER-3.1 on chrm 3 in a 3.9 Mb region. Zhang *et al*., (2018), mapped qSE7 between RM5436 and RM5499 markers, within a physical distance of 1000-kb, with the use of INDEL markers it was finally mapped to a region of 322.9-kb between InDel SER4-1 and RM5436. Rahman *et al*.,(2017) dissected a major QTL qSE11on chrm 11 to a region of 350.7-kb. Liu *et al*., (2019) fine mapped qSER7 on chrm 7 and narrowed it down to a region of 28.4-kb between the markers RM3859 and Indel4373. Rahman *et al*., (2016) identified 8 QTLs (qSES6, qSSE11, qDSE1a, qDSE1b, qDSE10, qDSE11, qTSE1 and qTSE11) for single, dual and total stigma exsertion. Bakti and Tanaka, (2019) mapped QTLs for stigma exsertion ratio on chrms 2, 3, 4, 8, and 11 from *Oryza rufipogon* accession 'W0120'. Although many QTLs have been identified for stigma exsertion, their expression may vary one genetic back ground to other. We have attempted to map QTLs in the back ground of popularly used maintainer lines in hybrid breeding programme in India. Hence, the present investigation was carried out with an objective to identify the QTLs for stigma exsertion traits.

Materials and Methods

Plant material

The F_2 mapping population consisting of 188 lines was developed from a cross between IR68897B as a recipient parent with low stigma exsertion and BF-16B as a donor parent with high stigma exsertion **(Figure 2).** IR68897B is an early duration maintainer line for wild abortive male-sterile cytoplasm with long slender grain type developed by IRRI, Philippines and BF-16B is also a maintainer line for WA-CMS improved for stigma exsertion trait by Barwale Company. Hybrid nature of F_1s was confirmed using reported markers (**Table 1**) which are polymorphic between parents and true F_1s were raised in the field to develop F_2 population for QTL mapping.

Field experiments

A total of 188 F_2 mapping population along with its parents was grown in field at Research Farm, Indian Council of Agriculture Research (ICAR)- Indian Institute of Rice Research (IIRR), Hyderabad during *kharif* 2014 for phenotypic evaluation of stigma exsertion trait.

Phenotyping of stigma exsertion trait

For phenotypic evaluation of stigma exsertion trait, panicles were collected at post anthesis. Immediately after collecting panicles, panicles were wrapped in water soaked blotting paper and stored at -20°C. This is to avoid damage to stigma and to keep panicles afresh. For assessing the type of stigma exsertion in each spikelet of a panicle by the whole panicle method, all the individual spikelets in each panicle were separated and observed under illuminated magnifier lens to categorize them into dual (DStgE), single (SStgE) (**Figure 1**) or no stigma exsertion (NStgE) types. Then, these counts were converted to:

SStgE (%) = [SStgE / (SStgE + DStgE + NStgE)] x100

DStgE (%) = [DStgE/ (SStgE +DStgE +NStgE) x100

Total stigma exsertion [TStgE] (%) = SStgE (%) + DStgE (%) and NStgE (%) =100 - TStgE (%).

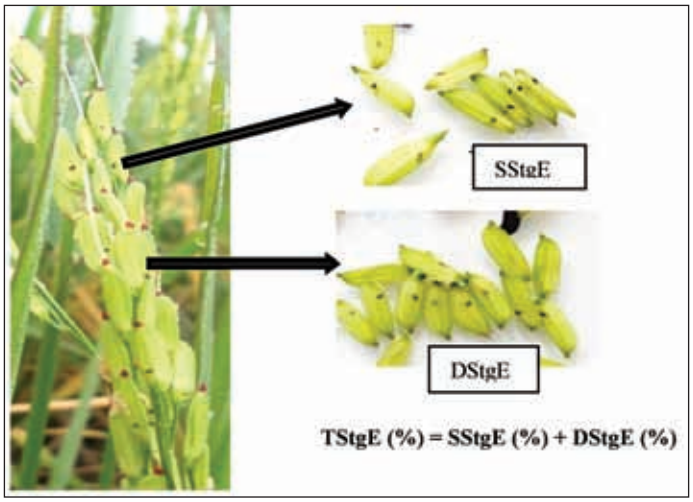

Figure 1: Figure showing the phenotyping of stigma exsertion trait

Genotyping

Genomic DNA was isolated from total 188 F_2 population and the parents using CTAB method of Zheng *et al.*, (1991) with minor modifications. Parental polymorphism survey was done using 513 SSR markers, which are distributed across 12 chrm. Out of 513 SSR markers only 124 markers showed (**Table 3**) polymorphism between parents. PCR amplification was carried out using thermal cycler (Veriti Thermo Cycler, Applied Bio systems). Master mix was prepared for 10 µl reaction volume containing 2 µl DNA template, 4.5 µl of nuclease free water, 1.0 µl 10X buffer, 1 µl dNTPs, 1 µl for both forward and reverse primers and 0.5 µl of 2U/ µl Taq DNA polymerase. PCR thermal profile is as followed: initial denaturation cycle of 95ºC for 5 min was followed by 35 cycles at 95ºC for 30 sec, 55°C for 30 sec , 1 min of extension at 72ºC with an additional step of 10 min at 72ºC. PCR products were resolved in 3% agarose gel in 0.5X TBE buffer stained with Ethidium Bromide and documented using gel documentation system (Bio-Rad, USA).

Linkage map construction and QTL mapping

The polymorphic 56 SSR (**Table 2**) markers were used to analyze the genomic DNA of the parents, IR68897B and BF-16B and F_2 population (188 plants). Each gel was scored for maternal (A), paternal (B) and heterozygous (H) banding pattern and later these converted into 2 (A), 0 (B) and 1 (H) score. QTL and

their effects were obtained using framework linkage map constructed for the genotypic data of 188 F_2 mapping population using 56 polymorphic markers and phenotypic data of 188 F_2 population using QTL ICI (Integrated Composite Interval Mapping) Mapping V4.2 (Meng *et al.*, 2015) employing Inclusive Composite Interval Mapping (ICIM) method. The LOD profiles were used to identify most of the likely position for a QTL in relation to the linkage map, which was the position where the highest LOD value was obtained. For QTL mapping, logarithm of the odds (LOD) threshold value was set to 2.5 using 1000 permutations at 0.05 level of significance.

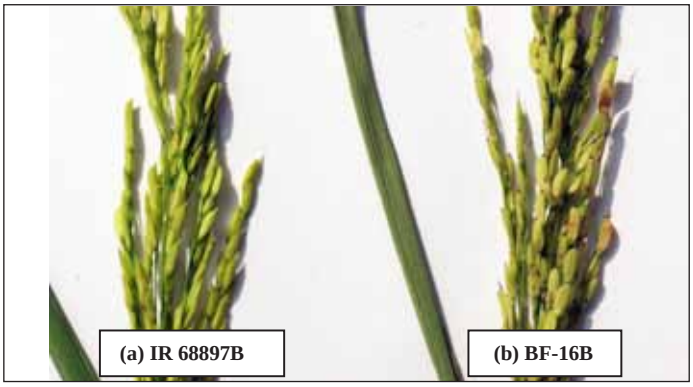

Figure 2: Figure showing the panicle type of parents;
(a) Recipient (IR 68897B) and (b) donor parent (BF-16)

Results and discussion

Phenotypic performance of stigma exsertion in the F_2 mapping population

The two parents used in the cross had contrasting stigma exsertion rates, *viz*., 36.78 and 80.25 %, while the F_1 was lower than better parent and higher than recipient parent *i.e*., intermediate with 60.44 % stigma exsertion rate.

Frequency distribution of stigma exsertion in F_2 population

The frequency distribution of the stigma exsertion rate in the F_2 population showed a continuous variation. The total phenotypic data were divided as per the scale of IRRI. The scale of stigma exsertion plotted against X-axis and number of plants on Y-axis. The graph showed a bell-shaped normal distribution curve and skewed towards low stigma exsertion. The frequency distribution of stigma exsertion (**Figure 3**) indicated that both parents have several chromosomal regions increasing the frequency of stigma exsertion and the trait is affected by the environment. In the present cross transgressive segregants were observed in the F_2 population.

Genotyping

Of the 513 SSR markers screened for parental polymorphism between IR 68897B and BF-16B, only 124 (24.17 %) showed clear polymorphism. A set of 56 polymorphic SSR markers, evenly distributed on rice genome covering all 12 chrm and showing clear polymorphism between stigma exsertion recipient and donor parents were used for genotyping of 188 F_2 population.

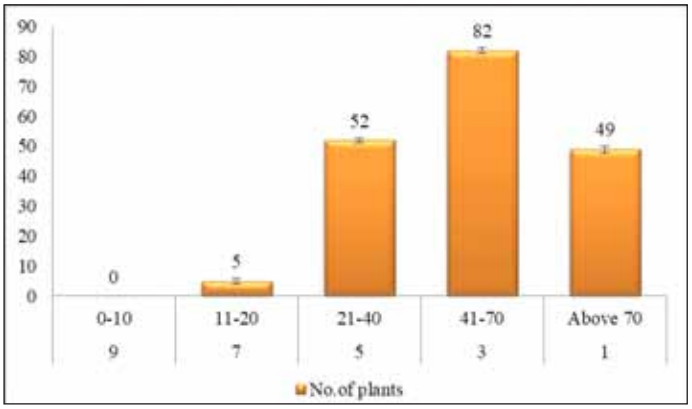

Figure 3: The graph dipicts the frequency distribution of stigma exsertion trait in 188 F_2 mapping population; 1, 3, 5, 7 and 9 the scale given by IRRI for phenotyping of stigma exsertion trait.

QTL mapping of stigma exsertion in F_2 mapping population

A total of 15 QTLs were identified for total stigma exsertion rate which included single, dual stigma exsertion using 56 polymorphic SSR markers with 188 F_2 mapping population and they were distributed on all 12 chrm except on chrm 1, 7, 9 and 10. The phenotypic variance and LOD value explained by each QTL ranged from 1.19 to 5.83% and 2.54 to 9.29, respectively. The majority of QTLs were contributed from the donor parent. On chrm 2, three QTLs were identified. The QTL, *qTSE2-1* was mapped at 24 cM and flanked by RM151 and RM12398 which accounts for 4.5% of the phenotypic variance with a LOD value of 3.75, the *qTSE2-2* was mapped at 129 cM, flanked

by markers RM13131 and RM208 and showed 4.94% of the phenotypic variance with a LOD value of 3.30 and the third QTL on chrm 2, *qTSE2-3* was flanked by RM208 and RM13238 at 218 cM map position and explained 4.98% of the phenotypic variance with 4.69 LOD value.

The QTL cluster *qTSE-3-1*, *qTSE-3-2* and *qTSE-3-3* on chrm 3 were flanked by RM1350-RM15466, RM1350-RM15466 and RM15466-RM7000 respectively and collectively explained 16.12% of the phenotypic variance with LOD value 6.83, 5.66 and 4.66 respectively.

A single QTL, *qTSE-4-1* was identified on chrm 4 at map position of 47 cM flanked by RM16338 and HRM17405 and explained 4.84 % of phenotypic variance with a LOD score of 3.5. On chrm 5, two QTLs, *qTSE-5-1 and qTSE-5-2* were identified at map positions of 117 and 184 cM flanked by the markers RM5592- HRM18222 and HRM18222-RM17950. Two QTLs *qTSE-5-1 and qTSE-5-2* on chrm 5 collectively accounted for 6.51 % of phenotypic variance with LOD scores of 2.54 and 2.59, respectively.

Two QTLs *qTSE-6-1 and qTSE-6-2* were identified on chrm 6 flanked by RM 20615-RM19569 with LOD value of 5.16 and 6.30 and explaining phenotypic variance of 1.19 and 5.76 %, respectively. Two QTLs *qTSE-8-1 and qTSE-8-2* on chrm 8 flanked by the markers RM22977-RM23643 and RM5493-RM6925 with LOD values of 3.88 and 3.77 and explaining 5 and 1.43 % of phenotypic variance, respectively. A single QTL *qTSE-11-1 was* detected on chrm 11 flanked by RM27183-RM26213 and explained 3.10 % phenotypic variance with LOD value of 9.29. A single QTL *qTSE-12-1* on chrm 12 flanked by RM7102-RM28157 and explained 4.28% phenotypic variance with LOD value of 2.72. The study detected contributing alleles for the trait were distributed in both i.e., donor and recipient parents. Eleven QTLs distributed on chrm 3, 4, 5, 6, 8, 11 and 12 from donor parent (BF-16B) and four QTLs present on chrm 2 and 8 were from recipient parent (IR 68897B). The QTL clusters identified on chrm 2(3 QTLs), 3(3 QTLs), 5(2 QTLs), 6(2 QTLs), 8(2 QTLs) collectively explained 14.42%, 16.12%, 6.51%, 6.95%, and 6.43%, respectively. The details of the putative QTLs for stigma exsertion were given in the **Table 4 and Figure 4.**

Table 4. Putative QTLs for total stigma exsertion detected in the F_2 population derived from IR 68897B and BF-16B

S.No.	QTL	Chr.	Position (cM)	Left Marker	Right Marker	LOD	PVE (%)	Add	Dom
1	qTSE-2-1	2	24	RM151	RM12398	3.75	4.50	0.87	21.17
2	qTSE-2-2	2	129	RM13131	RM208	3.30	4.94	1.31	21.46
3	qTSE-2-3	2	218	RM208	RM13238	4.69	4.98	4.23	-20.08
4	qTSE-3-1	3	28	RM1350	RM15466	6.83	5.19	-13.84	6.05
5	qTSE-3-2	3	94	RM1350	RM15466	5.66	5.83	-3.91	22.96
6	qTSE-3-3	3	153	RM15466	RM7000	4.66	5.10	-2.23	21.92
7	qTSE-4-1	4	47	RM16338	HRM17405	3.50	4.84	-3.86	-21.48
8	qTSE-5-1	5	117	RM5592	HRM18222	2.54	1.79	-7.98	-6.05
9	qTSE-5-2	5	184	HRM18222	RM17950	2.59	4.72	-0.35	21.37
10	qTSE-6-1	6	0	RM20615	RM19569	5.16	1.19	-1.02	-10.42
11	qTSE-6-2	6	58	RM20615	RM19569	6.30	5.76	-2.12	23.28
12	qTSE-8-1	8	27	RM22977	RM23643	3.88	5.00	-1.79	22.06
13	qTSE-8-2	8	155	RM5493	RM6925	3.77	1.43	0.16	12.45
14	qTSE-11-1	11	96	RM27183	RM26213	9.29	3.10	-10.42	3.97
15	qTSE-12-1	12	35	RM7102	RM28157	2.72	4.28	-1.10	20.05

PVE- Phenotypic variance explained by each QTL, LOD-Logarithm of odds ratio, qTSE-QTL for total stigma exsertion. Add-the additive effect of each QTL and dominant effect of each QTL

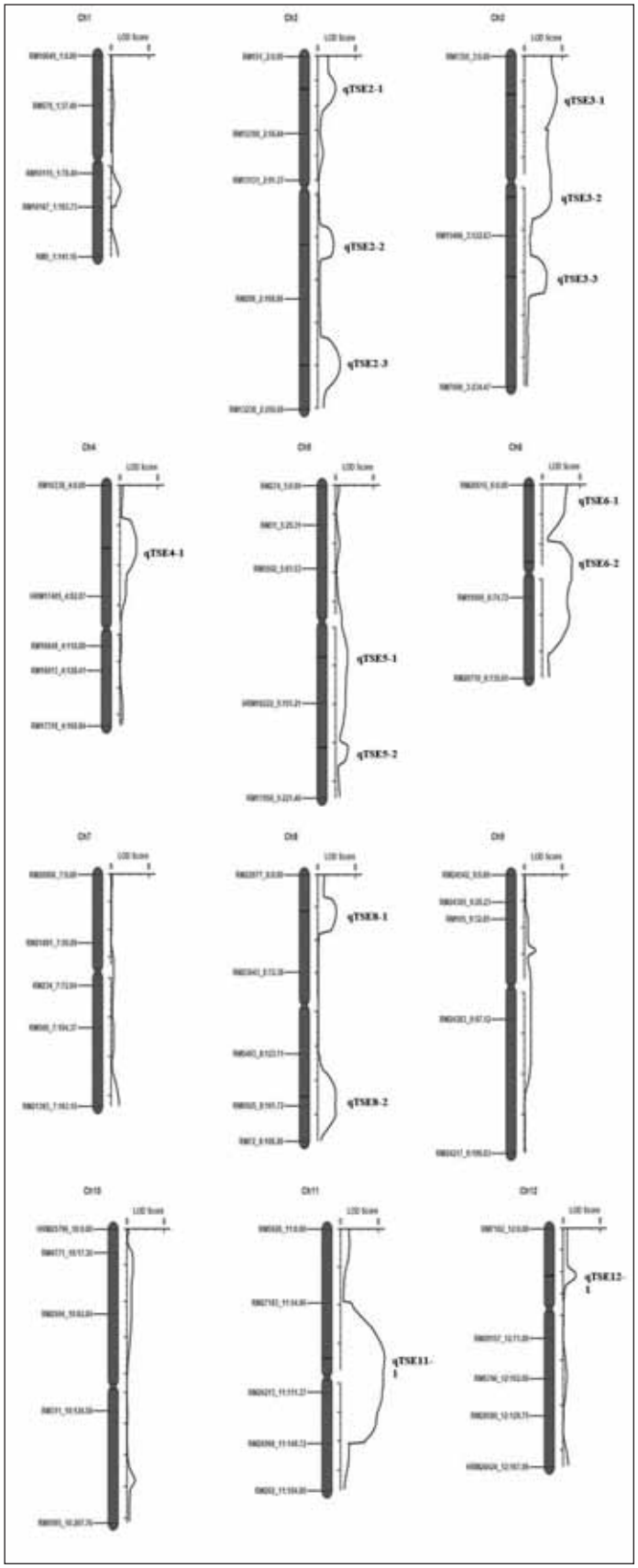

Figure 4: Distribution of QTLs for stigma exsertion trait on the linkage map

Breeding for hybrid rice is one of the pragmatic solutions for addressing the problem of food shortage, caused by marked increase in - global population with decreasing trend in available resources, land and water for agriculture. Breeding approaches may solve this problem by increasing yield per unit area through hybrid system of breeding. But the large scale adoption of hybrid rice has been hampered by low F_1 seed set and the consequent high price of hybrid seed. To overcome the extremely low seed set percentage of CMS line, there is a need to improve the parental lines for flowering behavior and floral traits and modifications in seed production practices are useful for pushing seed yields beyond 2.5 tonnes per hectare and also bring down the F_1 seed cost.

In our study, transgressive segregants were observed in both the directions signifying complementary action of the genes with additive effect that were dispersed in both the parents and a normal distribution for the phenotype was observed. According to Singh and Narayanan (1993) transgressive segregations are only possible from the crosses between two parents with mean values for a quantitative trait. Such segregants are not possible in case of qualitative traits. De Vicente and Tanksley, (1993) studied that the transgressive segregation is commonly observed in the population segregating for quantitative trait. Whereas Ricks and Smith (1953), mentioned that transgressive segregation in certain progeny is because of accumulation of complementary alleles at multiple loci inherited from the two parents.

These results were in conformity with the results of Virmani and Athwal, (1974), Li and Chen, (1985), Wang *et al*., (2008), Ling *et al.,* (1989), Miyata *et al*., (2007), Lou *et al*., (2014), Li *et al*., (2014) and Sruthi *et al*., (2014) who observed wide continuous variation with normal distribution and concluded that the stigma exsertion trait of rice is a quantitative trait, controlled by polygenes. Similarly, in tomato, stigma exsertion has been reported to be a quantitative trait and controlled by a few genes (Rick and Dempsey, 1969; Scott and George, 1978; Levin *et al*., 1994). However, earlier reports on the genetics of stigma exsertion in rice showed contradiction to the present finding. For instance, Hassan and Siddiq, (1984)

reported that fully exserted stigma to be monogenic and dominant over partially exserted stigma. Xu and Shen, (1987) also studied the inheritance of the stigma exsertion trait and reported that it was a dominant trait and in their study F_2 population showed continuous variation for stigma exsertion, this may be due to some minor genes supplementing the major gene in the expression of the trait. Experimental evidence suggested that environmental effects would also produce a continuous variation even if the number of genes governing a character was very small or even one.

We constructed 188 lines of the F_2 mapping population using IR68897B x BF-16B cross and genotyped these 188 lines with 56 polymorphic molecular markers to identify the QTLs for the trait of interest (stigma exsertion). Results showed that 15 QTLs were associated with stigma exsertion traits and were located on chrm 2, 3, 4, 5, 6, 8, 11 and 12. The phenotypic variance and LOD value explained by each QTL ranged from 1.19 to 5.83% and 2.54 to 9.29 respectively. The present QTL analysis results for stigma exsertion were in accordance with the result of Tan *et al*., (2020). They mapped the major QTLs for stigma exsertion rate from *Oryza glumaepatula* on chrm 1 and 3. Xu *et al.*, (2019) identified a major QTL on chrm 3 using double haploid population. Lou *et al*., (2014) identified a total of 5 QTLs and 9 epistatic QTL pairs were found to associate with the stigma trait in F_2 population of Huhan 1b and K17B. Li *et al.*, (2001) reported QTL on Chrm 5 and 8 using back cross-population of Dongxiang and Guichao 2. Li *et al.*, (2001) identified QTLs on chrm 2 and 3 using DH population of Zaiyeqing 8 and Jingxi 17. Uga *et al.*, (2003) identified QTL for the rate of stigma exsertion on chrm 5 and 10 using RILs of Pei-Kuh and a wild rice W1944 and Deng *et al*., (2011) observed on chrm 1, 2, 3 and 5 using F_2 population of (CMS) maintainer line II-32B and G46B. Miyata *et al*., (2007) reported QTLs for stigma exsertion on chrm 3 using F_2 population of Koshihikari and IR24 and Yue *et al*., (2009) demonstrated on chrm 3, 4, 7 and 9 using F_2 population of CMS maintainer line Huhan 1B and II-32B. Wang *et al*., (2008) studied high SE in chrm 2, 5 and 8 by introducing the genes of high stigma exsertion rate from *indica* into *japonica*.

The National plant biology symposium proceeding in China (2011) confirmed the QTLs for PES (percentage of exerted stigma) to be present on all chrm except on chrm 7. The studies on stigma exsertion by Li *et al*., (2014) reported the presence of the trait in chrm 3, 6, 7, 9, 10 and 12 using RIL population of ZX and CX29B. Lou *et al*., (2014) also reported the presence of PSE in chrm 5 and 6, 7 using F_2 population of CMS lines, Huhan1B and K17B, which are similar to the results of the present study. Li *et al*., (2014) resolved exserted stigma determined by a few main and epistatic pairs QTLs, indicating mainly influenced by minor QTLs in three-way cross/backcross population of Zhenshan 97B and 9311. In Rahman *et al*., (2016) studies showed a total of two QTLs for TSE located on chrm 1 and 11 in two environments and QTL on chrm 11 is novel to date for exserted stigma in our result also.

In addition, results detected contributing alleles for trait were distributed in both i.e., donor and recipient parents. Eleven QTLs distributed on chrm 3, 4, 5, 6, 8, 11 and 12 from BF-16B and four are on chrm 2 and 8 from IR 68897B. Previous studies (Rahman *et a.*, 2016; Li *et al*., 2014; Yamamoto *et al.*, 2003; Yu *et al*., 2006) reported that favorable alleles present and contribute to trait expression by both the parents. Hence, for improvement of exserted stigma trait it is very much essential to select suitable breeding program for pyramiding of traits in parental lines from different sources.

In the present study one QTL cluster has been identified on chrm 3 flanked between RM 1350 and RM 7000 for TSE, it collectively explaining 16.12 % phenotypic variance. Earlier also reported by Lou *et al*., (2014) for PDES on chrm 5 and by Yu *et al*., (2006), mapped the region by Li *et al*., (2001) and Uga *et al*., (2003b). Li *et al*., (2014) also detected two QTLs clusters on chrm 1 and 6 for extruded stigma.

Many QTL identified by different researchers for stigma exsertion rate (SER), Percentage of exserted stigma (PES), Percentage of single exserted stigma (PSES) and Percentage of double exserted stigma (PDES) in rice. In the present investigation, 15 QTLs were identified, of which 11 were contributed from

the donor parent (BF-16B) and 4 from the recipient parent (IR 68897B) for stigma exsertion to the progeny. Both the parents contributed towards the stigma exsertion. However, more number of QTLs was contributed by the donor parent to the progeny for total stigma exsertion (TSE) in *indica* rice. All the15 QTLs identified for total stigma exsertion in this study were minor affect QTLs accounting for less than 10 % of the phenotypic variance. This clearly indicates the polygenic nature of the trait controlled by several genomic regions with small effects. Stigma exsertion is highly influenced by environment which might be affecting the QTL expression. In general, QTLs with large effects are more stable across environments than QTLs with smaller effects. stigma exsertion is controlled by many genes and has low heritability. Hence, it is advisable to phenotype the trait in multiple environments in order to identify the stable QTLs. In the present study, the markers flanked by the QTLs can be utilized to screen the uncharacterized germplasm for stigma exsertion trait to identify new donors and can be used in marker assisted breeding programmes to improve maintainer lines. These maintainers can be converted into new CMS lines with improved stigma exsertion rate to increase hybrid seed production efficiency. The QTLs identified in the present study need validation before they can be utilized in Marker-Assisted breeding programs for improvement of the maternal parent in hybrid rice technology. This may exsertion help in overcoming the pollination barriers like time (asynchronous flowering) and will allow accumulation of more pollen on stigmatic surface of female parent improving the hybrid seed production efficiency.

Authors Contribution

Conceptualization of research (KKB, SMM & ASH): Designing of the experiments (KKB, SMM & ASH): Contribution of experimental material (Barwale Foundation): Execution of field /lab experiments and data collection (GS & JK): Analysis of data and interpretation (GS, JK, KKB, SMM & SK): Preparation of the manuscript and correction (GS, JK, KKB, SMM, SK, VS, SJ, AKS, PK, LS, SP, GC, AMS, RMS & ASH).

Compliance with Ethical Standards: The authors declare the research work is in Compliance with Ethical Standards

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Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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ORIGINAL RESEARCH ARTICLE

Assessment of the migration of the operational taxonomic units (OTUs) from rice rhizosphere to endosphere

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Abstract

An experiment was conducted to understand the migration of the OTUs from rice rhizosphere to endosphere at vegetative and flowering stages of the crop. The soil and plant samples were studied through culture independent molecular PCR-DGGE technique involving denaturing gradient gel electrophoresis (DGGE) of PCR-amplified V3 region of 16S rRNA gene. The DGGE analysis revealed higher species richness in soil, followed by root, stem and leaf. The analyses for range-weighted richness and Shannon diversity index indicated vegetative stage carries more diverse microflora in soil and root samples than stem and leaf samples. The Sorenson's similarity index indicates the migration of bacterial population from rhizosphere to endosphere. The soil samples shared about 28 to 35 % similarity with different plant compartments *viz*., root, stem and leaf at both crop stages. The fingerprinting of rhizosphere and endosphere samples would be a useful resource for plant microbe breeding through understanding of the associated microbial community.

Keywords: Rice, rhizosphere, DGGE, OTU, 16S rRNA, migration

Introduction

Microorganisms influence agriculture through their presence in rhizosphere and plant endosphere. It is crucial to understand the factors influencing the microbial communities, considering the enormous importance of plant microbe interactions in agricultural systems. Studies have revealed beneficial effects of endophytes in rice crop through N_2 fixation, phosphate solubilisation and anti-fungal or anti-bacterial properties (Kumar *et al.,* 2009; Naik *et al.* 2009; Ramesh and Mathivanan, 2009). Studies on the species diversity of bacterial endophytes have been mainly approached by cultivation-based methods. The study on movement of *gfp*-tagged rhizobia indicated surface colonization of the rhizoplane, followed by endophytic colonization within roots, and then ascending endophytic migration into the stem base, leaf sheath and leaves (Chi *et al.* 2005). However, a range of bacteria is not accessible to cultivation methods, because of their unknown growth requirements or their entrance into a viable but not culturable state. Diverse endophytic bacteria are present in various parts of rice plants as revealed by culture dependent and non-culture-based methods (Mano and Morisaki, 2008).

The 16S rRNA gene (rDNA) has become a frequently employed phylogenetic marker to describe microbial diversity in natural environments without the need for cultivation. Amplified ribosomal DNA restriction analysis (ARDRA) could reveal diverse taxa of endophytic bacteria in the SSU rDNA library of rice roots (Sun *et al.* 2007). Denaturing gradient gel electrophoresis (DGGE) analysis revealed bacterial and fungal communities in the intercellular fluid of rice leaf blades and sheaths were distinct from that in the surface washing fluids (Takahashi *et al*. 2011). Terminal restriction fragment length polymorphism (T-RFLP) and 16S rDNA cloning in the leaves of three rice varieties revealed that 74 % of communities were similar in all three rice varieties (Ferrando *et al.* 2012).

In an analysis of the actinomicrobiome present in rice, of the 393 actinobacterial OTUs discovered in root samples, 29 OTUs shared commonness in the grains and stem samples (Wang *et al*. 2016). Investigation on temporal changes in the root-associated microbial communities throughout the plant life cycle in field-grown rice indicated that root microbe composition varies with the developmental stage of the plants (Edwards *et al*. 2018).

However, our understanding of microbial structure in the rhizosphere and endosphere is still very poor. Although root-associated microbes are known to have the potential to be utilized to promote crop productivity, their exploitation has been hindered by a lack of understanding of the compositional dynamics of these communities. The rice root endophyte community suggested high potential for plant-growth promotion, improvement of plant stress resistance, biocontrol against pathogens and bioremediation, regardless of their culturability (Sessitsch *et al.* 2012). The objective of the present study was to understand the migration of the bacterial microflora from rice rhizosphere to endosphere at vegetative and flowering crop stages. Polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) analysis is preferable over other methods because of its sensitivity, ability to monitor community shifts and cluster analysis. The results highlight relationship between plant growth stage and associated microbes that can be considered in strategies for the successful manipulation of microbial communities to enhance crop performance.

Materials and Methods

Seed and soil material

The seeds of rice genotype BPT5204 (Samba Mahsuri) were collected from the Agricultural Research Station (ARS), Mugad. Plants were raised in garden soil for conducting the pot experiments in greenhouse conditions.

Sampling details

Soil, root, stem and leaf samples were collected at vegetative and flowering stages of rice plants for studying the migration of soil microflora from rhizosphere to the plants. The soil samples were collected near the root zone at a depth of 10-15 cm and stored at -20 °C till further processing.

Protocol for surface sterilization of plant samples

Surface sterilization of plant samples for efficient removal of the rhizoplane bacteria was done according to the optimized method (Suryawanshi, 2018). Potted rice plants sown in garden soil were harvested and washed thoroughly under tap water. Roots were placed in sterile 50 ml Falcon tubes containing 25 ml phosphate buffer. Further, the Falcon tubes were vortexed at maximum speed for 15 s to remove any adhered rhizosphere soil from the roots. The roots were placed in fresh tubes containing phosphate buffer and sonicated at low frequency for 3 min (three 30 s bursts followed by three 30 s rests in between). The roots were chemically sterilized using 1.5 % sodium hypochlorite for 2 min by intermittent manual shaking and then rinsed twice with sterile distilled water. The roots were transferred to fresh bottles containing glass beads in 0.9 % NaCl solution and the rhizoplane bacteria were removed by vortexing at maximum speed for 10 min.

Extraction of DNA from plant samples

The genomic DNA was extracted from paddy leaves by using cetyltrimethylammonium bromide (CTAB/ NaCl) method of DNA isolation (Murray and Thompson, 1980).

Protocol for DNA isolation from soil sample

The extraction of DNA from soil samples was done using the lab protocol developed (Pasha *et al*, 2020) and standardized (Suryawanshi, 2018) earlier. Calcium chloride was added as a chemical flocculant suspended in DNA isolation buffer to remove humic substances from the soil. For cell lysis, 200 mg soil sample was taken in each 2 ml micro-centrifuge tube and 1 ml of soil DNA isolation buffer containing 100 mM Tris (pH 9.0), 100 mM Na_2EDTA (pH 9.0), 1.5 M NaCl, 80 mM $CaCl_2$, 1.25 % PVPP and 20 % SDS was added. The samples were incubated in the ThermoMixer (Eppendorf, Germany) at 1400 rpm and

70 °C for 50 min. After incubation, soil samples were centrifuged at 13,000 rpm for 10 min. The supernatant was transferred to fresh 2.0 ml micro-centrifuge tube, mixed with equal volume of chloroform: isoamyl alcohol (24: 1) and centrifuged at 13,000 rpm. The upper aqueous layer obtained was transferred to 1.5 ml micro-centrifuge tube, mixed with equal volume of chilled isopropanol, and incubated overnight at -20 °C. The DNA was pelleted by centrifugation at 13,000 rpm at 4 °C for 10 min. The DNA pellet was washed with 70 % ethanol, air dried and dissolved in $T_{10}E_1$ buffer for further use.

PCR amplification

For the PCR amplification, the hypervariable region (V3) of 16S rDNA was targeted and amplified using PRBA338F (5´ACTCCTACGGGAGGCAGCAG3´) and PRUN518R (5´ATTACCGC GGCTGCTGG3´) primer pair (Nakatsu *et al.,* 2000) with 40 bp GC clamp added to forward primer. Each PCR reaction contained 1X PCR buffer, 250 µM of each dNTP (GeNei, Bangalore), 5 µM of each primer (Sigma-Aldrich, USA), 1-unit *Taq* DNA polymerase (NEB, USA) and 100 ng of template DNA. The template DNA was denatured at 95 °C for 5 min followed by 30 cycles of denaturation at 94 °C for 50 s, annealing at 55 °C for 30 s, extension at 72 °C for 50 s and final extension of 5 min.

Denaturing Gradient Gel Electrophoresis (DGGE)

DGGE was carried out using 12 % polyacrylamide gel prepared with 30 % to 70 % chemical denaturant (40 % formamide and 7 M urea corresponding to 100 % denaturant) (Muyzer *et al*, 1993) as resolving gel, which was topped with 5 % polyacrylamide without any denaturant. The PCR product was loaded in each well carefully without contaminating adjacent wells. The gel was run in 1X TAE buffer for 17 hours at 100 Volts in DGGE system (Cleaver Scientific, UK) as per manufacturer's instructions. After the run, the gel was carefully removed and placed between two sheets of transparent plastic for proper handling during staining. The gel was stained by the silver staining protocol given by Gustavo and Gresshoff (1994).

Analysis of DGGE profiles

The DGGE profile was documented in Syngene G box gel documentation unit and processed by GeneTools software (Syngene). The faint band was scored as 1 (or the brightest band was scored as 10) and used as reference for the densitometric based scoring of other bands in profile. The number of bands was taken as measure of different operational taxonomic units (OUT's) and the respective intensity as their proportion in the population.

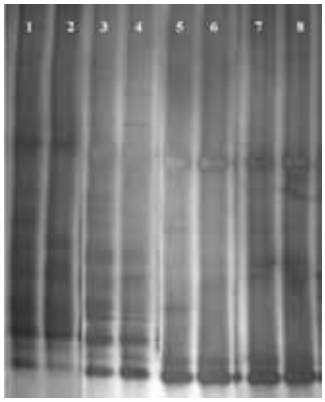
Species richness was calculated by range-weighted richness (Rr) = ($N^2 \times Dg$) (Marzorati *et al*, 2008), where N is the total number of bands in the pattern and Dg is the denaturing gradient in which the uppermost and lowermost bands were obtained. Shannon diversity index (H) was calculated using the equation: $H = -\Sigma Pi \times \log (Pi)$ (Shannon, 1948), where Pi is the proportional intensity of each band or OTU. The statistical analysis for Shannon diversity index was performed according to Hutcheson's modified t test (Hutcheson, 1970). Sorenson's similarity index (Cs) was calculated by formula $Cs = 2j/(a+b)$ (Sorensen, 1948), where j is the number of OTUs common for both samples, a and b are the number of OTUs present in first and second samples respectively.

Results

The soil and plant samples were studied through DGGE analysis to understand the migration of the OTUs from rhizosphere to leaf tissues at two crop growth stages *viz*., vegetative and flowering. The amplification of approximately 220 bp size product (including GC clamp) by PRBA338-PRUN518 primer pair for V3 region of 16S rDNA was obtained from all the samples. The DGGE profiles generated from amplified products for all the samples studied were distinct (**Figure 1**). Different bands, each representing an OTU were observed in all the samples. The number of OTUs was higher in soil followed by roots, stems and leaves. The vegetative stage had a relatively higher number of OTUs than the flowering stage in all the samples.

The range-weighted richness (*Rr*) for the analysed samples varied from 61.44 to 156.88 (**Figure 2A**). Soil showed higher *Rr,* followed by root, stem and leaf. For any sample, vegetative stage had higher *Rr* than flowering stage; implying broader carrying capacity at vegetative stage. The Shannon diversity index for the analysed samples ranged between 1.95 and 2.44 (**Table 1, Figure 2B**). The highest diversity index (2.44) was observed in soil at vegetative stage, while lowest diversity index (1.95) was observed in leaf at flowering stage. In soil and root samples, the Shannon diversity index at vegetative stage was significantly higher than flowering stage. No significant difference in the Shannon diversity index was observed in stem and leaf samples at vegetative stage and flowering stage.

The similarity index of soil with root, stem and leaf was compared within the stage, to determine the migration of bacterial population from soil to plant tissues. The similarity between samples was calculated using Sorenson's similarity index (**Table 2**). At vegetative stage, soil had 34.92 %, 32.97 % and 32.77 % similarity with root, stem and leaf respectively. At flowering stage, soil had 31.64 %, 29.10 % and 28.37 % similarity with root, stem and leaf respectively.



1: Soil (Vegetative stage)
2: Soil (Flowering stage)
3: Root (Vegetative stage)
4: Root (Flowering stage)
5: Stem (Vegetative stage)
6: Stem (Flowering stage)
7: Leaf (Vegetative stage)
8: Leaf (Flowering stage)

Figure 1: DGGE profile for assessment of migration of microflora from soil to plants

Table 1. Shannon diversity index of soil and plant samples

Stage vs Sample	Soil	Root	Stem	Leaf
Vegetative stage	2.44**	2.4*	2.13	1.98
Flowering stage	1.97	2.26†	2.07	1.95

*Significant at 1 % within sample, ** Significant at 5 % within sample, † Significant at 1 % within stage

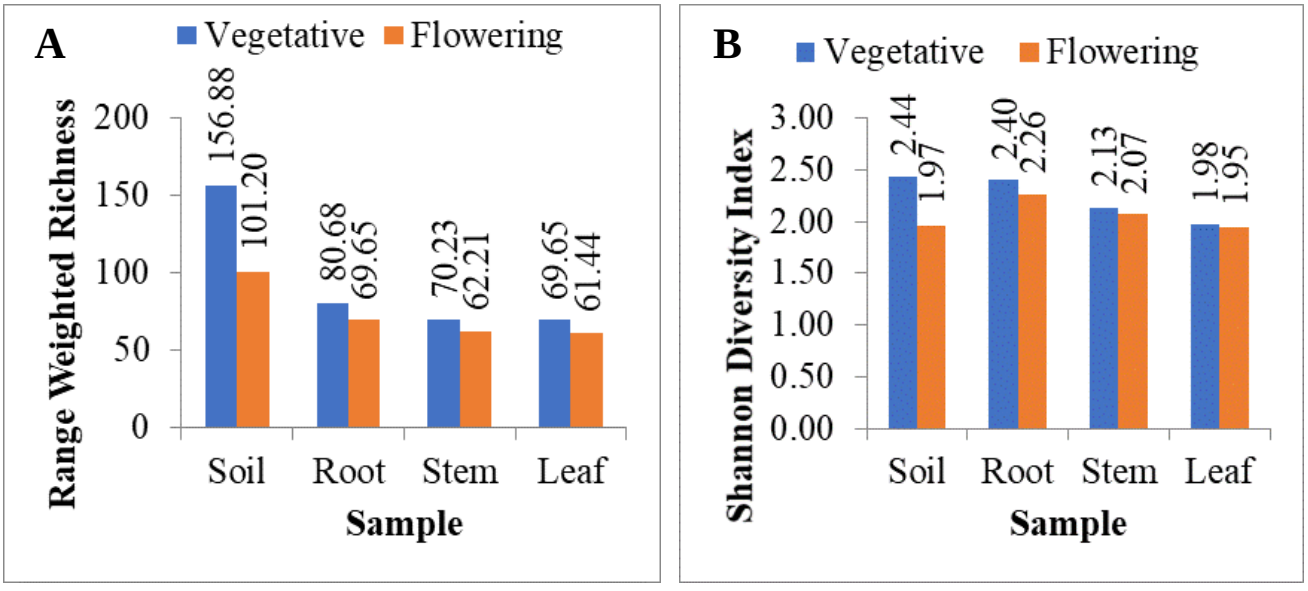


Figure 2: Assessment of migration of bacterial community from soil to plants

A. Range weighted richness, B. Shannon diversity index

Table 2. Sorenson's similarity index for migration of OTUs in soil and plant tissues

Sample	Stage	Soil		Root		Stem		Leaf	
		V	F	V	F	V	F	V	F
Soil	Vegetative	1							
	Flowering	38.06	1						
Root	Vegetative	**34.92**	31.59	1					
	Flowering	36.92	**31.64**	41.60	1				
Stem	Vegetative	**32.97**	29.41	39.44	30.42	1			
	Flowering	28.29	**29.10**	25.04	31.80	31.78	1		
Leaf	Vegetative	**32.77**	28.86	24.78	30.25	40.51	39.85	1	
	Flowering	27.36	**28.37**	24.47	31.51	28.13	44.67	46.38	1

V: Vegetative, F: Flowering

Discussion

The soil and plant samples were studied through culture independent DGGE analysis to understand the migration of the OTUs from rhizosphere to leaf tissues at two crop stages *viz.*, vegetative and flowering. Here in migration we assume, the soil as source of the bacteria, some of which would move into the root and further into the stem and leaf.

The number of OTUs was higher in soil samples followed by roots, stems and leaves. The vegetative stage samples had relatively higher number of OTUs than at flowering stage in all the soil and plant samples. The range-weighted richness (*Rr*) > 30 is typical of habitable environments with broad carrying capacity. Soil showed higher *Rr,* followed by root, stem and leaf. This implies decreased microbial diversity from soil to leaves; and leaves have relatively narrow carrying capacity than soil habitat. Similar results were observed by Ramond *et al.* (2013), Bodenhausen *et al.* (2013) and Wang *et al.* (2016) during their studies in sorghum, *Arabidopsis* and rice respectively. Soils are rich in carbon sources and other nutrients, which supports higher richness and diversity (Mendes *et al.*, 2013). On the other hand, plants represent relatively stable environment for microbial survival due to limited availability of carbon sources and other growth promoting factors (Moronta *et al*., 2018). Among all the samples herein, vegetative stage had higher *Rr* than flowering stage, implying broader carrying capacity at vegetative stage.

A Shannon diversity (H) index is a mathematical measure of species diversity in a community based on the species richness (the number of species present) and species abundance (the number of individuals per species) (Shannon, 1948). All the samples in current study showed moderate Shannon diversity index. In soil and root samples, the Shannon diversity index at vegetative stage was significantly higher than flowering stage samples; indicating more diverse bacterial population is present in soil and root during vegetative stage. This indicates that the microbial diversity (species richness and abundance) at flowering stage is lower than vegetative stage. Andreote *et al.* (2010) and Hussain *et al*. (2012) also reported reduced microbial diversity in potato and rice roots at reproductive stage as compared to vegetative stage. Edwards *et al*. (2018) investigated changes in the root-associated microbial communities throughout the plant life cycle in field-grown rice. Results indicated that root microbe composition varies with the developmental stage of the plants, suggestive of distinct root microbe associations for the juvenile and adult plant phases. The reduced microbial diversity at reproductive stage may be due to lesser rhizodeposition (Mougel *et al.*, 2006).

The Sorenson's similarity index indicates the percent of bacterial population that might have been shared between samples. In other words, it helped to determine the migration of bacterial population from rhizosphere to endosphere. The soil samples shared 28 to 35 % similarity with different plant compartments at both crop stages in current study. Wang *et al*. (2016) analysed the actinomicrobiome present in rice indicating more diverse OTUs were present in roots than in stems. The grains and stem samples shared 7 % commonness with root samples.

Conclusion

The carrying capacity of soil is higher than root, followed by stem and leaf. The vegetative stage carries more diverse microflora in terms of species richness and abundance in soil and root samples. There is huge similarity in bacterial population of rice rhizosphere and endosphere. Further studies on the identification of soil and plant bacterial communities would be a useful resource for improvement of soil and plant health.

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ORIGINAL RESEARCH ARTICLE

Bio-efficacy of new insecticides against paddy earhead bug, *Leptocorisa oratorius* (Fabricius)

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Abstract

The study was conducted to evaluate new insecticide molecules for the management of paddy earhead bug in field condition at College of Agriculture, V. C. Farm, Mandya, University of Agricultural Sciences Bangalore during *kharif* 2020. Nine different insecticides and one untreated control were evaluated. Number of earhead bugs per hill at day before spray (DBS), 1st, 3rd, 5th and 10th day after spray (DAS) were recorded. The data revealed that among different treatments fipronil 5 SC @ 2.00 mL L^{-1} was most effective with significantly lower population (0.10 bugs/hill) compared to rest of the treatments at 10 DAS. The grain and fodder yield were also significantly higher in fipronil 5 SC @ 2.00 mL L^{-1} treated plot followed by thiamethoxam 25 WG @ 0.30 g L^{-1}. The results of the cost economics for the management of earhead bug revealed that fipronil 5 SC @ 2.00 mL L^{-1} recorded highest net returns with maximum benefit cost ratio (3.02: 1).

Keywords: Earhead bug**,** management, cost economics, fipronil, thiamethoxam

Introduction

Rice (*Oryza sativa* Linn.) is the principal food for more than half of the world's population and contributes about 40 per cent of the total food grain production. Rice plays a vital role in the human diet, economy, employment, culture and history. The rice crop is subject to attack by more than 100 species of insects and twenty of them can cause economic damage (Pathak and Khan,1994).

Approximately 52 per cent of the overall global rice production is affected annually due to biotic agents, out of which 21 per cent is due to insect pest attacks (Brookes and Barfoot, 2003; Sarao *et al.*, 2015). In India also insect pest damage at various stages of crop growth is a major constraint in rice production. Rice earhead bug or Rice gundhi bug, *Leptocorisa oratorius* (Hemiptera: Alydidae) is an important pest of rice (Rao and Prakash, 1995). Both nymphs and adults cause damage by feeding on the sap of milky grain and make them partial or completely chaffy under severe infestation. At the site of feeding, small yellowish-brown spot is developed initially and enlarge later to form yellowish brown elliptical spot with greyish centre. Both nymphs and adults emit pungent odour when disturbed. Rice gundhi bug is considered as sporadic pest of rice and one of the serious pests of rice in India and sometimes reduce the yield by as much as 30 per cent. The adults are slender and brown-green, measure 19-16 mm long. The early instars are pale in colour. The nymphs have long antennae. The older instars measure 1.8-6.2 mm long, yellowish green. The eggs are oval, shiny, and reddish brown laid in batches of 10-20 in one to three rows along the midrib on the upper surface of the leaf (Tiwari *et al.*,2014).

The incidence and damage caused by earhead bug across rice ecosystem is increasing day by day in major rice growing areas of southern Karnataka particularly Cauvery command area. In view of this, the present investigation was conducted to evaluate different new insecticides against paddy earhead bug.

Materials and methods

The field experiment was conducted during late *kharif* 2020 at "A" block, College of Agriculture, V. C. Farm Mandya to evaluate new and conventional insecticides against paddy earhead bug.

Experiment was laid-out in Randomized Completely Block Design (RCBD) with 10 treatments, including an untreated control (**Table 4**) and replicated thrice. The popular and susceptible variety IR-64 was sown with a spacing of 20 X 15 cm, between rows and plants, respectively. For each replication, a plot size of 3 X 3 m was maintained. All packages of practices were followed in the plot except plant protection measures (Anon., 2017).

The observations were recorded by counting the number of bugs visually on 10 hills per plot at random in each replication. Observations were made on a day before spray, and at 1st, 3rd, 5th and 10th days after spray. Per cent reduction over untreated control was worked out using modified Abbot's formula. Harvest was made at physiological maturity; grain yield and fodder yield were recorded treatment wise. The data on grain yield per plot was converted into quintal per hectare. In each treatment the additional gain yield over untreated control was calculated as below

$$\% \text{ Additional gain yield} = \frac{\text{Yield in treatment- yield in control}}{\text{Yield in control}} \times 100$$

Data were subjected to ANOVA (Gomez and Gomez, 1984; Hosmand, 1988) and means were separated by Tukey's HSD (Tukey, 1965). Further, cost economics of each treatment was worked out as per market price, labour wages and additional costs during the course of study and benefit cost ratio was calculated.

Results and discussion

A day before spray, the population of earhead bug in each treatment varied between 1.10 to 2.53 bugs/hill and there was no significant difference among the treatments. At 1DAS, population of earhead bug among treatments ranges from 0.23 and 1.80 per hill. significantly lower population (0.23 bugs/hill) was recorded in fipronil 5 SC @ 2.0 mL L^{-1} followed by thiamethoxam 25 WG @ 0.30 g L^{-1} (0.30 bugs/hill). These treatments were followed by flonicamid 50 WG @ 0.25 g L^{-1}, imidacloprid 17.8 SL @ 0.30 mL L^{-1}, lambda cyhalothrin 5 EC @ 1.00 mL L^{-1}, acetamiprid 20 SP @ 0.30 g L^{-1} and deltamethrin 2.8 EC @ 1.00 mL L^{-1} which recorded 0.37, 0.40, 0.40, 0.43 and 0.47 bugs/hill, respectively and were on par with each other. The next best treatments were dinotefuran 20 SG @ 0.30 g L^{-1} and dimethoate 30 EC @ 2.00 mL L^{-1} which recorded 0.63 and 1.07 bugs/hill, respectively and were significantly differed. However, a significantly higher earhead bug population (1.80 bugs/hill) was recorded in untreated control (**Figure 1**).

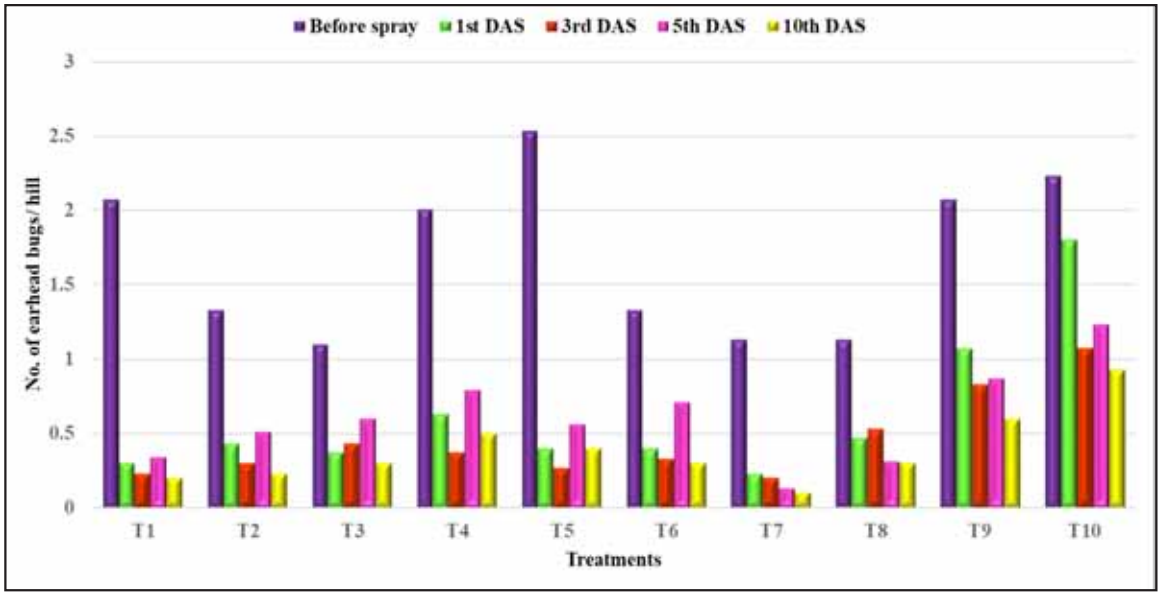

Figure 1: Earhead bug population in different treatments

Likewise, at 3 DAS, similar trend was observed and lower population (0.20 bugs/hill) observed in fipronil 5 EC @ 2.0 mL L^{-1} followed by thiamethoxam 25 WG @ 0.30 g L^{-1} (0.23 bugs/hill). The next best treatments were imidacloprid 17.8 SL @ 0.30 mL L^{-1}, acetamiprid 20 SP @ 0.30 g L^{-1}, lambda cyhalothrin 5 EC @ 1.00 mL L^{-1}, dinotefuran 20 SG @ 0.30 g L^{-1} and flonicamid 50 WG @ 0.25 g L^{-1} which recorded 0.27, 0.30, 0.33, 0.37 and 0.43 bugs/hill, respectively and were on par with each other. Likewise, the earhead bug population in deltamethrin 2.8 EC @ 1.00 mL L^{-1} and dimethoate 30 EC @ 2.00 mL L^{-1} was 0.53 and 0.83 bugs/hill, respectively and were on par with each other. However, an increase in earhead bug population was observed in untreated control (1.07 bugs/hill).

At 5 DAS, significantly higher population of earhead bug was observed in untreated control (1.23 bugs/hill) and was followed by dimethoate 30 EC @ 2.00 mL L^{-1} (0.87 bugs/hill) and were on par with each other. The next best treatments were dinotefuran 20 SG @ 0.30 g L^{-1}, lambda cyhalothrin 5 EC @ 1.00 mL L^{-1}, flonicamid 50 WG @ 0.25 g L^{-1} and imidacloprid 17.8 SL @ 0.30 mL L^{-1} which recorded 0.79, 0.71, 0.60 and 0.56 bugs/ hill, respectively and were found on par with each other.

Significantly lower population (0.13 bugs/hill) was observed in fipronil 5 SC @ 2 mL L^{-1} and superior over rest of the treatments followed by thiamethoxam 25 WG @ 0.30 g L^{-1} and deltamethrin 2.8 EC @ 1.00 mL L^{-1} (0.34 and 0.31 bugs/hill respectively) and were on par with each other. The lowest earhead bug population.

At 10 days after spraying, significantly lower population was observed in fipronil 5 SC @ 2.00 mL L^{-1} (0.10 bugs/hill) and but was on par with thiamethoxam 25 WG @ 0.30 g L^{-1} (0.20 bugs/hill), acetamiprid 20 SP @ 0.30 g L^{-1}, flonicamid 50 WG @ 0.25 g L^{-1}, lambda cyhalothrin 5 EC @ 1.00 mL L^{-1} and deltamethrin 2.8 EC @ 1.00 mL L^{-1} (0.23, 0.30, 0.30 and 0.30 bugs/hill, respectively). Likewise, the earhead bug population in imidacloprid 17.8 SL @ 0.30 mL L^{-1}, dinotefuran 20 SG @ 0.30 g L^{-1} and dimethoate 30 EC @ 2.00 mL L^{-1} were 0.40, 0.50 and 0.60 bugs/hill, respectively and were on par with each other. However, significantly higher earhead bug population (0.93 bugs/hill) was recorded in untreated control **(Table 1).**

Among the insecticides tested, the highest per cent reduction of earhead bug population (78.78 %) over untreated control was recorded in fipronil 5 SC @ 2.0 mL L^{-1} and this was followed by thiamethoxam 25 WG @ 0.30 g L^{-1} (76.83 %) and imidacloprid 17.8 SL @ 0.30 mL L^{-1} (62.09 %). Likewise, the per cent reduction of earhead bug population over untreated control in acetamiprid 20 SP @ 0.30 g L^{-1}, lambda cyhalothrin 5 EC @ 1.00 mL L^{-1}, dinotefuran 20 SG @ 0.30 g L^{-1}, deltamethrin 2.8 EC @ 1.00 mL L^{-1}, flonicamid 50 WG @ 0.25 g L^{-1} and dimethoate 30 EC @ 2.00 mL L^{-1} was 58.53, 45.91, 40.05, 36.53, 34.60 and 30.39 per cent, respectively (**Table 16; Figure 7**).

The insecticides in the decreasing order of their efficacy were fipronil 5 SC @ 2.0 mL L^{-1} > thiamethoxam 25 WG @ 0.30 g L^{-1} > imidacloprid 17.8 SL @ 0.30 mL L^{-1} > acetamiprid 20 SP @ 0.30 g L^{-1} > lambda cyhalothrin 5 EC @ 1.00 mL L^{-1} > dinotefuran 20 SG @ 0.30 g L^{-1} > deltamethrin 2.8 EC @ 1.00 mL L^{-1} > flonicamid 50 WG @ 0.25 g L^{-1} > dimethoate 30 EC @ 2.00 mL L^{-1}.

Sharma *et al.* (2019) who found that the plots treated with fipronil 5% + buprofezin 20% SC recorded the lowest number of rice earhead bug population (2.10 and 3.51 nos./5 sweep nets) after first and second insecticidal sprays, respectively, followed by indoxacarb 10% + thiamethoxam 10% WG (2.47 and 4.25 nos./5 sweep nets, respectively). Seni and Naik (2017) reported that thiamethoxam 25 WG @ 37.50 g a.i /ha treated plot recorded significantly higher % reduction of hoppers (>60% over untreated control) and fipronil 5 SC @ 75 g a.i/ha treated plot had lower number of silver shoot (2.6%) incidence.

The results are in conformity with the results of Rath *et al.* (2015), who reported imidacloprid 17.8% @ 300 g/ha treatment recorded lowest percentage of DH (3.3%), WEH (3.33%), gundhi bug damage (7.16%). Thiamethoxam 25 WG @ 0.3 g/l, registered its superiority over rest of the treatments by recording lowest ear head bug population and higher grain yield followed by malathion 5D @ 20

kg/ha. In other insecticidal treatments the level of ear head bug population was comparatively high (Girish and Balikai, 2015). Ashokappa *et al*. (2015) also reported that insecticides, imidacloprid 17.8 SL @ 0.25 ml/l, thiamethoxam 25 WG @ 0.3 g/l and malathion D @ 20 kg/ha recorded earhead bug lowest population (0.05,0.14 and 0.18 bugs/hill). Likewise, Krishna and Ashwani (2017) who found that the treatment Imidacloprid was recorded lowest population of gundhi bug with (0.91 bug/hill) and found to be superior among all other treatments. This was followed by thiamethoxam (1.22 bug/hill). Similar results were reported by Shafia and Singh (2016), where they tested various test concentrations of chlorpyrifos 35%+fipronil 3.5% EC against rice gundhi bug, *Leptocorisa varicornis* (Fabr) and reported that a mean reduction in population of rice gundhi bug was found to be 4.78, 5.11, 5.17 and 9.73 for chlorpyrifos 35%+Fipronil 3.5% EC @ 875+87.5, 525+52.5, 437.5+43.75 and 350+35 g a.i./ha treatments, respectively when compared to untreatedcontrol (15.98).

In the present study, grain yield was significantly varied from 39.56 to 59.33 q ha^{-1}. Among the different treatments, significantly highest grain yield was recorded in fipronil 5 SC @ 2 mL L^{-1} of 59.33 q ha^{-1} and this was followed by thiamethoxam 25 WG @ 0.30 g L^{-1}, imidacloprid 17.8 SL @ 0.30 mL L^{-1} and acetamiprid 20 SP @ 0.30 g L^{-1} which recorded 57.33, 54.67 and 53.44 q ha^{-1}, respectively and were on par with each other. The treatment, lambda cyhalothrin 5 EC @ 1.00 mL L^{-1} recorded 51.44 q ha^{-1} was on par with dinotefuran 20 SG @ 0.30 g L^{-1} recorded 44.89 q ha^{-1}. Whereas, flonicamid 50 WG @ 0.25 g L^{-1} recorded 43.67 q ha^{-1} and was on par with deltamethrin 2.8 EC @ 1.00 mL L^{-1} (41.33 q ha^{-1}), which was the next best treatment in recording grain yield. The treatment, dimethoate 30 EC @ 2.00 mL L^{-1} recorded 41.11 q ha^{-1}. However, significantly lowest yield of 39.56 q ha^{-1} was recorded in untreated control (**Table 2**). Plant biomass yield did not vary significantly among different treatments in the present study. However, highest biomass yield of 64.55 q ha^{-1} was recorded in fipronil 5 SC @ 2 mL L^{-1} followed by acetamiprid 20 SP @ 0.30 g L^{-1} and deltamethrin 2.8 EC @ 1.00 mL L^{-1} which recorded 62.00 and 60.33 q ha^{-1}. The lowest biomass yield (54.11 q ha^{-1}) was recorded in untreated control.

The results of the present findings are in close agreement with that of Ashokappa *et al.* (2015), who reported imidacloprid 17.8 SL @ 0.25 ml/l treated plot recorded highest grain yield of 7049.26 kg/ha followed by thiamethoxam 25 WG @ 0.3 g/l and malathion D @ 20 kg/ha which recorded 6461.11 and 6253.33 kg/ha, respectively. Similarly, Girish and Balikai (2015), observed highest grain yield in thiamethoxam 25 WG @ 0.3 g/l treated plot followed by malathion 5D @ 20 kg/ha. Likewise, Rath *et al.* (2015), who found that imidacloprid 17.8% @ 300 g/ha treatment recorded highest grain yield of 5.28 t/ha in variety Jaya followed by the treatment sulfoxaflor 24% @ 375 g/ha, 4.96 t/ha, thiamethoxam 25% @ 100 g/ha, 4.9 t/ha and triazophos 40% @ 625 g/ha, 4.78 t/ha.

The results of the cost economics for the management of earhead bug revealed that fipronil 5 SC @ 2 mL L^{-1} registered higher gross returns of Rs.122456 ha^{-1} resulting in maximum net profit of Rs.91998 ha^{-1}. This was followed by thiamethoxam 25 WG @ 0.30 g L^{-1}, imidacloprid 17.8 SL @ 0.30 mL L^{-1} and acetamiprid 20 SP @ 0.30 g L^{-1} which recorded gross returns of Rs.117712, Rs.112905 and Rs.110986 ha^{-1} with net profit of Rs.87799, Rs.83019 and Rs.81808 ha^{-1}, respectively. Likewise, lambda cyhalothrin 5 EC @ 1.00 mL L^{-1}, dinotefuran 20 SG @ 0.30 g L^{-1}, flonicamid 50 WG @ 0.25 g L^{-1}, deltamethrin 2.8 EC @ 1.00 mL L^{-1} and dimethoate 30 EC @ 2.00 mL L^{-1} were recorded gross returns of Rs.106007, Rs.94798, Rs.91349, Rs.87680 and Rs.87647 ha^{-1} respectively with net return of Rs.76734, Rs.63799, Rs.60022, Rs.58144 and Rs.57490 ha^{-1}, respectively. Whereas, untreated control recorded minimum net profit (Rs. 54072 ha^{-1}) compared to rest of the treatments.

Similarly, the highest benefit cost ratio (3.02:1) was recorded in fipronil 5 SC @ 2.00 mL L^{-1} followed by, thiamethoxam 25 WG @ 0.30 g L^{-1}, acetamiprid 20 SP @ 0.30 g L^{-1}, imidacloprid 17.8 SL @ 0.30 mL L^{-1} and lambda cyhalothrin 5 EC @ 1.00 mL L^{-1} which

recorded benefit cost ratio of 2.94:1, 2.80:1, 2.77:1 and 2.62:1, respectively. The next best benefit cost ratio observed in dinotefuran 20 SG @ 0.30 g L^{-1} and deltamethrin 2.8 EC @ 1.00 mL L^{-1} with benefit cost ratio of 2.05 and 1.96 respectively. However, very low benefit cost ratio among the treatment was recorded in flonicamid 50 WG @ 0.25 g L^{-1} and dimethoate 30 EC @ 2.00 mL L^{-1} with 1.92 and 1.90 respectively, although, in control it was the lowest (1.89:1) **(Table 2).**

The present findings are in accordance with reports of Gupta *et al*. (2019), who found highest benefit cost ratio in imidacloprid (1:2.66), followed by triazophos (1:2.53), monocrotophos (1:2.49), acephate (1:2.41), thiamethoxam (1:2.40), carbaryl (1:2.35), malathion (1:2.19) as compared to control (1:1.74). Similarly, Girish and Balikai (2015), reported thiamethoxam 25 WG @ 0.3 g/l treated plot recorded highest net profit of Rs. 65823.75 followed by malathion 5 D @ 20 kg/ha (Rs. 62070.63) against earhead bug in paddy.

Table 1. Bio-efficacy of insecticides against paddy earhead bug, *Kharif* 2020

Sl. No.	Treatments	Dose (mL or g L^{-1})	Number of earhead bugs/hills					Per cent reduction over control
			Before spray	1st DAS	3rd DAS	5th DAS	10th DAS	
1	Thiamethoxam 25 WG	0.30	2.07 (1.60)	0.30 (0.89)^{a}	0.23 (0.86)^{a}	0.34 (0.92)^{ab}	0.20 (0.84)^{ab}	76.83
2	Acetamiprid 20 SP	0.30	1.33 (1.35)	0.43 (0.97)^{ab}	0.30 (0.89)^{ab}	0.51 (1.00)^{bc}	0.23 (0.86)^{abc}	58.53
3	Flonicamid 50 WG	0.25	1.10 (1.26)	0.37 (0.93)^{ab}	0.43 (0.97)^{ab}	0.60 (1.05)^{bcd}	0.30 (0.89)^{abc}	34.60
4	Dinotefuran 20 SG	0.30	2.00 (1.58)	0.63 (1.06)^{b}	0.37 (0.93)^{ab}	0.79 (1.13)^{cd}	0.50 (1.00)^{cd}	40.05
5	Imidacloprid 17.8 SL	0.30	2.53 (1.54)	0.40 (0.95)^{ab}	0.27 (0.88)^{ab}	0.56 (1.03)^{bcd}	0.40 (0.95)^{bcd}	62.09
6	Lambda cyhalothrin 5 EC	1.00	1.33 (1.35)	0.40 (0.95)^{ab}	0.33 (0.91)^{ab}	0.71 (1.10)^{cd}	0.30 (0.89)^{abc}	45.91
7	Fipronil 5 SC	2.00	1.13 (1.28)	0.23 (0.86)^{a}	0.20 (0.84)^{a}	0.13 (0.80)^{a}	0.10 (0.77)^{a}	78.78
8	Deltamethrin 2.8 EC	1.00	1.13 (1.28)	0.47 (0.98)^{ab}	0.53 (1.02)^{bc}	0.31 (0.90)^{ab}	0.30 (0.89)^{abc}	36.53
9	Dimethoate 30 EC	2.00	2.07 (1.60)	1.07 (1.25)^{c}	0.83 (1.15)^{cd}	0.87 (1.17)^{de}	0.60 (1.05)^{d}	30.39
10	Untreated control	-	2.23 (1.65)	1.80 (1.52)^{d}	1.07 (1.25)^{d}	1.23 (1.31)^{e}	0.93 (1.20)^{e}	-
SE m±			NS	0.03	0.03	0.03	0.03	-
CD @ p=0.05				0.10	0.09	0.10	0.09	

Values in the column followed by common letters are non-significant at p=0.05 as per Tukey's HSD (Tukey, 1965); DAS: Days after spraying; NS: Non significant; Per cent reduction over untreated control as per Flemming and Ratnakaran, 1985; Figures in the parenthesis indicate √x+0.5 transformed values.

Table 2. Cost economics of different insecticides for the management of paddy earhead bug, *Kharif* 2020

Sl. No.	Treatments	Dose (mL or g L^{-1})	Yield (q/ha)		Gross return (Rs. /ha)	Cost involved (Rs)		Total cost (Rs./ ha)	Net profit (Rs./ ha)	B:C ratio
			Grain	Bio-mass		Cost of in-secticides	Other ex-penditure			
1	Thiamethoxam 25 WG	0.30	57.33	59.00	117712	1413.00	28500.00	29913	87799	2.94 : 1
2	Acetamiprid 20 SP	0.30	53.44	62.00	110986	678.00	28500.00	29178	81808	2.80 : 1
3	Flonicamid 50 WG	0.25	43.67	54.33	91349	2827.00	28500.00	31327	60022	1.92 : 1
4	Dinotefuran 20 SG	0.30	44.89	60.77	94798	2499.00	28500.00	30999	63799	2.05 : 1
5	Imidacloprid 17.8 SL	0.30	54.67	59.88	112905	1386.00	28500.00	29886	83019	2.77 : 1
6	Lambda cyhalothrin 5 EC	1.00	51.44	55.11	106007	773.00	28500.00	29273	76734	2.62 : 1
7	Fipronil 5 SC	2.00	59.33	64.55	122456	1958.00	28500.00	30458	91998	3.02 : 1
8	Deltamethrin 2.8 EC	1.00	41.33	58.22	87680	1036.00	28500.00	29536	58144	1.96 : 1
9	Dimethoate 30 EC	2.00	41.11	60.33	87647	1657.00	28500.00	30157	57490	1.90 : 1
10	Untreated control	-	39.56	54.11	82572	-	28500.00	28500	54072	1.89 : 1

Price of rice grain= Rs. 1868 per quintal; Price of fodder= Rs. 180 per quintal (As per APMC, Mandya, August 2021)

Conclusion

From the results of the present study, we can conclude that fipronil 5 SC @ 2.00 mL L^{-1}, thiamethoxam 25 WG @ 0.30 g L^{-1} and imidacloprid 17.8 SL @ 0.30 mL L^{-1} were found to be effective for the management of paddy earhead bug with highest monetary returns. However, fipronil 5 SC @ 2mL L^{-1} recorded higher grain yield, net returns and B:C ratio (3.02:1) compared to other treatments.

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ORIGINAL RESEARCH ARTICLE

An analysis of marketing channels and constraints faced by rice farmers in marketing of Telangana Sona variety in Nalgonda district of Telangana

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Abstract

The present study was undertaken in Nalgonda district of Telangana to analyse the marketing channels and constraints faced by rice farmers in the marketing of Telangana Sona variety. A total sample of 120 farmers from 6 villages of three mandals in Nalgonda district of Telangana was selected for the study. Producer → Miller → Retailer → Consumer was the major marketing channel in the study area. Garrett's ranking technique to identify the constraints faced by the farmers in marketing of Telangana Sona rice variety revealed that the most important problem perceived by the farmers in the study area was the low procurement price offered for Telangana Sona by the millers, compared to other popular varieties, followed by millers not buying the produce due to surplus stock. The other major problems for marketing the produce were lack of adequate and timely transportation and storage facilities and delay in payments. Since the procurement price offered for Telangana Sona by the millers was comparatively low, there is an urgent need to create awareness about the unique health benefits of the variety among the millers and consumers and support the farmers by providing price on par with the other popular varieties. Also, there is a need to devise a marketing strategy to help the farmers to realise a better price to accelerate the adoption of the variety in the state.

Keywords: Rice, Telanagana Sona, Marketing Channels, Constraints, Garretts' ranking

Introduction

In India, rice is the most important and extensively grown food crop, occupying nearly 43 million hectares, or nearly 44 per cent of the total area under cereals in the country. India has the largest area in the world accounting for nearly 28.2 per cent of the world area under rice. India is the second largest producer and consumer of rice in the world. The productivity of rice has increased from 1984 kg per hectare in 2004-05 to 2659 kg per hectare in 2019-20 due to improved technologies, irrigation facilities and government schemes and initiatives. Rice had a share of 43.5 per cent in the total cereals production in 2019. Major paddy growing states in India are West Bengal, Uttar Pradesh, Punjab, Telangana and Andhra Pradesh **(**Source: Directorate of Economics & Statistics, DAC&FW,2020).

Telangana has achieved a record procurement of 11 million tonnes of paddy in 2019-20 and became 'Rice Bowl' of the country. Total area under paddy in the state reached to 2.8 million hectares in 2019-20 (DES, Telangana, 2020). Total paddy production in the state increased to 10 million tonnes in 2019-20 from 6.2 million tonnes in 2017-18. Productivity increased to 3436 kg per hectare in 2018-19 against 3176 kg per hectare in 2017-18 in Telangana.

Telangana Sona (RNR 15048) is a new paddy variety developed by PJTSAU in the year 2015 with special characteristics such as short duration (125 days), water conserving, fine grain, high yielding and blast resistant. Due to these traits, it was suggested for the farmers to cultivate this variety. Also, it has low glycaemic index, making it ideal for rice eating diabetics (https://www.pjtsau.edu.in/files/Newlycrop2015.pdf). Considering the growing importance to the variety, Telanagan Sona, this study was undertaken to analyse the marketing channels and constraints in marketing of Telangana Sona variety in Telangana state.

Methodology

In Telangana, Nalgonda, Karimnagar, and Nizamabad are the major paddy growing districts (**Table. 1**). Nalgonda is the highest paddy growing district in the Telangana with 4.02 lakh acres of area in *Kharif-2020* (Source: Directorate of Economics and Statistics, Telangana 2020).

Table:1 Area under rice in major rice growing districts of Telangana *in Kharif*– 2020

S.No	District	Area (acres)
1	Nalgonda	4,01,684
2	Suryapet	4,01,580
3	Nizamabad	3,86,156
4	Khammam	2,83,942
5	Jagitial	2,83,107
6	Karimnagar	2,52,957
7	Peddapalli	2,05,089
8	Yadadri Bhuvanagiri	2,04,502

Source: Directorate of Economics and Statistics, Telangana, 2020

The study has been carried out in Nalgonda district as it is the highest paddy cultivating district in Telagana. The Nalgonda district is basically an agrarian ditrict with good irrigation sources and favourable climatic conditions. Approximately 75% of population depends directly or indirectly on agriculture in Nalgonda district. The major crops grown are paddy and cotton. Telangana state is the seed bowl of India and the contribution of Nalgonda district in this regard is sizeable.

Nalgonda district has 31 mandals and among them Miryalguda, Nidamanoor, Nalgonda, Kanagal, Thripuram, Thipparthy and Vemulapally are major paddy growing mandals. In Nalgonda, Telangana Sona, Chintu, MTU-1010, KNM-118, MTU-1156, BPT-5204 and HMT, are major paddy varieties grown in fine grain paddy production. Telangana Sona (RNR-15048) was the major cultivated variety in the study area, as it covered more than 80 percent of area in *kharif* 2020.

Telangana Sona occupies the major rice area in Nalgonda district. Among the 31 mandals in Nalgonda district, three major paddy cultivating mandals were selected. From each mandal two villages and from each village 10 farmers cultivating Telangana Sona variety and 10 farmers cultivating Chintu, the other popular variety grown in the study area, were selected as sample for the study. Total sample size was 120 farmers (**Table 2**).

Table 2: Mandal wise distribution of sample farmers

S. No	Mandal	Villages	Sample farmers who cultivated Telangana Sona	Sample farmers who cultivated Chintu variety
1.	Nidamanooru	Mupparam	10	10
		Errabelli	10	10
2.	Adavidevulapalli	Ulshaya palem	10	10
		Bangarigadda	10	10
3.	Kanagal	Dorepalli	10	10
		Shabdullapuram	10	10
		Total	60	60

Marketing channel reveals how the produce passes through different agencies from producer to final consumer. In marketing of Telangana Sona, the major marketing channels were identified through survey data collected from the sample farmers.

Constraints in marketing were studied by Garrets ranking technique. Constraints faced by farmers were grouped under following heads:

- ❖ Lower price compared to other popular varieties
- ❖ Transportation problem
- ❖ Storage problem
- ❖ Delay in payments

- ❖ Millers not buying produce due to surplus stock
- ❖ Problems at the miller level
- ❖ Malpractices at the market

Garett's ranking technique

Garett's ranking technique was used to indicate the constraints faced by the farmers in marketing of Telangana Sona. The individual rank was converted into percent position by using the formula given below.

$$\text{Percent position} = \frac{100 \text{ X } (R_{ij} - 0.5)}{N_j}$$

R_{ij} = Rank given to the i^{th} attribute by the j^{th} individual

N_j = Number of attributes ranked by the j^{th} individual.

The percent position of each rank was converted into scores using Garrett's table. For each constraint, scores of individual respondents were added together and were divided by total number of respondents for whom scores were added. Thus, mean score for each constraint was ranked by arranging them in descending order. Similar analysis was done by Nirmala *et al.*, (2013).

Results and Discussion

Marketing channels for Telangana Sona

Marketing channel reveals how the produce passes through different agencies from producer to final consumer. In marketing of Telangana Sona, the following channels were observed (**Table 3**).

Table No.3: Marketing Channels for Telangana Sona in the study area

Sl. No.	Marketing Channel	Percentage of farmers who adopted the marketing channel
I.	Producer Miller Retailer Consumer	80
II.	Producer Commission agent Miller Retailer Consumer	5
III.	Producer Milled by producer farmer Consumer	8.4
IV.	Producer Govt. procurement agencies Miller Retailer Consumer	6.6

Channel I (Producer → Miller → Retailer → Consumer) was the major marketing channel in the study area. Eighty percent of the sample farmers growing Telangana Sona were selling their produce directly to the rice millers. Very few farmers (8.4%) were milling their produce on their own and selling directly to their neighbour farmers or at nearby towns and gaining additional income. Five percent of the farmers were selling the produce to the commission agents and 6.6 % of the sample farmers were selling the produce to government procurement agencies.

The constraints faced by the farmers in the marketing of Telangana Sona were identified and listed. Their ranks based on Garrett's Ranking method are presented in the T**able 4.** The major problems faced by farmers in marketing of Telangana Sona were lower price offered by the millers for Telangana Sona compared to the other popular varieties (73.2%). During the survey, the sample farmers informed that few millers cited the comparatively high broken kernels in Telangana Sona as the reason for offering low price for Telangana Sona. Since the procurement price offered for Telangana Sona by the millers was comparatively low, there is an urgent need to create awareness about the unique health benefits of the variety among the millers and consumers and support the farmers by providing price on par with the other popular varieties. Millers not buying produce due to surplus stock (60.3%), lack of adequate and timely transportation (58.2 %) and lack of adequate storage facility (46.4%), delay in payment (38.35%), problem at miller's level (38.25%) and malpractices at market (31.03%) were the other constraints as opined by the sample farmers (**Figure 1**).

Table: 4. Constraints faced by farmers in marketing of Telangana Sona

Factor	Average score	Rank
Lower price compared to other popular varieties	73.2	1
Millers not buying produce due to surplus stock	60.3	2
Transportation problem	58.2	3
Storage problem	46.4	4
Delay in payments	38.35	5
Problem at miller's level	38.25	6
Malpractices in Market	31.03	7

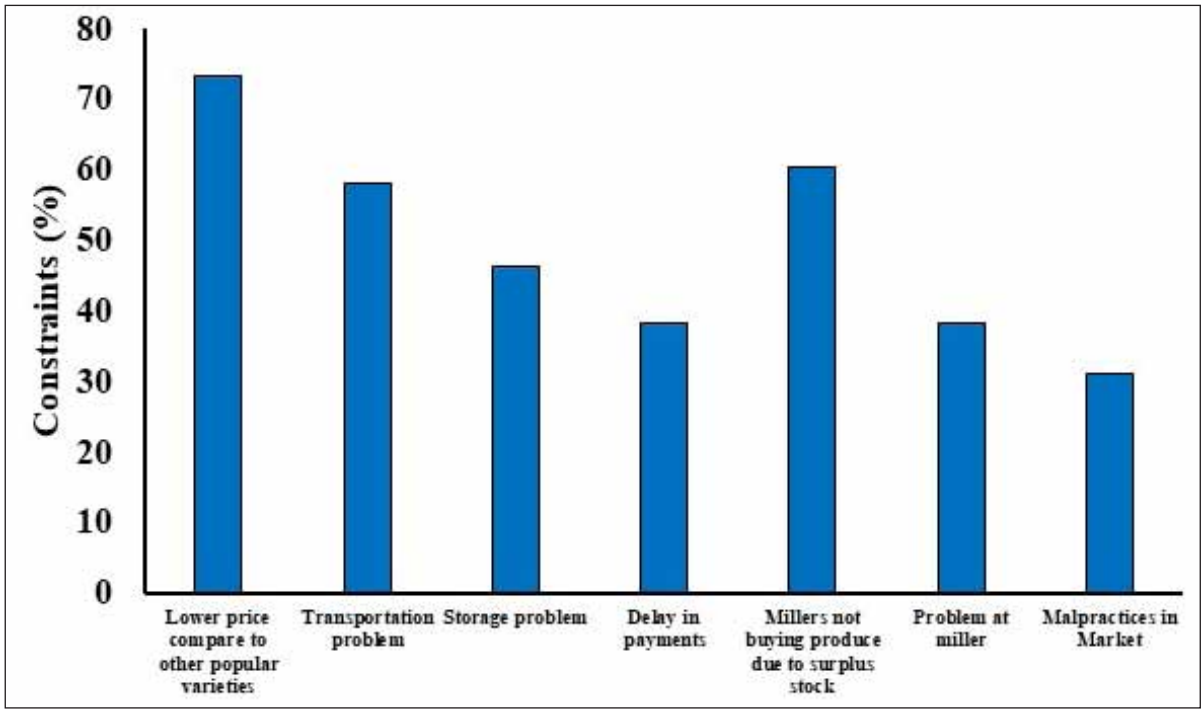

Figure 1: Constraints faced by farmers in marketing of Telangana Sona

Conclusion

(Producer → Miller → Retailer → Consumer) was the major marketing channel in the study area. Garrett's ranking technique to identify the constraints of farmers in marketing of Telangana Sona rice variety revealed that the most important problem perceived by the farmers in the study area was the low procurement price of Telangana Sona compared to other popular varieties, followed by millers not buying produce due to surplus stock. The other major problems were transportation, storage and delay in payments. The problems at miller's level and the malpractices at the market were other constraints as opined by the sample farmers. Since the procurement price offered for Telangana Sona by the millers was comparatively low, there is an urgent need to create awareness about the unique health benefits of the variety among the millers and consumers and support the farmers by providing price on par with the other popular varieties. Also, there is a need to devise a marketing strategy to help the farmers to realise a better price to accelerate the adoption of the variety in the state.

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SHORT COMMUNICATION

Telangana sona (RNR 15048): a short duration, low glycemic, super fine grain, high yielding rice variety

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Abstract

Telangana sona (RNR 15048) was derived from a cross between two released varieties, MTU 1010 and JGL 3855 through pedigree method of breeding. Telangana Sona is a short slender, super fine variety suitable for both *kharif* and *rabi* seasons. It is semi-tall, short duration (125 days), high yielding variety (6500-7000 kg/ha) having resistance to blast disease, suitable for late sowing conditions and has high head rice recovery (>67%). It is a low glycemic index variety with GI of 51.0. Telangana Sona was released by Professor Jaya Shanker Telangana State Agricultural University (PJTSAU) through 1st State Variety Release Committee (SVRC) meeting of Govt. of Telangana and was notified vide Indian Gazette Notification NO. S.O 2238€, dated 29.06.2016 for cultivation in the State of Telangana. Considering the uniqueness of the variety, Telangana Sona (RNR 15048) variety was also registered under PPV&FRA bearing Registration No. 196 of 2018 at Govt. of India.

Keywords: Telangana Sona, State variety, super fine grain, low glycemic index

Introduction

Rice is one of the major cereal crops feeding over more than half of the world's population. In India, rice crop is cultivated in 43.66 MHa producing 118.87 MT rice with productivity of 2722 kg/ha (Indiastat, 2019-20). Rice is being cultivated both in *kharif* and *rabi* seasons as one of the most important crop in Telangana. Rice being the staple food of Telangana, requires about 60-65 lakh tons annually to feed the population. During the year, 2019-20, rice crop was grown in Telangana in an area of about 31.78 lakh hectares producing 129 lakh tons of rice with the productivity of 4062 kg/ha (DES, 2020). Comparing the productivity of rice over other states, Telangana stood in second rank after Punjab which was evident in recent years with much emphasis paid on increasing irrigation facilities of the state.

Hitherto the proportion of coarse grain varieties was more in the state, in view of the ease of cultivation, mostly short duration and less prone to insect pests and diseases. However, huge demand exists from the consumers for fine grain varieties and more specifically to super fine (short slender) varieties. The popular fine grain varieties in the state are BPT 5204, JGL 1798, WGL 14, HMT Sona. Though they have very good physical and cooking quality grain parameters, most of them were medium to long in duration and susceptible to major insect pests and diseases.

In the context of limited resources of irrigation water and electricity, there was a dire need to develop a super fine grain, short duration, biotic stress tolerant varieties with good eating qualities to meet the demands of producers as well as consumers.

Keeping this in view, Rice Research Centre, ARI, PJTSAU (Formerly ANGRAU), Rajendranagar, Hyderabad initiated a breeding programme during the year, 2006 with an objective to evolve very fine grain, short duration, blast resistant, BPH tolerant, high yielding variety with good cooking quality comparable with BPT 5204. The pedigree method of breeding was followed involving two released varieties *viz*., MTU 1010 and JGL 3855 as parents (**Figure 1**). Female

parent, MTU 1010 was most popular long, slender, short duration variety having tolerance to blast and BPH, whereas the male parent, JGL 3855 was super fine grain, medium duration variety having tolerance to blast and gall midge.

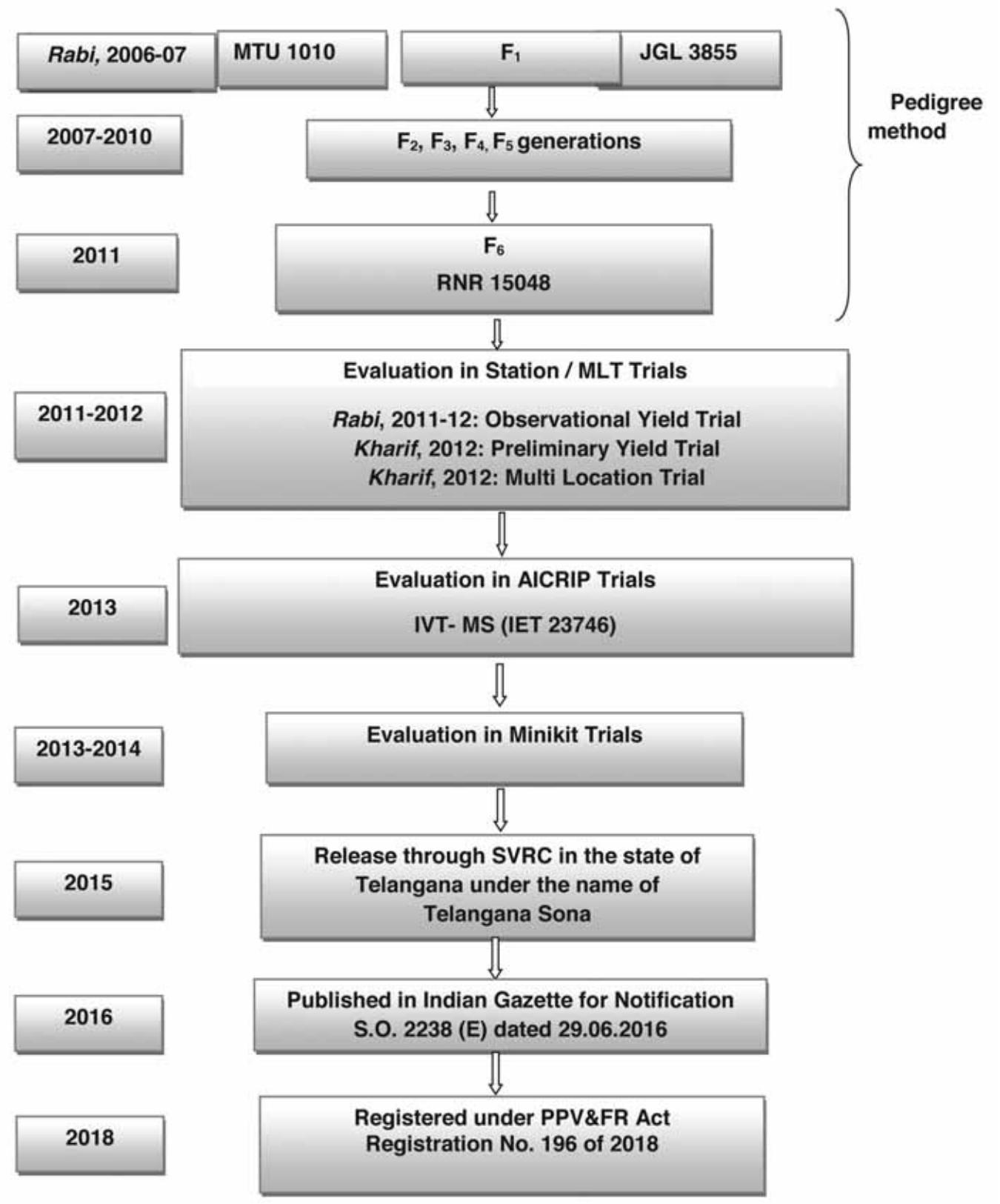

Figure 1: Pedigree flow chart of RNR 15048 (IET 23746) varietal development

Yield performance

During *kharif*, 2011, the F_6 plant to progeny row bearing number 15048 was identified as uniform with desirable phenotypic characters and promoted to Observation Varietal Trial (OVT) in *rabi*, 2011-12 with the designation RNR 15048. Considering its superior grain quality, yield and duration advantages, RNR 15048 was advanced to PVT and Multilocation Trials of PJTSAU during 2012. Simultaneously, RNR 15048 (IET 23746) was nominated to AICRIP trials during the year 2013. The mean data across years and trials clearly revealed that RNR 15048 (6085 kg/ha) has recorded 6.65% additional grain yield compared to local check (5706 kg/ha) **(Table 1).**

During the year 2013, the RNR 15048 has been approved for 1st year minikit (farmer's field) testing for both *kharif* and *rabi* seasons by the university authorities during state level annual workshop. Accordingly, RNR 15048 was evaluated in farmer's field during *kharif*, 2013 and 2014 in comparison to popular check, BPT 5204 and during *rabi*, 2013-14 with check, MTU 1010. The results showed that RNR 15048 had performed well with 11.48% and 7.65% yield superiority over checks, BPT 5204 and MTU 1010, respectively **(Table 2).**

Table 1. Grain yield of RNR 15048 in different trials across seasons and years

Trial Name	Season/year	No. of locations/ trials	Grain yield (kg/ha)		Per cent increase over Check
			RNR 15048	Local Check	
Station Trials	*Kharif* (2012)	4	6649	6199	7.26
Station Trials	*Rabi* (2011-12 & 2012-13)	2	6582	6438	2.24
Multi Location Trials	*Kharif* (2012)	6	6114	5663	7.96
AICRIP Trial (Telangana State)	*Kharif* (2013)	3	4945	4648	6.39
Weighted Average		**15**	**6085**	**5706**	**6.65**

(Source: SVRC Release Proposals of RNR 15048)

Table 2. Grain yield (kg/ha) of RNR 15048 in minikit trials (at farmers' fields) across seasons and years in Telangana during 2013 and 2014

Agro-climatic Zone	District	*Kharif (2013 & 2014)*		*Rabi (2013-14)*	
		RNR 15048	Check (BPT 5204)	RNR 15048	Check (MTU 1010)
Central Telangana	Warangal	6072	5651	6507	5734
	Khammam	6345	5739	6453	6264
	Medak	6298	5685	5725	5575
Southern Telangana	Nalgonda	6936	6008	7630	7620
	Mahabubnagar	6120	5132	6660	5669
	Rangareddy	6998	6062	6785	6071
Northern Telangana	Karimnagar	7228	6751	-	-
	Adilabad	3460	3420	-	-
	Nizamabad	3392	2958	-	-
Mean		**5872**	**5267**	**6627**	**6156**
Per cent increase over Check		**+11.48**		**+7.65**	

(Source: SVRC Release Proposals of RNR 15048)

RNR 15048 was also evaluated in agronomic trials at Rice Research Centre, ARI, Rajendranagar at different fertilizer levels as well as various dates of sowings during *kharif*, 2013. The data revealed that RNR 15048 has exhibited higher grain yield (6351 kg/ha) than BPT 5204 (4833 kg/ha) and on par yield with MTU 1010 (6475 kg/ha) at recommended levels of nitrogen fertilizer (120 kg N/ha) (**Table 3**). Conspicuously, days to 50% flowering was reduced drastically under late sowings compared to check varieties (**Table 4**). The same was also confirmed by other researchers at later date (Annual Report, 2016-17), indicating that RNR 15048 had photosensitivity and took more days to attain panicle initiation in case of early *kharif* sowings. Accordingly, it has been recommended for late sowings during *kharif* with optimum period as July 10th to July end.

Table 3. Grain yield of RNR-15048 in Agronomy Trials at Rice Research Centre, ARI, Rajendranagar during *Kharif 2014*

Variety	D1 (DS: 12.06.2014)			D2: (DS: 27.06.2014)			D3: (DS: 12.07.2014)			Mean		
	60 kg N/ha	120 kg N/ha	180 kg N/ha	60 kg N/ha	120 kg N/ha	180 kg N/ha	60 kg N/ha	120 kg N/ha	180 kg N/ha	60 kg N/ha	120 kg N/ha	180 kg N/ha
RNR 15048	**6095**	**6472**	**6174**	**5223**	**6274**	**5804**	**5488**	**6306**	**5954**	**5602**	**6351**	**5977**
BPT 5204	5260	5373	5477	4403	4878	4721	3846	4248	4125	4503	4833	4774
MTU 1010	6396	6592	6296	5613	6458	6045	5596	6374	6059	5868	6475	6133
Krishna	5603	5968	5747	4671	5634	5395	4333	5308	4952	4869	5637	5365

(Source: SVRC Release Proposals of RNR 15048)

Table 4. Days to 50% flowering of RNR 15048 as influenced by different dates of sowing at Rice Research Centre, ARI, Rajendranagar during *Kharif* 2014

Variety	D1 (DS: 12.06.2014)			D2: (DS: 27.06.2014)			D3: (DS: 12.07.2014)			Mean		
	60 kg N/ha	120 kg N/ha	180 kg N/ha	60 kg N/ha	120 kg N/ha	180 kg N/ha	60 kg N/ha	120 kg N/ha	180 kg N/ha	D1	D2	D3
RNR 15048	**111**	**112**	**112**	**95**	**95**	**97**	**94**	**95**	**96**	**112**	**96**	**95**
BPT 5204	117	117	117	114	115	115	114	116	118	117	115	116
MTU 1010	99	101	101	91	92	92	93	95	95	100	92	94
Krishna	111	113	113	107	107	107	105	105	106	112	107	105

(Source: SVRC Release Proposals of RNR 15048)

Reaction to biotic stresses

Reaction of RNR 15048 was evaluated against blast and sheath blight incidence during *kharif*, 2012 and 2014 at Rice Research Centre, ARI, Rajendranagar and the results clearly showed that RNR 15048 was resistant to blast and sheath rot (**Table 5**). Similarly, the data pertaining to entomology trials indicated that RNR 15048 was susceptible to stem borer compared to checks (**Table 6**).

Grain quality parameters

RNR 15048 was classified under super fine grain segment with more than 3 kernel length breadth ratio. RNR 15048 was extensively evaluated for cooking quality parameters and the data showed that it had very high percent head rice recovery compared to check, BPT 5204 (**Table 7**). Further, it had recorded intermediate amylose content with desirable eating quality.

Table 5. Reaction of RNR 15048 against diseases across years during *kharif* season at Rice Research Centre, ARI, Rajendranagar

Variety	Leaf Blast score		Neck Blast score		Sheath Rot score	
	2012	2014	2012	2014	2012	2014
RNR 15048	**0**	**0**	**0**	**0**	**0**	**0**
BPT 5204	9	-	7	-	9	-
NLR 34449	0	0	0	0	0	0
Swarna	9	9	7	9	7	7

(Source: SVRC Release Proposals of RNR 15048)

Table 6. Reaction of RNR 15048 against stem borer (white ear %) at Rice Research Centre, ARI, Rajendranagar

Variety	2013 *Kharif* (OPCT)	2013 *Kharif* (MRST-IIRR)	2013-14 *Rabi* (OPCT)	Mean
RNR 15048	**26.82**	**13.3**	**18.92**	**19.7**
BPT 5204	2.97	-	-	3.0
MTU 1010	13.28	-	9.27	11.3
W 1263	-	5.35	-	5.4
TN-1	-	16.5	-	16.5

(Source: SVRC Release Proposals of RNR 15048)

Table 7. Quality characters of RNR 15048 in comparison to Check, BPT 5204 conducted by ICAR-IIRR during 2013

Parameter	RNR 15048	BPT 5204
Hulling (%)	81.0	80.0
Milling (%)	71.6	71.2
Head rice recovery (%)	67.9	58.8
Kernel length (mm)	4.91	4.75
Kernel breadth (mm)	1.62	1.78
Kernel L/B ratio	3.03	2.66
Grain type	Short Slender	Medium Slender
Grain chalkiness	Absent	Absent
Volume expansion ratio	5.3	4.7
Water uptake	250	215
Kernel length after cooking	8.0	8.5
Kernel elongation ratio	1.62	1.78
Alkali spreading value	5.0	5.0
Amylose content	20.72	23.90
Gel consistency	22.0	22
Aroma	Non Scented	Non Scented

(Source: SVRC Release Proposals of RNR 15048)

Glycemic Index:

Generally, all high carbohydrate foods are rated as high in Glycemic Index (GI) which evaluates the standard (GI of glucose is 100). Diets which are higher in glycemic load implicated the development of various metabolic and chronic diseases such as diabetes and various cardiovascular diseases. Several studies have reported that higher intake of rice is strongly associated with type 2 diabetes. Systematic reviews from various parts of the world have shown that the GI of various varieties of rice ranged between 48 to 93. Foods are categorised as low-GI (≤ 55), medium-GI (56-69), high-GI (≥70), based on their ability to raise blood glucose.

The results conducted at Post Graduate & Research Centre, Department of Home Science, PJTSAU, Hyderabad on glycemic index revealed that, RNR 15048 has been classified as low glycemic Index variety with value of 51.0 whereas, BPT 5204 has been classified as moderate with GI of 56.5. Further, the results showed that RNR 15048 had recorded significant difference with respect to GI (blood glucose response) when compared with reference food. These results are akin to the reports of Prasanthi *et al.* (2019) where the GI of Telangana Sona rice was reported as 51.72.

Release and notification

Based on several advantages of the culture, RNR 15048 over existing varieties, PJTSAU has released RNR 15048 as ***Telangana Sona*** through 1st State Variety Release Committee meeting of Govt. of Telangana held during 2015. Subsequently, Telangana Sona (RNR 15048) variety was notified vide Indian Gazette Notification No.S.O. 2238 (E) dated 29.06.2016 and recommended for cultivation in the state of Telangana. Further, considering the uniqueness of the variety, Telangana Sona (RNR 15048) variety was also registered under PPV&FRA bearing Registration No. 196 of 2018 at Govt. of India (**Figure 2**).

Figure 2: Field view of Telangana Sona (RNR 15048) variety

Varietal spread

Keeping in view of its desirable traits with respect to farmer (high yield, blast tolerance, short duration, good market price), trader (super fine grain, high demand), miller (high head rice recovery), consumer (low glycemic index, good cooking quality), the variety was cultivated in about 13 lakh acres in the state of Telangana during 2020-21. Due to wider adoptability and preferences, the variety is being cultivated in many other states *viz*., Andhra Pradesh, Karnataka, Tamil Nadu, Maharashtra, Odisha, West Bengal etc. To meet the demand for supply of quality seed, about 250 quintals of breeder seed is being produced every year by PJTSAU and being supplied to various seed producers.

Further, a tripartite agreement was signed on strategic branding and marketing of Telangana Sona (RNR 15048), super fine rice between Dept. of Agriculture., Govt. of Telangana, PJTSAU and Indian School of Business (ISB) on 14.08.2020. This agreement assists in improving the brand image of Telangana Sona rice at national and international markets. The University is also popularising the benefits of low GI rice through MOAs with various firms for branding and marketing of super fine rice variety, Telangana Sona. Due to branding with private companies Telangana Sona rice is being exported to other countries also. In order to take advantage of this unique rice variety, many companies are looking forward to sign MOA with PJTSAU which would certainly assist in further popularisation and making the quality and healthy rice available to the consumer.

Salient features of Telangana Sona (RNR 15048) variety:

- It is a short slender (super fine) variety suitable for both *kharif* and *rabi* seasons.
- It is a semi-tall, short duration (125 days) high yielding variety having resistance to blast.
- It has grain yield potential of about 6500 to 7000 kg/ha.
- Suitable for late sowing conditions and hence provision to raise green manure crops immediately after the onset of monsoon which certainly assists

in improving the soil physical and chemical properties for sustainable rice production.

- It has high Head Rice Recovery (> 67 %) and hence even suitable during *rabi* season, which is a major advantage to the famers and millers.
- It became popular on account of its unique grain size and shape (short slender) and cooking qualities on par with BPT 5204 as evident from equal values of amylose content, alkali spread (ASV) and gel consistency.
- RNR 15048 has been classified as low Glycemic Index variety with GI of 51.0.

Impact

- With adoption of Telangana Sona (125 days) in place of BPT 5204 (145 days) in an area of 10 lakh acres, Telangana state could save 28 TMC water during 2020-21.
- Telangana Sona being resistant to blast, less BPH incidence than BPT 5204 could save ₹2000 per acre on plant protection cost accounting ₹20.0 cr. per every 1 lakh acres.
- 5 % higher milling recovery than BPT 5204 resulted in 1 lakh tonnes additional rice yield to millers of Telangana State during 2020-21.
- Being super fine grain, fetching ₹150 – 200 more per quintal than other fine grain varieties generating ₹500 - 600 cr. additional income to Telangana farmers annually.
- Large scale adoption of Telangana Sona played key role in increasing the productivity of the State.

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SHORT COMMUNICATION

Telangana Vari 4 [IET 27869 (JGL 25958)] – Mid early duration, long slender, high yielding rice variety

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Abstract

Telangana Vari 4 was derived through pedigree method of breeding from a cross MTU 1010 and NLR 34449 with an aim to develop a high yielding short duration variety. It has long slender grains with mid early duration and suitable for both *kharif* and *rabi* seasons. It was evaluated during 2018 to 2020 in AICRIP trials and recorded +10.51, +63.73 and +21.18 percent increase in yield over national, zonal and local checks, respectively in overall mean. Telangana Vari 4 was found to have moderate resistance to neck blast and rice tungro virus. Telangana Vari 4 was recommended by Varietal Identification Committee (VIC) during 2021 and approved by Central Sub-Committee on Crop Standards, Notification and Release of Varieties for irrigated conditions in the states of Odisha, Bihar and Jharkhand (Zone III) and notified vide Gazette Publication No. S.O. 8 (E) Dated 24.12.2021.

Keywords: Telangana, Rice, high yielding, duration, moderate resistance

Introduction

Short duration rice varieties in general have an advantage of suiting to different seasons and sowing windows. Long slender grain varieties have more preference and occupy more area than medium slender or bold grain varieties owing to fetching better market price. Accordingly, a cross was attempted between MTU 1010 (High yielding, short duration variety) and NLR 34449 (High yielding, fine grain variety with blast resistance) during the year 2007 with an objective to develop high yielding short duration, long slender variety at Regional Agricultural Research Station, Jagtial, Telangana. The pedigree method of breeding was followed and handled the segregating material from 2008 to 2014. Telangana Vari 4 was identified in the year 2014 at F_7 generation and promoted for replicated yield testing during *rabi*, 2014-15.

Morphological traits

Telangana Vari 4 is a short duration (115-120 days) variety suitable for *kharif* and *rabi* seasons. It has semi-erect, medium dwarf plant type, long and well exerted panicles (**Figure 1**). It has erect to semi erect and medium size flag leaf with no pigmentation on

Figure 1: Field view of Telangana Vari 4 (JGL 25958) variety

any plant part. The grains are devoid of awns, long slender in shape with 6.27 mm kernel length, 2.01 mm kernel breadth and 3.12 L/B ratio. Matured grains have straw coloured glumes, translucent kernel with no abdominal white (**Figure 2**). The test weight ranges from 21 to 22 g.

Figure 2: Grain, rice and panicle of Telangana Vari 4 (JGL 25958) variety

Yield performance

Telangana Vari 4 was tested in AICRIP yield trials *viz*., IVT ETP, AVT 1 ETP and AVT 2 ETP during 2018 to 2020 and exhibited 32.08, 45.57 and 21.33 per cent yield increase in Bihar, 4.19, 68.51 and 14.41 per cent yield increase in Jharkhand, 79.96 and 25.54 per cent yield increase in Odisha over zonal and local checks, respectively. The test entry also exhibited 12.24, 0.61 and 2.98 per cent yield increase over hybrid check in Bihar, Jharkhand and Odisha, respectively.

Telangana Vari 4 has recorded 10.51, 63.73 and 21.18 percent increase in yield over national, zonal and local check, respectively in overall mean in the states proposed *viz*., Bihar, Jharkhand and Odisha. It also exhibited 5.24 per cent yield increase over the hybrid check. The entry has shown superior performance in Zone III (Eastern) over national, zonal and local checks with 12.24, 44.12 and 12.80 per cent increase, respectively.

In Agronomy trials with different nutrient levels conducted at different locations indicated the significant yield reduction by application of only 50% RDN (Low input) at Coimbatore (4.87 t/ha), Faizabad (3.95 t/ha), Karjat (2.80 t/ha), Maruteru (3.38 t/ha), Rewa (4.70 t/ha), Sabour (4.16 t/ha), Vadgaon (3.78 t/ha) and Varanasi (3.15 t/ha) compared to 100% RDN, whereas, 50% RDN recorded numerical yield advantage at Hazaribagh (4.75 t/ha) and Jagdalpur (3.02 t/ha) over 100% RDN.

Reaction to biotic and abiotic stresses

Based on NSN1 and NSN2 screening trials data of AICRIP, Telangana Vari 4 was found to have moderate resistance to neck blast, rice tungro virus and grain discoloration.

Grain quality parameters

Telangana Vari 4 has good quality parameters of high milling percentage (70.2%) and head rice recovery (61.35%), It has intermediate amylose content of 22.38%, alkali spreading value of 4.0, gel consistence of 72.5 with very occasional chalkiness.

Release and notification

Telangana Vari 4 was recommended by Varietal Identification Committee (VIC) during 2021 and approved by Central Sub-Committee on Crop Standards, Notification and Release of Varieties for cultivation in the states of Odisha, Bihar and Jharkhand (Zone III) and notified vide Gazette Publication No. S.O. 8 (E) Dated 24.12.2021.

SHORT COMMUNICATION

Rajendranagar Vari-1 [IET 27077 (RNR 11718)] - Multiple stress resistant, high yielding rice variety

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Abstract

Rajendranagar vari-1 was derived through pedigree method of breeding from a cross MTU 1010 x NLR 34449 with an aim to develop high yielding multiple stress resistant variety. It is a medium duration variety having medium slender grain. It has recorded 6.81% and 9.7% increase in yield over check variety in station trials and minikits, respectively. Rajendranagar vari-1 (IET 27077) was evaluated during 2017 to 2020 in AICRIP trials and recorded 23.29, 18.61, 39.69, 40.12 and 12.46 percent increase in yield over alkaline, inland saline, saline tolerant, sensitive and local check, respectively in in zone VI and VII states (Puducherry, Telangana and Gujarat). It is identified as fertilizer responsive with potential to yield about 8.0 t/ha. It has multiple resistance to different insect pests and diseases (BPH, Blast, Brown Spot, Sheath Rot, grain discoloration) besides having salinity tolerance. Rajendranagar vari-1 was released through Telangana State Variety Release Committee and notified vide gazette number S.O. 8 (E) Dated 24.12.2021.

Keywords: Rice, variety, multiple resistance, high yielding, Telangana

Introduction

In Telangana, majority of the famers prefer to grow medium duration, high yielding varieties. However, the available medium duration varieties are susceptible to major insect pests and diseases resulting in high cost of cultivation and realizing less profits to the farmers. Moreover, due to rice-rice system of cultivation, the area under salinity is also being increased in Telangana state. Hence, there was a need to develop a medium duration variety having resistance to multiple insect pests and diseases apart from salinity tolerance. Accordingly, a cross was attempted between MTU 1010 (High yielding, short duration variety) and NLR 34449 (High yielding, fine grain variety with blast and salinity tolerance) during the year 2006 with an objective to develop high yielding multiple resistant variety and carried forward the breeding material through pedigree method. Rajendranagar vari-1 was identified during 2010 in F6 generation and promoted for replicated yield testing.

Morphological traits

Rajendranagar vari-1 is a medium duration (135-140 days), medium slender grain culture suitable for *kharif*. It has erect, semi dwarf nature, dark green foliage and good tillering habit with good panicle exertion (**Figure 1**). It has erect and medium size flag leaf with

Figure 1: Field view of Rajendranagar vari-1 (RNR 11718) variety

no pigmentation on any plant part. The grains have 5.43 mm kernel length, 2.08 mm kernel breadth and 2.61 L/B ratio. Matured grains have straw coloured glumes, translucent kernel with no abdominal white. The test weight ranges from 17 to 19 g (**Figure 2**).

Figure 2: Grain, rice and panicle of Rajendranagar vari-1 (RNR 11718) variety

Yield performance

Rajendranagar vari-1 recorded 6.81% yield superiority over check, MTU 1001 in station trials (2011 to 2015). It recorded 9.7 per cent higher yield (6555 kg/ha) compared to check, MTU 1001/BPT 5204 (5975 kg/ha) in minikit testing over three years across 173 test locations at farmers' fields. Rajendranagar vari-1 recorded 16.7% higher grain yield (4400 kg/ha) than saline tolerant check, CSR 23 (3770 kg/ha) and identified as salinity tolerant, nominated to AICRIP trials. It was evaluated during 2017 to 2020 under IET 27077 and recorded 23.29, 18.61, 39.69, 40.12 and 12.46 percent increase in yield over alkaline, inland saline, saline tolerant, sensitive and local checks, respectively in zone VI and VII states (Puducherry, Telangana and Gujarat). It was found to be fertilizer responsive with higher yields at high nitrogen doses and recorded significantly high grain yield (7256 kg/ha) compared to checks, MTU 1010 (6097 kg/ha), BPT 5204 (4413 kg/ha) and RNR 15048 (5792 kg/ha). Agronomy trials conducted at different locations with different nutrient levels indicated highest Grain Yield Efficiency Index (GYEI) of 1.27 for Rajendranagar vari-1 followed by checks, CSR 36 (1.05), CSR 23 (1.0), CSR 10 (0.84) and local check (1.04).

Reaction to biotic and abiotic stresses

Perusal of data on the reaction of Rajendranagar vari-1 against different diseases in station and AICRIP trials, indicated that it has moderate resistance to leaf blast, neck blast, bacterial leaf blight and brown spot. Similarly, it has moderate resistance to insect pests BPH, stem borer and leaf folder. However, it was found susceptible to gall midge. It can be concluded that Rajendranagar vari-1 offers multiple insect pest and disease resistance, which can help in reducing the plant protection costs of the farmers by Rs. 3000 to 5000/- per acre. Performance of Rajendranagar vari-1 under alkalinity and inland salinity trials clearly depicts that it has salinity tolerance.

Grain quality parameters

Rajendranagar vari-1 has good grain quality parameters of high milling percentage (67.7%) and head rice recovery (60.5%). It has intermediate amylose content of 23.7%, alkali spreading value of 6.0, gel consistence of 39.0 with very occasional chalkiness. The kernels have 10.35% crude protein and found to be nutritionally rich.

Release and notification

Rajendranagar vari-1 was recommended for released through State Sub-Committee on Crop Standards and Release of Varieties during the year 2021 and notified by Government of India vide Gazette Publication No. S.O. 8 (E) Dated 24.12.2021 for cultivation in Telangana state.

SHORT COMMUNICATION

Rajendranagar Vari-2 [IET 26143 (RNR 15435)] – An aromatic long grain rice variety

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Abstract

A cross was performed between RNR 17818 and Vasumathi with an aim to develop aromatic long grain rice varieties suitable for Telangana and forwarded the generations following pedigree of breeding. Rajendranagar vari-2 was identified in F_7 generation during the year 2011 and evaluated in yield and screening trials. It is an aromatic long grain, medium duration (135-140 days) culture suitable for *kharif*. It has recorded 58.8%, 13.19%, 17.4% yield superiority over check, Sumathi in station trials, multilocation trials and minikit trials, respectively. Rajendranagar vari-2 has moderate resistance to blast and brown spot. It also has good cooking quality parameters with aroma, intermediate amylose and more kernel length after cooking. Rajendranagar vari-2 was notified for cultivation in the state of Telangana vide Indian Gazette No. S.O. 8 (E) Dated 24.12.2021.

Keywords: Aromatic rice, Telangana, long grain, high yield

Introduction

In Telangana, with concerted efforts of the government to improve irrigation water availability, rice area has increased enormously and occupied about 1 crore acre annually with the production of 218 lakh tons. However, the demand for rice has not increased as that of production in the state and accordingly, a decrease in the rice area is being advocated in the state. In this context, farmers are interested to grow speciality rices like aromatic long and short grain varieties to fetch good market price. Hitherto, only one aromatic long grain variety, Sumathi was available for cultivation in the state but it has poor cooking quality. Hence, there is a need to develop aromatic long grain variety with good eating quality. Accordingly, a cross between RNR 17818 (short duration, non-aromatic, tolerance to BPH) and Vasumathi (aromatic variety) was attempted during the year 2005 and studied different generations through pedigree method of breeding with an objective to developing aromatic long grain variety. Rajendranagar vari-2 was identified during 2011 in advanced F_7 progeny and promoted for yield evaluation.

Morphological traits

Rajendranagar vari-2 is an aromatic long grain, medium duration (135-140 days) culture suitable for *kharif*. It has erect, semi tall plant type, light green drooping foliage with good panicle exertion and medium tillering habit (**Figure 1**). Panicle is long and semi-compact with grains having short awns at upper half of the panicle. Grains ripen to bright straw colour and classified as long slender. The kernels have 7.19 mm length, 1.59 mm breadth and 4.52 L/B ratio. The test weight ranges from 17 to 19 g **(Figure 2).**

Yield performance

Rajendranagar vari-2 recorded 58.8%, 13.19% yield superiority over check, Sumathi in station trials (2012 to 2017) and multilocation trials (2017), respectively. It recorded 17.4 per cent higher yield (5101 kg/ha) compared to the check, Sumathi (4435 kg/ha) in minikit testing over three years across 190 test locations at farmers' fields (2018-2020). Rajendranagar vari-2 was evaluated during 2016 under IET 26143 and recorded mean yield of 3039 kg/ha over 9 test locations across the country.

Figure 1: Field view of Rajendranagar vari-2 (RNR 15435) variety

Figure 2: Paddy and rice of Rajendranagar vari-2 (RNR 15435) variety

Reaction to biotic and abiotic stresses

Station and AICRIP screening trials data against insect pests and diseases revealed that Rajendranagar vari-2 has moderate resistance to blast and brown spot. However, it was found to be susceptible to BPH, stem borer, gall midge, sheath blight and sheath rot.

Grain quality parameters

Rajendranagar vari-2 has good milling percentage (69.81%), head rice recovery (48.9%), intermediate amylose content (23.98%), alkali spreading value of 2.5, gel consistence of 49.5 with very occasional chalkiness. It has very good kernel length after cooking (11.23 mm) with aroma.

Release and notification

Rajendranagar vari-2 was recommended for released through State Sub-Committee on Crop Standards and Release of Varieties during the year 2021 and notified by Government of India vide Gazette Publication No. S.O. 8 (E) Dated 24.12.2021 for cultivation in Telangana state.

MEMOIR

Overview of entomology research under AICRIP –An experiential learning

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Introduction

In India, rice ecosystems are highly diverse and pose varied challenges to the rice farmers in terms of abiotic and biotic stresses limiting rice production in the country. In the context of ecosystem complexities, destabilizing pest problems as well as production requirements of different rices consumed, Indian Council of Agricultural Research (ICAR) had the foresight to come up with the concept of All India Coordinated Rice Improvement Project (AICRIP) way back in 1965. Since then, a nation-wide network of rice researchers actively supported by progressive rice farmers has been in vogue leading to development of high yielding varieties supported by suitable crop production and protection technologies for the rice farming community.

Entomology Programme

The Entomology programme under AICRIP was initiated during 1970s with major thrust on research areas related to host plant resistance (HPR) and Chemical control of insect pests of rice. A National Screening Nursery (NSN) trial was constituted to evaluate the breeding material for their reaction against major insect pests at multi-locations. The focus was on identification of donors and breeding lines for resistance to the key prevailing pests, gall midge and stem borer. Chemical control programme involved screening and evaluation of new chemicals/ insecticides for their efficacy against rice pests and compatibility of effective ones with other recommended pesticides for application in fields.

During 1980's, with the introduction of high yielding varieties and input intensive management practices, some of the insect pests like brown planthopper, cutworm and leaf folder which were hitherto considered to be of minor importance, assumed major pest status leading to the need based constitution of location specific trials on these pests. Efforts were also made to develop effective, economical and ecologically sound techniques of insecticide application. Explorative studies related to ecosystem approach were started through trials on pest management and ecology. HPR programme received global focus with the initiation of International collaborative trials such as International Rice Brown Planthopper Nursery (IRBPHN), International Rice Gall Midge Nursery (IRGMN), International Rice Stem Borer Nursery (IRSBN), and International White Backed Planthopper Nursery (IRWBPHN) across international locations to ensure utilising wider genetic base in identification of pest resistant germplasm lines. Installation of light traps in different locations also commenced to monitor changing pest scenario through assessment of trap catches throughout the year.

During the 1990s, the Entomology research programme under AICRIP continued with major focus on HPR followed by Chemical Control. The screening trials were continued for major insect pests of rice both under greenhouse conditions and at insect pests hotspot locations. Evolution and identification of field biotypes of gall midge led to initiation of gall midge biotype monitoring based on the pest reaction to a set of host plant differentials. Concerted research efforts were also initiated to investigate into quantification of natural biological control in in rice fields. A new multi-location field trial (Natural Biocontrol in Rice Ecosystem – NBRE) was designed to quantify the

pest as well as natural enemy incidence in different rice ecosystems. Insecticide screening and pesticide compatibility trials were continued along with optimum pest control experiments highlighting the role of moderate pest resistant lines. On farm IPM trials were also carried out at various locations to develop and evaluate location specific IPM modules mainly consisting of HPR and Chemical control components.

In the 21st century, during the first decade (2000-2010), the focus shifted to evolving innovative eco-friendly pest management approaches like trap crop for stem borer management and use of semio-chemicals. Utility of sex pheromone for monitoring as well as mass trapping of yellow stem borer was evaluated and verified at different locations through field trials. Also, widespread incidence and importance of planthoppers in India and other Asian countries led to special studies on planthoppers for identification of genes and insecticide resistance. Investigations into Pest and Natural enemy compositions prevailing in different rice ecosystems of the country was another initiative launched during this period. Chemical control studies were continued with shift in attention to the role of biopesticides including botanicals. Changing rice cultivation practices with the introduction of newer methods of planting such as System of Rice intensification (SRI), aerobic rice and direct seeding resulted in change in insect pest scenario. Hence, studies were initiated to know the impact of these practices on pest incidence and damage potential. Efforts were also undertaken to quantify the yield losses due to key pests, particularly stem borer and leaf folder at different locations.

In the second decade (2010-20), HPR continued being the core component of rice IPM, pest specific screening trials like Planthopper screening (PHS), Gall midge screening (GMS), gall midge special screening (GMSS) Leaf folder Screening Trial (LFST) and Stem Borer Screening Trial (SBST) were constituted to evaluate material derived from landraces and germplasm. Entries found promising were further screened in the Multiple Pest Resistance Screening Trial (MRST) to generate multiple pest resistant material. Evaluation of National Screening Nurseries (NSN1, NSN2, and NSNH & NHSN) continued across locations against major insect pests. This trial included promising entries from plant breeding trials *viz.,* Advance Variety Trial (AVT 2) material in NSN1, Initial Variety Trial (IVT) material in NSN2, entries bred for hill region in NSNH and experimental hybrids in NSNH. Besides, special complementary research network activities on evaluation of rice germplasm collection of National Bureau of Plant Genetic Resources (NBPGR) as well as material generated from rice biotechnology progressed with emphasis on rapid development of entries resistant to multiple biotic stresses including insect pests and diseases. Concomitantly, biodiversity and biological control studies were strengthened through initiation of studies on in situ conservation of biocontrol agents through ecological engineering for pest management (EEPM) and biointensive pest management (BIPM). A new initiative on fortnightly monitoring of pest incidence (Pest Survey Report – PSR) at different locations was also undertaken for development of year wise database on incidence of pests across rice ecosystems. Another new development involved formulation of an all-encompassing participatory multi location on farm IPM trial with a holistic approach of managing all the pests including insects, diseases and weeds, initiated involving multidisciplinary team of entomologists, agronomists and plant pathologists.

Salient findings and achievements

Changing pest scenario

Insect pest scenario in rice has changed drastically in the last five decades. During 1965, only three pests' i.e, gall midge, stem borer and green leafhopper were of serious concern. After the Green Revolution period fertilizer responsive high yielding varieties were introduced and subsequently there were substantial alterations in rice cultivation methods, input resource use and intercultural practices leading to remarkable shifts in insect pest scenario in terms of diversity and numbers. At present, about twenty insect species are categorised into pests of national and regional significance as well as emerging ones, resulting in significant yield loss. Short- and long-term assessment of pest populations through light trap catches under

AICRIP have revealed that among the pests of national significance, stem borers and planthoppers, followed by leaf folder continue to be the most widespread pests in terms of numbers and spread across the rice ecosystems. Among the pests of regional significance, swarming caterpillar (*Spodoptera mauritia*) along with other species, *Mythimna separata,* rice hispa and caseworm have been found increasingly prevalent.

Integrated Pest Management as the viable alternative

Integrated Pest Management with its holistic approach remains a sustainable and realistic option for rice farmers to manage the pests across different rice based cropping systems. IPM provides the ideal ecologically sound framework for managing pests under which crop management specialists can intelligently integrate available pest management components such as the use of pest resistant varieties, biological suppression or natural regulation, modification of agronomic practices and habitat manipulation or deploying, only as last resort, eco-friendly insecticides to tackle the pests in times of unexpected outbreaks or emergency situations. This long term strategy also has the potential to minimize the risks to the human health, environment as well as ensure economic and social gains for the farmers. IPM strategies have evolved over the years from reliance on single approach of chemical control to the present multi-faceted approach aided by the synergy of developments in scientific research and discoveries related to biotechnology and other fields. The newer technological innovations have offered novel opportunities for reducing dependency on chemical pesticides leading to the evolution of a holistic refinement of IPM for sustainable rice production. AICRIP has singularly provided an ideal research platform for the evolution and development of different components of IPM for adoption across rice systems, discussed briefly as under:

Pest surveillance

Pest surveillance forms the most vital cog of rice IPM strategy. Conventionally, farmers have been habituated to identify or diagnose pest problem only after pest appearance and still find it challenging to differentiate the damage symptoms arising out of an insect pest or disease attack or nutrient deficiency. Reliable and immediate identification of all the rice pests is now possible through the coordinated efforts of National Agricultural System organizations, Agricultural Universities under AICRIP and State Departments of Agriculture aided by farmers' active cooperation and response. Vast data sets on light trap catches of rice pests along with macro weather data collated by cooperating centres in the last fifty years has enabled the rice researchers to guide the farmers to monitor the onset and development of the pest population dynamics in the field. Advanced research on semio-chemicals has provided a valuable tool for accurately monitoring specific pest populations. Sex pheromones mediated traps are now popularly used by the farmers as monitoring tools to take timely actions for preventing yellow stem borer in rice. There is further scope for deployment of this technique in case of other species of stem borers like pink and white stem borers and pests such as leaf folder.

In situations where it is not possible to prevent pest attacks, regular monitoring of pest populations becomes unavoidable and even essential to avert recurrences of pest outbreak particularly in view of unpredictable disruptions due to climate change scenario. Comprehending the shifts in pest profiles over time and space needs accurate and reliable tools of pest surveillance. Efforts are now in progress under AICRIP to generate relevant data related to changes in pest populations, soil profile, plant phenology and other phytofactors along with weather parameters over diverse ecosystems for a long period using Information Technology (IT) tools. Pest forewarning systems aided by Geographical information system (GIS) and weather data driven pest distribution maps are being developed to provide real time action advisories to farmers as part of decision support system.

Host plant Resistance (HPR)

Resistant varieties are the most efficient, economical and practical tools for encountering the pest problems and are ideally compatible with other components of IPM. However, it has been observed that the pest populations are quite capable of evolving adaptive biotypes or strains to overcome the effect of resistant

varieties. Hence, concerted efforts have to be continuously in place to refine and develop varieties to withstand the newly evolving strains. Also, due to changing pest scenario and situations of altered pest profiles in different cropping systems with multiple biotic stresses occurring simultaneously in rice, developing multiple pest resistant varieties is always the need of the hour.

Enormous amount of genetic material has been screened under AICRIP leading to development of rich data base on sources of pest resistance in rice. Detailed findings on the resistant varieties/donors of pest resistance through concerted efforts till 2010, have been well documented by Bentur *et al* (2011). Research efforts undertaken in the last decade (2010-20) have further led to identification of more promising entries for utilization as resistant sources by the plant breeders.

Pest	Trial	Promising entries (source of resistance)
BPH & WBPH	PHS,GEMP, MRST, NSN, IRBPHN	5-B-3-B-4, NDR 9210, CRK 26-1-2-1, KAUM 95-1, RP 4510-75, IC Nos 346849, 347612, 343060, 311865, 346889, GRH 33, KAUM 166-2, KAUM 168-1, CR 3005-77-2, CR 3006-8-2, CR 3005-230-5, IR 65482-7-216-1-2-B, IC # 449784, 450029, IET# IET 22489, 22989, 21709, 22218, 21423, 22345, 23000, 23396, 22984, 22951, 21765, CR 3006-8-2, IR 65482-7-216-1-2-B, RP Bio 4919-501, CR 2711-149, KAUM 179-1, KAUM 179-2, KAUM 182-1, IET Nos 23118, 22486, 23073, 23110, 23083, 23101, 23132, 23130, IET Nos 23887, 23888, 23919, 23921, 23939, 23612, 23613, 23175, 23874, 23875, IET 24158, *KAUM 166-2, RP Bio 4918-236, RP Bio 4918-221 (S), RP Bio 4918-228(S)*, IC Nos 463924, 578140, 578142, 578916, 578920 & Dhanrasi, CR 2711-149, KAUM 179-1, KAUM 179-2, KAUM 182-1, CR3006-8-2,RP 4918-228(S), JGL 19618, IET 23739(NSN1-51), IET 23081, IET 23052, IET 22055, IET 22302 and IET 22648, IET Nos 23150, 24452, 23918, 24485, 24490, 24493, 24503, 23906, 23929, 24424, 24537, 24367, 24393, 24629, 24714 and Swarna-dhan, BPT 2671, CB 05 022, CB 09 123, CB 12 701, CN 1231-11-7, CR 2711-149, IR 65482-7-216-1-2-B, CN 2072, CR 1898-32-69-CN 12-2, CR 2711-149*, KAUM 179-1, KNM 113, RP BIO 5478-166 M, RP BIO 5478-176 M, RP BIO 5478-196 M, CN 1231-11-7, IET24989, IET 25220 and IET 25419, IET 23906, 23934, 23053,23066, 24425, 24441, 24419, 24424, 24385, 24412, 25675, 25676, 25677, 24481(Repeat),CO 43(RP), Sabita(NC), Swarna (Recurrent Parent), IR 81896-B-B-195 (DP), BPT 2611, JGL 27371, MTU 1245, MTU 1247, IET 25835, IET 25846, Vivekdhan 62, IET No 25750, IET Nos 23934, 24426, 25086, 25053, CSR 23, 25512 and 25676
Gall midge	GMS, GMSS, MRST	JGL 18044, JGL 18080, JGL 19618, IC363753, CAUR-1, Madhuri 9, RCM-10, CORG 24, JGL 19618, CORG 15, KNM 113, KNM 563, SKL-3-22-19-31-55-11, NP 3113-7, KNM 134, KNM 489, KNM 539, KNM 557, KNM 637, IC 462402, IC 577036, CB-07-540, SB143, SB 319, DRR H2, RP Bio 4918-236, RP BIO 4918-221(S), PTB 33, IET No.s 23185, 23411, 23421,22764, 23459, 23464, 23383, 23913, 23972, 23525, 24159 & Lalat, IC# 462336, 463240, 353834, RP Patho-01, CB 07-540 IET # 22096, 21842, 21841, 22100, 22144, 22698, 22155, 22835, 22763, 23375, 23169, 23074, 23121, 23194, 23234, 23247, 23262, KNM 637, NP 3113-7, KNM 113, KNM 539, IC 578133, COGR-2, IET 22698, IET 23194 (NSN1-93), IET 24237, IET 24320, IET 24667 and IET 23536, KNM 1623, KNM 1625, KNM 1638, RDR 1181, RDR 1188, Vellaiilankalyan, RMSG7 (DRR 17B with *Gm4 + Gm8)*, RP 5925-24, (B95-1 with *Gm8*), TH BR 69, TH BR 70 and TH BR 71(*Gm4* gene), KNM113 and KNM 339, IET 247441 and 25563, JGL 3828, JGL 21831, JGL 25998, JGL 27058, JGL 27075, KNM 1632, KNM1724, KNM 2275, KNM 1623, KNM 1638, WGL-825, WGL-1062, ASD 7,KAKAI (K 1417), PTB 12 and WGL 1145, KNM113, NP3113-7 Varalu, IET Nos 23610, 25051, 25519 and KNM 1638

Pest	Trial	Promising entries (source of resistance)
Stem borer	GEMP, MRST, NSN	IC Nos 463445, 577293, 577566, 578116, 578672 , 463175, 466430, 578996, KAUM 166-2, RP Bio 4918-142, IET No.s 23004, 23009,23185, 23308, 22894, 22568, 23431, 23440, 22752, 22763, 22777, 22289, 23459, 23081, 23088, 23118, 23073, 23919, 23600, 23604, 23574, 23589, 23839,24007, 23961, 21936, 23923, 23935, 23003, 24083, 24114 & Jalmagna, W 1263, LF 293, Anjali, RP 4645-688, LF 270, TKM 6, SB 436, IC 114978 & CSR 23, IC Nos 462271, 578136, 578912, 578942, 578943 & 579029, IET No. 23604, 24062, 24071, 24114, NSN-H-03, NSN-H-05, NSN-H-06, NSN-H-08, NSN-H-43, NSN-H-47, RP 5587-B-B-B-258-1, RP 5588-B-B-B-B-32, RP 5588-B-B-B-B-63,IET 23642, IET 23053, IET 23596, IET 23413, IET 24601, IET 24673, IHRT 06, IHRT M-7 and HRT MS16 (IET 24894), JGL 23655, JGL 23824, JGL 23746, IIRR-BIO-SB-3, RP 5893-259-17-13-6-1-B-B-4, JGL 21836, RP 5588-B-215 and IIRR-BIO-SB-9, CR 1898-32-69-CN 12-2, RP 5588-B-B-B-B-63*, KNM 113, RP Bio 4918-142 S* and RP BIO 5478-166 M, IIRR-BIO-SB-8, RP 5588-B-B-B-B-38, RP 5588-B-B-B-B-159-2, JGL 23835, RP 5588-B-B-B-B-45, RP 5588-B-B-B-B-48, RP 5588-B-B-B-B-51, RP 5588-B-B-B-B-54, RP 5588, JGL 23848, JGL 23746, IIRR-BIO-SB-2, CN 2069, RP 5893-382-54-8-2-1-B-B, JGL 23848, JGL 23746 and RP 5893-382-54-8-2-1-B-B-5,
Leaf folder	LFST, GEMP, MRST, NSN	W 1263 (CBT), PTB 12, IC 449877, CR 2711-76, RP Patho-04, TNRH 206, IET # 22548, 21850, 22568, 22552, 21858, 22222, 22552, 22155, 22199, 22223, 22439, 22449, 22486, 22489, MTU 1162, RP Bio 4918-24k, IET 22222, IET 22155, JGL 21133, JGL 21828, MTU 1155, MTU 1160, IET 22155, RP 5588-B-B-B-B-76, RP5588-BBBB177-2 and RNT 42-1-1-1, IET 22489, JGL 21078, MTU 1153, MTU 1163, RP Bio 4918-236, RP Bio 4918-24K, RP Bio 4918-50-13, IR 65482-7-216-1-2-B and RP BIO 5478-196 M, IET24814, IET 25394, 23596 and 25041, MP 11, MP 209, NWGR-13108, Mahisagar
Multiple pests	GEMP	IC# 346207, 545441, 459646, 17065, 86004, 145397, 449784, 450029, 449994, 413645, IC 463924, 462407, 463445, 578116, 578148, 578406,
	MRST	RP 4680-1-2-23, RP 4681-16-2-569, RP 4684-35-1-732, RP 4686-48-1-937CR 2711-76, HR-DRR-02, RP 4918-212(S), RP 4918-228(S), RP Bio 4918-236, RP Bio 4918-228(S) & DRRH-2, CR3006-8-2, RP 4918-228(S) and JGL 19618, KNM 113, IR 65482-7-216-1-2-B, CR 2711-149, NP 3113-7, RP Bio 4918-142 S, CR 1898-32-69-CN 12-2 CN 2072, RP 5588-B-B-B-B-63, KNM 539 and RP BIO 5478-166 M, Co50, Bahadur, Varalu and KNM113
	NSN -1	IET # 22489, 22096, 22155, 22439, 22486, IET No.s 23185, 23118, 23073, 22989, 23081, 23440, 22752, 22486, 23009, 22565, 23083, 23132, 23078, 23004, Jalmagna (NC), Salivahana
	NSN-2	IET # 23000, 23148, 23033, 23040, IET No.s 23919, 23939, 23620
	NSN H	IET 22950, HPR 2143, IET No.s 22281, 22283, 22974, 23536, 23537, 23524, 23525, 23526
	NHSN	IET 22941 (IHRTMS11), IET No.s24111, 24159, 24131, 24149, 24151

Source: AICRIP Progress Reports, Vol. 2 (Crop Protection)

As a futuristic strategy, the versatile tool of biotechnology has progressively provided novel and more powerful alternatives to cumbersome and time taking conventional resistance breeding. Work on transformation of plant systems with expression of multiple toxins in transgenic plant varieties through gene stacking and using genetic markers and DNA marker technology to tag and map several major

resistance genes, has also been successful in conferring resistance to multiple pest problems. Disrupting gene function by the use of RNAi is another well-established technique in host plant resistance, while in the last few years, CRISPR/Cas9-based gene editing system has been another exciting means of exploiting genome intelligence for resistance breeding. Biotechnology research network projects involving the cooperating centres spread across the country have the potential to herald a new and more efficient strategy under AICRIP, in the coming years.

Cultural management

Appropriate manipulation of cultural practices offers the viable means for the resource deficient small and marginal farmers to indirectly suppress pest populations through resource use efficient techniques, particularly in rainfed rice. Simple practices like early and synchronous planting can help in either escaping damage or alleviating the effects of many pests and diseases. Similarly, water management and field sanitation measures can take care of biotic stresses through the removal of alternate hosts or creating conditions difficult for pest survival.

AICRIP studies have revealed that Integrated soil health and plant nutrient management strengthens plant system through induced resistance to withstand the insect populations preventing them from either reaching to 'pest' level status or enabling the plants to yield well despite stress impacts. Vigorous plant health also results in a substantial reduction of pesticide use ensuring cost optimization. Development of farmer friendly practices like application of Nitrogen in splits along with slow release fertilisers such as Neem coated urea help to meet the dual goal of higher yields and lower pest incidence. Novel practice of using leaf colour charts is recommended for optimized use of nitrogenous fertilizers. Site specific nutrient management is another practical means available to rice farmers to get direct benefits of pest suppression at no additional cost. Application of organic manures like FYM or vermicompost facilitates build-up of beneficial populations of detrivorous and plankton feeders as well as natural enemies or pest antagonists, both below and above the water.

Precision farming based on intensive grid-sampled information obtained by GIS and global positioning system (GPS) is the futuristic dimension initiative under AICRIP towards development of efficient soil and plant health management systems in rice.

Conservation and utilization of bio control agents

AICRIP studies have led to a comprehensive understanding of rich and diverse wealth of beneficial biological control agents and their natural *in situ* interactions in rice ecosystems, for optimum use as key components of IPM. Augmentative releases of natural enemies can further be made to supplement the efforts for their natural conservation. Release of egg parasitoids, *Trichogramma japonicum* adults against yellow stem borer and *T. chilonis* against leaf folder are recommended to supplement the already existing natural populations of these parasitoids and thus increase the per cent parasitism in rice fields (Gururaj Katti *et al*, 2007).

In the last decade, Biointensive pest management strategies (BIPM) have been developed to strengthen the natural regulatory mechanisms for *in situ* management of pest populations with least disturbance to the balance of nature. BIPM focusses more on measures to restructure the agricultural ecosystem towards conservation of natural enemies to the disadvantage of a pest. Habitat manipulation through naturally innovative strategies such as use of trap crop and ecological engineering, are few of the attempts designed to protect rice crop with minimum damage to the environment. Modifying rice habitat by growing aromatic rice, preferably Pusa Basmati 1 as a trap crop can effectively help in the management of yellow stem borer as this pest prefers scented rice as host over the non-scented varieties. The technique was systematically evaluated through multi-locational testing under AICRIP and is recommended for stem borer endemic areas. It is particularly useful in rice-rice cropping system of the peninsular region (Padma Kumari *et al* 2017).

Similarly, Ecological engineering for natural enemy impact / conservation biological control has also been found successful for the management of planthoppers (Chitra Shanker *et al*, 2016). Growing flowering

plants such as marigold, pulses like cowpea soybean *etc*. on bunds surrounding paddy fields can effectively help in reducing planthopper pests in paddy fields in an eco-friendly way. These crops serve as reservoirs of natural enemies by acting as pollen and nectar sources to attract natural enemies of planthoppers.

Behaviouristic manipulations using sex pheromones

Pheromones are the chemicals produced by one species that affect the behaviour of other members of the same species. They are usually very specific to the species that produce them. Pheromones have no adverse effects on the biota or the environment, are unaffected by rain fall and hence would be fully compatible with an integrated pest management (IPM) approach to control rice pests.

Sex pheromones have been found promising for the management of yellow stem borer (YSB), in monitoring as well as direct control through male annihilation by mass trapping. The rationale of pheromone mediated mass trapping technique is to place enough traps to concentrate pest insects into a restricted space (catch enough males) and leave the females of the species without mates. Extensive multi location trials have revealed that mass trapping technique offers great promise against monophagous pests like yellow stem borer, particularly in areas where the crop is cultivated extensively and contiguously (Krishnaiah *et al* 2004).

Chemical management

Pest management using chemicals with its curative effects and ease of application continues to be an important choice of the farmers for managing insect pest populations in rice. Regular screening and evaluation of newer insecticide molecules for their efficacy against rice pests under AICRIP has helped in the identification of suitable chemical options in different cropping system regimes depending on pest prevalence (Krishnaiah *et al* 2008). In the last decade, Chemical control studies have shown that newer chemicals and botanicals with novel modes of action and effectiveness at very low doses are compatible with other pesticides and have the potential to fit well into rice IPM programmes.

Newer Insecticide	Target pest
i. Fipronil @ 50 g a.i./ha, ii. Sulfoxaflor 24% SC w/v (21.8% w/w) @ 75 & 90 g a.i./ha, iii. Imidacloprid plus ethiprole (Glamore 80 SG) @ 100 g a.i./ha, iv. Triflumezopyrim (DPX-RAB 55 106 SC)., @ 25 g a.i./ha, v. Dinotefuran (Token 20 SC) @ 40 g a.i./ha	Planthoppers
i. Coragen 20% SC (Rynaxypyr) at 30 g a.i./ha, ii. Acephate 95% SG (Acephate) @ 500 g a.i./ha iii. Spinetoram 6% w/v (5.66% w/w) + Methoxyfenozide 30% w/v (28.3% w/w) SC @ 135 & 144 g a.i./ha	Stem borer. leaf folder and other lepidopteran pests
i. Buprofezin 20% + Acephate 50% WP (RIL- 049/F1) @ 1000 g/ha ii. Flubendiamide 4% plus buprofezin 20% SC(RIL-IS-109) @ 1000 g a.i./ha iii. Flubendiamide 240% g/L plus Thiacloprid 240% % g/L (Belt Expert 480 SC-g/L) @ 120 g a.i./ha	Stem borer and planthoppers

Source: AICRIP Progress Reports, Vol. 2 (Crop Protection)

However, use of pesticides is recommended only in situations of pest outbreaks or resurgences or only as a last resort when alternate options do not yield results. Also, farmers are advised to use only the Government approved insecticides as per the recommended list available on the website of Directorate of Plant Protection, Quarantine & Storage, Faridabad, under the Ministry Of Agriculture & Cooperation (major use of pesticides as on 30.11.2021.pdf ppqs.gov.in). For effective chemical use, the correct choice of active ingredient, suitable formulation, time of application and application techniques need to be made based on pest biology and crop phenology. Ongoing research on the uses of unmanned aircraft (drones) for various agricultural activities, including surveying fields; crop health and watering; application of pesticides and fertilizers provides the scope of using chemicals in more effective and environment friendly manner.

Use of biopesticides and botanical pesticides though advocated as environment friendly component of IPM, is another potential area in need of a fresh relook in the light of new developments in advanced chemistry

and formulation technology. Newer analytical standardization tools can improve and refine their performance as effective, cheap and widely available alternative products for ready use by farmers.

IPM evaluation and verification

IPM is the most appropriate approach to overcome biotic stresses and obtain sustainable rice yield with least damage to the environment. Earlier workers verified and demonstrated the efficiency as well as cost effectiveness of location specific IPM technology on farmers' fields compared to conventional farmers' practices. However, in order to make IPM more adaptive, there is need to develop more than one IPM modules at every location thereby addressing to the plant protection needs of diverse farmers' situations within and across the rice ecosystems. AICRIP has provided an ideal mechanism to evaluate and demonstrate location specific IPM modules for superior performance and cost effectiveness at each location in different rice ecosystems of the country. Multi-disciplinary team of scientists have contributed towards more efficient and practical IPM modules differing in their package of optimized pest management components to address the requirements of farmers across the rice ecosystems (Gururaj Katti *et al*, 2022; AICRIP Progress Reports 2015-2020). The IPM modules have shown clear superiority to the farmers' practices in terms of higher benefit cost ratios.

Conclusion – A personal Note

AICRIP is a unique and broad based programme with a well thought out set of objective criteria to evaluate rich rice breeding material of the country involving local landraces, diverse germplasm, advanced breeding material across multi locations representing the different rice ecosystems of the country. The ultimate aim is to develop high yielding and nutritionally fortified rice varieties along with suitable production and protection technologies to ensure economic and social benefits to the rice farmers and meet the rice consumption needs of general public in the country. The uniqueness of AICRIP lies in the holistic teamwork of multi-disciplinary rice research workers belonging to ICAR, Central and State Agricultural Universities, Private Sector agencies and State Departments of Agriculture in tandem with progressive farmers contributing to the strength and authenticity of the programme in producing realistic outputs. In AICRIP, all the cooperating centres are provided with adequate opportunities to contribute and test breeding material along with research infrastructure support across multi disciplines to efficiently carry out the programme. AICRIP's framework of multi location and multi-disciplinary generation and evaluation of research material through a healthy spirit of competition and exchange of scientific information across multiple stakeholders, has been instrumental in rapid progress of rice research over time and space in India. Recently, Government of India (GOI) has endorsed the need to develop an efficient Natural Farming based sustainable programme to tackle ill effects of modern intensive agriculture. AICRIP offers an efficient and holistic mechanism to evaluate and assess the potential of this chemical-free alias traditional farming method's role in the future rice production roadmap of the country.

On a personal note, AICRIP has profoundly enriched my experiential learning through exposure to very diverse range of rice ecosystems and constant interaction with vastly talented, experienced and committed rice research workers across the country. AICRIP is very well equipped and ever ready to embrace the fast paced advances in cutting edge technologies in rice research heralding a potentially promising future.

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

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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. At least one of the authors (in case of joint authorship) should be member of the Society for Advancement of Rice Research (SARR) and not in arrears of subscription. Authors of invited articles are exempted from this.

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

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

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

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

Research papers

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

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

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

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

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