ISSN 2319-3670

Journal of Rice Research

Volume 15, No. 1

June 2022

Articles with DOI

Society for Advancement of Rice Research

Society For Advancement of Rice Research

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

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

Aims and Objectives

- To advance the cause of rice research and development in the country.
- To disseminate knowledge on latest developments in rice research through publications, seminars, lectures and training programmes.
- To provide consultancy in rice production and development.
- To facilitate research and industry collaboration and public private partnership at national level.
- To honour outstanding achievers in rice research and development.
- To cooperate with other organizations having similar aims and objectives.
- To promote any other scientific/professional activities conducive for the advancement of science of rice and rice improvement.

Executive Council (2019-2022)

President	:	Dr. RM Sundaram, Director, ICAR-IIRR, Hyderabad
Vice - Presidents	:	Dr. ON Singh, Vice-Chancellor, Birsa Agril. University, Ranchi
		Dr. Y Chandra Mohan, ARI, PJTSAU, Hyderabad
Secretary	:	Dr. R Mahender Kumar, ICAR-IIRR, Hyderabad
Joint Secretary	:	Dr. S Gopalakrishnan, ICAR-IARI, New Delhi
Treasurer	:	Dr. B Nirmala, ICAR-IIRR, Hyderabad
Zonal Councillors	:	Dr. VK Srivastava, BHU, Varanasi - North India
		Dr. PB Patel, Navsari Agril. University, Gujarat – West India
		Dr. Vijay Pal Bhadana, ICAR-IIAB, Ranchi – East India
		Dr. BNVSR Ravi Kumar, RARS, Maruteru, Andhra Pradesh – South India

Journal of Rice Research is an official publication of Society for Advancement of Rice Research (SARR), ICAR-Indian Institute of Rice Research, Hyderabad - 500030 and published twice a year i.e., June and December. All correspondence related to SARR should be addressed to secretary, Dr. R M Kumar (kumarrm213@gmail.com).

Membership: Membership of the Society is open to all persons interested in furthering the aims and objectives of the Society and in the opinion of the executive committee, have the necessary qualification and experience.

Membership and Journal Subscription fee w.e.f August 2019

	Subscription price	
Membership category	**Annual**	**Life**
A. Individual membership		
Indian (Rs.)	500 (300*)	3000 (2000*)
Foreigner (US $ or its equivalent)	50	300
B. Corporate members/Patrons-one time (Rs.)	----	20,000
C. Libraries and Institutions (Annual)		
Indian (Rs.)	2500	
Foreign (US $ or its equivalent)	550	

Admission fee of Rs. 100/- will be charged in addition to the above subscription fee; *For Students

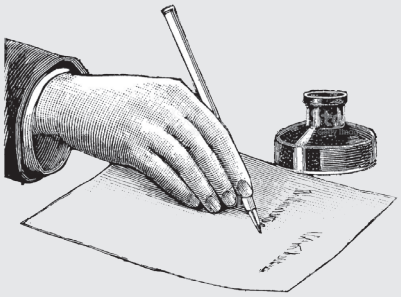
All remittances should be made in favour of Society for Advancement of Rice Research, Hyderabad with a mail to treasurer, Dr. B Nirmala (treasurersarr@gmail.com).

ISSN 2319-3670

Journal of Rice Research

Volume 15, No. 1 June 2022

Society for Advancement of Rice Research



From the SARR President's desk....

India is the second largest producer and largest exporter of rice Worldwide. Rice production in India increased from 54 million tons in 1980's to 120 million tons in 2020-21. The southwest monsoon season began on time in June and I am happy to inform you that the rice plantings are going on all over the country.

All India Coordinated Research Project on Rice (AICRP on Rice) Annual Workshop was held during 25-27 April 2022 in hybrid mode and many of our co-operators participated physically after two years gap due to COVID 19 pandemic. For the first time in India, ICAR is planning for Crop Production & Protection Technologies Identification Committee (CPPTIC) and ICAR-IIRR prepared and presented the guidelines and proforma for evaluation, notification and release of CPPT.

I am glad to inform that from this June issue onwards, all our research articles will get internationally recognized Digital Object Identifier (DOI). We thank Cross-reference, the enabler. You or anybody to whom you send the DOI can access your paper and catch up with your publications in any part of the world.

I congratulate the executive committee and the editorial board for their efforts in getting the DOI. I request all the researchers in India to contribute their articles of rice research and development for publication in this Journal.

I am happy to inform you that we are going to organize an International Conference on System of Crop Intensification (ICSCI 2022) for Climate-Smart Livelihood and Nutritional Security during 12-14, December 2022. I invite all of you to participate in the International Conference and make it a grand success.

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

Journal of Rice Research

Volume 15 : Issue No. 1 June 2022

Contents

RESEARCH ARTICLE https://doi.org/10.58297/PACY8180

Phenotypic evaluation of seedling vigour-related traits in a set of rice lines

Padmashree R[1,2], Nakul D Magar[1], Kalyani M Barbadikar[1*], Amol Phule[1], Honnappa[1,2], Senguttuvel P[1], Sheshu Madhav Maganti[1], Anantha M Siddaiah[1], Divya Balakrishnan[1], Gireesh Chanappa[1], Manasa V[1] and Lokesha R[3]

[1]ICAR-Indian Institute of Rice Research, Hyderabad 500030

[2]University of Agricultural Sciences, Raichur, Karnataka 584104

[3]Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka 577204

* Corresponding author Email: kalyani.mb@icar.gov.in, kalyaniaau@gmail.com

Received: 28th April 2022; Accepted: 24th June 2022

Abstract

Under the changing climatic conditions, depleting nutrients and water scarcity, the dry-direct seeded rice (DDSR) with an aerobic system of cultivation is gaining ground in India. The uniform establishment of seedlings is critically dependent on the seedling vigour and high seedling vigour provides better uptake of nutrients with uniform plant growth. With an aim to identify rice lines exhibiting high seedling vigour traits, we phenotyped the seedling vigour index-related traits *viz*., germination percentage (GP), mesocotyl length (ML), coleoptile length (CL), root and shoot fresh and dry weights in a set of rice lines consisting of introgression lines, landraces, mutant lines and popular varieties using the paper towel method. ANOVA revealed high significant variation for all the traits including seedling vigour index-I (SVI-I) and seedling vigour index-II (SVI-II). Significant positive intercorrelation was recorded between two sub-traits for establishment of seedling *viz*. coleoptile length and mesocotyl length. The rice lines *viz*., ATR-486, ATR-473, ATR-385, ATR-279, ATR-472, ATR-397, ATR-275, KK-12 and ATR-387 exhibited comparatively higher CL, ML, SVI-I and SVI-II than all the checks used in the present study. The identified lines exhibiting better seedling vigour traits are promising genetic resources that can be deployed in breeding programs for improving adaptability under DDSR conditions.

Keywords: Mesocotyl, coleoptile, early seedling vigour, seedling vigour index-I (SVI-I), seedling vigour index-II (SVI-II)

Introduction

The uniform establishment of a crop is critically dependent on seedling vigour and seedling development (Singh *et al*., 2017). Seedling vigour is determined by various traits *viz*., coleoptile length, germination rate and early germination, so that weed competitiveness in the crop can be enhanced with the help of seedling vigour and associated traits (Okami *et al*., 2011). Varieties having the maximum mesocotyl length are preferred for improving rice seedling emergence rate, predominantly under deep sowing and submergence, because mesocotyl length has the beneficial effect of vigour enhancement by pushing the buds from the rhizosphere soil.

Mesocotyl (an organ between the coleoptile node and the basal part of the seminal root) and coleoptile elongation are vital traits influencing seedling emergence and establishment in direct-seeded rice cultivation (Alibu *et al*., 2012, Wang *et al*., 2021). The rice lines with long mesocotyl have stronger germination and a higher rate of seedling emergence than those with short ones. Such rice lines have a better chance of overcoming the problems faced during direct seeding of rice like weed competitiveness, emergence rate, and poor seedling establishment (Zhang *et al*., 2005; Alibi *et al*., 2011, Zhan *et al*., 2020). Rice lines with inherently longer coleoptile and mesocotyl perform better than those with shorter ones.

In the present study, we have screened phenotypically a set of rice lines (introgressed lines, mutant lines, landraces, and varieties) for seedling vigour index-related traits.

Materials and Methods

The experimental material comprised of introgression lines, mutant lines, landraces and varieties (**Table 1**). The seeds were kept at 50°C for 48 hours to overcome possible dormancy, followed by rinsing with sterile water to ensure aseptic conditions and surface sterilized with 1% sodium hypochlorite solution for 15 minutes and later rinsed three times with sterile water to ensure aseptic conditions. The brown paper towels, size (30 × 20 cm) were autoclaved and made wet with sterile distilled water. Ten surface-sterilized seeds were placed at equidistance on the wet brown paper towels in two replications (three plants in each replication) with a completely randomized design (CRD). The paper towels with seeds were covered with a polythene sheet to avoid wear and tear of paper towels and to avoid moisture loss. The paper towels with polythene cover were folded from both ends towards the centre and rubber bands were tied to ensure unfolding. Each setup was properly labelled

Table 1. Rice lines used in the study

Sl. No.	Rice lines	Biological status of accession
1	ATR-284	Azucena × KMR3
2	ATR-286	
3	ATR-373	
4	ATR378	
5	ATR-380	
6	ATR-385	
7	ATR-387	
8	ATR-391	
9	ATR-393	
10	ATR-394	
11	ATR-397	
12	KK-6	
13	KK-7	
14	KK-8	
15	KK-9	
16	KK-10	
17	KK-11	
18	KK-15	
19	KK-12	Azucena × IR20
20	KK-13	Azucena × IR20
21	ATR-472	Azucena × Bala
22	ATR-473	
23	ATR-275	Azucena × BPT-5204
24	ATR-279	
25	KK-25	
26	KK-2	BPT 5204 × Azucena
27	KK-3	
28	KK-4	
29	KK-16	Azucena × RPHR 1005
30	KK-17	
31	KK-18	
32	KK-23	
33	KK-24	
34	ATR-486	Azucena × Dular
35	PUP1	MTU 1010 × Vandana
36	PUP2	
37	PUP3	
38	PUP4	
39	KK-14	MTU 1010 × MDU 5
40	Rasi	TN1 × C029
41	Sahbhagi Dhan	IR74371-70-1-1(IR5541-04 ×WayRarem)
42	NH-1	N22 EMS mutant
43	NH-2	
44	NH-4	
45	Azucena	Landrace
46	Swarna	Vasista/ Mahsur
47	N22	*Aus* landrace
48	IR-64	IR5657-33-2-1/IR2061-465-1-5-5
49	KMR-3	IR 78937-B-4-B-B-B

with the rice line name and was placed in a water container in a slanting position.

Seedling vigour traits *viz*., germination percentage (GP) was calculated as the total number of normal seedlings counted to the total number of seeds kept for germination and expressed in percentage; mesocotyl length (ML), coleoptile length (CL), shoot length (SL), root length (RL) and total seedling length (TSL) were measured manually using a centimetre scale. Shoot fresh weight (SFW), root fresh weight (RFW), total fresh weight (TFW), shoot dry weight (SDW), root dry weight (RDW) and total dry weight (TDW) ratios were calculated using an electronic balance (IGene, India). The SVI-I and SVI-II were obtained using the formulae mentioned by Addanki *et al*., (2019) as SVI-I = Germination percentage × seedling length, SVI-II = germination percentage × total dry weight (g). The SVI-I and SVI-II were recorded at 14th days after sowing (DAS) in two replications. The CRD ANOVA analysis of variance was carried out in R studio (*version* 3.5.2) using R-scripts for statistical analysis (Aravind *et al*., 2019) and correlation analysis was carried out in past-3 using Spearman correlation coefficient (http://folk.uio.no/ohammer/past).

Results and Discussion

Analysis of variance (ANOVA) for seedling vigour traits

The mean sum of squares for all the seventeen seedling vigour traits has been presented in **Table 2**. The ANOVA revealed a significant mean sum of square (MSS) at p<0.01 and p< 0.05 for all the traits except

Table 2. Analysis of variance for seedling vigour traits in rice lines

Traits	Mean sum of squares	Error	C.D.	C.V. (%)
	Lines			
Degrees of freedom	48	49		
GP	190.07**	50.94	14.21	7.65
CL	0.30**	0.09	0.60	28.75
ML	0.90**	0.09	0.62	7.65
SL	19.98**	1.70	2.62	10.03
RL	19.78**	3.37	3.69	19.48
RSLR	0.142**	0.023	0.30	20.06
TSL	50.65**	6.43	5.09	11.29
SFW	1134.30**	21.43	9.30	8.16
RFW	356.38**	9.35	6.14	9.96
RSFWR	0.19NS	0.12	NA	59.60
TFW	2409.80**	30.69	11.13	6.30
SDW	80.82NS	52.00	NA	77.79
RDW	110.94**	28.29	10.69	93.80
RSDWR	4.05**	0.08	0.57	32.43
TDW	218.95*	126.05	22.57	75.12
SVI-I	56.23**	7.44	5.48	13.13
SVI-II	193.78**	97.46	19.84	71.54

The * and ** indicate the mean sum of squares are significant at P<0.05 and P<0.01 respectively, NS indicates non-significance

CV: Co-efficient of variation; CD: Critical difference; GP: Germination (%); ML: Mesocotyl length (cm); CL: Coleoptile length (cm); SL: Shoot length (cm); RL: Root length (cm); TSL: Total seedling length (cm); RSLR: Root to shoot length ratio; SFW: Shoot fresh weight (mg); RFW: Root fresh weight (mg); TFW: Total fresh weight (mg); RSFWR: Root to shoot fresh weight ratio; SDW: Shoot dry weight (mg); RDW: Root dry weight (mg); TDW: Total dry weight (mg); RSDWR: Root to shoot dry weight ratio; SVI-I: Seedling vigour index-I and SVI-II: Seedling vigour index-II

for root to shoot fresh weight ratio and shoot dry weight which was highly variable for the traits under study for seedling vigour traits.

Estimates of correlation coefficients for seedling vigour traits

Among the seventeen seedling vigour traits studied, SVI-I reported the highest significant positive correlation with total seedling length, followed by shoot length, shoot fresh weight, root length, total fresh weight, mesocotyl length, total dry weight, germination percentage, root dry weight, root fresh weight, shoot dry weight and coleoptile length, similarly SVI-II exhibited positive significant association with total dry weight followed by shoot dry weight, shoot fresh weight, total fresh weight, root dry weight, root fresh weight, SVI-I, total seedling length, shoot length, mesocotyl length, coleoptile length and root length (**Table 3** and **Figure 1**). Further, inter-correlation among the important traits, such as coleoptile length recorded a significant positive correlation with shoot length, mesocotyl length, shoot fresh weight, total

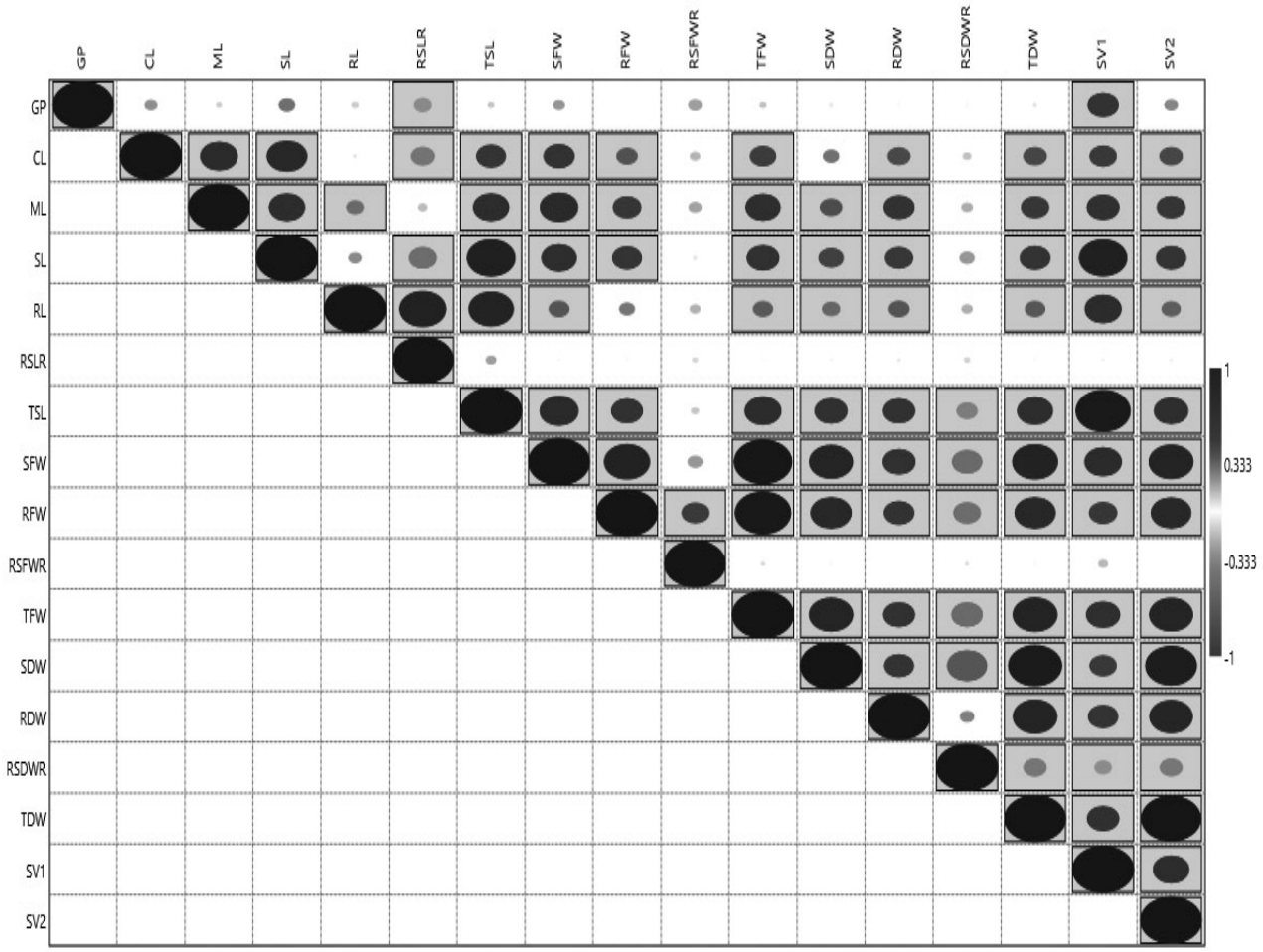


Figure 1: Correlation among seedling vigour traits in the rice lines

GP: Germination (%);ML: Mesocotyl length (cm); CL: Coleoptile length (cm); SL: Shoot length (cm); RL: Root length (cm); TSL: Total seedling length (cm); RSLR: Root to shoot length ratio; SFW: Shoot fresh weight (mg); RFW: Root fresh weight (mg); TFW: Total fresh weight (mg); RSFWR: Root to shoot fresh weight ratio; SDW: Shoot dry weight (mg); RDW: Root dry weight (mg); TDW: Total dry weight (mg); RSDWR: Root to shoot dry weight ratio; SVI-I: Seedling vigour index-I and SVI-II: Seedling vigour index-II

Boxed rectangles indicate significant correlation. Red box: Indicates negative correlation and **Blue box:** Indicates positive correlation; the clockwise direction of the boxes indicates the intensity of positive correlation, and the anticlockwise direction of the boxes indicates the intensity of negative correlation

Table 3. Estimation of correlation coefficients for seedling vigour traits in rice lines

Traits	GP	CL	ML	SL	RL	RSLR	TSL	SFW	RFW	RSFWR	TFW	SDW	RDW	RSDWR	TDW	SVI-I	SVI-II
GP	1	0.196	0.084	0.259	-0.103	-0.277*	0.095	0.181	-0.008	-0.213	0.101	-0.051	0.020	-0.027	0.050	0.493**	0.212
CL		1	0.601**	0.650**	0.047	-0.375**	0.472**	0.495**	0.335*	-0.157	0.416**	0.255	0.362**	-0.128	0.370**	0.431**	0.370**
ML			1	0.578**	0.274*	-0.141	0.578**	0.614**	0.454**	-0.202	0.558**	0.350*	0.492**	-0.176	0.446**	0.522**	0.456**
SL				1	0.204	-0.438**	0.783**	0.567**	0.475**	-0.054	0.520**	0.397**	0.446**	-0.237	0.487**	0.777**	0.482**
RL					1	0.754**	0.728**	0.327*	0.248	-0.164	0.315*	0.285*	0.329*	-0.172	0.320*	0.591**	0.303*
RSLR						1	0.162	-0.021	-0.019	-0.082	0.013	0.022	-0.045	-0.090	-0.019	0.028	-0.037
TSL							1	0.624**	0.507**	-0.117	0.588**	0.524**	0.511**	-0.331*	0.575**	0.888**	0.551**
SFW								1	0.741**	-0.235	0.940**	0.704**	0.523**	-0.478**	0.737**	0.598**	0.721**
RFW									1	0.425**	0.908**	0.659**	0.484**	-0.433**	0.659**	0.448**	0.650**
RSFWR										1	0.061	0.020	-0.007	0.053	-0.021	-0.147	-0.012
TFW											1	0.711**	0.506**	-0.496**	0.720**	0.545**	0.706**
SDW												1	0.473**	-0.641**	0.870**	0.432**	0.834**
RDW													1	0.224	0.714**	0.479**	0.698**
RSDWR														1	-0.364**	-0.271	-0.357**
TDW															1	0.516**	0.971**
SVI-I																1	0.582**

Note: *and ** indicate the mean sum of squares were significant at P<0.05 and P<0.01 respectively

GP: Germination (%);ML: Mesocotyl length (cm); CL: Coleoptile length (cm); SL: Shoot length (cm); RL: Root length (cm); TSL: Total seedling length (cm); RSLR: Root to shoot length ratio; SFW: Shoot fresh weight (mg); RFW: Root fresh weight (mg); TFW: Total fresh weight (mg); RSFWR: Root to shoot fresh weight ratio; SDW: Shoot dry weight (mg); RDW: Root dry weight (mg); TDW: Total dry weight (mg); RSDWR: Root to shoot dry weight ratio; SVI-I: Seedling vigour index-I and SVI-II: Seedling vigour index-II

seedling length, total fresh weight, total dry weight, root dry weight, and root fresh weight. The mesocotyl length exhibited a significant positive correlation with shoot fresh weight, coleoptile length, shoot length, total seedling length, total fresh weight, root dry weight, root fresh weight, total dry weight, shoot dry weight and root length, similar correlation study was reported earlier (Yang *et al*., 2020, Dilday *et al*., 1990). Root length showed a significant positive correlation with mesocotyl length, root to shoot length ratio, total seedling length, shoot fresh weight, total fresh weight, shoot dry weight, root dry weight, total dry weight, SVI-I, and SVI-II. Similarly, total dry weight exhibited a significant correlation with coleoptile length, mesocotyl length, shoot length, root length, total seedling length, shoot fresh weight, root fresh weight, total fresh weight, shoot dry weight, root dry weight, SVI-I, and SVI-II. The positive and significant correlation among component traits indicated that these related traits could be used in combination for selection or initial phenotyping for seedling vigour.

The rice lines ATR-473, ATR-385, ATR-279, ATR-472, and ATR-486, recorded higher CL, ML, SVI-I, and SVI-II than the checks Swarna, Sahbhagi Dhan, Azucena, KMR-3 and IR-64. KK-12 reported higher CL, SVI-I, and SVI-II, ATR-397 and ATR-275 recorded higher ML, SVI-I, and SVI-II. The line, ATR-387 exhibited the highest CL and SVI-II **(Figures 2, 3 and 4)**, whereas the N22 mutant NH-4 and rice line KK-6 showed higher ML, SVI-II, and SVI-I respectively than the checks. These lines with the superior potential of ML and CL have a strong inherent genetic ability for seedling emergence (Turner, *et al*., 1982, Yang *et al*., 2020, Dilday *et al*., 1990). Longer mesocotyl is an important characteristic for the breeder to select as it is beneficial for germination under deep sown conditions and primarily responsible for seedling emergence under DDSR (Gao *et al*., 2012; Hu *et al*., 2010; Watanabe *et al*., 2001; Zhang *et al*., 2005, Zhou *et al*., 2006; Finch-Savage *et al*., 2010, Wu *et al*., 2015). Luo *et al*., (2007) reported a significant positive interactive effect of mesocotyl elongation and sowing depth on seedling emergence and showed that mesocotyl elongation increasingly influenced seedling establishment when sowing depth was increased, but only extremely long coleoptiles affected seedling establishment.

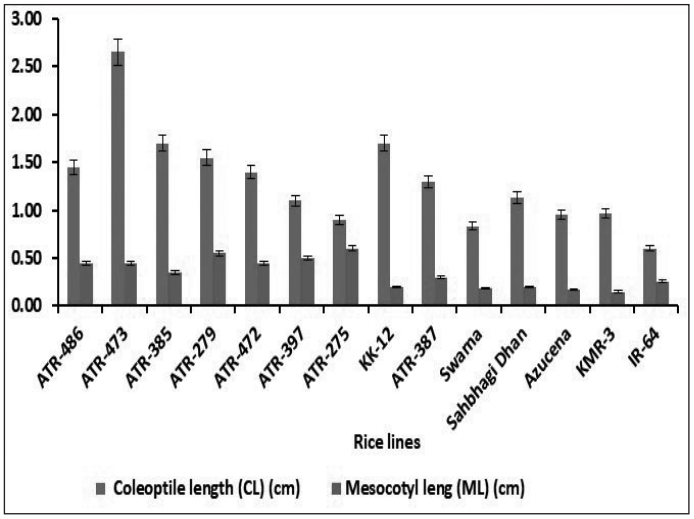


Figure 2: Better performing rice lines for coleptile length and mesocotyle length

The bars on top of indicate standard deviation

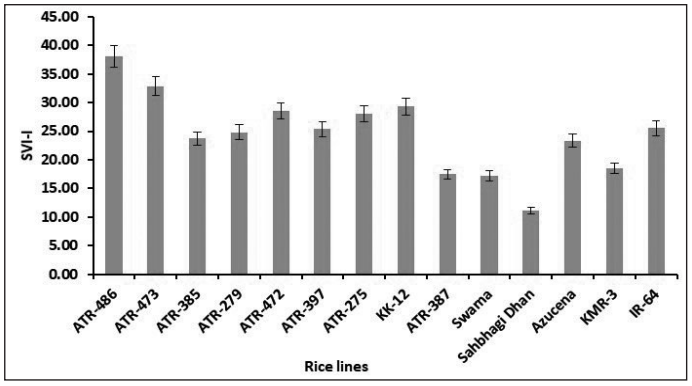


Figure 3: Better performing rice lines for seedling vigour-I

The bars on top of indicate standard deviation

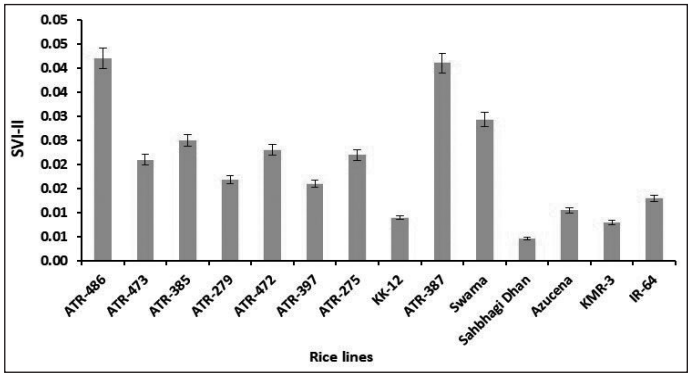


Figure 4: Better performing rice lines for seedling vigour-II

The bars on top of indicate standard deviation

In this study, the lines *viz*., ATR-486, ATR-473, ATR-385, ATR-279, and ATR-472 had a better seedling

vigour index, mesocotyl, and coleoptile length. Our study revealed that SVI-I had the highest positive significant positive correlation with mesocotyl length and coleoptile length. Similar results were reported by Chung (2010) wherein a significant positive relationship between mesocotyl elongation in weedy rice and seedling emergence was recorded but found no correlation between coleoptile length and emergence. In literature Yang *et al*., (2020) identified 20 QTLs for mesocotyl length by using GWAS on chromosomes 4, 6, 7, 8, 10, and 12. Mesocotyl and coleoptile are important for seedling emergence from deeper levels of soil and lines with the maximum mesocotyl length are preferable for improving rice seedling emergence rate, mostly under deep sowing and submergence (Lee *et al*., 2017, Zhan *et al*., 2020). It has been reported by Lu *et al*., (2016) that natural genetic variation for mesocotyl elongation exists and provides the basis for developing rice varieties with long mesocotyl and the identification of genes through linkage mapping or genome-wide association studies (GWAS). Also, studies have reported that long mesocotyl is important for the uniformity of seedling emergence (Turner *et al*., 1982; Mgonja *et al*., 1993; Chung 2010). Thus, mesocotyl length has been projected as a key trait in developing rice varieties for DDSR cultivation. Yang *et al*., (2020) reported a significant difference in the mesocotyl length of 290 rice accessions, and the trend of change in mesocotyl length for each accession is highly correlated to the sowing depths. The interaction between coleoptile and mesocotyl elongation in effective seedling emergence must be explored further for breeding (Zhan *et al*., 2020). The introgressed lines identified as having comparatively higher mesocotyl and coleoptile lengths can be further evaluated in the field under DDSR conditions.

Conclusion

The identified rice lines exhibiting the highest seedling vigour traits can be used for improving seedling vigour and adaptability under DDSR conditions. This method of phenotyping can be the first line of screening for large sets of germplasm before subjecting to field-level evaluation. The lines with high seedling vigour index can be registered as a novel genetic resource used in breeding programs.

Acknowledgements

The authors are thankful to the Director, ICAR-IIRR for providing research facilities (Institute Research Council Project Codes ABR/CI/BT/15).

Authors' contribution

The study was planned by KMB, phenotyping the panel and analysis was done by KMB, PR, NDM, AP, H; supervision and timely inputs were given by DB, PS, VM; critically edited the manuscript MSM, AMS, CG, LR.

References

Addanki KR, Balakrishnan D, Yadavalli VR, Surapaneni M, Mesapogu S, Beerellik and Neelamraju S. 2019. Swarna × *Oryza nivara* introgression lines: a resource for seedling vigour traits in rice. *Plant Genetic Resources*, 17(1): 12-23.

Alibi S, Saito Y, Shiwachi H and Kenji IR. 2011. Relationship between coleoptile and mesocotyl elongation of upland rice (*Oryza sativa* L.) seedlings under submergence and soil-sand culture. *African Journal of Agricultural Research*, 6(31): 6463-6472.

Alibu S, Saito Y, Shiwachi H and Kenji IR. 2012. Genotypic variation in coleoptile or mesocotyl lengths of upland rice (*Oryza sativa* L.) and seedling emergence in deep sowing. *African Journal of Agricultural Research*, 7(47): 6239-6248.

Aravind J, Shankar MS, Wankhede DP and Kaur V. 2019. Analysis of Augmented Randomised Complete Block Designs, pp 1-17.

Chung NJ. 2010. Elongation habit of mesocotyls and coleoptiles in weedy rice with high emergence ability in direct-seeding on dry paddy fields. *Crop and Pasture Science*, 61(11): 911-7.

Dilday RH, Mgonja MA, Amonsilpa SA, Collins FC and Wells BR. 1990. Plant height *vs*. mesocotyl and coleoptile elongation in rice: linkage or pleiotropism? *Crop Science*, 30: 815-818.

Finch-Savage WE, Clay HA, Lynn JR and Morris K. 2010. Towards a genetic understanding of seed vigour in small-seeded crops using natural variation in Brassica oleracea. *Plant Science*, 179(6): 582-589.

Gao S, Xie X, Yang S, Chen Z and Wang X. 2012. The changes of GA level and signaling are involved in the regulation of mesocotyl elongation during blue light mediated de-etiolation in *Sorghum bicolor*. *Molecular Biology Reports*, 39(4): 4091-4100.

Hu Z, Yan H, Yang J, Yamaguchi S, Maekawa M, Takamure I, Tsutsumi N, Kyozuka J and Nakazono M. 2010. Strigolactones negatively regulate mesocotyl elongation in rice during germination and growth in darkness. *Plant and Cell Physiology*, 51(7): 1136-42.

Lee HS, Sasaki K, Kang JW, Sato T, Song WY and Ahn SN. 2017. Mesocotyl elongation is essential for seedling emergence under deep-seeding condition in rice. *Rice*, 10(1):1-11.

Lu Q, Zhang M, Niu X, Wang C, Xu Q, Feng Y, Wang S, Yuan X, Yu H and Wang Y. 2016. Uncovering novel loci for mesocotyl elongation and shoot length in *Indica* rice through genome-wide association mapping. *Planta*, 243(3): 645-57.

Luo J, Tang SQ, Hu PS, Louis A, Jiao GA and Jian TA. 2007. Analysis on factors affecting seedling establishment in rice. *Rice Science*, 14(1): 27-32.

Mgonja MA, Dilday RH, Skinner SL and Collins FC. 1988. Association of mesocotyl and coleptile elongation with seeding vigour in rice. *Journal of the Arkansas Academy of Science*, 42(1): 52-55.

Mgonja MA, Ladeinde T and Aken Ova ME. 1993. Genetic analysis of mesocotyl length and its relationship with other agronomic characters in rice (*Oryza sativa* L.). *Euphytica*, 72: 189-195.

Okami M, Kato Y and Yamagishi J. 2011. Role of early vigour in adaptation of rice to water-saving aerobic culture: effects of nitrogen utilization and leaf growth. *Field Crops Research*, 124(1): 124-31.

Singh UM, Yadav S, Dixit S, Ramayya PJ, Devi MN, Raman KA and Kumar A. 2017. *QTL* hotspots for early vigour and related traits under dry direct-seeded system in rice (*Oryza sativa* L.). *Frontiers in Plant Science*, 8: 1-14.

Turner FT, Chen CC and Bollich CN. 1982. Coleoptile and mesocotyl lengths in semi-dwarf rice seedlings. *Crop Science*, 22(1): 43-46.

Wang Y, Liu J, Meng Y, Liu H, Liu C and Ye G. 2021. Rapid identification of *QTL* for mesocotyl length in rice through combining *QTL-seq* and Genome-Wide Association Analysis. *Frontiers in Genetics*, 12 https://www.frontiersin.org/articles/ 0.3389/fgene.2021.713446

Watanabe H, Takahashi K and Saigusa M. 2001. Morphological and anatomical effects of abscisic acid (ABA) and fluoridone (FLU) on the growth of rice mesocotyls. *Plant Growth Regulation*, 34(3): 273-275.

Wu JH, Feng FJ, Lian XM, Teng XY, Wei HB and Yu H. 2015. Genome-Wide Association Study (GWAS) of mesocotyl elongation based on the re-sequencing approach in rice. *BMC Plant Biology*, 15: 218, https://doi.org/10.1186/s12870-015-0608-0

Yang C, Wang H, Tang X, Guo Q, Li Y, Liu Y, Li X, Qin P, Tu B, Chen W and Yuan H. 2020. Identification of quantitative trait loci for mesocotyl length of rice seedling in different sowing depths using genome-wide association study. *Genes*, https://doi.org/10.21203/rs.3.rs-101899/v1

Zhan J, Lu X, Liu H, Zhao Q and Ye G. 2020. Mesocotyl elongation, an essential trait for dry-seeded rice (*Oryza sativa* L.): a review of physiological and genetic basis. *Planta*, 251(1): 1-4.

Zhang GH, Lin JR, Wu MG, Cao LY and Cheng SH. 2005. Analysis on germinating dynamic source of rice (*Oryza sativa* L.). *Chinese Journal of Rice Science*, 19(1): 59-62.

Zhou L, Wang J, Yi Q, Wang Y, Zhu Y and Zhang Z. 2006. Quantitative trait loci for seedling vigour in rice under field conditions. *Field Crops Research*, 100(2-3):294-301.

RESEARCH ARTICLE https://doi.org/10.58297/SRNZ2394

Line x Tester analysis for deducing heterosis in rice (*Oryza sativa* L.)

Modunshim Maring K[1], Madhu Bala[1] and Patel PB[2]

[1]Genetics and Plant Breeding, [2]Main Rice Research Centre, N.M. College of Agriculture, Navsari Agricultural University, Navsari-396 450, Gujarat, India

Corresponding author email: *modunshim123@gmail.com*

Received: 11th May 2022; Accepted: 13th June 2022

Abstract

An experiment consisting of twelve genotypes (four lines and eight testers) along with their thirty-two crosses was conducted in Line x Tester design to study the heterobeltiosis and standard heterosis for grain yield per plant and its component traits in rice at Main Rice Research Centre, Navsari. The result indicated that significant heterosis in a desirable direction was observed in all the characters except days to 50% flowering where none of the crosses showed significant heterobeltiosis in a negative direction for this trait. The best heterobeltiosis and standard heterosis for grain yield per plant was exhibited by the cross NVSR-453 x NVSR-475 followed by Gurjari x NAUR-1 and NVSR-453 x NVSR-409.

Keywords: Heterosis, Hybrid, Line x Tester, Rice, Yield.

Introduction

Rice (*Oryza sativa* L., 2n = 2x = 24) is one of the most consumed food grains in the world. It is regarded to be first cultivated in South-East Asia. Rice belongs to the family poaceae and the genus *Oryza*. The genus *Oryza* has twenty-one wild and two cultivated species. i.e., *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice). Based on the number of morphological, physiological, biochemical and molecular traits, Asian rice cultivars are broadly grouped into two major subspecies, i.e., *Oryza sativa indica* and *Oryza sativa japonica* (Glaszmann, 1987).

Rice is mainly a self-pollinating crop, hydrophilic, short-day and C_3 plant. It is best suited to moist humid weather during vegetative growth and dry sunny weather during the ripening period. With optimum temperature requirement between 25°C to 35°C and optimum humidity between 60-80%, rice cultivation extends from 40°S to 45°N latitude, 70°W to 140°E longitude at an altitude range of 1500-2200 m amsl (above mean sea level). Rice serves as a good source of B-vitamins, iron, manganese and magnesium. Rice bran oil is used as medicine as well as cooking oil and its straw is used as fodder in many countries. Global production of milled rice is 450.38 million tonnes from 162.43 million ha area and its productivity is 2.77 kg/ha during 2018-19 (Anonymous, 2020). In India, rice is cultivated in 44.16 million ha along with the production of 105.67 million tonnes and productivity of 2.39 kg/ha during 2018-19 (Anonymous, 2020). The major rice producing countries in Asia are India, China, Indonesia, Thailand, Myanmar and Bangladesh (IRRI-World Rice Statistics, 2010).

Heterosis breeding ensures extensive and detailed genetic assessment of existing germplasm as well as newly evolved promising lines which could be used in future breeding programme or could be directly released as cultivars after proper evaluation. Line x Tester analysis (Kempthorne, 1957) is the most popular method for the exploitation of heterosis in self-pollinated crops, especially in rice breeding programme (Peng and Virmani, 1990). Therefore, the present investigation was undertaken to study heterosis for yield and its component traits in rice.

Materials and Methods

The crossing programme was carried out at Main Rice Research Centre (M. R. R. C.), N.A.U., Navsari during *rabi*-2019 and evaluation was carried out during *kharif*-2019. The experimental materials consisted of 45 entries including 4 lines (Gurjari, NVSR-452, NVSR-453 and GNR-3) and eight testers (NVSR-6157, NVSR-473, NVSR-475, NVSR-486, Dandi, NVSR-403, NVSR-409 and NAUR-1), their 32 crosses (Line × tester mating design) and one check variety (GNR-5). The crossing was carried out by hand emasculation and pollination. The experiment was laid out in a randomized block design with three replications. Each entry was planted in a single row consisting of 10 plants with a spacing of 20 cm x 15 cm. The standard agronomical practices were followed to raise the good experimental crop. Five competitive plants excluding the border plants were randomly selected in each replication to record all the observations for its components characters *viz*., Plant height (cm), productive tillers per plant, panicle length (cm), grains per panicle, kernel length (mm), kernel width (mm), L: B ratio, 100 grain weight (g), protein content (%) by Kjeldahl method, amylose content (%) and straw yield per plant. The traits days to 50 % flowering and grain yield were recorded on a net plot basis, taking border plants into consideration. Heterosis was estimated over better parent and standard check by using the formula suggested by Fonseca and Patterson (1968).

Results and discussion

The highest percent contribution of line has been noticed for days to 50 % flowering, followed by 100 grain weight, kernel length, plant height, grain yield per plant and grains per panicle. The highest contribution of tester was shown in protein content, followed by L : B ratio, kernel width, straw yield per plant, kernel length and 100 grain weight. The highest percent contribution of line x tester was exhibited by productive tillers per plant, followed by amylose content, panicle length, grain yield per plant, grains per panicle and plant height.

Significance in the negative direction is desirable for the trait days to 50 % flowering. The heterosis for these characters ranged from -5.88 % (Gurjari x NVSR-6157) to 19.09 % (NVSR-452 x NVSR-6157) over the better parent while it ranged from -14.83 % (Gurjari x NVSR-6157) to 10.87 % (NVSR-452 x NAUR-1) over the standard check. Significant negative standard heterosis was observed for eight crosses, of which the three highly significant cross combinations were Gurjari x NVSR-6157 (-14.83 %), Gurjari x NVSR-473 (-14.45 %), and Gurjari x Dandi (-12.93 %). But no hybrids showed significant heterobeltiosis in a negative direction. The above results were in accordance with the findings of Bhati *et al*. (2015), Sala *et al*. (2016), Kumari and Jaiswal (2017), Sahu *et al*. (2017a), Patel *et al*. (2018), Prajapati and Kathiria (2018), Thakor *et al*. (2018) and Sundaram *et al*. (2019). Besides days to 50 % flowering, a negative direction is also desirable for the trait plant height. The heterobeltiosis for this trait ranged from -17.19 % (NVSR-453 x Dandi) to 35.75 % (NVSR-453 x NVSR-475). Only one hybrid exhibited significant negative heterobeltiosis *i.e*. NVSR-453 x Dandi. Whereas the value of standard heterosis ranged from -22.36 % (GNR-3 x NVSR-486) to 24.65 % (NVSR-453 x NVSR-475). The crosses NVSR-453 x Dandi and GNR-3 x NVSR-486 had the least heterobeltiosis and standard heterosis, respectively. The present findings are in accordance with results reported by Bhati *et al.* (2015), Dar *et al*. (2015), Waza *et al*. (2016), Kumari and Jaiswal (2017), Sahu *et al*. (2017a), Thorat *et al*. (2017), Prajapati and Kathiria (2018), Thakor *et al*. (2018) and Sundaram *et al*. (2019).

The trait productive tillers per plant is associated with high grain yield. So, it is an important indirect trait for consideration while determining yield. The crosses that showed the highest percentage of heterosis over better parent and standard check are NVSR-453 x NVSR-475 and NVSR-452 x NVSR-486, respectively. Bano and Singh (2018), Patel *et al*., (2018), Prajapati and Kathiria (2018), and Thakor *et al*., (2018) also reported similar results in rice. For panicle length, two and eight crosses expressed significant and positive heterosis over better parent and standard check, respectively. The cross NVSR-453 x NVSR-475 exhibited the highest heterobeltiosis as well as the highest standard heterosis for this character. The results were in uniformity with the results reported by

Table 1. Estimates of heterobeltiosis and standard heterosis for days to 50 % flowering, plant height (cm), productive tillers per plant, panicle length, grains per panicle and kernel length in rice.

Sr. No..	Crosses	Days to 50 % flowering		Plant height (cm)		Productive tillers per plant		Panicle length (cm)		Grains per panicle		Kernel length (mm)	
		BP	SC	BP	SC	BP	SC	BP	SC	BP	SC	BP	SC
1.	Gurjari x NVSR-6157	-5.88	-14.83**	-11.80	-15.67*	-13.06	-12.39	-15.67*	-13.06	-12.39	-19.77**	-3.44	10.38**
2.	Gurjari x NVSR-473	-5.46	-14.45**	-14.16	-24.33**	-20.56**	-16.64*	-24.33**	-20.56**	-16.64*	-25.28**	18.57**	22.77**
3.	Gurjari x NVSR-475	-0.84	-10.27*	-2.19	-0.50	-1.82	2.63	-0.50	-1.82	2.63	-11.35	20.31**	11.31**
4.	Gurjari x NVSR-486	1.68	-7.98*	-8.30	-7.96	-9.18	-6.00	-7.96	-9.18	-6.00	-18.80**	-3.67	20.32**
5.	Gurjari x Dandi	-3.78	-12.93**	6.63	3.48	12.80	7.06	3.48	12.80	7.06	0.81	-14.05**	16.22**
6.	Gurjari x NVSR-403	-2.10	-11.41**	0.04	-2.85	-1.32	6.38	-2.85	-1.32	6.38	-8.10	41.62**	31.02**
7.	Gurjari x NVSR-409	2.10	-7.60	14.20	9.62	14.85*	9.22	9.62	14.85*	9.22	6.03	1.93	21.25**
8.	Gurjari x NAUR-1	5.04	-4.94	28.41**	10.97	26.44**	20.87**	10.97	26.44**	20.87**	22.24**	-30.86**	-1.75
9.	NVSR-452 x NVSR-6157	19.09**	9.13*	0.46	1.03	4.15	5.84	1.03	4.15	5.84	-3.08	-1.67	12.40**
10.	NVSR-452 x NVSR-473	12.10**	5.70	8.09	5.90	11.17	11.39	5.90	11.17	11.39	-0.16	-0.26	3.28
11.	NVSR-452 x NVSR-475	11.49**	10.65**	-9.30	-13.85*	-13.24	-11.48	-13.85*	-13.24	-11.48	-21.23**	-11.72**	-9.94**
12.	NVSR-452 x NVSR-486	9.20*	8.37*	19.58*	15.13*	15.94*	25.36**	15.13*	15.94*	25.36**	11.54	-5.64*	17.86**
13.	NVSR-452 x Dandi	12.25**	7.98*	8.71	3.16	12.44	8.81	3.16	12.44	8.81	2.46	-37.96**	-16.11**
14.	NVSR-452 x NVSR-403	9.20*	8.37*	2.40	0.80	2.38	6.92	0.80	2.38	6.92	-4.86	-5.57*	-3.66
15.	NVSR-452 x NVSR-409	8.14*	6.08	0.65	1.26	6.09	2.00	1.26	6.09	2.00	-0.97	-8.82**	8.47**
16.	NVSR-452 x NAUR-1	11.72**	10.87**	-8.27	-16.58**	-4.94	-14.74*	-16.58**	-4.94	-14.74*	-13.78*	-29.44**	0.27
17.	NVSR-453 x NVSR-6157	7.47	-1.52	3.32	3.05	12.09	1.28	3.05	12.09	1.28	0.49	-6.55**	6.83*
18.	NVSR-453 x NVSR-473	2.82	-3.04	22.04*	12.14	-13.06	15.03*	12.14	-13.06	15.03*	14.13*	18.14**	22.34**
19.	NVSR-453 x NVSR-475	1.57	-1.90	35.75**	24.65**	34.48**	19.10	18.14**	28.51**	22.55**	21.59**	-0.06	-14.04**
20.	NVSR-453 x NVSR-486	3.94	0.38	-0.40	-9.37	10.00	-1.49	-9.93	-2.03	-9.01	-9.72	-9.14**	13.49**
21.	NVSR-453 x Dandi	0.00	-3.80	-17.19*	-18.71*	-8.33	-17.91	-25.02**	-18.27**	-21.75**	-22.37**	-22.21**	5.19
22.	NVSR-453 x NVSR-403	6.69	3.04	6.24	-2.44	-17.43	-26.87*	-0.68	8.03	-0.19	-0.97	-3.62	-17.09**
23.	NVSR-453 x NVSR-409	7.48	3.80	18.45*	16.27*	-16.89	-14.93	8.97	18.53**	17.16**	16.24*	7.30**	27.64**
24.	NVSR-453 x NAUR-1	5.12	1.52	7.17	5.20	0.00	13.43	0.62	14.65*	4.04	5.22	3.73	47.41**
25.	GNR-3 x NVSR-6157	-0.83	-9.13*	-7.00	-13.45	-16.05	-11.94	-8.05	-5.21	-8.14	-15.88*	-19.30**	-7.76**
26.	GNR-3 x NVSR-473	2.02	-3.80	2.06	-6.03	-24.59*	-20.90*	-3.31	1.50	3.62	-7.13	-9.34**	-6.12*
27.	GNR-3 x NVSR-475	-1.20	-6.08	-6.21	-13.88	-24.59*	-20.90*	-1.69	-5.65	0.98	-16.53*	6.41	-9.39**

Sr. No..	Crosses	Days to 50 % flowering		Plant height (cm)		Productive tillers per plant		Panicle length (cm)		Grains per panicle		Kernel length (mm)	
		BP	SC	BP	SC	BP	SC	BP	SC	BP	SC	BP	SC
28.	GNR-3 x NVSR-486	5.20	0.00	-14.67	-22.36**	-18.90	-14.93	-15.79*	-22.33**	-11.76	-27.07**	-3.24	20.86**
29.	GNR-3 x Dandi	-5.60	-10.27*	16.15	8.09	2.76	7.79	6.32	15.89*	16.56*	9.76	-39.46**	-18.13**
30.	GNR-3 x NVSR-403	1.20	-3.80	-3.32	-11.21	18.10	23.88*	-3.81	-2.29	2.45	-11.83	12.51**	-4.21
31.	GNR-3 x NVSR-409	0.80	-4.18	3.24	-3.92	-21.74*	-17.91	-0.67	4.06	-1.34	-4.21	1.79	21.08**
32.	GNR-3 x NAUR-1	12.40**	6.84	10.78	3.09	4.61	18.66	0.31	14.30*	5.64	6.84	-32.01**	-3.39
	S.E. (d) ±	3.44	3.44	7.79	7.79	1.15	1.15	1.25	1.25	13.02	13.02	0.17	0.17
	C.D. at 5%	6.88	6.88	15.57	15.57	2.31	2.31	2.50	2.50	26.02	26.02	0.34	0.34
	C.D. at 1%	9.14	9.14	20.70	20.70	3.07	3.07	3.32	3.32	34.59	34.59	0.45	0.45
	Range	-5.88 to 19.09	-14.83 to 10.87	-17.19 to 35.75	-22.36 to 24.65	-34.21 to 34.48	-28.36 to 26.87	-25.02 to 18.14	-22.33 to 28.51	-21.75 to 25.36	-27.07 to 22.24	-39.46 to 41.62	-18.13 to 47.41

*-Significant at 5 % and **-Significant at 1 %, BP-Better Parent, SC-Standard Check

Table 2. Estimates of heterobeltiosis and standard heterosis for kernel width (mm), L : B ratio, 100 grain weight (g), protein content, amylose content, grain yield per plant and straw yield per plant in rice.

Sr. No.	Crosses	Kernel width (mm)		L : B ratio		100 grain weight (g)		Protein content (%)		Amylose content (%)		Grain yield per plant (g)		Straw yield per plant (g)	
		BP	SC	BP	SC	BP	SC	BP	SC	BP	SC	BP	SC	BP	SC
1.	Gurjari x NVSR-6157	-3.49*	10.09**	-19.05**	0.14	-10.44**	4.69*	-3.03**	29.67**	12.92**	20.64**	-22.12*	-28.93**	18.84	-20.18*
2.	Gurjari x NVSR-473	-6.98**	6.11**	20.57**	15.62**	3.85	9.74**	-14.78**	3.60*	-18.83**	-13.06**	-26.50*	-33.18**	3.05	2.36
3.	Gurjari x NVSR-475	-8.15**	4.78**	30.91**	6.16*	-3.38	2.10	-28.48**	-13.06**	-8.18*	-7.34	9.48	-11.94	6.95	-9.55
4.	Gurjari x NVSR-486	-3.38*	10.23**	-10.16**	9.04**	6.64**	14.80**	-17.89**	-0.18	-17.21**	-16.45**	-11.68	-29.21**	59.98**	7.45
5.	Gurjari x Dandi	-3.38*	10.23**	-17.58**	5.34**	2.68	8.51**	-17.67**	0.09	0.76	13.64**	23.04*	13.61	11.71	-24.97**
6.	Gurjari x NVSR-403	-6.87**	6.24**	52.03**	23.29**	12.02**	18.37**	-13.93**	4.64**	7.88*	18.84**	-0.58	-10.60	1.38	-31.91**
7.	Gurjari x NVSR-409	-28.87**	-18.86**	18.09**	49.32**	-16.80**	-12.08**	-27.04**	-11.30**	-3.88	2.29	23.41*	25.26*	40.14**	7.45
8.	Gurjari x NAUR-1	-9.55**	3.19	-29.15**	-4.79	-11.67**	-6.66**	-24.70**	-8.46**	-5.63	2.45	34.10**	38.87**	93.74**	55.37**
9.	NVSR-452 x NVSR-6157	-4.36**	4.78**	-13.40**	7.12**	-12.24**	2.59	-4.85**	27.24**	-23.35**	-12.09**	-8.48	-5.98	-9.26	3.78
10.	NVSR-452 x NVSR-473	-29.58**	-22.84**	39.57**	33.84**	-19.69**	-30.09**	-3.45**	13.46**	0.67	15.44**	0.98	3.74	-37.09**	-28.04**

Sr. No.	Crosses	Kernel width (mm)		L : B ratio		100 grain weight (g)		Protein content (%)		Amylose content (%)		Grain yield per plant (g)		Straw yield per plant (g)	
		BP	SC	BP	SC	BP	SC	BP	SC	BP	SC	BP	SC	BP	SC
11.		0.24	9.83**	-11.93**	-18.08**	5.67*	-8.01**	-17.32**	-2.84*	-7.91*	5.61	-33.65**	-31.83**	-58.04**	-52.00**
12.	NVSR-452 x NVSR-486	1.09	10.76**	-12.30**	6.44**	5.50*	13.56**	-18.47**	-4.19**	-0.80	13.76**	31.23**	34.82**	-48.28**	-40.85**
13.	NVSR-452 x Dandi	3.03	12.88**	-41.91**	-25.75**	-18.31**	-14.18**	-20.88**	-7.02**	-6.77*	6.91	10.66	13.68	-8.87	4.23
14.	NVSR-452 x NVSR-403	-2.67	6.64**	-2.95	-9.73**	5.83*	-5.92*	-1.95	15.22**	-0.13	14.53**	10.66	13.68	-27.33**	-16.89
15.	NVSR-452 x NVSR-409	-7.88**	0.93	-15.06**	7.40**	-3.87	-2.10	-37.78**	-26.88**	-3.20	11.01**	-8.09	-5.58	-52.54**	-45.71**
16.	NVSR-452 x NAUR-1	5.70**	15.80**	-35.58**	-13.42**	16.86**	1.73	-27.62**	-14.95**	4.57	19.92**	-21.81*	-19.03	-23.01**	-11.94
17.	NVSR-453 x NVSR-6157	1.99	8.76**	-20.60**	-1.78	-12.97**	1.73	-11.85**	17.87**	11.92**	19.57**	4.34	8.34	-3.96	-26.54**
18.	NVSR-453 x NVSR-473	-17.34**	-10.76**	42.86**	36.99**	12.32**	-2.22	12.69**	21.52**	7.78*	15.44**	31.69**	36.75**	-26.20**	-26.69**
19.	NVSR-453 x NVSR-475	0.74	9.03**	-2.38	-21.23**	4.92	-18.50**	-10.20**	-16.34**	-5.26	-2.60	36.25**	41.48**	20.41	1.83
20.	NVSR-453 x NVSR-486	2.49	9.30**	-14.45**	3.84	-1.37	6.17**	-14.71**	-19.32**	10.35**	13.46**	-14.82	-11.55	-10.62	-31.64**
21.	NVSR-453 x Dandi	0.50	7.17**	-23.26**	-1.92	-5.05*	-0.25	-2.85	-9.50**	5.56	19.05**	-35.23**	-32.74**	-29.52*	-46.09**
22.	NVSR-453 x NVSR-403	-2.12	4.38*	-1.70	-20.68**	-12.76**	-22.44**	-1.90	9.32**	-2.97	6.88	-0.10	3.74	8.47	-17.03
23.	NVSR-453 x NVSR-409	-6.97**	-0.80	1.63	28.49**	5.69*	7.64**	-21.27**	-26.65**	11.21**	18.35**	31.99**	37.06**	-31.54*	-47.51**
24.	NVSR-453 x NAUR-1	9.84**	17.13**	-6.42**	25.75**	39.80**	20.84**	-17.64**	-23.28**	-7.46*	0.46	19.45*	24.04*	38.66**	11.19
25.	GNR-3 x NVSR-6157	0.73	9.56**	-32.00**	-15.89**	-20.25**	-6.78**	-13.03**	16.30**	12.32**	20.00**	-14.19	-21.69*	-18.12	-45.86**
26.	GNR-3 x NVSR-473	-17.70**	-10.49**	9.43**	4.93	-36.06**	-27.62**	-2.09	5.58**	-0.50	6.57	-1.15	-10.13	-30.42**	-30.89**
27.	GNR-3 x NVSR-475	-1.71	6.91**	8.42*	-15.34**	-23.09**	-12.95**	-5.07**	-19.95**	12.57**	11.47**	-6.13	-24.49*	6.33	-10.07
28.	GNR-3 x NVSR-486	3.05	12.08**	-11.29**	7.67**	2.83	16.40**	-15.56**	-20.13**	6.73	1.38	-8.81	-33.72**	-13.25	-42.64**
29.	GNR-3 x Dandi	3.05	12.08**	-42.87**	-26.99**	-28.98**	-19.61**	8.63**	-10.45**	-27.20**	-17.89**	39.89**	29.17**	-25.14	-50.51**
30.	GNR-3 x NVSR-403	-5.13**	3.19	16.12**	-7.26**	-22.00**	-11.71**	-0.65	10.72**	3.55	14.07**	-7.78	-17.07	116.76**	43.32**
31.	GNR-3 x NVSR-409	-22.22**	-15.41**	13.22**	43.15**	-16.99**	-6.04**	-17.26**	-31.79**	-0.43	5.96	-7.09	-5.69	33.69**	2.51
32.	GNR-3 x NAUR-1	-22.34**	-15.54**	-14.98**	14.25**	-37.15**	-28.85**	-13.60**	-28.77**	-3.24	5.05	10.19	14.11	57.89**	26.62**
	S.E. (d) ±	0.04	0.04	0.06	0.06	0.06	0.06	0.10	0.10	0.81	0.81	2.37	2.37	4.13	4.13
	C.D. at 5%	0.09	0.09	0.12	0.12	0.12	0.12	0.21	0.21	1.61	1.61	4.73	4.73	8.26	8.26
	C.D. at 1%	0.12	0.12	0.16	0.16	0.16	0.16	0.28	0.28	2.15	2.15	6.29	6.29	10.99	10.99
	Range	-29.58 to 9.84	-22.84 to 17.13	-42.87 to 52.03	-26.99 to 49.32	-37.15 to 39.80	-30.09 to 20.84	-37.78 to 12.69	-31.79 to 29.67	-27.20 to 12.92	-17.89 to 20.64	-35.23 to 39.89	-33.72 to 41.48	-58.04 to 116.76	-52.00 to 55.37

*-Significant at 5 % and **-Significant at 1 %, BP-Better Parent, SC-Standard Check

Patel *et al*., (2018), Prajapati and Kathiria (2018), Thakor *et al*., (2018), and Sundaram *et al*., (2019).

Grains per panicle is an important trait to determine the grain yield in rice crop. The range of heterobeltiosis for grain per panicle was from -21.75 % (NVSR-453 x Dandi) to 25.36 % (NVSR-452 x NVSR-486). The estimates of standard heterosis varied from -27.07 % (GNR-3 x NVSR-486) to 22.24 % (Gurjari x NAUR-1). Six and four hybrids exhibited significant positive heterobeltiosis and standard heterosis, respectively in the desired direction for this trait. The results were akin to the findings of Rukmini (2014), Shinde and Patel (2014), Bhati *et al*., (2015), Sala *et al*., (2016), Kumari and Jaiswal (2017). The cross, Gurjari x NVSR-403 (41.62 %) recorded the maximum heterobeltiosis and the cross NVSR-453 x NAUR-1 (47.41 %) recorded maximum standard heterosis for kernel length. A total of six and seventeen crosses expressed significant heterobeltiosis and standard heterosis in positive direction, respectively. Positive heterosis for this trait was also reported by Sanghera and Hussain (2012), Sarial (2014), Bhatti *et al*., (2015), and Prajapati and Kathiria (2018).

The range of heterobeltiosis for kernel width was from -29.58 % (NVSR-452 x NVSR-473) to 9.84 % (NVSR-453 x NAUR-1). The estimates of standard heterosis varied from -22.84 % (NVSR-452 x NVSR-473) to 17.13 % (NVSR-453 x NAUR-1). Two and twenty-three crosses exhibited significant positive heterobeltiosis and standard heterosis, respectively. The results were similar to the findings of Sanghera and Hussain (2012), Bhatti *et al*. (2015), and Prajapati and Kathiria (2018). The L: B ratio is an important trait as it has a greater contribution towards seed quality. The cross combination Gurjari x NVSR-403 exhibited the maximum heterobeltiosis and the cross-combination Gurjari x NVSR-409 exhibited the maximum standard heterosis among all the crosses. The results were in agreement with the results reported by Shinde and Patel (2014), Dar *et.al.,* (2015) and Bano and Singh (2018). The heterobeltiosis for 100 grain weight varied between -37.15 % (GNR-3 x NAUR-1) to 39.80 % (NVSR-453 x NAUR-1). The standard heterosis ranged from -30.09 % (NVSR-452 x NVSR-473) to 20.84 % (NVSR-453 x NAUR-1). With regard to 100 grain weight, heterosis over better parent and standard check were exhibited by nine and ten crosses, respectively. Utharasu and Anandakumar (2013), Waza *et al*., (2016) and Sahu *et al*., (2017b) reported similar results.

The protein content of the hybrids is positively correlated with that of the protein content of the parents. However, it has a negative correlation with the grain yield per plant. The results for protein content revealed that only two crosses had significant and positive heterosis over better parent while twelve crosses had significant and positive heterosis over the standard check. The cross NVSR-453 x NVSR-473 had reported the highest magnitude of heterosis over better parent and the cross Gurjari x NVSR-6157 reported the highest heterosis over the standard check. Comparable harmony for this character was also recorded by Patel *et al*., (2018) and Thakor *et al*., (2018). The heterobeltiosis for amylose content ranged from -27.20 % (GNR-3 x Dandi) to 12.92 % (Gurjari x NVSR-6157). The value of standard heterosis ranged from -17.89 % (GNR-3 x Dandi) to 20.64 % (Gurjari x NVSR-6157). The amylose content of the hybrids has a positive correlation with that of the parents. Eight crosses depicted positive and significant heterosis over better parent and sixteen crosses over the standard check for amylose content. These results were in accordance with the results of Bano and Singh (2018), Patel *et al.,* (2018) and Thakor *et al.,* (2018).

Grain yield per plant is an attribute of economic importance which breeders attempt to improve by evolving new high yielding hybrids. The grain yield per plant for the hybrids is positively correlated with that of the parents. It also has positive correlation with the amylose content and negative correlation with the protein content. Among thirty-two hybrids, nine and eight hybrids exhibited significant and positive estimates of heterobeltiosis and standard heterosis, respectively for grain yield per plant. The crosses GNR-3 x Dandi and NVSR-453 x NVSR-475 expressed the highest positive and significant estimates for the heterosis over better parent and over the standard check, respectively. The results were in accordance with the findings of Sravan *et al*. (2016), Kumari and Jaiswal (2017), Sahu *et al*., (2017a), Sahu *et al*., (2017b), Bano and Singh (2018), Patel *et al*., (2018), Thakor *et al*., (2018) and Sundaram (2019). The range of heterobeltiosis for straw yield per plant was between -58.04 % (NVSR-452 x NVSR-475) to 116.76 % (GNR-3 x NVSR-403). The standard heterosis ranged from -52.00 % (NVSR-452 x NVSR-475) to 55.37 % (Gurjari x NAUR-1). Seven and three hybrids exhibited significant positive heterobeltiosis and standard heterosis, respectively. Similar results have been reported by Kumar *et al*., (2012), Patel *et al*., (2018) and Thakor *et al*., (2018).

In the present study, the magnitude of the heterosis varied from cross to cross. The top five crosses *viz.,* NVSR-453 x NVSR-475, Gurjari x NAUR-1, NVSR-453 x NVSR-409, NVSR-453 x NVSR-473 and NVSR-452 x NVSR-486 showed significant positive heterosis over better parent and check variety for grain yield and various yield component characters. So, these crosses can fully be exploited through the pedigree method to obtain higher yielding transgressive segregates.

References

1. Anonymous.2020. United State Department of Agriculture.
2. Bagheri N and Jelodar NB. (2010). Heterosis and combining ability analysis for yield and related-yield traits in hybrid rice. *International Journal of Biology*, 2(2): 222-231.
3. Bano DA and Singh SP. 2018. Heterosis for yield and yield related traits in aromatic rice [*Oryza sativa* (L.)] genotypes. *Current Journal of Applied Science and Technology*, 30(6): 1-18.
4. Bhati PK, Singh SK, Singh R, Sharma A and Dhurai SY. 2015. Estimation of heterosis for yield and yield related traits in rice [*Oryza sativa* (L.)]. *SABRAO Journal of Breeding and Genetics*, 47(4): 467-474.
5. Bhatti S, Pandey DP and Singh D. 2015. Combining ability and heterosis for yield and its component traits in rice [*Oryza sativa* (L.)]. *Electronic Journal of Plant Breeding*, 6(1): 12-18.
6. Dar SH, Rather AG, Najeeb S, Ahanger MA, Sanghera GS and Talib S. 2015. Heterosis study in rice [*Oryza sativa* (L.)] under temperate conditions. *International Journal of Agriculture Sciences,* 7(6): 540-545.
7. Fonseca S and Patterson FC. 1968. Hybrid vigour in a seven parent diallel cross in common winter wheat. *Crop Sciences*, 8(1): 85-88.
8. Glaszmann JC. 1987. A varietal classification of Asian cultivated rice (*Oryza sativa* L.) based on isozyme polymorphism. In: Rice genetics. *International Rice Research Institute*, pp 83–90.
9. http://ricestat.irri.org: World Rice Statistics.2010.
10. Kempthorne O. 1957. An introduction to genetic statistics. John Wiley and Sans Inc., New York, U.S.A.
11. Kumari P and Jaiswal HK. 2017. Heterosis studies for yield and yield related traits over seasons of boro rice. *International Journal of Pure and Applied Bioscience*, 5(6): 1000-1009.
12. Kumar S, Pratap N and Dwivedi DK. 2012. Estimation of heterosis for grain yield and yield attributing traits in rice. *Plant Archives*, 12(1): 159-164.
13. Patel A, Rathava K, Gajjar PD, Intwala CG and Patel P. 2018. Study of heterosis for grain yield and its components in aerobic rice [*Oryza sativa* (L.)]. *International Journal of Pure Applied Bioscience*, 6(6): 358-368.
14. Peng JY and Virmani SS. 1990. Combining ability for yield and four related traits in relation to breeding in rice. *Oryza*, 27(1): 1-10.
15. Prajapati KN and Kathiria KB. 2018. Heterosis studies for grain yield and related characters in rice [*Oryza sativa* (L.)]. *International Journal of Genetics,*10(3): 380-386.
16. Sanghera GS and Hussain W. 2012. Heterosis and Combining Ability Estimates using Line X Tester Analysis to Develop Rice Hybrids for Temperate Conditions. *Notulae Scientia Biologicae*, 4(3):131-142.
17. Rukmini DK, Parimala K and Cheralu C. 2014. Heterosis for yield and quality traits in rice [*Oryza sativa* (L.)]. *The Journal of Research of ANGRAU*, 42(1): 1-11.
18. Sahu PK, Sharma D, Singh S, Khute IK and Sao FC. 2017a. Heterotic expressions evaluation for grain yield and quality traits in CMS based rice hybrids. *International Journal of Genetics*, 9(3): 257-262.
19. Sahu R, Singh SK, Singh DK, Vennela PR, Reddy YS, Kumar D, Singh M and Rathan ND. 2017b. Heterosis studies for yield & yield traits in rice

[*Oryza sativa* (L.)] under rainfed condition. *International Journal of Agriculture, Environment and Biotechnology,* 10(1): 1-10.

20. Sala M, Shanti P, Selvi B and Ravi V. 2016. Heterosis study for yield and yield components in rice [*Oryza sativa* (L.)]. *International Journal of Plant Sciences*, 11(1): 98-102.
21. Sarial AK. 2014. Heterosis and combining ability analysis for grain quality and physico-chemical traits involving fertility restorers with basmati background in rice (*Oryza sativa* L.). *Electronic Journal of Plant Breeding*, 5(4): 615-625.
22. Shinde DA and Patel PB. 2014. Study of heterosis for improvement in aerobic rice [*Oryza sativa* (L.)]. *The Bioscan,* 9(2): 739-743.
23. Sravan T, Jaiswal HK, Waza SA and Kumari P. 2016. Heterosis for yield and yield attributes in rice [*Oryza sativa* (L.)]. *Journal of Applied and Natural Sciences*, 8(2): 622-625.
24. Sundaram MK, Rajeswari S, Saraswathi R and Jeyaprakash P. 2019. Heterosis and combining ability analysis for yield related traits in rice hybrids involving land races. *Electronic Journal of Plant Breeding*, 10(1): 92-100.
25. Thakor RP, Balat JR, Delvadiya IR. and Rathva SR. 2018. Heterosis and heterobeltosis studies for grain yield and yield attributing traits in rice [*Oryza sativa* (L.)]. *Electronic Journal of Plant Breeding*, 9(4): 1286-1294.
26. Thorat BS, Kunkerkar RL and Bagkar TA. 2017. Studies on heterosis for yield and its contributing traits in hybrid rice [*Oryza sativa* (L.)]. *International Journal of Chemical Studies*, 5(5): 07-12.
27. Utharasu S and Anandakumar CR. 2013. Heterosis and combining ability analysis for grain yield and its component traits in aerobic rice [*Oryza sativa* (L.)] cultivars. *Electronic Journal of Plant Breeding*, 4(4): 1271-1279.
28. Waza SA, Jaiswal HK, Sravan T, Bano DA, Priyanka K and Umesh. 2018. Heterosis for yield and quality traits in rice (*Oryza sativa* L.). *Journal of Applied and Natural Science*, 8(3): 1510-1522.

RESEARCH ARTICLE https://doi.org/10.58297/BIYU9236

Impact of crop establishment methods and mineral nutrition on the productivity of rice (*Oryza sativa* L.) in North-West India

Buta Singh Dhillon[1], Gurpreet Kaur Gill[1], Mahender Kumar R[2] and Jasvir Singh Gill[3]

[1]Department of Plant Breeding and Genetics, [3]Department of Farm Power and Mechanical Engineering, Punjab Agricultural University, Ludhiana, Punjab – 141001
[2]Indian Institute of Rice Research, Hyderabad- 500030
Corresponding author email: kumarrm21364@gmail.com

Received: 21th March 2022; Accepted: 11th May 2022

Abstract

Due to scarcity of labour and declining underground water resources, there is a need to shift from the conventional transplanting method of rice to other methods which save on irrigation water and labour while maintaining similar productivity. Hence, studies to evaluate the comparative performances of different rice establishment methods under varied nutrition were conducted during *Kharif* 2017 and 2018 on Sandy Loam soil. The experiment was laid out in split plot design with three crop establishment methods (Manual transplanting, Mechanical transplanting and Direct seeding) in main plots, and five mineral nutrition (NPK) treatments {T_1-120:60:40- (Through chemical fertilizer), T_2-120:60:40- (75 % N through chemical fertilizer + 25 % N through farmyard manure), T_3-180:90:60 (Through chemical fertilizer), T_4-150:0:0 (Through chemical fertilizer) and T_5- Control (No fertilizer)} in subplots replicated thrice. Direct seeding recorded the highest panicles/m^2 but panicle weight was the highest under manual transplanting leading to similar grain and straw yields under all the establishment methods. Among mineral nutrition treatments, N: P: K 150:0:0 applied through chemical fertilizer recorded the highest grain and straw yields with respective increase of 56.4 and 59.0% than no fertilizer treatment (control), but former treatment was statistically similar to all other nutrition treatments. Interactive effects reveal statistical parity among all the establishment methods under different mineral nutrition treatments except control (no fertilizer), where manual transplanting treatment out yielded mechanical transplanting and direct seeding. Correlation studies revealed significant positive correlation of seed yield with plant height, panicle weight, filled grains, 1000 grain weight, SPAD and dry matter accumulation by crop, whereas, grain yield and unfilled grains were found to be negatively correlated.

Keywords: crop establishment methods, grain yield, mineral nutrition, rice, SPAD

Introduction

Paddy is cultivated in Punjab on a large area for more than half century due to the availability of plenty of groundwater as well as surface water resources (Srivastava *et al.,* 2014). The conventional system of rice production i.e., manual transplanting of rice in puddled fields is water and energy intensive, thereby, leading to increased pumping cost and water scarcity (Kaur and Vatta, 2015; Dhillon and Mangat, 2018; Vijaya kumar *et al*., 2018, 2019).

Repeated puddling destroys soil structure and creates shallow hard pan, affecting the performance of rice as well as the succeeding wheat crop. It also makes the conditions favorable for emission of methane (Bhardwaj and Sidana, 2019). Rice transplanting requires 150-200 man-hr ha^{-1}, which forms the major labour requirement for rice crop production. Moreover, the non-availability of sufficient labour at an appropriate time delays the transplanting of rice, causing yield reduction (Rakesh *et al.,* 2017). Hence, the productivity and sustainability of rice production

systems are threatened because of increasing resource scarcity (Nayak *et al*., 2020, Vijayakumar *et al*., 2021b). To sustain the production of rice, alternative methods of rice production needed to be explored. Punjab, a state with a geographical area of 1.53 % of India contributed 30-48 % to the national buffer stock and played a key role in the food security of India (Chauhan *et al.,* 2012). Therefore, the sustainability of the rice production system in Punjab is important for ensuring the food security of India. Among the rice production systems, puddled transplanted rice (PTR) is the most widely adopted system while globally; about 23% of rice is direct-seeded rice (Rao *et al.,* 2007). To check the fall in ground water and diminishing labour availability, technological intervention is needed. Direct seeding of rice claims to reduce water footprints by 10-20% and labour requirements by 80% besides increasing water recharge (Anonymous, 2022, Chakraborty *et al.,* 2017; Vijayakumar *et al*., 2019a, 2022). Pathak *et al.,* (2009) reported that there was negligible methane emission in aerobic rice fields as compared to that in the transplanted rice. Similarly, the mechanical transplanting technique also addresses labour problem along with shortening of transplanting window on account of higher field efficiency of mechanical transplanters as compared to manual labour (Manes *et al.,* 2013; Vijayakumar *et al*., 2021). Changing the rice ecosystem from stagnated water (reducing conditions) to aerobic system as in case of DSR, even within two transplanting methods, where row spacing automatically got varied as in recommended practice of manual transplanting row spacing of 20 cm but paddy transplanter plants seedlings at the spacing of 30 cm. Under variable ecosystems and planting geometries, plant nutrition acquired our attention (Subramanian *et al*., 2020). Changes in crop establishment methods have some variations for farm operations like tillage, seedbed preparation, sowing or planting method, weed, water and nutrient management, that cause a major effect on the growth and development of rice (Dhillon and Mangat, 2018; Pooja *et al*., 2021, 2021a, Saravanane *et al*., 2021). Meagre information on the nutrition requirements of rice under different rice establishment methods is available for north-western India. Keeping these facts in mind, present studies were planned to evaluate the effect of different doses and sources (organic or inorganic) of mineral nutrition in relation to crop establishment methods of rice.

Materials and Methods

A field experiment was conducted at the research farm of Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana located at 30° 54' N and 75° 48' E and 247.0 m above mean sea level with subtropical climatic conditions during summer (*Kharif*) seasons of 2017 and 2018. The monthly average maximum, minimum temperature and rainfall during the *Kharif* seasons of both years (2017 and 2018) has been given in **Figure 1**. The soil of the experimental field was sandy-loamy in texture, high in available–P (27.0 kg ha^{-1}), medium in available–K (167 kg ha^{-1}) and soil organic carbon (0.42%) but low in available N (270 kg ha^{-1}) status. The electrical conductivity and pH of the soil were within the normal range.

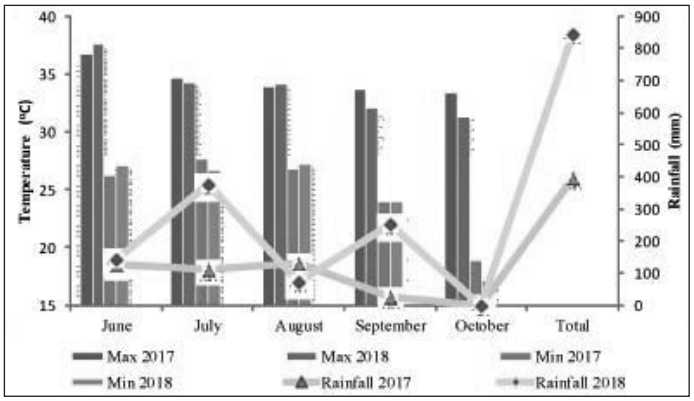


Figure 1: Maximum, minimum temperature and rainfall received during crop seasons of 2017 and 2018

The field trial was laid out in split plot design with three crop establishment methods (Manual transplanting, Mechanical transplanting and Direct seeding) in main plots, and five mineral nutrition (NPK) treatments {T_1: 120:60:40- (through chemical fertilizer), T_2: 120:60:40- (75% N through chemical fertilizer + 25% through farmyard manure), T_3: 180:90:60 (through chemical fertilizer), T_4: 150:0:0 (through chemical fertilizer) and T_5: control (no nutrients)}in sub plots replicated thrice. The farmyard manure (FYM) applied under T_2 treatment was analyzed for nitrogen,

phosphorus and potassium content. It was found that FYM contained 0.5 N, 0.21 P_2O_5 and 0.36% K_2O. Hence, out of 120 kg N ha^{-1}, 25% i.e., 30 kg was applied through FYM @ 6 t/ha, which also supplied about 12.6 kg/ha of P_2O_5 and 21.6 kg/ha of K_2O. So, remaining 47.4 kg/ha P_2O_5 and 18.4 kg/ha K_2O was applied through chemical fertilizers. The sowing of rice nursery for manual and machine transplanting as well as direct sowing of rice in the field was done on the same day (June 5, 2017 and June 8, 2018) and was transplanted 25 days after sowing. The nursery of rice for manual and mechanical transplanting was sown as per the recommendations of Punjab Agricultural University, Ludhiana (Anonymous, 2022). For direct seeded rice, 25 kg seed ha^{-1} was sown in lines spaced at 20 cm. Irrigation was applied immediately after sowing. Manual transplanting of rice seedlings was done at 20 cm x 15 cm spacing and machine transplanting was carried out using a self-propelled paddy transplanter at 30 cm x 12 cm spacing. Crop was raised as per the recommendations of Punjab Agricultural University given in the package of practice for *kharif* crops (Annonymous, 2022). Urea (46% N), single super phosphate (16% P_2O_5) and muriate of potash (60% K_2O) were used as sources of NPK, respectively and were applied as per treatment. The plant height, tillers m^{-2}, days to 50 percent flowering, panicles m^{-2}, panicle weight, filled and unfilled grain $panicle^{-1}$, 1000 grain weight were recorded as per the standard procedures in all the treatments. SPAD was estimated from five randomly selected leaves of different plants with a portable chlorophyll meter (SPAD-502). For estimating grain yield, a net area of 8.0 m^2 [4.0 m X 2.0 m] was harvested from each plot and then threshed, sun dried, winnowed, cleaned, and weighed on an electronic balance. For valid comparison of different treatments, moisture in grains was estimated using a digital moisture meter (Kett's RICETER J Handheld grain moisture meter). Grain yield was adjusted at 14% moisture and expressed as q ha^{-1}. The weight of the straw from each net plot was also recorded three days after harvest for estimation of straw yield, which was expressed as q ha^{-1}. Data were subjected to the analysis of variance (ANOVA) using the Proc GLM procedure of SAS software (SAS 9.3.). Least significant difference (LSD) at 5% level of probability was computed to compare the statistical significance of different treatments.

Results and Discussion

Planting methods

The pooled analysis of two *Kharif* seasons indicated that plant height at harvest, number of tillers per metre square, days taken to attain 50 % flowering stage, panicle per square metre, panicle weight and grain number per panicle differed significantly among various establishment methods of rice (**Table 1 and 2**). Crop attained significantly the highest plant height (97 cm) under manual transplanting whereas, machine transplanting (88.4 cm) and direct seeding (87.4 cm) attained statistically similar plant height. A similar trend was observed for tillers per square metre. Direct seeding had significantly the highest density of tillers (406.1) per meter square followed by machine transplanting, however, manual transplanting had the lowest density of tillers (290.3) per meter square. Machine transplanted crop took significantly the highest number of days (97.9) to attain 50% flowering followed by manual transplanting (91 days), whereas, the direct seeded crop took the least number of days for attaining 50% flowering stage, which was about 15 days earlier than machine transplanting of rice seedlings (**Table 1**). Data presented in **Figure 2** indicate that manually transplanted crops recorded significantly the highest SPAD value (37.8) whereas, direct seeded crop recorded the least, although differences between direct seeding (33.9) and mechanical transplanting (33.9) were not significant. Dry matter accumulation by crop and weed did not vary under the influence of establishment method.

Manual transplanting recorded the highest panicle weight (3.31g) and number of filled grains per panicle (149.5), which were 32.4 and 30.7% higher than that obtained under direct seeding. Direct seeding and machine transplanting of rice remained statistically similar w.r.t. panicle weight and number of filled grains per panicle (**Table 2**). The differences in 1000 grain weight among different establishment methods were not significant. Similar results were also reported by Gill and Walia (2013). It is further evident that

Table 1. Effect of establishment method and mineral nutrition on plant height, tiller count and days to 50% flowering of rice

Treatments	Plant height (cm) (at harvest)			Tillers/ m² (at harvest)			Days to 50 percent flowering		
	2017	2018	Pooled	2017	2018	Pooled	2017	2018	Pooled
Planting method									
M_1: Manual transplanting	99.8	94.2	97.0	299.4	281.2	290.3	92.7	89.3	91.0
M_2: Mechanical transplanting	92.4	84.3	88.4	387.7	349.4	368.6	96.3	99.5	97.9
M_3: Direct seeding	83.1	91.7	87.4	340.0	472.1	406.1	85.3	80.3	82.8
LSD (p=0.05)	9.4	7.3	6.1	31.9	49.6	29.6	2.5	1.3	2.0
Mineral nutrition									
T_1: 120:60:40- (Through chemical fertilizer)	94.6	91.6	93.1	337.3	386.0	361.7	90.2	89.2	89.7
T_2: 120:60:40- (75% N through chemical fertilizer + 25 % N through FYM)	89.0	92.8	90.9	329.9	367.0	348.5	90.8	89.1	90.0
T_3: 180:90:60 (Through chemical fertilizer)	95.9	93.8	94.9	367.7	408.0	387.9	92.4	88.4	90.4
T_4: 150:0:0 (Through chemical fertilizer)	94.8	91.0	92.9	373.9	376.0	375.0	90.3	90.1	90.2
T_5: Control (No nutrient)	84.3	81.0	82.7	287.9	296.1	292.0	93.3	91.7	92.5
LSD (p=0.05)	3.2	4.4	3.0	22.8	19.3	18.2	1.3	1.5	1.2

panicles per square meter showed the reverse trend with direct seeding recoding the highest (377 per square meter), whereas, manually transplanted cop recording the least (265 per square meter). Hence the superiority of panicle weight and the number of filled grains per panicle under manual transplanting over direct seeding was superseded by significantly the highest number of panicles per square meter under direct seeding, resulting into statistically similar grain yield under all establishment methods (**Table 3**). Similar grain yield under different establishment methods has also been reported by Dhillon *et al.,* (2020). The differences in the data were found to be non-significant for straw yield and harvest index among various crop establishment methods **(Table 3).** Our results are in contradiction to the findings of Kumar *et al.,* (2018) who reported more grain yield of rice under manual transplanting in puddled conditions than in direct seeded rice.

Mineral Nutrition

Mineral nutrition plays an important role in the growth and development of crop plants. The various mineral nutrition treatments had a significant effect on the growth, yield attributes and yield of rice crop as is indicated in pooled data of 2 years **(Table 1, 2, 3).** Data reveal that the control (no nutrition) treatment recorded the least plant height, tiller density, SPAD

Table 2. Effect of establishment method and mineral nutrition on yield attributes of rice

Treatments	Panicles/ m^2			Panicle weight (g)			Filled grain/ panicle			Un filled grains/ panicle			1000 grain weight (g)		
	2017	2018	Pooled	2017	2018	Pooled	2017	2018	Pooled	2017	2018	Pooled	2017	2018	Pooled
Planting method															
M_1: Manual transplanting	260	269	265	3.35	3.26	3.31	163	135.9	149.5	15.7	22.4	19.1	22.1	20.0	21.1
M_2: Mechanical transplanting	300	310	305	2.89	2.66	2.78	141.5	98.2	119.9	13.1	21.1	17.1	21.9	20.2	21.1
M_3: Direct seeding	323	431	377	2.52	2.48	2.50	123.5	105.2	114.4	22.1	22.4	22.3	21.4	20.1	20.8
LSD (p=0.05)	35	48	31	0.42	0.32	0.30	14.9	7.3	8.9	5.2	NS	NS	NS	NS	NS
Mineral nutrition															
T_1: 120:60:40- (Through chemical fertilizer)	292	366	329	3.12	2.90	3.01	151.8	120.8	136.3	15.0	18.8	16.9	22.0	20.8	21.4
T_2: 120:60:40- (75% N through chemical fertilizer + 25 % N through FYM)	289	353	321	3.03	2.86	2.95	148.6	124.4	136.5	16.3	18.5	17.4	22.2	20.6	21.4
T_3: 180:90:60 (Through chemical fertilizer)	325	388	357	3.05	2.96	3.01	153.2	115.8	134.5	19.6	19.3	19.5	21.5	20.6	21.1
T_4: 150:0:0 (Through chemical fertilizer)	325	355	340	3.12	2.98	3.05	149.6	120.2	134.9	15.5	20.1	17.8	21.9	20.8	21.4
T_5: Control (No nutrient)	242	222	232	2.29	2.32	2.31	110	84.1	97.1	18.4	33.1	25.8	21.4	17.9	19.7
LSD (p=0.05)	30	36	28	0.24	0.22	0.19	13.0	4.4	7.4	NS	9.6	6.6	NS	NS	NS

value, dry matter accumulation by crop, panicles per square meter, panicle weight and filled grains per panicle but under the same treatment (i.e., control) crop took the significantly highest number of days to attain 50% flowering stage along with the highest dry matter accumulation by weeds. All other nutrition treatments recorded similar growth and yield attributes of rice but significant improvement in tiller density along with the concurrent reduction in dry matter accumulation by weeds was noted under higher levels of nutrition i.e., T_3 and T_4 (**Figure 2**). Although there was an improvement in tiller density under higher nutrition the panicles per square meter did not improve on account of the higher level of fertilizer application.

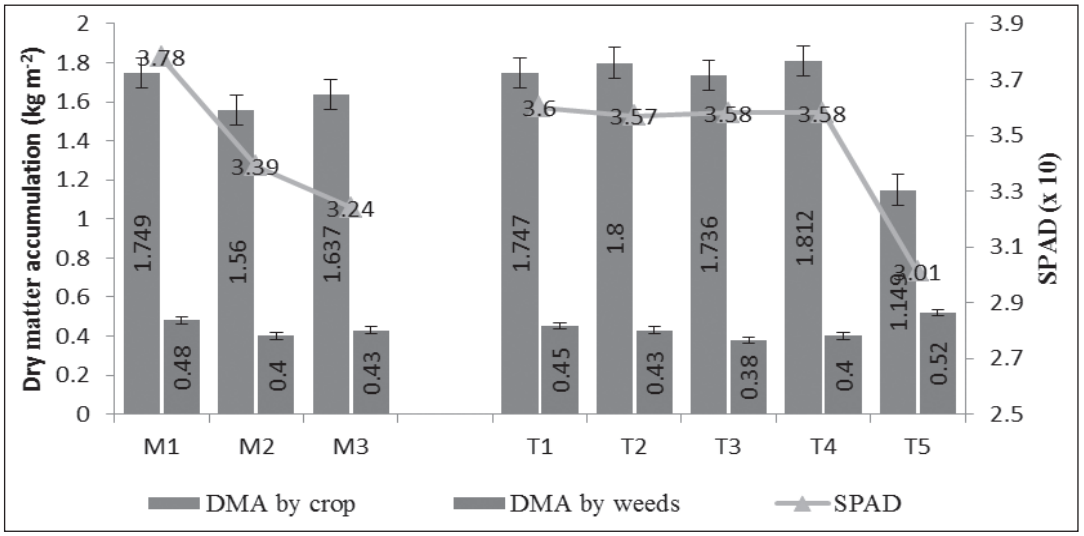


Figure 2: Effect of crop establishment methods and mineral nutrition on SPAD value at 50% floweing stage; dry matter accumulation by crop and weeds at active tillering stage (pooled data)

All the treatments irrespective of the source and amount of nutrition had a significantly higher number of filled grains and lower unfilled grains per panicle than the control treatment (no fertilizer) (**Table 3**). In pooled data differences for 1000 grain weight and harvest index were found to be non-significant. All the nutrition treatments had significantly higher grain and straw yields than the control. Increase in grain and straw yield with different sources of nutrition might be due to better growth attributes of rice like plant height, tillers, dry matter accumulation and higher chlorophyll content in leaves by the vigorous plants of rice with nutrients in comparison to control (no fertilizer). It is interesting to note that in the foregoing studies, even non-use of phosphatic and potassic fertilizers (T_4) did not affect the growth and yield of rice crop, which can probably be ascribed to the sufficient status of available phosphorus and potassium in the soil of the experimental field. Similar results have been reported by Tripathi *et al.,* (2019) and Dai *et al.,* (2010.)

Planting Methods x Mineral Nutrition

The interactive effects of planting methods x mineral nutrition on grain and straw yields have been presented in **Figures 3 and 4**. Data revealed that all the nutrition treatments performed significantly better than the control (no nutrient) under all methods of crop establishment. Data revealed statistical parity among all the establishment methods under different mineral nutrition treatments except control (no fertilizer), where manual transplanting treatment out yielded mechanical transplanting and direct seeding. Manual transplanting treatment having NPK 150:0:0 (through chemical fertilizer) gave significantly higher grain yields and statistically on par with all other treatments combination except for the control of all planting methods and machine transplanting with NPK 120:60:40- (75% N through chemical fertilizer) + (25 % N through FYM). Mitali *et al*., (2019) reported that the grain yield of rice increased with increasing N dose under manual transplanting and direct seeding of rice. Similarly, Ranjan and Yadav, (2019) also found

that the establishment methods and appropriate doses of N fertilizer are needed to improve the nitrogen use efficiency in rice. The various crop establishment methods also performed significantly different at the different levels of nutrition for straw yields. In general, machine transplanting gave more straw yield than direct seeding and manual transplanting. Significantly higher straw yields were obtained under machine transplanting with NPK 180:90:60 (through chemical fertilizer) than the control of all planting methods

Table 3. Effect of establishment method and mineral nutrition on grain, straw yield and harvest index of rice

Treatments	Grain yield (q/ha)			Straw yield (q/ha)			Harvest index (%)		
	2017	2018	Pooled	2017	2018	Pooled	2017	2018	Pooled
Planting method									
M_1: Manual transplanting	65.5	69.7	67.6	80.1	87.3	83.7	45.0	44.4	44.7
M_2: Mechanical transplanting	62.0	59.8	60.9	81.5	81.1	81.3	43.2	44.3	43.8
M_3: Direct seeding	60.9	64.0	62.5	78.9	80.8	79.9	43.6	44.5	44.0
LSD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS
Mineral nutrition									
T_1: 120:60:40- (Through chemical fertilizer)	65.2	69.4	67.3	84.4	91.5	88.0	43.6	43.2	43.4
T_2: 120:60:40- (75% N through chemical fertilizer + 25 % N through FYM)	63.7	68.3	66.0	82.5	90.5	86.5	43.5	43.0	43.3
T_3: 180:90:60 (Through chemical fertilizer)	67.2	71.8	69.5	86.6	96.2	91.4	43.7	42.7	43.2
T_4: 150:0:0 (Through chemical fertilizer)	68.3	72.5	70.4	85.4	89.3	87.3	44.4	44.9	44.7
T_5: Control (No nutrient)	49.7	40.3	45.0	61.8	48.0	54.9	44.6	47.9	46.3
LSD (p=0.05)	6.2	5.3	5.0	8.5	8.4	8.1	NS	3.3	NS

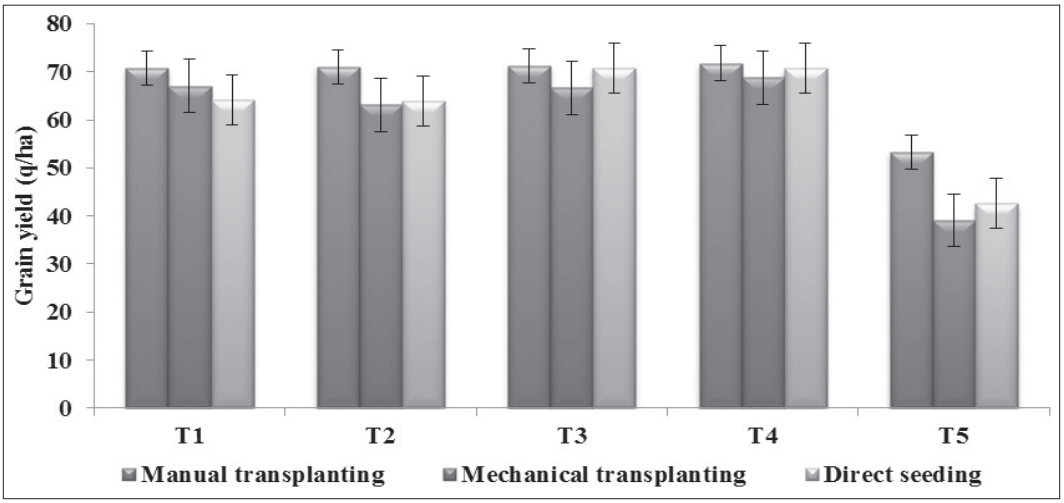


Figure 3: Interactive effect of crop establishment methods and mineral nutrition on grain yield of rice (pooled data)

but were statistically at par with all other treatment combinations for varied planting methods (**Figure 4**).

Correlation studies

Correlation studies revealed significant positive correlation of seed yield with plant height, panicle weight, filled grains, 1000 grain weight, SPAD and dry matter accumulation by crop at active tillering stage, whereas, grain yield and unfilled grains were found to be negatively correlated (**Table 4**). Cursory look at the data also reveal that panicles m^{-2} and tiller density had significant negative correlation with dry matter accumulation by weed at active tillering stage thereby indicating the smothering potential of more tiller density on weeds. It is also evident that DMA by crop and SPAD had significant positive correlation with panicle weight and filled grains, ultimately with grain yield.

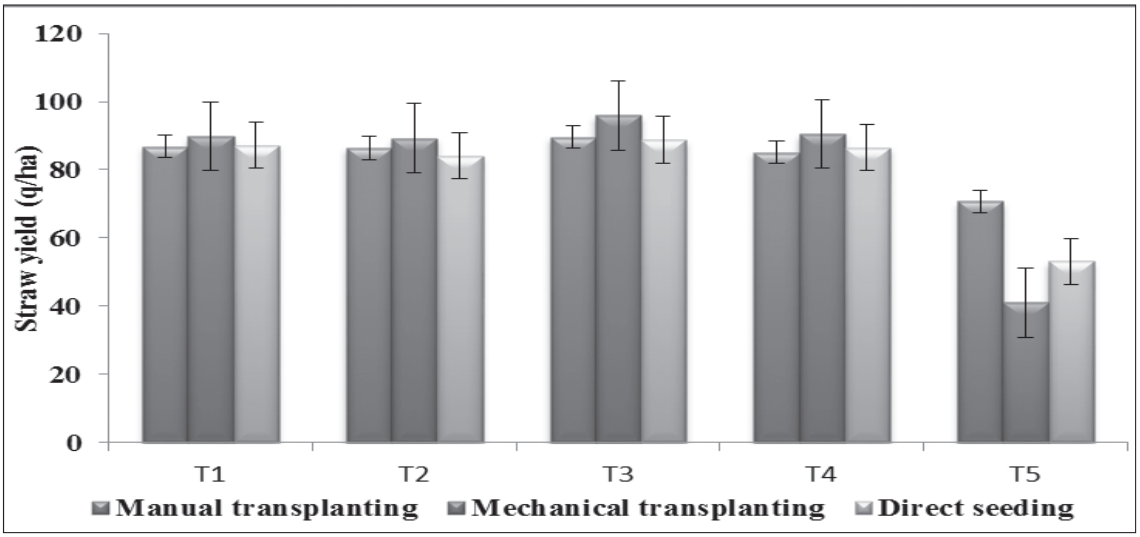


Figure 4: Interactive effects of crop establishment methods and mineral nutrition on straw yield of rice (pooled data)

Conclusions

It is concluded that all the methods of crop establishment performed statistically similar and one can practice any method of crop establishment method depending upon the local situations like availability of labour, groundwater, energy, sowing window and other resources. Rice crop responds more to nitrogen fertilizer in sandy loam soil having sufficient phosphorus and potash status. Hence, one can omit addition of phosphorus and potassium fertilizers to rice if soil status for these nutrients is sufficient.

Acknowledgements

Authors thankfully acknowledge the funding and technical guidance for the conduct of the experiment received under the All India Coordinated Rice Improvement Project.

References

Anonymous. 2022. *Package and Practices for Kharif Crops*, Punjab Agricultural University, Ludhiana

Bhardwaj S and Sidana BK. 2019. Ground water depletion and role of direct seeded rice in water saving: A move towards sustainable agriculture of Punjab. *Economics Affairs*, 64(1):19-28.

Chakraborty D, Ladha JK, Rana DS, Jat ML, Gathala MK, Yadav S, Rao AN, Ramesh MS and Raman A. 2017. A global analysis of alternative tillage and crop establishment practices for economically and environmentally efficient rice production. *Scientific Reports,* 7, 9342, https://doi.org/10.1038/s41598-017-09742-9

Chauhan BS, Mahajan G, Sardana V, Timsina J, and Jat ML. 2012. Productivity and sustainability of Rice-wheat cropping system in Indo-gangetic plains of

Table 4. Correlation matrix between growth, yield attributes and yield of rice (on the basis of pooled data)

Parameters	PH	TD	DTF	Pm^{-2}	PW	FG	UFG	1000GW	SPAD	DM-crop	DM-weeds	GY
PH	1.000											
TD	0.070^{NS}	1.000										
DTF	-0.058^{NS}	-0.378^{NS}	1.000									
Pm^{-2}	0.282^{NS}	0.952^{**}	-0.557^{NS}	1.000								
PW	0.965^{**}	-0.050^{NS}	0.114^{NS}	0.142^{NS}	1.000							
FG	0.964^{**}	0.021^{NS}	-0.042^{NS}	0.244^{NS}	0.981^{**}	1.000						
UFG	-0.671^{NS}	-0.325^{NS}	-0.259^{NS}	-0.382^{NS}	-0.764^{*}	-0.763^{*}	1.000					
1000GW	0.758^{*}	0.466^{NS}	-0.056^{NS}	0.591^{NS}	0.791^{*}	0.834^{*}	-0.942^{**}	1.000				
SPAD	0.969^{**}	0.029^{NS}	0.031^{NS}	0.233^{NS}	0.991^{**}	0.996^{**}	-0.792^{*}	0.841^{**}	1.000			
DM-crop	0.835^{**}	0.451^{NS}	-0.277^{NS}	0.642^{NS}	0.816^{*}	0.883^{**}	-0.810^{*}	0.954^{**}	0.872^{**}	1.000		
DM-weed	-0.386^{NS}	-0.830^{*}	-0.017^{NS}	-0.794^{*}	-0.335^{NS}	-0.342^{NS}	0.615^{NS}	-0.677^{NS}	-0.374^{NS}	-0.630^{NS}	1.000	
GY	0.883^{**}	0.478^{NS}	-0.226^{NS}	0.652^{NS}	0.841^{**}	0.882^{**}	-0.794^{*}	0.932^{**}	0.883^{**}	0.980^{**}	-0.685^{NS}	1.000

PH: plant height; TD: tiller density; DTF: days to flowering; Pm^{-2}:Panicles m^{-2}; PW: Panicle weight; FG: filled grains; UFG: Un filled grains; 1000GW: 1000 grain weight; SPAD: SPAD value; DM-crop: Dry matter accumulation by crop at active tillering stage; DM-weed: Dry matter accumulation by weed at active tillering stage; GY: Grain yield.

Indian sub-continent: problems, opportunities and strategies. *Advances in Agronomy*, 117: 315-369.

Dai XQ, Zhang HY, Spiertz JHJ, Yu J, Xie GH and Bouman BAM. 2010. Crop response of aerobic rice and winter wheat to nitrogen, phosphorus and potassium in a double cropping system. *Nutrient Cycling in Agroecosystem*s, 86(3): 301–315

Dhillon BS, Bhullar MS and Sarao P S 2020. Crop Establishment Method and Planting Density Effects on Weeds, Insects and Productivity of Rice. *Indian Journal of Weed Science 52*. https://doi.org/10.5958/0974-8164.2020.00031.3.

Dhillon BS and Mangat, GS 2018. Direct Seeded Rice In Punjab: Opportunities and Challenges. *In. Technology for Rice Production*. Technical papers of National seminar on "Sustainable Rice Production Technology for Increasing the Farmers' Income" held during January 20-21, 2018 at Indira Gandhi Krishi Vishva Vidyalaya, Raipur (Chhatisgarh). *Pp* 535-38

Gill JS and Walia SS. 2013. Quality and grain yield of basmati rice as influenced by different establishment methods and nitrogen levels. *Asian Journal of Soil Science*, 8(2): 311-318.

Kaur S and Vatta K. 2015. Groundwater depletion in central Punjab: Pattern, access and adaptations. *Current Science*, 108 (4), 485-490.

Kumar V, Singh S, Sagar V and Maurya ML. 2018. Evaluation of different crop establishment method of rice on growth, yield and economics of rice cultivation in agro-climatic condition of eastern Uttar Pradesh. *Journal of Pharmacognosy and Phytochemistry*, 7(3): 2295-2298.

Manes GS, Dixit A, Singh A, Mahal JS and Mahajan G. 2013. Feasibility of mechanical transplanter for paddy transplanting in Punjab. *Agricultural Mechanization in Asia, Africa and Latin America*, 44(3):14-17.

Mitali KS, Shah P, Yadav R, Sah NJ and Ranjan R. 2019. Interaction of nitrogen doses and establishment methods in lowland rice. *Archives of Agriculture and Environmental Science*, 4(1): 113-118.

Nayak AK, Chatterjee D, Tripathi R, Shahid M, Vijayakumar S, Satapathy BS, Kumar A, Mohanty S, Bhattacharyya P, Mishra P and Kumar U. 2020. Climate smart agricultural technologies for rice production system in Odisha. ICAR-National Rice Research Institute, Cuttack, Odisha, 753006, India. pp 366. ISBN: 81-88409-14-6.

Pathak H, Saharawat YS, Gathala MK, Mohanty S and Ladha JK. 2009. Simulating environmental impact of resource-conserving technologies in the rice wheat system of the Indo-Gangetic Plains.In: *Integrated crop and resource management in rice-wheat system of south Asia.* (JK Ladha, Y Singh, O Erenstein and B Hardy, Eds.), International rice research institute, Los Banos, Philippines, pp. 321-333.

Pooja K, Saravanane P, Nadarajan S, Sundaravaradhan S, Muthukumarasamy S and Vijayakumar S. 2021. Effect of cultivars and different weed management practices on yield, nutrient uptake and soil micro-flora in dry direct-seeded rice under coastal deltaic ecosystem. *Oryza* 58(1): 21-25. https://doi.org/10.35709/ory.2021.58.1.4

Pooja K, Saravanane P, Sridevi V, Nadarajan S and Vijayakumar S. 2021a. Effect of cultivars and weed management practices on productivity, profitability and energetics of dry direct-seeded rice. *Oryza,* 58(3): 442-447. https://doi.org/10.35709/ory.2021.58.3.11

Rakesh S, Mahajan G, Mukesh S, Angrej S and Chauhan BS. 2017. Performance of drip-irrigated dry-seeded rice (*Oryza sativa* L.) in South Asia. *Paddy Water Environment*, 15:93-100.

Ranjan R and Yadav R. 2019. Targeting nitrogen use efficiency for sustained production of cereal crop. *Journal of Plant Nutrition*, 51(2): 85-91.

Rao AN, Johnson DE, Shivaprasad B, Ladha JK and Mortimer AM. 2007. Weed management in direct-seeded rice. *Advances in Agronomy,* 93: 153-255.

Saravanane P, Pavithra M and Vijayakumar S. 2021. Weed management in direct seeded rice Impact of biotic constraint and its sustainable management options. *Indian Farming*, 71(4): 61–64.

Srivastava SK, Ghosh S, Kumar A and Anand PSB. 2014. Unravelling spatio-temporal pattern of irrigation development and its impact on Indian agriculture. *Irrigation and Drainage*, 63 (1), 1-11.

Subramanian E, Aathithyan C, Raghavendran VB and Vijayakumar S. 2020. Optimization of nitrogen fertilization for aerobic rice (*Oryza sativa*). *Indian Journal of Agronomy*, 65(2):180-184.

Tripathi SC, Chander S and Meena RP. 2019. Phosphorus skipping effect on productivity, profitability and sustainability of rice-wheat cropping systems in Indo-Gangetic plains of India. *Archives of Agronomy and Soil Science,* 65(8): 1134-1146.

Vijayakumar S, Dinesh K, Shivay YS, Sharma VK, Sharma DK, Saravanane P, Poornima S and Nain S. 2019b. Energy budgeting of aerobic rice (*Oryza sativa*)-wheat (*Triticum aestivum*) cropping system as influenced by potassium fertilization. *Indian Journal of Agricultural Sciences*, 89(11): 1911-5.

Vijayakumar S, Jinger D, Parthiban P and Lokesh S. 2018. Aerobic rice cultivation for enhanced water use efficiency. *Indian Farming*, 68(6): 3-6.

Vijayakumar S, Kumar D, Jinger D, Bussa B and Panda BB. 2021b. 4R nutrient stewardship based potassium fertilization for dry-direct seeded rice-wheat cropping system. *Indian Farming*, 71(1): 22-25

Vijayakumar S, Kumar D, Ramesh K, Jinger D and Rajpoot SK. 2022. Effect of potassium fertilization on water productivity, irrigation water use efficiency, and grain quality under direct seeded rice-wheat cropping system. *Journal of Plant Nutrition* 45(13): 2023-2038. https://doi.org/10.1080/01904167.2022.2046071

Vijayakumar S, Kumar D, Shivay YS, Anand A, Saravanane P, Poornima S, Jinger D and Singh N. 2019a. Effect of potassium fertilization on growth indices, yield attributes and economics of dry direct seeded basmati rice (*Oryza Sativa* L.). *Oryza*, 56(2): 214-220. https://doi.org/10.35709/ory.2019.56.2.6

Vijayakumar S, Subramanian E, Saravanane P, Gobinath R and Saha S. 2021. Farm mechanisation in rice cultivation: Present status, bottlenecks and potential. *Indian Farming*, 71(4): 04–07.

RESEARCH ARTICLE https://doi.org/10.58297/QZMA2757

Effect of new generation herbicides on mixed weed flora and yield of direct seeded rice (*Oryza sativa* L.)

Swathi S*, Babu S, Karthikeyan A and Dhanasekaran K

Department of Agronomy, Annamalai University, Annamalai Nagar- 608 002, Tamil Nadu, India
*Corresponding author email: swathiselvasekaran1@gmail.com

Received: 29th March 2022; Accepted: 12th May 2022

Abstract

A field experiment was conducted during the "Navarai" season of 2019 and 2020 at Experimental Farm of the Department of Agronomy, Annamalai University, Annamalai Nagar, to find out the effect of new generation herbicides on direct seeded rice (*Oryza sativa* L.). The herbicide used were pre-emergence application of pyrazosulfuron-ethyl 10% WP at 200 g ha^{-1}, pre-emergence application of metsulfuran-methyl 10% + chlorimuron-methyl 10% WP at 20g ha^{-1}, pre-emergence application of pretilachlor 50% EC at 1250 ml ha^{-1}, Early post-emergence application of pretilachlor 6% + pyrazosulfuran-ethyl 0.15% G at 615g ha^{-1}, Early post-emergence application of bispyribac-sodium at 300 ml ha^{-1}. The experimental result revealed that the new generation herbicide has a significant influence on weed flora and yield attributes and yield of direct seeding rice. The result indicated that application of Bispyribac-sodium 10% SC at 300 ml ha^{-1} at 15 DAS + one Hand weeding at 30 DAS recorded the lowest weed population, weed dry matter production and higher weed control index (WCI) and yield. Then twice hand weeding at 15, and 30 DAS was next in order. The lowest weed control index and yield were recorded with un-weeded control.

Keywords: Direct seeded rice, grain yield, weed control index, herbicide

Introduction

Rice is the staple food of more than 60% of the world's population. It is globally grown in 155.62 m ha area with a production of 432.4 m tonnes. India ranks first in the acreage with 43.81 m ha but second in production with 96.43 m t, after china (Singh *et al*., 2019). Direct seeded rice (DSR) is one of the oldest methods of rice cultivation. In the 21st century, scarcity of agricultural land and water and a continuing shortage of labour would encourage a shift toward a direct-seeding method of the rice production system (Mortimer *et al*., 2005). Manual removal of weeds is labour intensive and sometimes, rice mimicking certain grassy weeds, hand weeding is not effective. Weed removal at the critical stage of the crop-weed competition is not possible due to the non-availability of labour and sometimes bad weather conditions. Hence the chemical method of weed control is considered to be an alternative to hand weeding (Singh *et al*., 2007). The chemical method of weed control is effective in controlling the weeds; besides, it reduces the total energy requirement for rice cultivation. The present study was undertaken to study the effect of pre and early post-emergence on weed management and yield in direct-seeded rice.

Materials and Methods

A field experiment was conducted during the "Navarai" season of 2019 and 2020 at the Experimental farm of the Department of Agronomy, Annamalai University, Annamalai Nagar, to find out the influence of pre- and early post-emergence herbicide on weed management in direct sown rice (*Oryza sativa* L.) under wet condition. The soil was clay loam, low in available nitrogen (232.5 kg ha^{-1}), medium in available phosphorus (19.2 kg ha^{-1}) and high in potassium (324.6 kg ha^{-1}), with organic carbon 0.70 % and pH 7.5. The Experiment was laid out in a randomized block design with three replications using a variety of CO-51 as

the test crop. The treatments include T_1- Un-weeded check, T_2- Twice Hand weeding at 15 and 30 DAS, T_3- Pre-emergence application of Pyrazosulfuron-ethyl 10% WP at 200g ha^{-1} at 7 DAS + one Hand weeding at 30 DAS T_4- Pre-emergence application of Metsulfuran-methyl 10% + Chlorimuron-methyl 10% WP at 20g ha^{-1} at 7 DAS + one Hand weeding at 30 DAS T_5-Pre-emergence application of Pretilachlor 50% EC at 1250 ml ha^{-1} at 7 DAS + one Hand weeding at 30 DAS T_6- Early post-emergence application of Pretilachlor 6% + Pyrazosulfuran-ethyl 0.15% G at 615g ha^{-1} at 15 DAS. T_7- Early post-emergence application of Bispyribac-sodium 10% SC at 300 ml ha^{-1} 15 DAS T_8- Early post-emergence application of Pretilachlor 6% + Pyrazosulfuran-ethyl 0.15% G at 615g ha^{-1} at 15 DAS + one Hand weeding at 30 DAS T_9- Early post-emergence application of Bispyribac-sodium 10% SC at 300 ml ha^{-1} at 15 DAS+ one Hand weeding at 30 DAS. The rice variety …… was raised under optimum conditions for agronomic practices and plant protection measures in the field. Observation of individual weed count, total weed flora and weed biomass were taken at 30 and 60 days after sowing (DAS) and also the final yield was taken at the time of harvesting.

Results and Discussion

The major weed flora of the experimental field consist of grasses *Echinochloa colonum* (L.), *Echinochloa crus-Galli* (L.), and *Leptochloa Chinensis* (L.) followed by sedges, *Cyperus rotundus* (L.) and *Cyperus difformis* (L.). Under the category of broad-leaved weeds, *Marsilea quadrifolia* and *Eclipta alba* (L.) were predominant.

Effect of weed control measures on weed population

Different weed control measures significantly influenced the weed population. The data on weed population recorded at 30 and 60 DAS in 2019 and 2020 is furnished in **Table 1**. Among the various weed control measures, early post-emergence application of Bispyribac-sodium 10% SC at 15 DAS + one Hand weeding at 30 DAS recorded the lowest weed population in both the years (6.77 and 8.83 on 30 and 60 DAS in 2019 and 5.12 and 6.58 on 30 DAS and 60 DAS in 2020) and significantly superior to the other treatments. Twice Hand weeding at 15 and 30 DAS was next in order. This might be due to the mode of action of bispyribac-sodium which is selective, systemic, post-emergence herbicide, and it is been absorbed by foliage and roots. Spraying of bispyribac-sodium efficiently destroyed the weeds and it may be ascribed to the trans-laminar activity of bispyribac-sodium. According to Madhulika and Paikra (2014), Bispyribac-sodium is translocated in the plant both by the downward movement to the roots and rhizomes and also an upward movement to the meristem; once the bispyribac-sodium arrives in the meristematic region, it attacks EPSP synthase, an enzyme of the tyrosine, phenylalanine and tryptophan; these amino acids are essential to protein synthesis and cell wall formation. This enzyme blockage might have led to a massive phytotoxic build-up of shikimic acid and benzoic acid, which inhibits respiration, bud development, chlorophyll synthesis and transpiration, leading to the eventual death of the plants, so the weed population is reduced. The highest weed population was recorded in un-weeded control. Because weeding operations are not carried out in un-weeded plots, the weed population is not reduced.

Effect of weed control measures on weed dry matter production (DMP)

All the treatments significantly influenced the weed DMP. The data on weed dry matter production recorded at 60 DAS in 2019 and 2020 is furnished in **Table 2**. Application of early post-emergence herbicide Bispyribac-sodium 10% SC at 15 DAS + one Hand weeding at 30 DAS recorded the lowest weed DMP of 24.87 at 60 DAS on 2019 and 18.53 at 60 DAS on 2020, respectively. Hand weeding twice at 15 and 30 DAS was next in order. This might be due to the fact that the better placement of herbicides on the interspacing provided and the better efficacy of herbicides in controlling the emerging weeds led to the suppression of weeds from the beginning. Bispyribac effectively controlled all categories of weeds and reduced the weed population. There was no phytotoxicity symptom observed during the observation, even at higher doses of this herbicide (Gosh *et al*., 2013). The highest dry matter production was recorded in un-weeded control. Because the weed population was not reduced the weed dry matter also increased.

Table 1. Effect of new generation herbicides on weed population

Treatments	2019		2020	
	Weed population on 30 DAS	Weed population on 60 DAS	Weed population on 30 DAS	Weed population on 60 DAS
T_1- Un-weeded control	106.08 (10.29)	143.29 (11.99)	92.18 (9.60)	121.31 (11)
T_2- Twice Hand weeding at 15 and 30 DAS	12.99 (3.60)	17.74 (4.27)	10.01 (3.16)	13.19 (3.63)
T_3- Pre-emergence application of pyrazo sulfuron-ethyl 10 % WP at 200g ha^{-1} at 7 DAS + one Hand weeding at 30 DAS	44.67 (6.68)	59.45 (7.74)	38.17 (6.17)	45 (6.70)
T_4- Pre-emergence application of metsulfuron-methyl 10% + chlorimuron-ethyl 10 % WP at 20 g ha^{-1} at 7 DAS + one hand weeding at 30 DAS	39.10 (6.25)	53.03 (7.31)	32.53 (5.70)	39.15 (6.25)
T_5- Pre-emergence application of pretilachlor 50 % EC at 1250 ml ha^{-1} at 7 DAS + one Hand weeding at 30 DAS	42.70 (6.53)	56.01 (7.51)	36.15 (6.01)	42.67 (6.53)
T_6- Early post-emergence application of pretilachlor 6% + pyrazosulfuron-ethyl 0.15% G at 615g ha^{-1}at 15 DAS	59.81 (7.73)	88.36 (9.42)	52.33 (7.26)	76.82 (8.76)
T_7- Early post-emergence application of bispyribac-sodium 10% SC at 300 ml ha^{-1} at 15 DAS	74.82 (8.64)	93.67 (9.70)	63.10 (7.94)	81.10 (9.0)
T_8- Early post-emergence application of pretilachlor 6% + pyrazosulfuron-ethyl 0.15% G at 615g ha^{-1}+ one hand weeding at 30 DAS	24.09 (4.91)	30.29 (5.54)	20.18 (4.49)	22.33 (4.72)
T_9- Early post-emergence application of bispyribac-sodium 10% SC at 300 ml ha^{-1} + one Hand weeding at 30 DAS	6.77 (2.60)	8.83 (3.05)	5.12 (2.26)	6.58 (2.56)
S.Ed	0.47	0.59	0.35	0.51
CD (p=0.05)	0.97	1.21	0.73	1.03

Figures in parenthesis are original values; values are square root transformed ($\sqrt{x} + 0.5$)

Effect of weed control measures on weed control index (WCI)

All the treatments significantly influenced the weed control index (WCI). The data on the weed control index in 2019 and 2020 is furnished in **Table 2**. Application of early post-emergence herbicide Bispyribac-sodium 10% SC at 15 DAS + one Hand weeding at 30 DAS recorded the highest weed control index of 92.40% on 2019 and 93.31% on 2020, respectively. Followed by Hand weeding twice at 15 and 30 DAS was next in order. This might be due to the synergistic and cumulative effect of the application of early post-emergence herbicide followed by mechanical weeding. This herbicide effectively controlled the weeds, reduced the weed population and dry matter production and hence the weed control index was high. The last weed control index was recorded with un-weeded control.

Yield parameters

Number of panicle m^{-2}

The effect of herbicide treatment on the number of filled grains panicle^{-1} was found to be highly significant. The data on the number of panicles m^{-2} in 2019 and 2020 is furnished in **Table 3**. Thus, the highest number of panicles was recorded from the plots sprayed with bispyribac-sodium 10% SC on 15

Table 2. Effect of new generation herbicides on weed dry matter production and weed control index

Treatments	2019	2020	2019	2020
	Weed dry matter production on 60 DAS	Weed dry matter production on 60 DAS	Weed control index (WCI)	Weed control index (WCI)
T_1- Un-weeded control	327.38	276.16	---	---
T_2- Twice Hand weeding at 15 and 30 DAS	37.53	27.90	88.53	89.93
T_3- Pre-emergence application of pyrazosulfuron-ethyl 10 % WP at 200g ha^{-1} at 7 DAS + one Hand weeding at 30 DAS	114.26	86.49	65.09	68.80
T_4- Pre-emergence application of metsulfuron-methyl 10% + chlorimuron-ethyl 10 % WP at 20 g ha^{-1} at 7 DAS + one hand weeding at 30 DAS	109.43	80.79	66.57	70.85
T_5- Pre-emergence application of pretilachlor 50 % EC at 1250 ml ha^{-1} at 7 DAS + one Hand weeding at 30 DAS	112.11	85.41	65.75	69.18
T_6- Early post-emergence application of pretilachlor 6% + pyrazosulfuron-ethyl 0.15% G 615g ha^{-1} at15 DAS	157.54	136.96	52.53	50.58
T_7- Early post-emergence application of bispyribac-sodium 10% SC at 300 ml ha^{-1} at 15 DAS	155.39	134.54	51.87	51.46
T_8- Early post-emergence application of pretilachlor 6% + pyrazosulfuron-ethyl 0.15% G 615g ha^{-1}+ one hand weeding at 30 DAS	46.72	34.44	85.72	87.57
T_9- Early post-emergence application of bispyribac-sodium 10% SC at 300 ml ha^{-1} + one Hand weeding at 30 DAS	24.87	18.53	92.40	93.31
S.Ed	3.27	2.81	---	---
CD (p=0.05)	6.65	5.72	---	---

Figures in the parenthesis indicate the original values

Table 3. Effect of new generation herbicides on number of panicle m^{-2}, thousand-grain weight, grain yield (kg ha^{-1})

Treatments	2019	2020	2019	2020	2019	2020
	Number of panicle m^{-2}	Number of panicle m^{-2}	Thou-sand-grain weight	Thou-sand-grain weight	Grain yield (kg ha^{-1})	Grain yield (kg ha^{-1})
T_1- Un-weeded control	216.90	235.10	16.41	16.58	2289.7	2474.66
T_2- Twice Hand weeding at 15 and 30 DAS	403.79	419.97	16.69	16.76	5287.37	5869.52
T_3- Pre-emergence application of pyrazosulfuron-ethyl 10 % WP at 200g ha^{-1} at 7 DAS + one Hand weeding at 30 DAS	385.64	354.45	16.51	16.59	4654.81	5017.63
T_4- Pre-emergence application of metsulfuron-methyl 10% + chlo-rimuron-ethyl 10 % WP at 20 g ha^{-1} at 7 DAS + one hand weeding at 30 DAS	352.38	371.31	16.58	16.63	4784.34	5218.28
T_5-Pre-emergence application of pretilachlor 50 % EC at 1250 ml ha^{-1} at 7 DAS + one Hand weeding at 30 DAS	346.09	363.86	16.53	16.60	4701.2	4936.13
T_6- Early post-emergence applica-tion of pretilachlor 6% + pyrazo-sulfuron-ethyl 0.15% G 615g ha^{-1} at15 DAS	318.53	336.02	16.48	16.59	3804.76	4066.85
T_7-Early post-emergence applica-tion of Bispyribac-sodium 10% SC at 300 ml ha^{-1} at 15 DAS	312.34	329.10	16.46	16.61	3633.85	3925.44
T_8- Early post-emergence applica-tion of pretilachlor 6% + pyrazo-sulfuron-ethyl 0.15% G 615g ha^{-1}+ one hand weeding at 30 DAS	385.64	403.09	16.60	16.70	5045.92	5638.46
T_9- Early post-emergence applica-tion of Bispyribac-sodium 10% SC at 300 ml ha^{-1}+ one Hand weeding at 30 DAS	415.42	436.11	16.72	16.75	5674.91	6230.71
S.Ed	6.64	7.71	NS	NS	90.71	104.50
CD	13.49	15.65	NS	NS	184.16	212.14

DAS + one Hand weeding at 30 DAS, which produced 415.42 panicle m^{-2} in 2019 and 436.11 panicle m^{-2} in 2020 and these treatments followed by Hand weeding twice at 15 and 30 DAS produced maximum panicles. The lowest number of filled grains panicle^{-1} were found in the case of weedy check plots. These results are in conformity with that of Iqbal *et al.,* (2017).

1000 grain weight

The results showed that the thousand-grain weight was not influenced by the treatments. Since thousand-grain weight is mainly governed by the inherent genetic makeup of the cultivar, the treatment effect was not reflected in character.

Grain yield

The yield of rice crop was significantly improved by the application of herbicides in direct-seeded rice. The data on grain yield in 2019 and 2020 is furnished in **Table 3**. Application of Bispyribac-sodium 10% SC at 15 DAS + one Hand weeding at 30 DAS recorded maximum yield of 5674 kg ha^{-1} in 2019 and 6230 kg ha^{-1} in 2020 than all other treatments. The application of this combination improved the availability of natural resources and critical inputs for the establishment of rice crop by reducing the germination of weeds as well as suppressing the weed growth with a proper, efficient mode of action in the initial days of critical crop weed competition. In direct seeded rice, yield and yield attributes were tremendously increased due to the timely control of weeds in a critical period of crop weed competition that has enhanced the availability of nutrients, light and moisture to the crop and also increased the crop yield with timely application of these broad-spectrum herbicides combination. Rao *et al.,* (2019) reported similar results in herbicides usage in direct-seeded rice. The hand weeding twice at 15, and 30 DAS was next in order. The lowest yield was recorded with un-weeded control because of the highest weed population.

References

Gosh RK, S Mallick and S Bera 2013. Efficacy of Bispyribac-sodium 10% SC against weed complex under different rice ecosystems. *Pestology*, 37(9): 47-53.

Iqbal N, MU Saleem, TH Awan, UB Khaliq, S Iqbal, A Iram and M Akhter. 2017. Effective weed management in dry direct-seeded rice for sustainable productivity. *Applied Sciences and Business Economics,* 4(1): 1-8.

Madhulika Singh and PR Paikra. 2014. Bio-efficacy of post-emergence herbicides in transplanted rice of Chhattisgarh plains. The Bioscan, 9(3): 973-976.

Mortimer M, CR Richs, M Mazid, AS Pandey and DE Johnson. 2005. Issue related to direct rice seeding in the rainfed cropping system in North-West Bangladesh. p.2. In: Proceedings of Workshop on Direct Seeded Rice in the Rice-Wheat System of the Indo-Gangetic Plains, G.B. Pant University of Agriculture and Technology, Pantnagar.

Rao S, JH Kumar, M Venkataramulu and PR Reddy. 2019. Evaluation of different herbicides in direct seeded rice (*Oryza sativa* L.). *International Journal of Current Microbiology and Applied Sciences.* 8(12): 790-798.

Singh S, JK Ladha, RK Gupta, L Bhusan, AN Rao, B Sivaprasad and PP Singh. 2007. Evaluation of mulching, intercropping with sesbania and herbicide use for weed management in dry-seeded rice (*Oryza sativa* L.). *Crop Protection*, 26(4): 518-524.

Singh RS, Singh RP, Singh RK, Pathak, M Kumar and D Pandey. 2019. Effect of weed management practices on weed flora, growth attributes and yield of direct seeded rice (*Oryza sativa* L.). *Indian Journal of Agronomy,* 1(1): 27-34.

RESEARCH ARTICLE https://doi.org/10.58297/ZUNM1448

A preliminary investigation of cultivated and wild species of rice for tocopherol contents

Rajvir Kaur[1]*, Rupinder Kaur[1], Neerja Sharma[1], Gurjeet Singh[1], Renu Khanna[1], Neelam Kumari[2]

[1] *Department of Plant Breeding & Genetics, PAU, Ludhiana-141004, India*
[2] *School of Agricultural Biotechnology, PAU, Ludhiana-141004, India*
*Corresponding author email: rajvirdeol215@gmail.com

Received: 30th April 2022; Accepted: 19th May 2022

Abstract

Rice (*Oryza sativa* L.), which provides calories to more than half of the world's population, has been considered as a source of vitamins, minerals, and proteins. The portion of nutrition quality of rice is determined by the content of tocopherols which exhibit an antioxidant effect. In this study, a total of 63 genotypes, including 35 wild accessions belonging to AA genome (*O. glaberrima, O. barthii, O. rufipogon and O. meridionalis*) and CC genome (*O. officinalis*), 9 Basmati and 19 non-basmati genotypes were analyzed for the total tocopherol content in brown rice. Wild rice accessions had considerably high total tocopherol content and it ranged from 9.7 mg/kg (*O. rufipogon*, IR105491) to 45.3 mg/kg (*O. rufipogon* CR100368). For the non-Basmati genotypes, it varied from 13.6 mg/kg (BPT5204) to 22.4 mg/kg (PR 128). Similarly, for Basmati cultivars, it ranged from 18.2 mg/kg (Basmati 370) to 25 mg/kg (Pusa Basmati 1509). The wild species that had high total tocopherol content could be used as donors to generate interspecific crosses which will ultimately lead to the development of nutritionally rich rice cultivars.

Keywords: Basmati, Non-basmati, Tocopherols, Wild rice , Nutrition

Introduction

Rice (*Oryza sativa* L.) is a staple food which provides up to 70% of daily calories to more than 3.5 billion population. The genus *Oryza* has 11 genome types, including six diploid species ($n = 12$) with AA, BB, CC, EE, FF and GG genomes and five polyploid species ($n = 24$) with BBCC, CCDD, HHJJ, HHKK and KKLL genomes. Rice contains phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid, all of which have antioxidant action (Goufo and Trindade, 2014). The quality of rice is also determined by the tocopherol content which exhibits an antioxidant effect in human body, and has long been regarded as one of the most potent natural antioxidants (Quereshi *et al.,* 2000). Tocopherols are categorized as, alpha tocopherol (α), beta tocopherol (β), gamma tocopherol(γ), and delta tocopherol(δ) (Goufo and Trindade, 2014). Vitamin E is a compound made up of tocotrienols and tocopherols. Tocopherols make about 20–53% of total vitamin E content, while tocotrienols make up 47–80%. The recommended dietary allowance (RDA) for vitamin E (α-tocopherol) for males and females is 7.5-10 mg (FSSAI 2017). Only α-tocopherol has the highest biological activity and has been found to meet human requirements. Contents of α-tocopherol in rice husk, rice bran, rice whole grain, rice endosperm, and rice germ are reported to be ranged from 0.06–2.13mg/kg, 7.34–107.7mg/kg, 2.40–49.14mg/kg, 0.17–5.43mg/k, and 60.61–457.9mg/kg, respectively. Overall, in rice varieties, the total tocopherol content ranged from 3 to 105.5 mg/kg in brown rice (Goufo and Trindade, 2014).

In relation to tocopherols and tocotrienols, hexane (Xu *et al*., 2001), methanol (Jeng *et al*., 2012), acetone (Gunaratne *et al*., 2013) and ethanol (Ghasemzadeh *et al*., 2015), are most often used for their extraction from rice grains. Tocopherols

and tocotrienols have also been extracted from rice using supercritical fluids (Imsanguan *et al*., 2008), ultrasonication (Moongngarm *et al*., 2012), soxhlation (Mohanlal *et al*., 2012) and vortexing (Gunaratne *et al*., 2013). The most extensively used technology for determining tocopherols is high-performance liquid chromatography (HPLC). After extraction, both normal phase-HPLC and reverse phase-HPLC have been used for the separation of tocopherols and tocotrienols (Goufo *et al.,* 2014). A spectrophotometric method for tocopherol analysis is simple to use in conventional laboratories for preliminary studies for breeding towards nutritional quality.

Different researchers have already reported on genetic diversity studies for vitamin E in various rice cultivars (Gunaratne *et al*., 2013; Kim *et al*., 2012; Lin and Lai, 2012; Kong and Lee, 2010). *OsγTMT* in rice is mainly responsible for the genetic diversity of the α-tocopherol content in rice (Wang *et al.,* 2015). In accordance with the consumer's preferences, grain and nutritional quality have become a primary goal for producers. In this perspective, it should provide all the essential minerals, vitamins and contain sufficient amount of proteins. Plant breeding is usually practiced to produce highly nutritious rice. So the first requirement of breeding program is to study variability in existing germplasm for target trait. Wild relatives of rice are also considered as a source of genetic variability for the enhancement of cultivated rice varieties or to develop new rice varieties. Hence, the present study was planned to study the variability for total tocopherol content in wild rice species, Basmati and non-Basmati cultivars. The present study is a continuation of our previous work (Kaur *et al.,* 2022), in which wild rice species, Basmati and non-Basmati cultivars were studied for protein content, fractionation of seed storage proteins and SDS gel electrophoresis. A total of 35 wild species which had high protein content (≥12%) along with Basmati and non-Basmati cultivars were selected for the analysis of total tocopherol content in brown rice as brown rice is nutritionally rich due to the presence of the bran layer, which is removed in case of polished rice.

Materials and Methods

The experimental material consisted of 63 genotypes, comprising 35 wild rice accessions from the AA (*O. glaberrima, O. barthii, O. rufipogon,* and *O. meridionalis*) and CC genome (*O. officinalis*), 9 Basmati and 19 non-Basmati genotypes. The seed of the wild rice accessions were procured from School of Agricultural Biotechnology, Punjab Agricultural University (PAU), Ludhiana. The Basmati and non-Basmati accessions were sown at experimental farm area of Rice section, Department of Plant Breeding and Genetics, PAU. Each entry was grown in a paired row with 10 plants per row with a uniform spacing of 20 cm between rows and 15 cm between plants. The crop was raised following the standard agronomic practices and harvested at maturity. The designations of genotypes are presented in table 1, table 2 and table 3.

The extraction of total tocopherols and their estimation was done by the method given by Kayden *et al.,* (1973). Seed of these 63 genotypes was dried to a moisture content of 13% and dehusked with hand dehusker to get brown rice and samples were ground to powder. For the extraction of tocopherols, about 50 mg of brown rice sample was homogenized with 4 ml of ethanol. The mixture was centrifuged at 4000 rpm for 30 min. The supernatant so obtained was used for the estimation of tocopherols. For estimation of tocopherols, 2.4 ml of the supernatant was pipetted in glass centrifuge tube and 2.4 ml of distilled water was added. The solution was vortexed properly. Then purified xylene (2.4 ml) was added in samples and again vortexed for 2 min and the glass tubes were then centrifuged at 5000 rpm for 5 min. About 1ml of xylene layer was taken into fresh glass tubes containing 0.8 ml of bathophenanthroline reagent (0.4% in absolute ethanol) and contents were mixed thoroughly. Then 0.8 ml ferric chloride reagent (60 mg $FeCl_3.6H_2O$ in 100 ml absolute ethanol) was added to tubes and contents were mixed thoroughly. To the above samples 0.9 ml *o*-phosphoric acid (0.5 ml of 85 % phosphoric acid in 100 ml of absolute ethanol) was added and mixed properly. The absorbance of the sample was read at 536 nm within 30 seconds. The sample should not be exposed to direct sunshine. The

Table 1. Variation in total tocopherols in wild species of rice (Mean ± SE, n = 3)

Sl. No.	Designation	Tocopherol content (mg/kg) (Mean ± SE)
1	*O. rufipogon* (CR100368)	45.3 ± 1.49
2	*O. rufipogon* (CR100372)	37.0 ± 1.43
3	*O. meridionalis* (IR105294)	33.9 ± 0.71
4	*O. rufipogon* (CR100334)	27.5 ± 0.89
5	*O. rufipogon* (IR80600)	25.6 ± 0.66
6	*O. glaberrima* (IR100983)	25.3 ± 0.39
7	*O. rufipogon* (CR100346A)	25.2 ± 1.03
8	*O. rufipogon* (CR100029)	24.9 ± 0.78
9	*O. rufipogon* (IR80610)	23.8 ± 0.76
10	*O. rufipogon* (CR100036)	23.1 ± 0.24
11	*O. barthii* (IR104103)	22.9 ± 0.53
12	*O. rufipogon* (IR80562)	22.5 ± 0.58
13	*O. rufipogon* (CR100334)	21.6 ± 1.06
14	*O. rufipogon* (IR104404D)	21.3 ± 0.64
15	*O. rufipogon* (CR 100201A)	21.2 ± 0.99
16	*O. meridionalis* (IR101146)	19.7 ± 0.72
17	*O. rufipogon* (CR100267)	19.2 ± 0.56
18	*O. rufipogon* (IR104433)	19.1 ± 0.60
19	*O. officinalis* (IR83809)	18.1 ± 1.02
20	*O. rufipogon* (CR 100201)	16.4 ± 1.23
21	*O. rufipogon* (CR 100309)	16.1 ± 0.47
22	*O. meridionalis* (IR105305)	15.7 ± 0.82
23	*O. meridionalis* (IR105300)	15.7 ± 0.34
24	*O. glaberrima* (IR102206)	15.5 ± 0.53
25	*O. meridionalis* (IR86538)	14.5 ± 0.31
26	*O. rufipogon* (CR100216)	14.2 ± 0.31
27	*O. barthii* (IR104076)	14.1 ± 0.35
28	*O. meridionalis* (IR86539)	13.6 ± 0.69
29	*O. meridionalis* (IR105290)	13.0 ± 0.38
30	*O. meridionalis* (IR93266)	12.7 ± 0.49
31	*O. glaberrima* (IR101800)	12.3 ± 0.27
32	*O. rufipogon* (CR100018A)	12.3 ± 0.59
33	*O. rufipogon* (IR105214B)	11.3 ± 0.72
34	*O. meridionalis* (IR104093)	10.7 ± 0.60
35	*O. rufipogon* (IR105491)	9.7 ± 0.56

SE=Standard error

Table 2. Variation in total tocopherols in Non-Basmati genotypes (Mean ± SE, n = 3)

Sl. No.	Designation	Tocopherol content (mg/kg) (Mean ± SE)
1	PR 128	22.4 ± 0.76
2	PR 127	21.4 ± 1.06
3	PR 122	21.0 ± 0.32
4	Pusa 44	20.5 ± 0.62
5	PR 126	20.2 ± 0.36
6	HKR 47	20.1 ± 0.26
7	PR 123	20.1 ± 0.30
8	PR 129	18.6 ± 0.64
9	PR 124	18.4 ± 0.60
10	PR 121	18.4 ± 0.36
11	RP5115-111-1	18.2 ± 0.72
12	PR 113	18.2 ± 0.44
13	PR 114	18.2 ± 0.46
14	IR82475-110-2	18.1 ± 0.41
15	R-RHZ-MI-81	17.8 ± 0.48
16	PAU 201	16.7 ± 0.69
17	IR64	14.9 ± 0.37
18	CR2826-1	14.5 ± 0.58
19	BPT5204	13.6 ± 0.54

SE=Standard error

Table 3. Variation in total tocopherols in Basmati genotypes (Mean ± SE, n = 3)

Sl. No.	Designation	Tocopherol content (mg/kg) (Mean ± SE)
1	Pusa Basmati 1509	25.0 ± 0.20
2	Punjab Basmati 4	24.8 ± 0.21
3	Pusa Basmati 1718	24.3 ± 0.18
4	Pusa Basmati 1637	24.3 ± 0.30
5	Pusa Basmati 1121	23.9 ± 0.14
6	Punjab Basmati 7	23.7 ± 0.27
7	Punjab Basmati 5	23.0 ± 0.18
8	CSR 30	18.7 ± 0.16
9	Basmati 370	18.2 ± 0.16

SE=Standard error

amount of tocopherols was estimated using a standard curve with tocopherol (2-10 g) as the reference.

The data presented in the tables represented the average of three observations, (±) standard error which was subjected to box plot analysis, one sample t-test and paired sample t-test using SPSS 20.0 at the 0.05 significance level.

Results and Discussion

The total tocopherol contents in brown rice of different accessions were significantly different among wild rice species, Basmati and non-Basmati cultivars (**Table 4**). The content of total tocopherols for wild, non-Basmati and Basmati genotypes is given in **Tables 1, 2 and 3**, respectively. For wild rice, it ranged from 9.7 mg/kg (*O. rufipogon*, IR105491) to 45.3 mg/kg (*O. rufipogon* CR100368). Seed morphology of wild rice accessions with highest and lowest tocopherol content is given in **Figure 1**. For the non-Basmati, it varied from 13.6 mg/kg (BPT5204) to 22.4 mg/kg (PR 128). Similarly, for Basmati cultivars, it ranged from 18.2 mg/kg (Basmati 370) to 25 mg/kg (Pusa Basmati 1509). Frequency distribution using box plots for total tocopherol content in wild, non-Basmati and Basmati genotypes is given in **Figure 2**. Paired t-test was applied to calculate significant differences between the different pairs *viz*: Wild-Basmati, Wild-Non-Basmati and Basmati–Non-Basmati. Significant differences were found to be present between means of these pairs (**Table 5**).

Table 4. Significant differences within wild rice species, Basmati and Non-Basmati genotypes for tocopherol content in present study

One-Sample Test			
Type	**t**	**df**	**p-value**
Wild	15.12	34	.000 (S)
Non-basmati	34.36	18	.000 (S)
Basmati	26.59	8	.000 (S)

Significant at p<0.05, S= significant

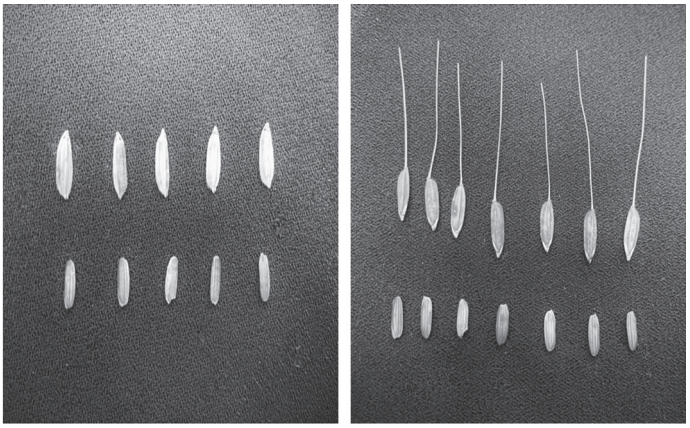


Figure 1: Seed morphology of wild rice accessions with highest and lowest tocopherol content

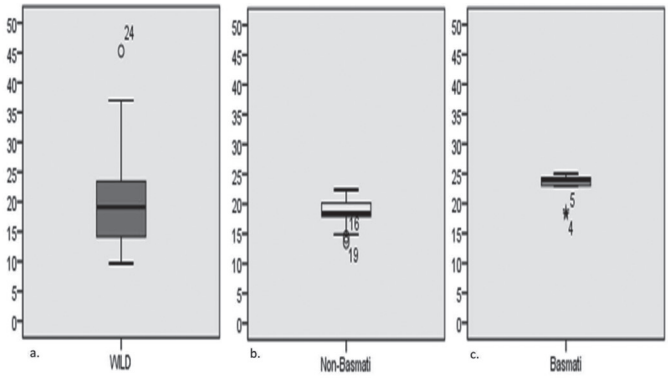


Figure 2: Frequency distribution using box plots for total tocopherol content (mg/kg) in wild, non-Basmati and Basmati genotypes

Total tocopherol content obtained in the wild rice accessions under study (9.7 to 45.3 mg/kg) was higher than (6.7 to 13.3 mg /kg, 10 to 32.5 mg/kg 6.24 – 12.52 mg/kg, 4.6 to 16.2 mg/kg, 9.3 to 19.9 mg/kg, 9.3 to 19.9 mg/kg, 6.52 to 21.51mg/kg and 9.74 to 30.64 mg/kg) previously reported by Aguilar-Garcia *et al.,* (2007), Heinemann *et al.,* (2008), Huang *et al.,* (2011), Fasahat *et al.,* (2012), Gunaratne *et al.,* (2013), Shammugasamy *et al.,* (2014) and Yu *et al.,* (2016), respectively. Brown rice from four varieties had total tocopherol content ranging from 31.3 to 48.7 mg/kg (Gopala Krishna *et al.,* 1984) and Zubair *et al.,* (2012) reported total tocopherol content ranged from 67.1 to 115.3 mg/kg which is higher than the observed range in the present study. The levels of total tocopherols observed in the present study for Basmati (25 to 18.2 mg/kg) and non-Basmati (13.6 to 22.4 mg/

kg) cultivars were in agreement with these previous studies. Furthermore, it is clear that differences in total tocopherol distribution may exist depending on the rice genotype and extraction method used. *O. rufipogon* accessions *viz*; CR100334 (27.5), CR100368 (45.3), CR100372 (37.0) and *O. meridionalis* accession, (IR105294) (33.9) had highest tocopherol contents.

With reference to our previous report (Kaur *et al.,* 2022), the results showed that the four accessions *viz*; *O. rufipogon* (CR100334), *O. rufipogon* (CR100368), *O. rufipogon* (CR100372) and *O. meridionalis* (IR105294) with high total tocopherol content also had high protein content (16.2%, 16.7%, 12.5% and 13.5%, respectively) in brown rice. Some high total tocopherol and high protein accessions along with their grain characteristics are given in **Table 6**. We cannot use these wild rice accessions directly for nutritional enhancement as they have short length, medium shape and low TGW as compare to Basmati and non-Basmati genotypes which are not preferred by consumers. But superior recombinants could be found by crossing these wild rice accessions with cultivated rice varieties which already had excellent grain quality and phenotypic acceptability.

In concusion, this study provides information on total tocopherol content of different rice wild accessions along with Basmati and non-Basmati cultivars**.** Wild rice accessions have high total tocopherol content and high protein content as compared to Basmati and non-Basmati accessions. Approximately 197g rice was available per person per day in India during 2021 (https://www.statista.com) and if rice contains 45.3

Table 5. Significant differences between the pairs of wild rice species, Basmati and Non-Basmati genotypes for tocopherol content

Paired Samples Test								
Pairs	Paired Differences					t	df	p-value
	Mean	SD	Mean SE	95% CI of the Difference				
				Lower	Upper			
Wild - Non-Basmati	6.63	5.08	1.17	4.18	9.08	5.68	18	.000 (S)
Wild - Basmati	6.96	6.20	2.07	2.19	11.73	3.36	8	.010 (S)
Non-Basmati - Basmati	-2.58	1.54	0.51	-3.76	-1.39	-5.02	8	.001 (S)

SD=Std. Deviation, SE=Std. Error, S= significant, NS= non-significant, Significant at p<0.05

Table 6. Wild rice accessions with high protein and high tocopherol content along with grain characteristics

S.No.	Wild accessions	Tocopherol	P (%)	GL	GB	L:B	TGW
1	*O. rufipogon* (CR100334)	27.5	16.2	5.5 (Short)	1.8	3.0 (Medium)	16.7
2	*O. rufipogon* (CR100368)	45.3	16.7	5.3 (Short)	2.1	2.6 (Medium)	15.0
3	*O. rufipogon* (CR100372)	37.0	12.5	5.3 (Short)	2.1	3.0 (Medium)	14.3
4	*O. meridionalis* (IR105294)	33.9	13.5	5.4 (Short)	1.7	3.2 (Medium)	10.8

Tocopherol (mg/kg), P=Protein%, GL=Grain Length (mm), GB=Grain Breadth (mm), L:B=Length/Breadth ratio, TGW= Thousand grain weight (gm)

mg/kg tocopherols (with reference to present study), thus tocopherol intake per person per day will be 8.92mg which is higher as compared to tocopherols provided by Basmati (4.92mg) and non-Basmati (4.41mg) genotypes. For instance, if general RDA of α-tocopherol for an average adult is 7.5-10 mg, the wild rice could assist to meet the required target in cultivated varieties in future rice breeding programs. So, this finding could provide rice breeders with new opportunities by allowing them to use wild rice species with high protein and tocopherol content as donors in interspecific crosses with elite rice cultivars to enhance their protein and tocopherol levels.

Acknowledgements

The authors gratefully acknowledge the research facilities provided by the Department of Science and Technology, India under the PURSE (Promotion of University Research and Scientific Excellence) Grant.

References

Aguilar-Garcia C, Gavino G, Baragano-Mosqueda M, Hevia P and Gavino V. 2007. Correlation of tocopherol, tocotrienol, γ-oryzanol and total polyphenol content in rice bran with different antioxidant capacity assays. *Food Chemistry*, 102(4): 1228–1232.

Fasahat P, Muhammad K, Abdullah A and Ratnam W. 2012. Proximate nutritional composition and antioxidant properties of' Oryza rufipogon', a wild rice collected from Malaysia compared to cultivated rice, MR219. *Australian Journal of Crop Science,* 6(11): 1502-1507.

FSSAI. 2017. Food Safety and Standards Authority of India.

Ghasemzadeh A, Jaafar HZ, Juraimi AS and Tayebi-Meigooni A. 2015. Comparative evaluation of different extraction techniques and solvents for the assay of phytochemicals and antioxidant activity of hashemi rice bran. *Molecules*, 20(6): 10822-10838.

Gopala Krishna AG, Prabhakar JV and Sen DP. 1984. Effect of degree of milling on tocopherol content of rice bran. *Journal of Food Science and Technology*, 21(4): 222–224.

Goufo P and Trindade H. 2014. Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid. *Food Science and Nutrition*, 2(2): 75-104.

Goufo P, Pereira J, Figueiredo N, Oliveira MBP, Carranca C, Rosa EA and Trindade H. 2014. Effect of elevated carbon dioxide (CO2) on phenolic acids, flavonoids, tocopherols, tocotrienols, γ-oryzanol and antioxidant capacities of rice (*Oryza sativa* L.). *Journal of Cereal Science*, 59(1): 15-24.

Gunaratne A, Wu K, Li D, Bentota A, Corke H and Cai Y. 2013. Antioxidant activity and nutritional quality of traditional red-grained rice varieties containing proanthocyanidins. *Food Chemistry*, 138(2-3): 1153–1161.

Heinemann RJB, Xu Z, Godber S and Lanfer-Marquez UM. 2008. Tocopherols, tocotrienols and γ-oryzanol contents in japonica and indica subspecies of rice (*Oryza sativa* L.), cultivated in Brazil. *Cereal Chemistry,* 85: 243–247.

Huang SH and Ng LT. 2011. An improved high-performance liquid chromatographic method for simultaneous determination of tocopherols, tocotrienols and γ-oryzanol in rice. *Journal of Chromatography*, 1218(29): 4709–4713.

Imsanguan P, Roaysubtawee A, Borirak R, Pongamphai S, Douglas S and Douglas PL. 2008. Extraction of α-tocopherol and γ-oryzanol from rice bran. *LWT-Food Science and Technology*, 41(8): 1417-1424.

Jeng TL, Shih YJ, Ho PT, Lai CC, Lin YW, Wang CS and Sung JM. 2012. γ-Oryzanol, tocol and mineral compositions in different grain fractions of giant embryo rice mutants. *Journal of the Science of Food and Agriculture*, 92(7): 1468-1474.

Kaur R, Kaur R, Sharma N, Kumari N, Khanna R and Singh G. 2022. Protein profiling in a set of wild rice species and rice cultivars: a stepping stone to protein quality improvement. *Cereal Research Communications*, https://doi.org/10.1007/s42976-022-00273-2.

Kayden H, Chow C and Bjornson L. 1973. Spectrophotometric method for determination of tocopherol in red blood cells. *Journal of Lipid Research,* 14(5): 533-40.

Kim H, Hwang IG, Kim TM, Woo KS, Park DS, Kim JH and Jeong HS. 2012. Chemical and functional components in different parts of rough rice (*Oryza sativa* L.) before and after germination. *Food Chemistry*, 134(1): 288–293.

Kong S and Lee J. 2010. Antioxidant in milling fractions of black rice cultivars. *Food Chemistry*, 120(1): 278–281.

Lin PY and Lai HM. 2011. Bioactive compounds in rice during grain development. *Food Chemistry*, 127(1): 86–93.

Mohanlal S, Parvathy R, Shalini V, Mohanan R, Helen A and Jayalekshmy A. 2013. Chemical indices, antioxidant activity and anti-inflammatory effect of extracts of the medicinal rice "njavara" and staple varieties: a comparative study. *Journal of Food Biochemistry*, 37(3):369-380.

Moongngarm A, Daomukda N and Khumpika S. 2012. Chemical compositions, phytochemicals, and antioxidant capacity of rice bran, rice bran layer and rice germ. *Apcbee Procedia*, 2: 73-79.

Qureshi AA, Mo H, Packer L and Peterson DM. 2000. Isolation and identification of novel tocotrienols from rice bran with hypocholesterolemic, antioxidant, and antitumor properties. *Journal of Agricultural and Food Chemistry,* 48(8): 3130–3140.

Shammugasamy B, Ramakrishnan Y, Ghazali HM and Muhammad K. 2015. Tocopherol and tocotrienol contents of different varieties of rice in Malaysia. *Journal of the Science of Food and Agriculture*, 95(4): 672-678.

Wang XQ, Yoon MY, He Q, Kim TS, Tong W, Choi BW, Lee YS and Park YJ. 2015. Natural variations in OsγTMT contribute to diversity of the α-tocopherol content in rice. *Molecular Genetics and Genomics*, 290(6): 2121-2135.

Xu Z, Hua N and Godber JS. 2001. Antioxidant activity of tocopherols, tocotrienols, and γ-oryzanol components from rice bran against cholesterol oxidation accelerated by 2, 2 '-azobis (2-methylpropionamidine) dihydrochloride. *Journal of Agricultural and Food Chemistry*, 49(4): 2077-2081.

Yu L, Li G, Li M, Xu F, Beta T and Bao J. 2016. Genotypic variation in phenolic acids, vitamin E and fatty acids in whole grain rice. *Food Chemistry*, 197: 776-782.

Zubair M, Anwar F, Ashraf M and Uddin M. 2012. Characterization of high-value bioactives in some selected varieties of Pakistani rice (*Oryza sativa* L.). *International Journal of Molecular Sciences*, 13(4): 4608-4622.

RESEARCH ARTICLE https://doi.org/10.58297/JIDJ8461

Mycofloral diversity of glutinous rice *Aghoni Bora* in storage condition

Bandana Saikia* and Ali MS

Department of Plant Pathology, Assam Agricultural University, Jorhat-13
*Corresponding author e-mail: saikiabandana800@gmail.com

Received: 7th May 2022; Accepted: 25th June 2022

Abstract

Rice grain is contaminated with various fungi depending on the storage condition. The present study was conducted to identify the possible contaminants of glutinous rice variety *Aghoni bora. Aghoni Bora* grain samples were collected from districts of Assam. Nine species of filamentous fungi from the genus *Acremonium, Aspergillus, Chaetomium, Cladosporium, Gibellula, Paecilomyces* and *Penicillium* were identified morphologically among which potential mycotoxin producers were also present.

Keywords: *Aghoni Bora,* storage fungi, morphology, mycotoxins, contaminants

Introduction

Rice (*Oryzae sativa* L.) is the most important staple food crop in India and the crop is cultivated during *kharif* or wet season. Like most of the states in the country, Assam is also a traditionally rice growing state. In Assam, rice is grown in an area of 25 lakh ha with a production of 2093 kg/ha. In India, rice is cultivated in 43.87 m ha with the production of 104.32 mt, and productivity of 2381 kg/ha 2016 (Ministry of Agriculture, GOI, 2016). Rice plays a pivotal role in the socio-cultural life of the people of the state. As a traditional practice, specific rice varieties are processed into rice products like *tilpitha, ghilapitha, sungapitha, chungachaol, chira, bhojabora, hurum, laru, bhoja chaul, sandahguri* etc. and even rice beer, which are of both ethnic and commercially important (Ahmed *et al*., 2010; Dutta *et al*., 2014). However, rice harbours many microorganisms specially fungi either at field conditions or/and during storage. Fungi such as *Alternaria* sp., *Cladosporium* sp., *Fusarium* sp., *Helminthosporium oryzae* and *Pullularia* sp., to name a few, invade seeds as they are developing on the plants in the field or after they have matured, but before they are harvested; and, for this reason, they have been designated "field fungi" (Christensen, 1957). In storage, the development of fungi, especially *Aspergillus* spp. and *Penicillium* spp., is an unsolved problem. These fungi are responsible for rice quantitative and qualitative losses and are also potential mycotoxin producers. Mycotoxin contamination in stored agricultural commodities like rice has been a serious concern for human and animal health. Mycotoxins are substances produced mostly as secondary metabolites by filamentous fungi that grow on seeds, grains, and feed in the field, or in storage. The major mycotoxin-producing fungi are species of *Aspergillus, Fusarium* and *Penicillium*. Important mycotoxins are *viz*., Aflatoxins, fumonisins, trichothecenes, ochratoxins, cyclopiazonic acid, patulin, deoxynivalenol, zearalenone, citrinin, gliotoxin, and sterigmatocystin. Several workers reported the dominance of *Aspergillus* and *Penicillium* under storage conditions (Ali and Deka, 1996; Amadi *et al*., 2009). The present study identifies and determines the presence of filamentous fungi associated with dehusked rice grains of Aghoni bora under storage.

Materials and Methods

Survey and sample collection

A roving survey was conducted in Jorhat (26°87′ N, 94°15′E), district of Assam to observe the rice storage practice and to collect dehusked rice samples. Rice grains (cultivar: *Aghoni Bora*) stored in gunny bags, plastic bags and at house hold storage bins were collected in sterilized plastic bags and were brought to laboratory for direct isolation of associated fungi.

Isolation and purification of fungi

Direct plating of stored rice grain to isolate fungi was done in potato dextrose (PDA) agar containing 200 ppm streptomycin sulphate. Any visible mycelial growth or spores were transferred to Potato Dextrose Agar (PDA) plates. The fungal cultures were purified by hyphal tip culture method in water agar media. The pure culture of the isolates was maintained on PDA slants throughout the experimental period by subsequent periodical sub-culturing on fresh medium and stored at 4°C in refrigerator.

Cultural or macromorphological studies

Apart from PDA, the isolated pathogens were also cultured on specific media, whenever needed, like Malt Extract Agar (MEA), Czapek Dox Agar(CDA), Rose Bengal Agar (RBA) and Oat Meal Agar (OMA) containing 200 ppm streptomycin sulphate for specific growth characteristics. The observations were recorded after incubation at 25±1°C for 5 days, on colony colour, diameter, elevation and type of margin. Colony colour was recorded with standard reference of Royal Horticultural Society (RHS) colour chart. The changes in pigmentation of the colonies were also recorded.

Micro-morphological studies

Colony structures of different isolates of fungal cultures were studied with different dyes *viz.,* lacto phenol; lacto phenol cotton blue; and basic fucshin. Care was taken to minimize the damage of structures during slide preparation. Structures *viz*., sporangial head, phialide, conidia etc. were measured under high power objectives using an ocular micrometer. The average size of the spores was determined by ocular and stage micrometer and shape of the spores was also recorded.

Identification

Identification and characterization of fungi were carried out with the help of relevant keys, monograph and literature (Thom and Church, 1926; Subramanium, 1971; Raper and Thom, 1984 and CBS database). For confirmation of the identity of isolated fungal cultures, pure cultures were sent to National Centre for Fungal Taxonomy (NCFT), New Delhi.

Results and Discussion

Nine filamentous fungi were isolated from stored rice samples and their macro and micromorphology were listed in **Table 1**. An isolate was identified as *Acremonium strictum* W. Gams [Synonyms: *Sarocladium strictum* (W. Gams) Summerbell (2011)] based on slow growing colony, attained 3 cm in 10 days. The mycelia of the colony showed fibrous pattern of growth. The front colour of the colony was recorded as white (155A RHS) along with the reverse colony colour of yellowish (16 B RHS) tinge on MEA. Conidia were single celled that arose from weakly branched conidiophores. The long slender phialides arose from hyphae, cylindrical to oval in shape and ranged from 4.8 to 7.0 micrometer (µm) in length and 2.0 to 3.0 µm in breadth (**Figure 1**). Perdomo *et al*., (2010); Summerbell *et al*., (2011) also described similar characteristics of *A. strictum* based on which the fungus was identified. However, the species was also confirmed as *A. strictum* by NCFT with the I.D. no. 1995.17.

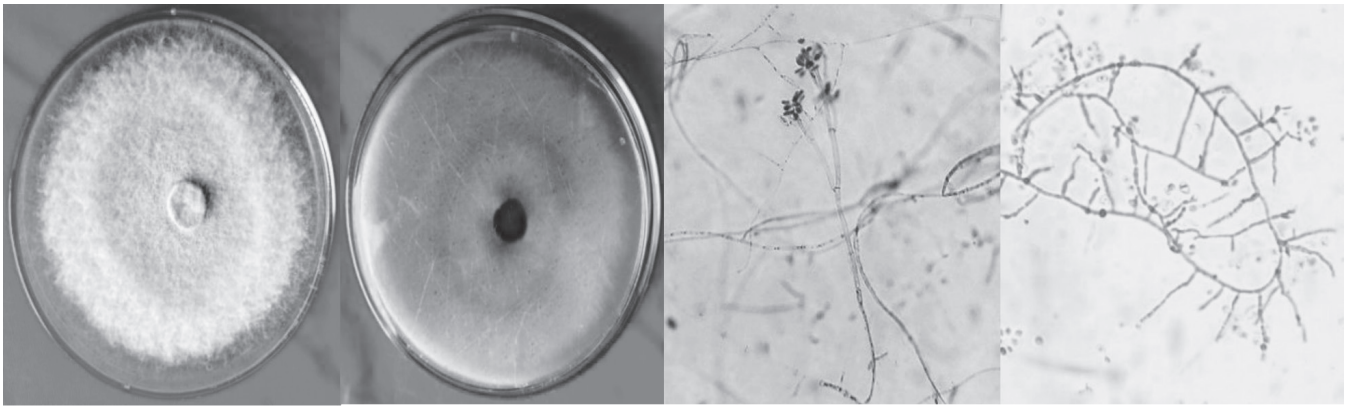


Figure 2: Macro and micromorphology of *Aspergillus niger*

(a) front view (b)reverse view on MEA (c) front view (d) reverse view on Oat Meal Agar
(e) hyaline smooth conidiophore, globose vesicle and biseriate conidial head (f) foot cell

Table 1. Macromorphology and micromorphology of filamentous fungi isolated from stored rice (*Aghoni Bora*)

Isolate	Macromorphology (Colony characters)		Micromorphology		
	Front Colour	Reverse colour	Conidia/spore shape	Conidia size (µm)	Conidium ontogeny
Acremonium strictum	White	Yellowish	cylindrical to oval, single celled	4.8 to 7.0 x 2.0 to 3.0	On Phialides
Aspergillus niger	Black	Yellow	globose to subglobose	3.5-5.0 diameter	On biseriate conidial heads with phialides
Chaetomium globosum	Grey	Grey	lemon-shaped ascospores	4.2 to 6.5 x 4.5 to 6.8	within clavate ascomata
Cladosporium sp.	Grey	Grey	Single celled or septate; oval to cylindrical	4.2 to 8.3 x 5.5 to 16.5	Budding present
Gibellula sp.	White	Whitish	cylindrical to clavate	4 to 9 x 2 to 4	On conidial heads
Paecilomyces sp.	White	Orange	spherical to ovoid	2.3 x 2.5-2.6	On awn shaped phialides
Penicillium chrysogenum	gray green	Yellowish	subglobose to elliptical	3.0 to 4 x 2.8 to 3.5	On biverticillate penicilli
Penicillium purpurogenum	Dark green	Orange red	subglobose	3.0 to 3.5 x 2.5 to 3.0	On biverticillate penicilli
Penicillium sp.	grey green	Dark green	globose to subglobose	3.5 to 4.8 x 3.2 to 4.5	On biverticillate penicilli

Another isolate was identified as *Aspergillus niger* van Tiegh. based on colonies on czapek dox agar, which consisted of a compact white or yellow (10C RHS) basal felt covered by dense layer of black conidial heads (202A RHS) **(Figure 2a-b)**. On oat meal agar the colony produced dark black spores (**Figure 2. c-d**). Conidiophores were smooth-walled, hyaline or changing dark in colour towards the vesicle. Conidial heads were 250-300 µm in diameter, with globose vesicle and dark brown in colour, radiate in nature and tend to split into several loose columns with age. Conidial heads were biseriate with the phialides borne on brown metulae. Conidia were globose to sub-globose (3.5-5.0 µm in diameter), dark brown to black color (**Figure 2.e**). These characteristics were compared with standard description of Thom and Church (1926) and the species was confirmed as *Aspergillus niger.1*

Chaetomium globosum Kunze grey coloured colony on PDA and recorded fast growth (156B RHS) (**Figure 3a-b**). Perithecial hairs were terminal, long and undulate and loosely coiled (**Figure 3c**). Sexual sporulation produced flat lemon-shaped ascospores **(Figure 3e)** , within the clavate ascomata (**Figure 3d**), ascus (up to 20-30 µm) with eight ascospores and a size varied from 4.2 to 6.5 by 4.5 to 6.8 µm. Girisham *et al*., (2016) described similar characteristics for *Chaetomium globosum* based on which isolated species was confirmed.

Cladosporium herbarum (Pers) Link produced single celled to septate small, lemon-shaped and smooth walled conidia. They formed long, fragile chains up to 10 conidia in length with distinctive darkened connective tissue between each spore. Budding was present and conidia gave rise to new conidia, ranged from 4.2 to 8.3 by 5.5 to 16.5 µm in measurement (**Figure 4**). Identification of isolated species was confirmed following the monograph of *Cladosporium* (Bensch *et al.*, 2012).

Gibellula sp. showed septate and verrucose conidiophores with reduced vesicle. Conidial structures aggregated into synnemata, conidia were more or less cylindrical to clavate, apiculate, 4-9 x 2-4 µm (**Figure 5**). Samson and Evans (1973) described similar characteristics for *Gibellula* reported from insect host, based on which the identity of the genus was confirmed.

Isolated *Paecilomyces* sp. was fast growing and white colour in nature (155A RHS) on PDA Produced orange pigment (25D RHS) on the reverse side of the colony. Conidiophore were septate, branched and bore the penicilate heads. Phialides are born in verticils on penicilate head. Phialides were awn shaped, swollen at the base with slender long neck. Conidia are spherical to ovoid and smooth in nature (**Figure 6**). Thus, the genus was identified as *Paecilomyces* sp. based on the description of Samson (1974).

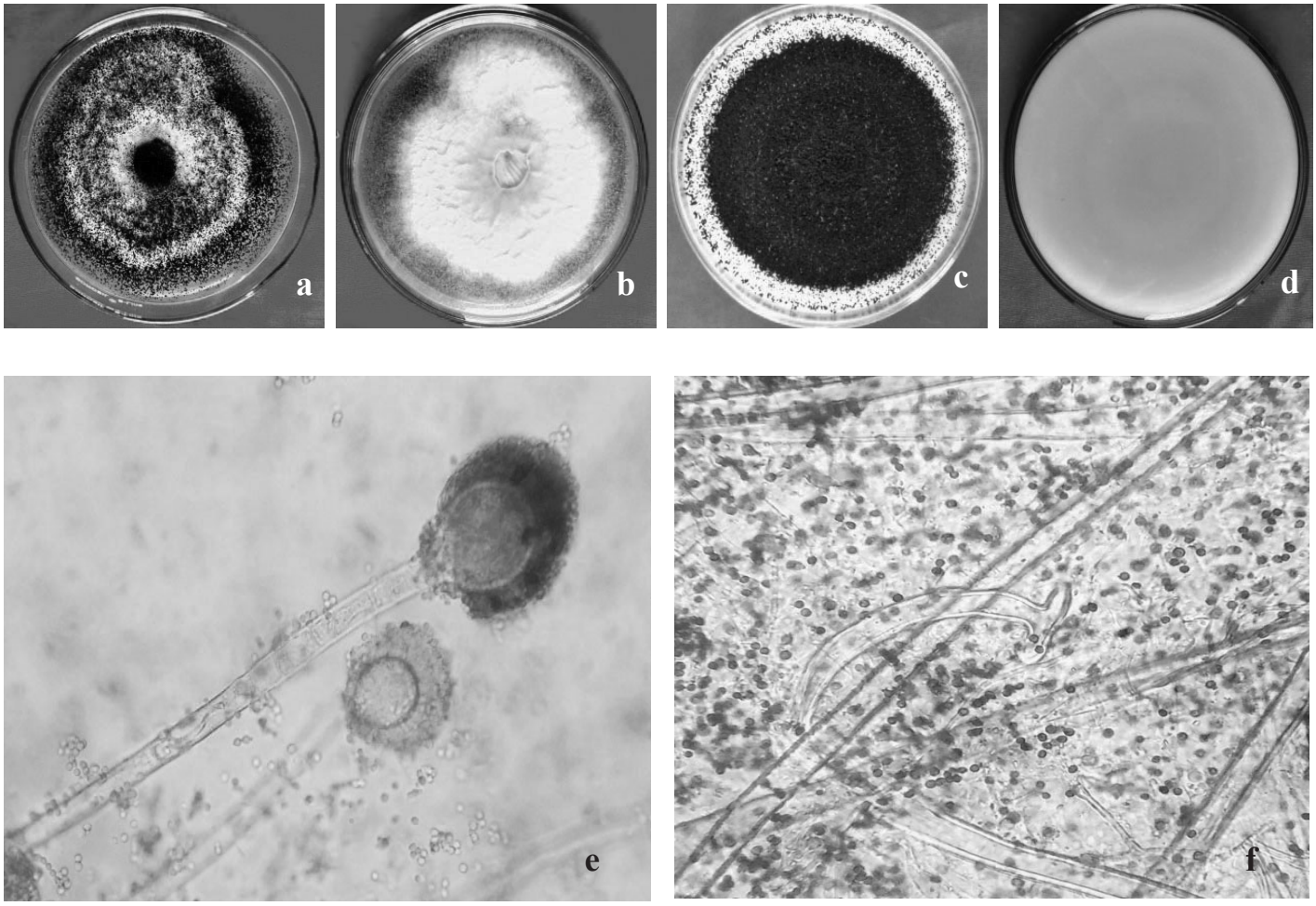


Figure 2: Macro and micromorphology of Aspergillus niger

(a) front view (b)reverse view on MEA (c) front view (d) reverse view on Oat Meal Agar

(e) hyaline smooth conidiophore, globose vesicle and biseriate conidial head (f) foot cell

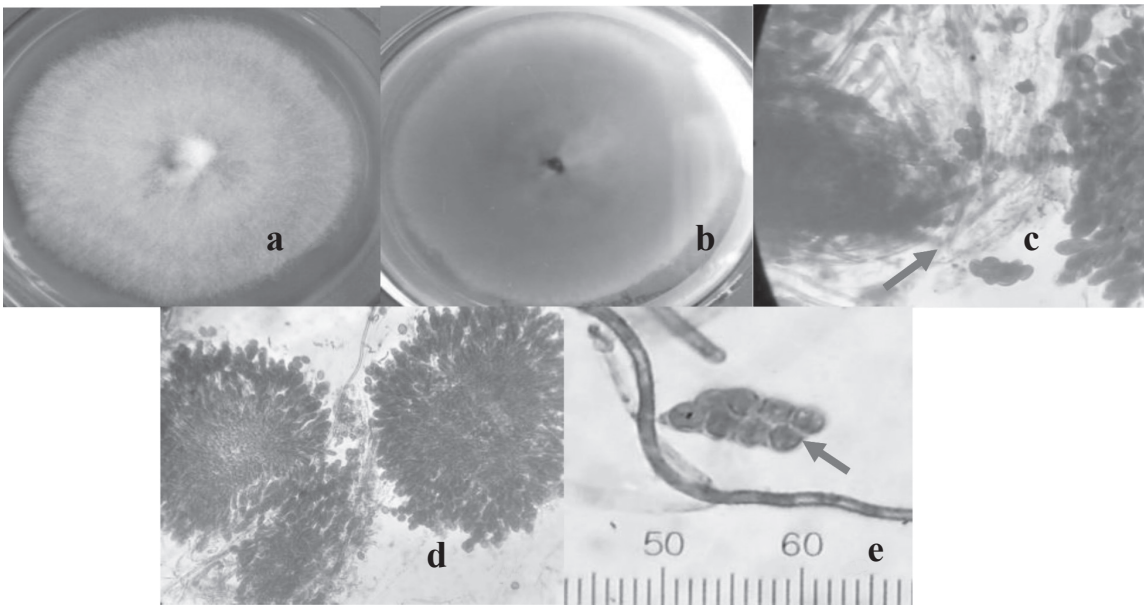


Figure 3: Macro and micromorphology of Chaetomium globosum
(a) front view (b) reverse view on PDA (c) perithecial hair (d) delinquished asci (e) ascospores

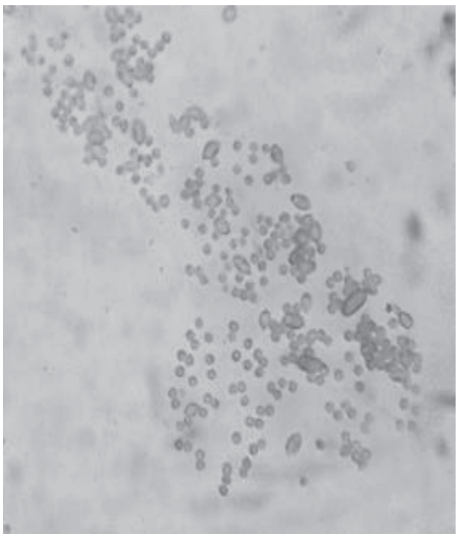


Figure 4. Conidia of *Cladosporium herbarum*

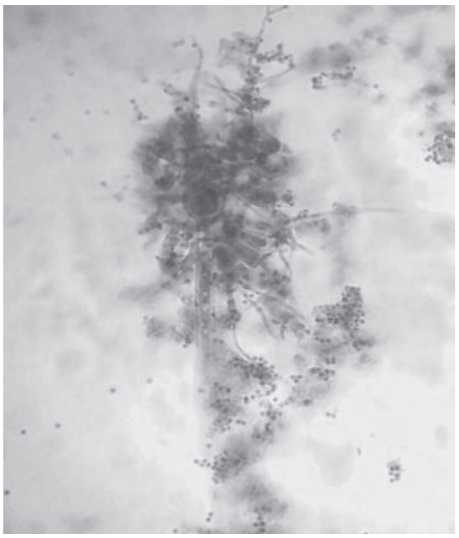


Figure 5. Conidial head of *Gibellula* sp.

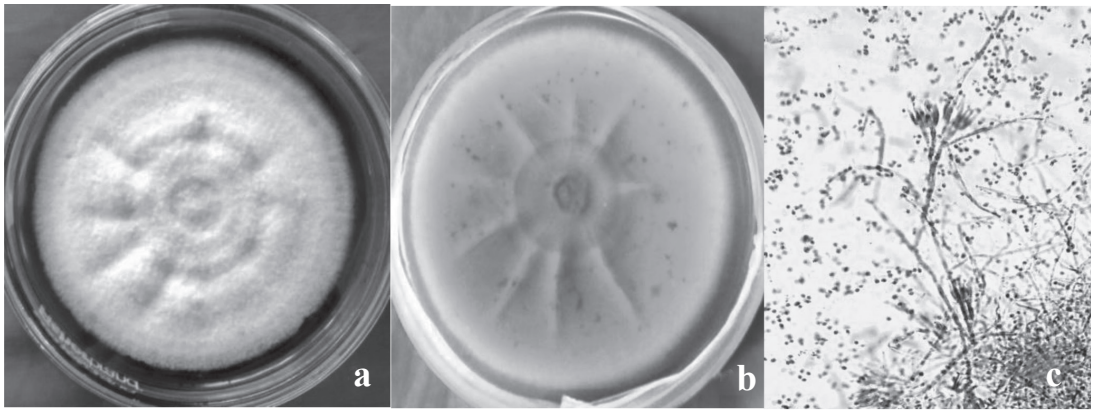


Figure 6: Macro and micromorphology of *Paecilomyces* sp.
(a) front view (b) reverse view on PDA (c) conidiophore with tenpin phialides

Three species of *Penicillium viz*., *Penicillium chrysogenum* Thom, *P. purpurogenum* Stoll and *Penicillium* sp. were identified based on their morphology.

Colonies of *P. chrysogenum* on PDA showed velvety, grey green colour, conspicuous radial furrows which lend the colony a wheel like appearance (**Figure 7a**). Colonies were yellow colour in reverse (**Figure 7b**). Penicilli were biverticillate; main axis terminated in verticils of 2 to 5 metulae, which bore sterigmata. Size of the metulae were ranged from 10 to 12 µm by 2-3 µm; sterigmata produced vertically, ranged from of 4 to 6, 8 to 10 µm by 2.0 to 2.5 µm in size. Conidial chains were well defined chains up to 200 µm in size, sub-globose to elliptical in nature and ranged from 3.0 to 4 µm by 2.8 to 3.5 µm (**Figure 7c**). Based on the reports of Raper and Thom (1984), the isolated species was identified. It was further confirmed by NCFT a with the ID. No. of 1998.17.

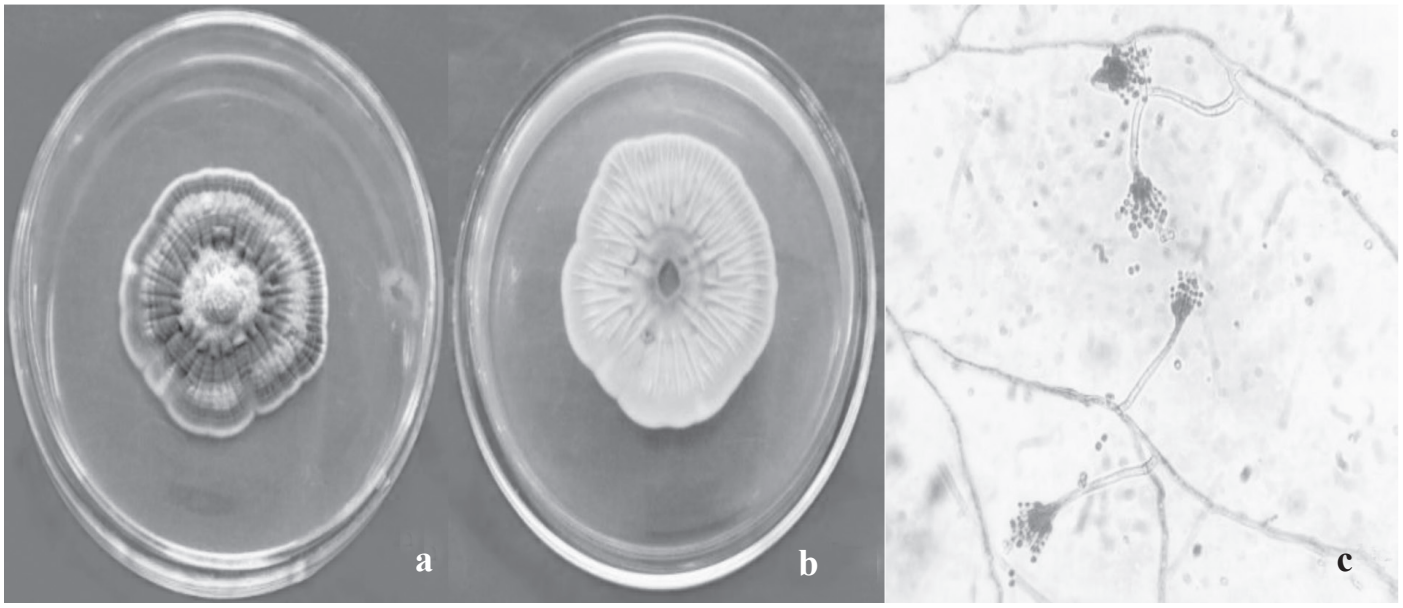


Figure 7: Macro and micromorphology of *Penicillium chrysogenum*
(a) front view (b)reverse view on PDA (c) conidiophore arising from hyphae

However, colonies of *Penicillium purpurogenum* Stoll were dark green along with (191A RHS) (**Figure 8a**), reverse colour of orange red shades (174 B RHS) (**Figure 8b**) on MEA where in the size of diameter recorded as 5 to 5.2 cm in 10 days at 25 ± 1° C. Conidiophores arose from the substratum and measured up to 100 to 120 µm in length by 2.5 to 3.0 µm in breadth. Penicilli were biverticillate symmetrical, each consisted of 5-6 metulae with successive 4 to 5 sterigmata. Metulae were 8 to 10 µm by 2.5 to 3.0 µm in size and sterigmata recorded a measurement of 10.0 to 12.0 µm by 2.0 to 2.5 µm. Conidia were arranged in short chain, smooth, sub-globose, measured 3.0-3.5 µm x 2.5-3.0 µm (**Figure 8c**). Raper and Thom (1984) described similar characteristics for *Penicillium purpurogenum* and the isolated culture identity was confirmed as *Penicillium purpurogenum*.

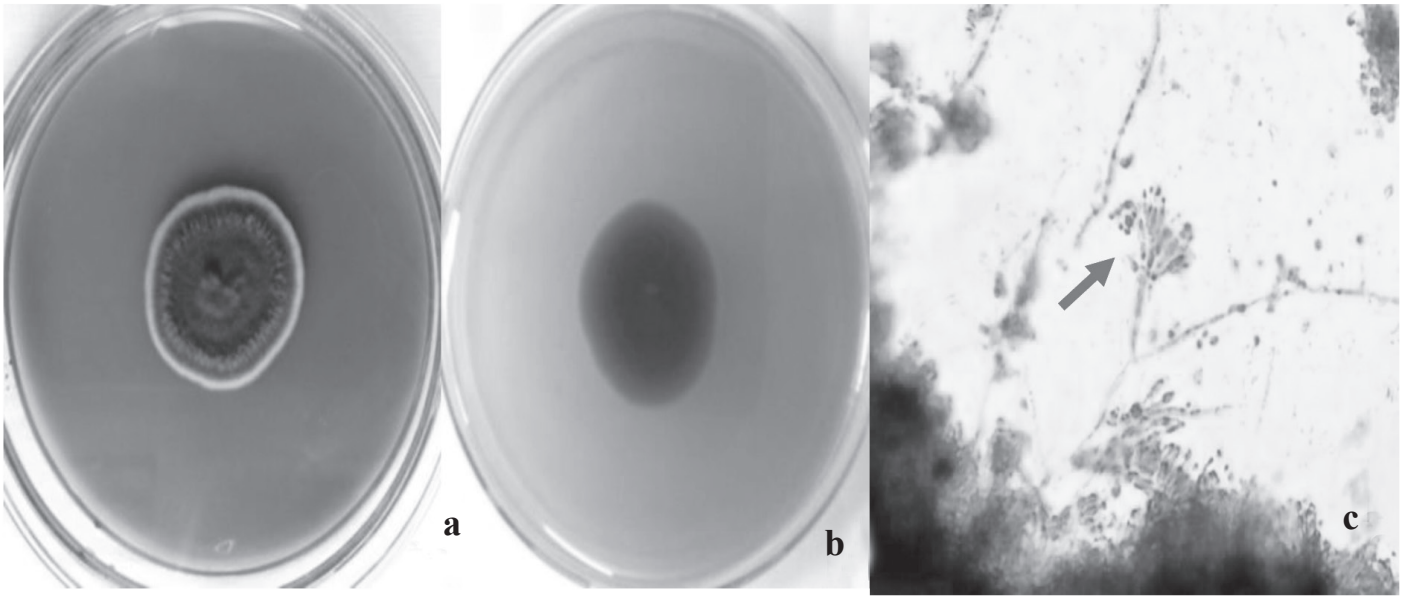


Figure 8: Macro and micromorphology *Penicillium purpurogenum*
(a) front view(b) reverse view on MEA (c) asymmetrical biverticilate penicilli

Another *Penicillium* sp. produced grey green colonies (191A RHS) (**Figure 9a**); with dark green colour (139A RHS) pigmentation on the reverse side (**Figure 9b**) on PDA. Penicilli were biverticillate and asymmetrical, with each major element bearing successive verticils of 2-5 metulae and 3 to 5 sterigmata and the size varied from9.0 to 15.0 µm by 2.5 to 3.5 µm and 9.0 to 15.0 µm by 2.5 to3.5 µm. Conidia smooth in nature and formed in chain, borne on the tip of phialides, globose to subglobose in shape and the size varied from 3.5-4.8 µm long to 3.2-4.5 µm wide (**Figure 9c**). The fungus was confirmed by following the standard descriptions of Raper and Thom (1984).

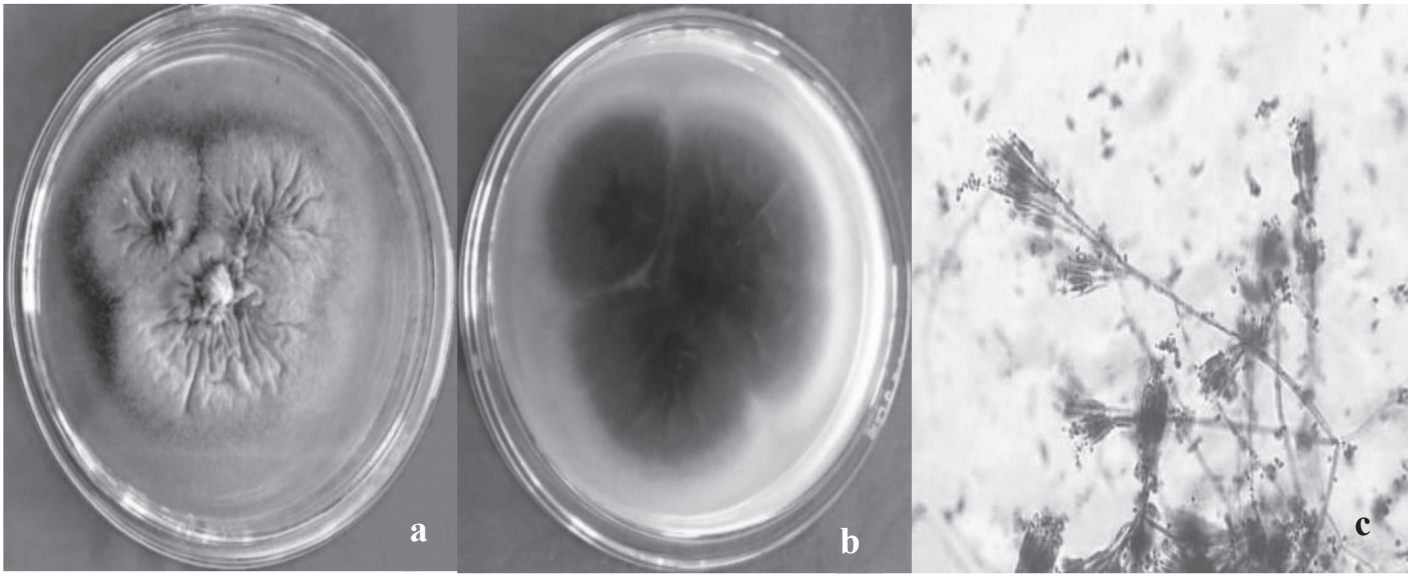


Figure 9: Macro and micromorphology *Penicillium sp.*
(a) front view (b) reverse view on PDA (c) biverticillate penicilli bearing globose conidia

Table 2. Identified fungi species from stored rice cultivar Aghoni Bora

Sl.No.	Samples	Storage type	Location/District	Fungi identified
1.	Stored rice grain (Aghoni Bora)	Storage bins	Titabar, Jorhat	*Penicillium chysogenum, Gibellula* sp., *Chaetomium globosum, Aspergillus niger, Cladosporium* sp.
		Gunny bag	Teok, Jorhat	*Penicillium* sp., *Acremonium strictum, Aspergillus niger*
		Plastic bags	Rowroiah, Jorhat	*Penicillium purpurogenum, Paecilomyces* sp.

Species wise distribution of fungi isolated from different storage structures where *Penicillium* is a common genus was found in all three types of storage *viz*., storage bins, gunny bags and plastic bags (**Table 2**). Furthermore, *Acremonium* is a potential toxin producer (Girisham *et al*., 2016) and its presence in stored rice may deteriorate the quality of the grain and may cause health hazards on consumption. *Sitophilus zeamais,* a stored grain pest, was reported to transmit *Acremonium* sp., along with other fungi including *A. niger, A. glaucus, A. candidus, Penicillium islandicum, P. citrinum, Paecilomyces, Epicoccum, F. semitectum*, yeasts and many others was reported by Mason and Mcdonough (2012). Perhaps the reason behind this was the rice sample infested by stored grain pests might have introduced the fungus to it.

Earlier scientists reported the presence of *Aspergillus niger* in the stored rice grains (Amadi *et al.,* 2009; Reddy *et al.,* 2009 and Akano 1990). Tamang (2003) and Joshi and Sandhu (2000) also reported the association of *A. niger* in stored rice. Conidia of *Aspergillus* are always present in air through which they may be introduced into the sample (Dube, 2015) and has the potential to cause allergic reaction. In addition, the fungal species was known for the production of harmful toxins like fumonisins and ochratoxins (Reddy *et al*., 2008; Frisvad *et al*., 2011). Hence, handling and consumption of rice contaminated with *A. niger* may lead to health issues.

The present study revealed that the association of *Chaetomium globosum* with stored rice grain. Similar results were reported by Ibiam *et al.,* (2008), Reddy *et al.,* (2009) and Surekha *et al*., (2011). *Chaetomium globosum* is a cellulose degrading fungus commonly present as indoor contaminants (Dube, 2015) and hence there is possibility of infection in the stored rice grains also. The only species of *Cladosporium* recorded in the present investigation was *Cladosporium herbarum*. It was reported as a common contaminant of food and food products by Reddy *et al.,* (2009) and Bensch (2012).

Gibellula sp., an entomopathogenic fungus, was also recorded in the study. The fungus reported as a habitat for spiders (Samson and Evans, 1973). It is probable that due to dual infection of both fungi and insect, the seed lot deteriorated beyond the tolerance limit and *Gibellula* was found as the predominant genus in that sample. Different insect species, such as *Sitophilus oryzae, Sitotroga cerelella, Rhizopertha dominica, Trogoderma granarium* and *Tribolium castaneum* were reported in stored samples of rice (Ali and Bhattacharya, 1991) which may harbour *Gibellula* like fungi.

One species of *Paecilomyces* was found to be associated with stored rice. Surekha *et al*., (2011) also found species of *Paecilomyces varioti* to be associated with stored rice during early storage period. However, Akano and Atanda (1990) reported *Paecilomyces varioti* from Nigeria in stored groundnut cake.

All the species of *Penicillium viz., P. chrysogenum, P. purpurogenum and Penicillium* sp. were found to be associated with stored rice. Ali and Deka (1996), Amadi *et al.,* (2009), and Ibiam *et al.,* (2008) reported species of *Penicillium* to be associated with stored rice. *Paecilomyces* sp. and *Penicillium* spp. spores were present abundantly in stored rice.

Conclusions

The study concludes that stored *Aghoni bora* rice grain samples collected from Jorhat found infected with nine fungal species *viz.*, *Acremonium strictum, Aspergillus niger, Chaetomium globosum, Cladosporium* sp., *Gibellula* sp., *Paceliomyces* sp., *Penicillium chrysogenum, Penicillium purpurogenum, Penicillium* sp. These fungi are frequently known to produce mycotoxins which may cause detrimental effect on human health.

Acknowledgements

The authors would like to acknowledge the support and facilities received from Assam Agricultural University, Jorhat to conduct the study.

References

Ahmed T, Chetia SK, Chowdhury R and Ali S. 2010. Status Paper on Rice in Assam. Retrieved from http://www.rkmp.co.in

Akano DA and Atanda O. 1990. The present level of aflatoxin in Nigerian groundnut cake (kulikuli). *Letter Applied Microbiol*ogy, 10(4): 187-189

Ali MS and Bhattacharya H. 1991. Biodeterioration of vegetations, storage grain and grain products in North East India. In: Biodeterioration, Culture Collections and Aspects of Applied Mycology. University of Mysore, Manasagangotri, Mysore.

Ali MS and Deka B. 1996. Role of fungi causing grain discoloration of rice and management under storage. *Indian Journal of Mycology and Plant Pathology*, 26(1): 79-85.

Amadi JE and Adeniyi DO. 2009. Mycotoxin production by fungi isolated from stored grains. *African Journal of Biotechnology*, 8(7):1219-1221

Bensch K, Braun U, Groenewald JZ and Crous PW. 2012. The genus *Cladosporium*. *Studies in Mycology*, 72(1): 1–401.

Christensen CM. 1957. Deterioration of stored grains by fungi. *The Botanical Review*, 23(2): 108-134.

Dube HC. 2015. An Introduction to Fungi (4th eds.). Scientific Publishers, New Delhi, pp. 603

Dutta H and Mahanta CL. 2014. Traditional parboiled rice-based products revisited: Current status and future research challenges. *Rice Science*, 21(4): 187-200

Frisvad JC, Larsen TO, Thrane U, Meijer M, Varga J, Samson RA and Nielsen KF. 2011. Fumonisin and ochratoxin production in industrial *Aspergillus niger* strains. *Plos one*, *6*(8), e23496.

Girisham S, Koteswara RV and Reddy SM. 2016. Taxonomy of mycotoxicogenic fungi. Scientific Publishers. p.317

Ibiam OFA, Umechuruba CI and Arinze AE. 2008. A survey of seed-borne Fungi associated with seeds of rice (*Oryzae sativa* l faro 12, 15, and 29) in storage and the field in Afikpo North Local Government Area of Ebonyi State. *Scientia Africana*, 7(2). **DOI:** 10.4314/sa.v7i2.46011

Joshi VK and Sandhu DK. 2000. Quality evaluation of naturally fermented alcoholic beverages, microbiological examination of source of fermentation and ethanol productivity of the isolates. *Acta Alimentaria*, 29(4): 323-334.

Magro A, Matos O, Bastos M, Carolino M, Lima A and Mexia A. 2010. The use of essential oils to protect rice from storage fungi. *Julius-Kühn-Archiv*, 10.5073/jka.2010.425.338.

Mason LJ and McDonough M. 2012. Biology, behavior, and ecology of stored grain and legume insects. In Stored Product Protection; Hagstrum, D.W., Phillips, T.W., Cuperus, G., Eds.; Kansas State University: Manhattan, KS, USA, pp. 7–20.

Perdomo H, Sutton DA, Garcia D, Fothergill AW, Cano J, Gene J, Summerbell RC, Rinaldi MG and Guarro J. 2010. Spectrum of clinically relevant Acremonium species in the United States. *Journal of Clinical Microbiology*. 49 (1): 243–256. doi:10.1128/jcm.00793-10

Raper KB and Thom C. 1984. A Manual of the Penicillia. The William and Wilkins Company, Baltimore. p. 875

Reddy CS. 2009. Mycotoxin contamination of rice. Rice Knowledge Management Portal

(RKMP) Directorate of Rice Research, Rajendranagar, Hyderabad, 500030.

Reddy KRN, Reddy CS, Abbas HK, Abel CA & Muralidharan K. 2008. Mycotoxigenic fungi, mycotoxins, and management of rice grains. *Toxin Reviews*, *27*(3-4), 287-317.

Reddy OR and Sathyanarayana N. 2001. Seed-borne fungi of rice and quarantine significance. pp. 331-345. In *Major Fungal Diseases of Rice*. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-2157-8_24

Samson RA. 1974. Studies in Mycology. No.6. In Paecilomyces and some allied Hyphomycetes, Institute of Netherlands, p119

Samson RA and Evans HC. 1973. Notes on entomogenous fungi from Ghana. I. The genera *Gibellula* and *Pseudogibellula. Acta Botanica Neerlandica,* 22(5): 522-528.

Subramanian CV. 1971. Hyphomycetes. Indian Council of Agricultural Research. New Delhi, p. 930

Summerbell RC, Gueidan C, Schroers HJ, Hoog GS, Starink M, Arocha RY, Guarro J, and Scott JA. 2011. Acremonium phylogenetic overview and revision of Gliomastix, Trichothecium and Sarocladium. *Studies in Mycology,* 68(68): 139–162.

Surekha M, Saini K, Reddy VK, Reddy AR and Reddy SM. 2011. Fungal succession in stored rice (Oryza sativa Linn.) fodder and mycotoxin production. *African Journal of Biotechnology*, 10(4), 550-555.

Tamang JP. 2003. Summary of ongoing projects: Microbial and biochemical studies of the traditional fermented beverages of the Darjeeling hills and Sikkim. Integrated Ecodevelopment Research Project (IERP) by G. B. Pant Institute of Himalayan Environment and Development, Almora, India.

Thom C and Church MB. 1926. The Aspergilli. Bailliere, Tindall and Cox, London. Pp. 4, 5, 165, 198.

RESEARCH ARTICLE https://doi.org/10.58297/VFNT1970

Assessing the molecular variability in *Ustilaginoidea virens*, the rice false smut pathogen with ISSR markers

Ladhalakshmi D[1]*, Yugander A[2], Laha GS[1], Vijayasamundeeswari A[3], Basavaraj K[1], Divya Balakrishnan[1], Preeti[4], Bhaskar M[1], Aparna MD[1] and Prasad MS[1]

[1]ICAR-Indian Institute of Rice Research, Hyderabad, Telangana, India
[2]Institute for Molecular Physiology, Heinrich Heine University, Düsseldorf, Germany
[3] Assistant Professor of Plant Pathology, TNAU
[4] University of Agricultural Sciences, Raichur, Karnataka.
*Corresponding author email: ladhasavitha@gmail.com, dladha.lakshmi@icar.gov.in

Received: 1st April 2022; Accepted: 30th May 2022

Abstract

Rice false smut disease is gaining importance because of its impact on the grain yield and its toxin production ability. Fifty-eight isolates of *Ustilaginoidea virens* were collected from different rice-growing regions of India. DNA of *U. virens* was isolated by the CTAB method and fifty ISSR primers were screened for molecular variability studies. Twelve primers *viz*., UBC series 807, 808, 809, 810, 811, 812, 834, 835, 836, 840, 841, and 842 were selected to study the genetic variability. Different parameters of tested primers *viz*., heterozygosity (Hn), polymorphism information content (PIC), effective multiplex ratio (EMR), marker Index (MI), and resolving power (RP) were calculated. Primers UBC 812 and UBC 809 recorded maximum heterozygosity (Hn). The PIC values ranged from 0.10 to 0.27 and UBC 807 recorded the maximum value of 0.27. The EMR value varied from 6.75 to 24.0, Similarly, UBC 807 recorded the highest value of MI (24) and RP (8.55). A dendrogram was generated using the DARwin software (version 6.0.21A) based on the unweighted neighbor-joining cluster method. All the fifty-eight *U. virens* isolates were grouped into three major clusters. Clusters I and II had 21 and 35 *U. virens* isolates respectively. Cluster III had only two isolates. The isolates showed genetic variations and there was no specific grouping based on the geographical distance.

Keywords: Rice, False smut, Molecular variability, ISSR, Isolate

Introduction

Rice crop is affected by fungal, bacterial and viral diseases. Among the fungal diseases, the false smut disease of rice has become one of the important grain diseases. The pathogen *Ustilaginoidea virens* (Cooke) (Takahashi) causes up to 50% yield loss under favorable environmental conditions. It infects the pollen and stigma and converts the rice grain into a ball-like structure that contains mycelia and chlamydospores. Initially, young smut balls are covered with a thin layer of a white membrane which later bursts, and upon maturity, the smut ball turns into yellow and green color. The exact mode of infection of the pathogen was less understood and management of the disease mainly depends on the application of fungicides. The pathogen produces both sexual (sclerotia) and asexual (chlamydospores) resting structures. The pathogen infects the pollen and produces the typical smut balls (Tang *et al*., 2013). Ustiloxins and Ustilaginoidins are the toxins produced by *U. virens* (Wang *et al*., 2019). A study on the genetic diversity of pathogen will give an idea about the survival strategy of the pathogen. The present study was carried out to know the existence of variability in the isolates of *U. virens* collected from different rice-growing regions of India using Inter Simple Sequence Repeats (ISSR) primers.

Materials and Methods

Isolation of *U. virens* and pathogenicity

Smut ball samples were collected from different rice-growing states of India. Care was taken to collect the samples from different fields and different varieties.

Yellow-coloured smut balls were surface sterilized with 1% sodium hypochlorite for two minutes and washed three times with sterile water. By using a sterile bacterial inoculation needle, the chlamydospores were streaked onto Petri dishes containing potato sucrose agar (PSA) medium amended with 100 ppm streptomycin sulfate (Ladhalakshmi *et al*., 2012). The plates were incubated at 27°C for seven days. Typical white fungal colonies of *U. virens* were transferred onto fresh PSA slants for purification and stored under 4°C. The injection method of inoculation (Ladhalakshmi *et al*., 2019) using a hypodermic syringe was adopted in the booting stage of the plants for proving the pathogenicity of the pathogen. Around 4 to 5 tillers were inoculated per plant. The identity of all the collected isolates was confirmed by using specific ITS primers (Zhou *et al*., 2003; Ladhalakshmi *et al*., 2012).

Genetic variability

DNA Isolation

Total fungal DNA was isolated by following Cetyl trimethyl ammonium bromide (CTAB) method (Zhou *et al*., 2003). *U. virens* mycelium was harvested by filtration and washed with sterile distilled water repeatedly. The mycelial mat was frozen in liquid nitrogen and ground into fine powder and to the powdered mycelia (50-60 mg), 750 µl of CTAB buffer was added and incubated at 65°C for 45 min. During the incubation period, the tubes were vortexed and spun at 10,000 rpm for 15 min and equal volume of chloroform and isoamyl alcohol (24: 1) (v/v) was added to the collected supernatant and again centrifuged at 13,000 rpm for 15 min. Supernatant was pooled, and an equal volume of chilled iso-propanol was added and incubated for an hour at −20°C. The DNA was precipitated by centrifugation at 10,000 rpm for 15 min and finally, the DNA pellet was washed with 70% ethanol, air dried and finally dissolved in 50 µl of the sterile distilled water.

PCR conditions and gel electrophoresis

Initially, 50 ISSR primers (UBC series from 801 to 850) were screened with a subset of samples. Among them, twelve primers that gave a scorable banding pattern were selected for the study. ISSR-PCR was performed in a total volume of 20 µl contained 2 µl of genomic DNA (50 ng), 1 µl of primer of 5 µM primer solution, 2 µl of 10x buffer (0.1 M *Tris* pH 8.3; 0.5 M KCl; 7.5 mM $MgCl_2$; 0.1% gelatin), 1 µl of 2.5 mM dNTPs and 1.0 unit of Taq polymerase. PCR amplifications were performed in a thermal cycler (Applied Biosystems, USA) with the following conditions: initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturing at 94°C for 1 min, annealing at 50°C for 1 min, extension at 72°C for 2 min and final extension at 72°C for 7 min. The amplified products were resolved in 2% agarose gel in 1X TBE buffer under room temperature at a constant voltage of 90 V. The molecular weight markers, 100 bp and 1 Kbp ladder (Banglore Genei Private Limited, India) were used for band sizing.

Data Analysis

Each amplified product/band was scored as 1 and 0 respectively based on the presence (1) and absence (0). Based on the scored data, various parameters *viz*., number of loci, number of polymorphic loci, polymorphism (%), polymorphism information content (PIC), and primer resolving power (Rp) were calculated. The PIC value for each primer was calculated. $PIC = 1 – (f^{2} + (1 - f)^{2})$, where *Pi* is the frequency of *i* th allele, *n* is the number of bands. Effective multiplex ratio (EMR) is calculated as a total number of polymorphic loci per primer multiplied by the rate of polymorphic loci from their total number. The Rp of each primer was calculated; $Rp = \Sigma Ib$, where $Ib = 1 – [2 \times (0.5 – Pi)]$, where *Pi* is the proportion of accessions containing band *i* (Chesnokov and Artemyeva, 2015). A distance-based unweighted neighbour-joining cluster tree was constructed based on the dissimilarity index and a dendrogram was constructed using the DARwin software version 6.0.21.

Results and Discussion

A total of 58 isolates of *U. virens* from 18 different rice-growing states of India were isolated and maintained (**Table 1**). The pathogen produced white coloured colony which later turned into yellow and green colour. All the collected isolates were confirmed by specific ITS primers. Pathogenicity of the false smut was proved and typical yellow-colored smut balls (up to 10 smut balls per panicle) were observed in the inoculated panicles 20 days after inoculation.

Fifty ISSR markers (801 to 850) were screened and among them, twelve markers were selected as potential makers *viz*., 807, 808, 809, 810, 811, 812, 834, 835, 836, 840, 841, and 842 based on their amplification banding pattern and reproducibility. Across the markers, the number of amplified loci varied from 9 to 24 (**Figure 1**). Twelve ISSR primers amplified a total of 6503 bands with an average number of 541 bands for 58 isolates of *U. virens*. The maximum heterozygosity (Hn) was shown by UBC 812 and UBC 809. The value of the polymorphism information content (PIC) indicates the ability of a marker to find the polymorphism in the population (Chesnokov and Artemyeva, 2015). In the present study, the polymorphism of the primers varied from 75% to 100%. The polymorphism information content (PIC) values ranged from 0.10 to 0.27. UBC 807 recorded the maximum PIC value of 0.27. Among the tested ISSR primers, the Effective multiplex ratio (EMR) varied from 6.75 to 24.0, and the maximum value was recorded with UBC 807 (24.0) and it can be considered an efficient marker. To estimate the utility of the markers, the Marker Index (MI) is used and the values ranged from 1.0 to 5.9 and the primer UBC 807 recorded the maximum value of 5.9. The parameter resolving power (RP) determines the ability of the primer to differentiate the tested isolates and tested primers recorded a range of values from 2.14 to 8.55 and UBC 807 recorded the highest value of 8.55 (**Table 2**).

Table 1. Details on the *Ustilaginoidea virens* isolates collected from different rice-growing states of India

S. No.	Isolate No.	Place of collection	Variety from which smut balls were collected
1	WB-1	RRS, Chinsurah, West Bengal	Not Known
2	WB-2	Chinsurah, West Bengal	Not Known
3	MH-1	Sakoli-1, Maharastra	RPN
4	MH-2	Sakoli-2, Maharastra	PKV Khamang
5	MH-3	Sakoli-3, Maharastra	Swarna
6	MH-4	Sakoli-4, Maharastra	Jai Sriram
7	MH-5	Sakoli-5, Maharastra	HMT
8	GJ-1	Nawagam, Gujarat	Not Known
9	HP	Malan, Himachal Pradesh	Not Known
10	PU-1	PAU-1, Ludhiana, Punjab	IRRI line
11	PU-2	PAU-2, Ludhiana, Punjab	Signet 5050
12	PU-3	PAU-3, Ludhiana, Punjab	NK6704
13	PU-4	PAU-4, Ludhiana, Punjab	HRI107
14	PU-5	PAU-5, Ludhiana, Punjab	Not Known
15	PU-6	PAU-6, Ludhiana, Punjab	PR-120
16	PU-7	PAU-7, Ludhiana, Punjab	GSK-37
17	PU-8	PAU-8, Ludhiana, Punjab	Not Known
18	PU-9	Kapurthala, Punjab	Not Known
19	HR-1	Kaul-1, Haryana	PR106
20	HR-2	Kaul-2, Haryana	HKR47
21	HR-3	Kaul-3, Haryana	Haryana Shankar Dhan
22	HR-4	Kaul-4, Haryana	HKR126

S. No.	Isolate No.	Place of collection	Variety from which smut balls were collected
23	HR-5	Gunthala	PAU201
24	HR-6	Kaul-5, Haryana	Not Known
25	HR-7	Kaul-6, Haryana	Not Known
26	HR-8	Kaul-7, Haryana	Not Known
27	HR-9	Karnal-1, Haryana	CSR-5
28	HR-10	Karnal-2, Haryana	PET-TR-2000-003
29	HR-11	Karnal-3, Haryana	IRSSTN-3
30	HR-12	Karnal-4, Haryana	Bulk
31	HR-13	Uchani, Haryana	HKR581
32	UK-1	Pantnagar, Uttarakhand	Not Known
33	UK-2	Kasipur, Uttarakhand	Not Known
34	UP-1	Nagina, Uttar Pradesh	Not Known
35	MP-1	Jabalpur, Madhya Pradesh	Not Known
36	OD-1	CRRI-1, Odisha	Not Known
37	OD-2	CRRI-2, Odisha	Not Known
38	TN-1	Aduthurai-1, Tamil Nadu	AD0812
39	TN-2	Aduthurai-2, Tamil Nadu	MDU4
40	TN-3	Aduthurai-3, Tamil Nadu	Not Known
41	TN-4	Aduthurai-4, Tamil Nadu	Not Known
42	TN-5	Aduthurai-5, Tamil Nadu	Not Known
43	TN-6	Ramanathapuram-1, Tamil Nadu	BPT 5204
44	TN-7	Ramanathapuram-2, Tamil Nadu	BPT 5205
45	TN-8	Thirurmangalam-1, Tamil Nadu	BPT 5206
46	TN-9	Thirurmangalam-2, Tamil Nadu	BPT 5207
47	KA-1	Karnataka	Not Known
48	KA-2	Mugad, Karnataka	Not Known
49	KL-1	Palakkad, Kerala	Not Known
50	KL-2	Pattambi, Kerala	Not Known
51	KL-3	Moncompu, Kerala	Not Known
52	TS-1	Medak, Telangana	Hybrid
53	TS-2	IIRR, Telangana	PAU1400
54	TS-3	Warangal, Telangana	BPT5204
55	AS	Titabar, Assam	Not Known
56	ML	Meghalaya,	Not Known
57	MN-1	Wangbal, Manipur	Not Known
58	MN-2	Imphal, Manipur	Not Known

Twelve potential ISSR markers were used to generate the unweighted Neighbor-joining dendrogram

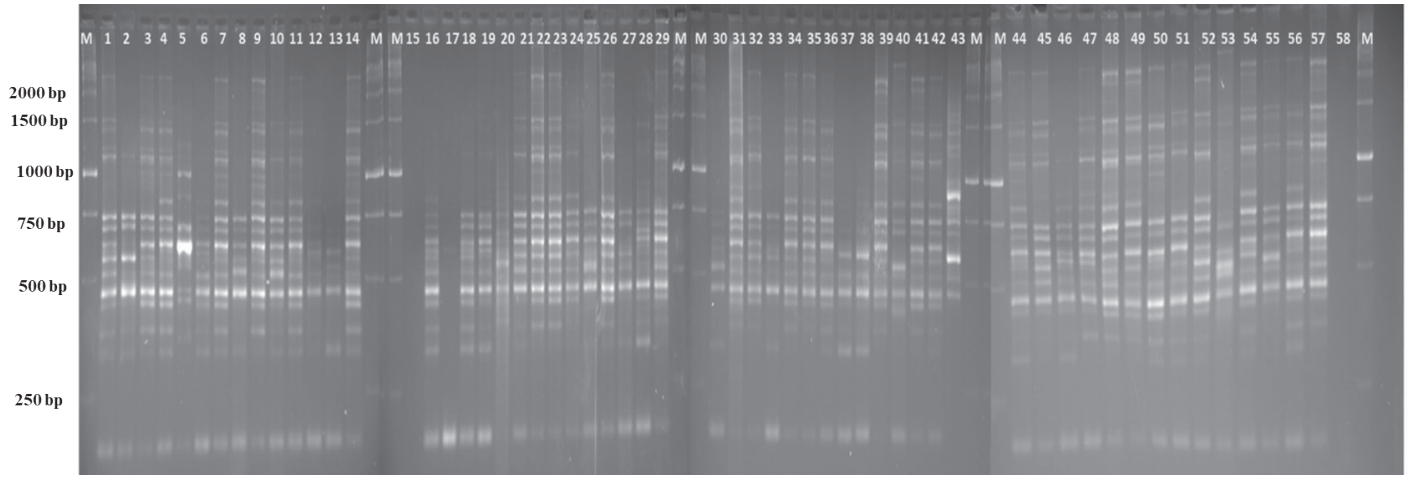


Figure 1: DNA amplified fragments profile of *U. virens* isolates using ISSR primer UBC 807; M – DNA Ladder; 1-58 – *U. virens* isolates

Table 2. Primer names with their sequences and different parameters of ISSR primers parameters

Sl. No	Primer	Primer Sequence	No of Loci	No of Polymorphic Loci	Polymorphism (%)	Hertero-zyosity	PIC	EMR	MI	RP Values
1	807	AGAGAGAGAGAGAGAGT	22	22	100	0.47	0.27	22.00	5.9	8.55
2	808	AGAGAGAGAGAGAGAGC	24	24	100	0.49	0.15	24.00	3.7	4.38
3	809	AGAGAGAGAGAGAGAGG	23	23	100	0.66	0.14	23.00	3.1	4.07
4	810	GAGAGAGAGAGAGAGAT	13	13	100	0.57	0.14	13.00	1.9	2.24
5	811	GAGAGAGAGAGAGAGAC	17	17	100	0.49	0.17	17.00	2.8	3.45
6	812	GAGAGAGAGAGAGAGAA	17	17	100	0.67	0.10	17.00	1.6	2.14
7	834	AGAGAGAGAGAGAGAGYT	15	15	100	0.34	0.21	15.00	3.1	3.93
8	835	AGAGAGAGAGAGAGAGYC	12	9	75	0.39	0.15	6.75	1.0	2.28
9	836	AGAGAGAGAGAGAGAGYA	23	22	95.65	0.55	0.24	21.04	5.0	7.83
10	840	GAGAGAGAGAGAGAGAYT	14	14	100	0.53	0.20	14.00	2.7	3.76
11	841	GAGAGAGAGAGAGAGAYC	20	20	100	0.36	0.24	20.00	4.8	6.97
12	842	GAGAGAGAGAGAGAGAYG	21	20	95.23	0.35	0.20	19.05	3.9	5.66

and the dendrogram grouped the 58 *U. virens* different isolates into three major clusters. Cluster I consisted of 21 *U. virens* isolates and it had many subclusters and the majority of the isolates belonged to Haryana and Punjab. Cluster II had 35 *U. virens* isolates were further divided into four subclusters. In this cluster, the majority of the isolates belong to Haryana and Tamil Nadu. Cluster III had only two *U. virens* isolates *viz*., TS-3 and TN-3. The cluster graph (**Figure 2**) revealed that that the variation among the isolates is minimum, except for few isolates. For example, the isolates collected from Punjab grouped together in cluster I whereas two of the Punjab isolates PU5 and 9 grouped in the cluster II. All the clusters had isolates collected from different geographical regions. Hence, the results revealed that no specific pattern of clustering was observed with respect to geographic region. ISSR markers were used

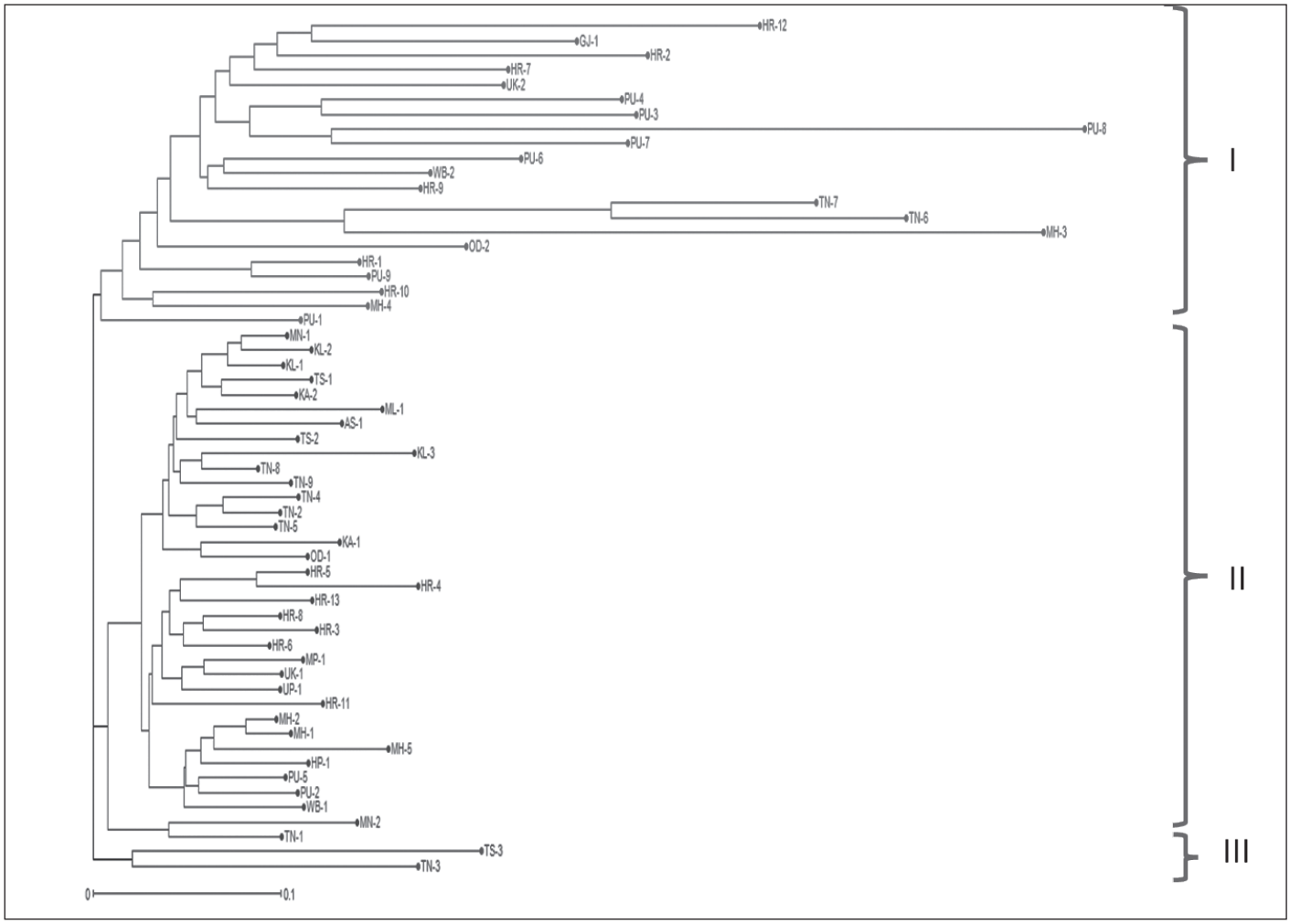


Figure 2: Dendrogram was generated using Jaccard's coefficient and UPGMA cluster analysis in DARwin for 58 *Ustilaginoidea virens* isolates based on 12 ISSR primers.

by many authors to study the molecular variability of the pathogens. Bag *et al*. (2021) reported the use of SSR markers to study the genetic variability of *U. virens* isolates collected from eastern and north-eastern India and stated that more genetic variation within populations and less among populations. Yugander *et al*. (2015) used ISSR primers to study the pathogenic and genetic variability and found that different isolates grouped based on their geographical regions. Kandan *et al*. (2015) reported that the combined use of universal rice primers (URPs), inter-simple sequence repeat (ISSR), and random amplified polymorphic DNA (RAPD) marker systems were more suitable to study the genetic variability in the rice brown spot pathogen *Bipolaris oryzae*. Anand *et al.* (2018) used ISSR markers and studied the population structure and virulence of *Alternaria carthami*, a causal agent of Alternaria leaf spot.

Conclusions

Understanding the molecular variability of plant pathogens will help to understand the nature of the existence of pathogens. With respect to false smut pathogen, twelve ISSR primers were used to understand the genetic variability. All tested primers are able to differentiate the variability and among them, UBC 807, 836 and 841 can be employed to study the genetic variability in *U. virens* isolates because of their high PIC, RP, and MI values. With respect to variability, clusters had isolates collected from different regions and there was no strong correlation based on geographical origin of

the isolates. The use of different pathogen genome-specific markers can reveal more depth in the genetic variation of the *U. virens* population.

References

Anand G, and R. Kapoor. 2018. Population structure and virulence analysis of *Alternaria carthami* isolates of India using ISSR and SSR markers. *World Journal of Microbiology and Biotechnology,* 31: 34(9):140. doi: 10.1007/s11274-018-2524-6

Bag MK, A Ray, P Masurkar, BN Devanna, C Parameswaran, M Baite, PC Rath and A Nayak. 2021. Genetic diversity and population structure analysis of isolates of the rice false smut pathogen *Ustilaginoidea virens* in India. *Plant Pathology*, https://doi.org/10.1111/ppa.13352

Chesnokov Yu V and AM Artemyeva. 2015. Evaluation of the measure of polymorphism information of genetic diversity. *Agricultural biology*, 50 (5): 571-578

Gilbert JE, RV Lewis, MJ Wilkinson and PD Caligari. 1999. Developing an appropriate strategy to assess genetic variability in plant germplasm collections. *Theoretical and Applied Genetics,* 98: 1125-1131(doi: 10.1007/s001220051176)

Kandan A, J Akhtar, B Singh, D Dixit, D Chand, A Roy, S Rajkumar and PC Agarwal. 2015. Molecular diversity of *Bipolaris oryzae* infecting *Oryza sativa* in India. *Phytoparasitica,* 43, 5–14. https://doi.org/10.1007/s12600-014-0430-5

Ladhalakshmi D, GS Laha, Ram Singh, A Karthikeyan, SK Mangrauthia, RM Sundaram, P Thukkaiyannan and BC Viraktamath. 2012. Isolation and characterization of *Ustilaginoidea virens* and survey of false smut disease of rice in India. *Phytoparasitica,* 40:171–176

Ladhalakshmi D, GS Laha, M Srinivas Prasad, RM Sundaram, N Sarla, B Divya, V Prakasam, PC Latha, D Sanjeeva Rao, D Krishnaveni and C Kannan. 2019. Artificial screening technique to identify the promising sources against rice false smut disease under field conditions. XIX International Plant Protection Congress, IPPC2019, 10-14 November 2019, Hyderabad, Telangana, India

Laha, GS, Sailaja B, Srinivas Prasad M, Ladhalakshmi D, Krishnaveni D, Ram Singh, Prakasam V, Yugander A, Kannan C, Valarmathi P. and Ravindra Babu V. 2016. Changes in Rice Disease Scenario in India. An Analysis from Production Oriented Survey. Technical Bulletin Number 91, ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad.

Tang YX, Jin J, Hu DW, Yong ML, Xu Y and He LP. 2013. Elucidation of the infection process of *Ustilaginoidea virens* (teleomorph: *Villosiclava virens*) in rice spikelets. *Plant Pathology*, 62:1-8. DOI: 10.1111/j.1365-3059.2012. 02629.x

Yugander A, D Ladhalakshmi, V Prakasham, SK Mangrauthia, MS Prasad, D Krishnaveni, MS Madhav, RM Sundaram and GS Laha. 2015. Pathogenic and Genetic Variation among the Isolates of *Rhizoctonia solani* (AG 1-IA), the Rice Sheath Blight Pathogen. *Journal of Phytopathology*, 163(6): 465–474.

Wang W, Fan J and Jeyakumar JMJ. 2019. 'Rice False Smut: An Increasing Threat to Grain Yield and Quality', in Y. Jia (ed.), Protecting Rice Grains in the Post-Genomic Era, Intech Open, London. 10.5772/intechopen.84862.

Zhou YL, K Izumitsu, R Sonoda, T Nakazaki, T Tanaka and M Tsuda. 2003. PCR-based detection of *Ustilaginoidea virens* and *Ephelis japonica*. *Journal of Phytopathology,* 151(9): 513–518.

RESEARCH ARTICLE https://doi.org/10.58297/CBJE8196

Evaluation of newer fungicides against neck blast disease of rice under field condition in south-eastern Rajasthan

Yadav DL*, Manoj Kumar, Sharma KM and Pratap Singh

Agricultural Research Station, Kota
Agriculture University, Kota, Rajasthan, India
*Corresponding author email: dlaau21@gmail.com

Received: 17th May 2022; Accepted: 5th June 2022

Abstract

Neck blast is considered as the most destructive phase of the blast disease in rice under south-eastern Rajasthan. Eight new generation fungicides *viz*., Tebuconazole, Difenconazole Propiconazole , Hexaconazole Azoxystrobin , Azoxystrobin + Tebuconazole , Azoxystrobin Mancozeb Trifloxystrobin +Tebuconazole were evaluated under field conditions during Kharif 2021. Result revealed that the combination of Trifloxystrobin (25%) + Tebuconazole (50 WG @ 0.4g/lit) gave minimum disease intensity (3.2%) and maximum disease control (80.8%) with higher yield (4482.3 kg/ha) which was at par with Propiconazole with a grain yield of 4400.0 kg/ha.

Keywords: Neck blast, Pyricularia, Trifloxystrobin 25%+Tebuconazole 50 WG, Difenconazole 25%, Propiconazole 25 % EC and Azoxystrobin 23% SC.

Introduction

Rice is one of the most important food crops that feeds more than 60 per cent population of India. India is one of the leading producers of rice in the world. Rice is the basic food crop and being a tropical species, it flourishes comfortably in a hot and humid climate. Rice is mainly grown in rain-fed areas that receive heavy rainfall. Rice blast disease caused by *Magnaporthe oryzae* (anamorph: *Pyricularia oryzae*) is the most serious constraint in all the rice ecosystems of the country. The fungus *P. oryzae* attacks at all stages of the crop and symptoms appear on leaves and nodes (Seebold *et al.,* 2004). Neck region of panicle develops a black colour and shrivels completely / partially, grain set inhibited, panicle breaks at the neck and hangs. Rice blast is by far the most important disease of rice. It is found wherever rice is grown and it is always a threat. Failures of entire rice crops have resulted directly from rice blast epidemics (Long *et al.,* 2001). The symptoms are more severe in case of neck blast that is characterized by the infection at the panicle base and its rotting (Bonman *et al.,* 1989).

Neck blast is considered as the most destructive phase of the disease and can occur without being preceded by severe leaf blast. (Zhu *et al.,* 2005). Yield reduction by neck blast infection is twice as severe as leaf blast (Hwang *et al.,* 1987). Heavy yield losses have been reported in many rice growing countries. For example, 75 percent grain loss may occur in India (Padmanabhan, 1965). Okhovot (1989) obtained maximum control of neck blast with Tricyclazole. Saikia (1991) found that the fungicides, Edifenphos, Thiophanate methyl and Carbendazim reduced leaf and neck blast infections by 7.5-80.8 and 60.5-64.5 per cent, respectively. Hence, continuous efforts are required to identify effective and safe molecules to manage this disease. The present study was, therefore, undertaken to evaluate new molecules effective against neck blast and their influence on rice yield.

Materials and Methods

The experiment was conducted at Agricultural Research Station, Agriculture University, Kota during *kharif* 2021 to evaluate the efficacy of various new fungicides against neck blast of rice under

field conditions. The experiment was laid out in a Randomized Block Design (RBD) with three replications and nine treatments. Variety PB-1121 was sown in 20 m^2 plot at 20 cm row to row distance. Crop was raised as per the recommended package of practices of the zone and irrigation was given as and when required. Fungicides were sprayed twice using a hand operated knapsack sprayer fitted with hollow cone nozzle and water volume of 500 lit/ha was maintained. First spraying was given just after the appearance of the disease and second was given 14 days after the first spray. Observations related to diseases were recorded 15 days after first application (at 15 days) and second 15 days after second application (at 30 days). One random tiller from each of the ten hills in each field was assessed for the neck blast incidence and expressed as per cent.

Calculation of Per Cent Disease intensity (PDI)

These grades are then utilized for the calculation of PDI by using the following formula of Wheeler (1969).

$$\text{Per cent Disease intensity (PDI)} = \frac{\text{Sum of individual rating}}{\text{No. of panicle examined}} \times \frac{100}{\text{Max. Disease rating}}$$

Yield assessment: The rice grains were harvested and weighed plot wise; the average seed yield per treatment was recorded and then calculated in to Kg/ha and statistically analyzed.

Neck blast disease scoring was done as suggested by Goto and Yamanaka, (1968); Mackill and Bonman, (1992) and Hayashi and Fukuta (2009).

Table 1. Neck blast disease rating scale

Neck blast score	Score description
0	No visible lesions or lesions only on few pedicles
1	Lesions on several pedicels or secondary branches
3	Lesions on a few primary branches or the middle part of panicle axis
5	Lesions partially around the base (node) or the uppermost internodes or the lower part of the panicle axis near the base
7	Lesion completely around the panicle base or uppermost internodes or panicle axis near the base with more than 30% of filled grains
9	Lesion completely around the panicle base or uppermost internodes or the panicle axis near the base with less than 30% of filled grains

Results and Discussion

In the present experiment, all applied fungicides were found significantly superior in reducing the disease severity as compared to check against neck blast disease of rice during *Kharif* 2021. Minimum disease intensity (3.2%) and maximum disease control (80.8%) was observed with foliar spray of fungicide combination Trifloxystrobin 25%+Tebuconazole 50 WG @ 0.4g/lit which was at par with Propiconazole 25 % EC @ 1ml/lit (PDI 4.1% & PDC 75.4%). Next best treatment was the combination of Azoxystrobin 11% + Tebuconazole 18.3% SC @ 1.5 ml/lit which recorded disease intensity *i.e.* 4.6% with 72.4% disease control. Minimum disease control (47.9%) was recorded under fungicide spray of Hexaconazole @ 5% SC as compared to other foliar spray of fungicides. However, maximum disease intensity (16.7%) was recorded in untreated plot (**Table 2**).

Maximum yield (4482.3 kg/ha) was recorded in foliar spray of combination Trifloxystrobin 25%+Tebuconazole 50 WG @ 0.4g/lit which was at par with Propiconazole 25 % EC @ 1ml/lit (4400.0kg/ha), Azoxystrobin 11% + Tebuconazole 18.3% SC @ 1.5 ml/lit (4340.0kg/ha) and Azoxystrobin 8.3%+ Mancozeb 66.7% WG @ 2.5gm/lit. (4240.0 kg/ha), while, minimum yield was recorded in control plot (3846.7 kg/ha.). Maximum cost benefit ratio was recorded with application of Propiconazole 25 % EC @ 1ml/lit.

Present findings are in accordance with Narayanswamy *et al.,* (2009) who reported that application of tebuconazole 50 % + trifloxystrobin 25 % (WG) was found most effective in controlling leaf blast as it controlled the disease up to the extent of 84 per cent compared to control. Mohan *et al.,* (2011) and Nirmalkar *et al.,* (2017) reported that, tebuconazole 50 % + trifloxystrobin 25 % (WG) and tebuconazole 25.9 % (EC) were found most effective against the leaf and neck blast of paddy. The strobilurin fungicides interfere with spore germination and germ tube development, absorbed into the leaf tissue and move in a translaminar manner (Sauter *et al*., 1999). However, the triazole fungicides are sterol inhibitors that interfere with sterol biosynthesis in fungal membranes and are absorbed into the leaf tissue (Tsuda *et al*., 2004).

Table 2. Bio-efficacy of new generation fungicides against neck blast and yield of rice

Treatments	Dose/ lit. water	Percent disease intensity	Per cent disease control	Yield kg /ha
T1: Tebuconazole 250 EC	0.5 ml/lit.	6.6	60.5	4021.7
T2: Difenconazole 25% EC	0.5 ml/lit.	6.3	62.3	4126.7
T3: Propiconazole 25 % EC	1 ml/lit.	4.1	75.4	4400.0
T4: Hexaconazole 5 SC	2 ml/lit.	8.7	47.9	3900.0
T5: Azoxystrobin 23% SC	1ml/lit.	8.5	49.1	4083.3
T6: Azoxystrobin 11% + Tebuconazole 18.3% SC	1.5 ml/lit.	4.6	72.4	4340.0
T7: Azoxystrobin 8.3%+ Mancozeb 66.7% WG	2.5gm/lit.	6.5	61.1	4240.0
T8: Trifloxystrobin 25%+Tebuconazole 50 WG	0.4g/lit	3.2	80.8	4482.3
T9: Control		16.7	--	3846.7
S. Em. ±		0.59	--	120.2
CD at 5 %		1.24	--	254.9

Table 3. Economics of treatments for the management of neck blast in paddy cultivation

Treatments details	Cost of cultivation (Rs/ha)	Cost of treatments (Rs/ha)	Cost of inputs (Rs/ha)	Yield kg /ha	Income (Rs./ha)	Net return (Rs/ha)	BC Ratio
T1: Tebuconazole 250 EC @ 0.5 ml/lit.	45000	1339	46339	4021.7	108586	62247	1.34
T2: Difenconazole 25% EC @ 0.5 ml/lit.	45000	2500	47500	4126.7	111421	63921	1.35
T3: Propiconazole 25 % EC @ 1 ml/lit.	45000	1200	46200	4400.0	118800	72600	1.57
T4: Hexaconazole 5 SC @ 2 ml/lit.	45000	1390	46390	3900.0	105300	58910	1.27
T5: Azoxystrobin 23% SC @ 1ml/lit.	45000	6670	51670	4083.3	110249	58579	1.13
T6: Azoxystrobin 11% + Tebuconazole 18.3% SC @ 1.5 ml/lit.	45000	4800	49800	4340.0	117180	67380	1.35
T7: Azoxystrobin 8.3%+ Mancozeb 66.7% WG @ 2.5gm/lit.	45000	3250	48250	4240.0	114480	66230	1.37
T8: Trifloxystrobin 25%+Tebuconazole 50 WG @ 0.4g/lit	45000	3148	48148	4482.3	121022	72874	1.51
T9: Control	45000	--	45000	3846.7	103861	58861	1.31

Conclusions

Among the eight fungicides tested under field conditions, the fungicides Trifloxystrobin 25%+Tebuconazole 50 WG @ 0.4g/lit and Propiconazole 25 % EC @ 1ml/litre were found to be the most effective against neck blast disease with great reduction in the per cent disease intensity and getting higher grain yield. Maximum cost benefit ratio was recorded with application of Propiconazole 25 % EC @ 1ml/lit followed by Trifloxystrobin 25%+Tebuconazole 50 WG @ 0.4g/lit.

Acknowledgements

The authors are highly thankful to the Zonal Director Research, ARS, Kota for encouragement and providing necessary facilities and other faculty members for facilitating required needs as well as rendering moral support during the entire research work.

Conflict of interests

The authors declare no conflict of interest.

References

Bonman JM, Estrada BA, Banding JM.1989. Leaf and neck blast resistance in tropical lowland rice cultivars. *Plant Disease.,* 73(5):388-390.

Goto K, and Yamanaka S. 1968. Studies on the race of blast fungus. *Bulletin of the Faculty of Agriculture*, Utsunomiya University, 7(2): 27-71.

Hayashi N and Fukuta Y. 2009. Proposal for a new international system of differentiating races of blast (*Pyricularia oryzae Cavara*) by using LTH monogenic lines in rice (*Oryza sativa* L.) in: JIRCAS Working Report No. 63. Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki, Japan, pp. 11-15.

Hwang BK, Koh YJ and HS Chung. 1987. Effects of adult-plant resistance on blast severity and yield of rice. *Plant Disease*, 71(11): 1035-1038.

Long DH, Correll JC, Lee FN and TeBeest DO. 2001. Rice blast epidemics initiated by infested rice grain on the soil surface. *Plant Disease,* 85(6): 612-616.

Mackill DJ and Bonman JM. 1992. Inheritance of blast resistance in near-isogenic lines of rice, *Phytopathology*, 82(7), 746-749.

Narayanswamy H, Syed sannaulla M and Kumar D. 2009. Evaluation of new fungicides against rice blast in cauvery delta. *Karnataka Journal of Agricultural Sciences*, 22(2): 450- 45.

Okhovot M. 1989. Effects of new fungicides on rice blast disease (*Pyricularia oryzae*) in rice paddies. *Iranian Journal of Agricultural Sciences,* 20:11-19.

Padmanabhan SY. 1965. Estimating losses from rice blast in India. In: The Rice Blast Disease:John Hopkins Press, Baltimore, Maryland. 203-221.

Saikia UN. 1991. Combating rice blast through chemicals. *Indian Phytopathology*, 44:40-44.

Sauter H, Steglich W and Anke T. 1999. Strobilurins: evolution of a new class of active substances. *Angewandte Chemie International Edition*, England. 38:1328-1349.

Seebold KW, Datnof JLE, Correa-Victoria FJ, Kucharek TA and Snyder GH. 2004. Effects of Silicon and fungicides on the control of leaf and neck blast in upland rice. *Plant Disease,* 88(3): 253-258.

Tsuda M, Itoh H and Kato S. 2004. Evaluation of the systemic activity of simeconazole in comparison with that of other DMI fungicides. *Pest Management Science,* 60(9):875-880.

Wheeler BEJ (1969). An Introduction to Plant Diseases, John Wiley and Sons Limited, London, P. 301.

Zhu YY, H Fang, YY Wang JX, Fan SS, Yang, TM Mew and CC Mundt. 2005. Panicle blast and canopy moisture in rice cultivar mixtures. *Phytopathology,* 95(4):433-438.

RESEARCH ARTICLE https://doi.org/10.58297/MIXG4169

Biochemical factors associated with vegetative phase resistance against yellow stem borer, *Scirpophaga incertulas* (Walker) in land races of rice

Megha CM[1], Vijaykumar L[2], Shivanna B[3], Anusha SB[4]

[1,2,4]Department of Agricultural Entomology, College of Agriculture, V. C. Farm, Mandya-571 405, Karnataka, India
[4]Department of Agricultural Entomology, University of Agricultural Sciences, GKVK, Bangalore-560065, Karnataka, India
[2]Corresponding author email: vkumaruasb@gmail.com

Received: 29th March 2022; Accepted: 12th May 2022

Abstract

A total of 50 local land races were screened against infestation of yellow stem borer, *Scirpophaga incertulas* (Crambidae: Lepidoptera) at vegetative phase under natural infestation (0 to 65%) during kharif 2019. Among them 18 land races were selected from each category of resistance based on standard evaluation system for rice. The estimation of biochemical constituents in rice stem (60 days old plants) of different categories was done to establish a relationship between various biochemical components with resistance/ susceptibility. Studies revealed that higher amounts of total sugars, reducing sugars and crude proteins were found in susceptible lines compared to resistant land races and were positively correlated with stem borer infestation. Total free amino acids, total phenols and tannins were found to be higher in resistant than susceptible varieties and were negatively correlated. The mineral content in the stem *viz.*, nitrogen was positively correlated whereas phosphorous and potassium were found in higher quantities in resistant categories (2.19 – 2.37%) than susceptible ones and were found to be negatively correlated with infestation of yellow stem borer (-0.95).

Keywords: Biochemicals, Resistance, Rice, Yellow stem borer

Introduction

Rice (*Oryza sativa* Linn.), an ancient crop domesticated more than 8000 years ago, evolved along with man and adapted to diverse ecological conditions. Production and productivity of rice yield depends on a number of biotic and abiotic stress factors. Insects, mites and nematodes are few of the key biotic stresses which limit rice production in India (Prakash *et al.*, 2006). The annual yield loss due to insect pests in India varies from 21 to 51 per cent (Singh and Dhaliwal, 1994) andit varies between 26 to 34 per cent globally (Widowsky and O` Toole, 1996). More than 1,000 species of invertebrate pests have been reported to infest rice and about a dozen species of insects are important as key pests. Major insect pests that cause significant economic losses in Asian countries are the yellow stem borer, *Scirpophaga incertulas* (Walker), *Cnaphalocrocis medinalis* (Guenee), brown planthopper, *Nilaparvata lugens* (Stal.), Green leafhopper, *Nephotettix virescens* (Distant) and Asian rice gall midge, *Orseolia oryzae* (Wood-Mason). In South-Asia, yellow stem borer, Asian rice gall midge and brown planthoppers are the major production constraints (Ramaswamy *et al*., 1996; Vijaykumar *et al*., 2009).

The plant strategy to deter feeding insect pests has become an important aspect of insect- plant interaction studies. Feeding activities of insect pests results in physiological, morphological and chemical changes in the form of accumulation of toxic compounds with defensive properties (Amsagowri *et al*., 2018). The biochemical factors are chemicals that affect behaviour, physiology and growth of insect. Some biochemical factors are associated with repellence, deterrence or adverse effects on insect pests. Several control strategies such as field sanitation, introduction of parasitoids and use of synthetic pesticides have been employed for control of *S. incertulas* but their

deployment have not given satisfactory control particularly when the larvae were feeding inside the stalks (Kfir *et al*., 2002). In the present study the biochemical constituents in rice stem of land races were correlated with dead heart damage by yellow stem borer in so as to understand the role of biochemical basis of defense.

Materials and Methods

A total of 50 local land races were collected from Zonal Agricultural Research Station, V. C. Farm, Mandya 12^0 32'N, 760 53'E, and 690 m AMSL under AICRP (Rice) were evaluated against rice yellow stem borer under field conditions during *Kharif* 2019 at 'A' block V. C. Farm, Mandya. The estimation of biochemical constituents *viz*., total sugars, reducing sugars, total phenols, crude proteins total free amino acids, tannins and minerals *viz.,* nitrogen, phosphorous and potassium in rice stem collected from 60 days old plants were carried to establish the relationship between various biochemicals with selected resistant and susceptible genotypes *viz.,* Malpali samba- 1, Rajboga, Kari kagga, Neermullare, Malgudisanna -2, Jenugudu, Kalajeera, Mara batta- 2, China ponni, Kavekantak, Gangadale, Neermulka, Naweli, Kana kunja, Punkattkodi -1, Kundipullan, Krishna leela, Puttabatta-2.

Preparation of plant samples for analysis

The samples of stem were dried at 35 °C in hot air oven for 24 to 48 hrs. The dried samples were ground using mixer grinder. The powdered samples were stored in plastic covers until analysis.

Extraction of plant tissues in alcohol

The stem samples of 18 selected rice land races were collected and analyzed from the pooled sample of 3 replications. The samples were thoroughly washed with distilled water and dried under shade. 10 gram of plant sample was taken in a separate conical flask and 150 ml of 80 per cent ethanol was added and refluxed for 30 minutes on hot water bath. After boiling, the extract was cooled and tissues were ground thoroughly in a mortar with pestle in slight amount of ethanol. The supernatant was decanted into another flask and the residue was again re-extracted with small quantity of hot ethanol and decanted. This extract was filtered through Whatman's No.1 filter paper and made up to a known volume with 80 per cent ethanol. The ethanol part of (alcoholic) extract was stored in refrigerator at 4 °C, and used for the estimation of total sugars, reducing sugars and phenols.

The total and reducing sugars in each test genotype were estimated by following the method suggested by Somogyi (1952). Estimation of total phenols in stem samples of test genotypes was done by following Folin-Ciocalteau method suggested by Bray and Thorpe (1954). The amount of total free amino acids present in the samples was estimated by following Ninhydrin method developed by Moore and Stein (1948). Estimation of tannins in the stem samples of test genotypes was done by following Folin-Ciocalteau method suggested by Bray and Thorpe (1954). Further, the data collected were subjected to Analysis of Variance (ANOVA) and means were separated by Tukey's HSD test (Tukey, 1953).

Estimation of nitrogen and crude proteins

Finely powdered oven dried Na_2CO_3 samples (0.5 g) weretaken in the digestion tubes. To this 1-2 g of digestion mixture and 10-15 ml of concentrated sulphuric acid was added and digested. Then the samples were placed in Kjeldahl digestion assembly till a light bluish green residue is obtained. Then the content was cooled by adding 5 to 10 ml of distilled water. The receiving flask was placed at the receiving end of distillation unit. The digested mixture was loaded on tube for distillation apparatus one at a time. By keeping all reserve tanks loaded with appropriate reagents such as 4% boric acid with mixed indicator and 40% NaOH, the content was distilled for 6 minutes and the released ammonia was collected in boric acid solution by programming the Kjeldahl distillation unit (make: Borosil KDI 1300W, BLFAKDI010). Once the distillation was completed, the receiving flask was removed and titrated against standard H_2SO_4 till the color changed from green to pink. Titer value (TV) was noted and nitrogen content was calculated using the equation

$$\% \text{ N in plant sample} = \frac{\text{TV x N.of H2S04x0.0014}}{\text{wt.of sample}} \text{ x } 100$$

Crude protein was calculated by the formula:

Crude protein (g %) = % N x 5.95 (conversion factor for rice)

Then, the percentage crude protein was expressed in terms of mg/gm of the sample.

Phosphorous content

Five ml of digested sample was pipetted out into a 25 ml volumetric flask and 5 ml of vanadomolybdate reagent was added. The volume was made up with distilled water and the content was mixed thoroughly. The absorbance was read after 30 minutes at 420 nm on UV double beam spectrophotometer (make: Shimadzu UV-1900i). The phosphoric acid (Himedia) was used for the preparation standard curve. Further, the phosphorous concentration in the stem samples was estimated with the help of standard curve (Graph ppm) by using the following formula, and expressed in percentage.

$$\% \text{ Phosphorous} = \frac{\text{Graph ppm} \times \text{vol. of digested sample} \times \text{vol. made} \times 100}{\text{Weight of sample} \times \text{Aliquot taken} \times 10^6}$$

Potassium content

The potassium content in di-acid digested plant sample was determined by flame photometric method after appropriate dilution (10^6). Flame photometer (Systronics 130) was switched on and K filter was selected. The blank was fed to the instrument and the reading was adjusted to zero with the blank in the instrument. Then 40 ppm K solution (standard Himedia) was fed to the flame photometer and adjusted to 100 to run the standards. After this, readings were taken for other intermediate concentrations for standardization. The acid digested plant sample was fed into the atomizer and the flame photometer reading was noted down. If the concentration of the sample exceeded the range, the sample was diluted to the suitable concentration range so that final concentration lies between 0 to 40 ppm. The curve was plotted using graph sheet by taking K concentration on X-axis and flame photometer readings on Y-axis. Acid digested plant sample reading was located on the standard curve, which will give the K concentration in the extract (Graph ppm). From this graph concentration, the amount of K in the sample was calculated by using the following formula.

$$\% \text{ Potassium} = \frac{\text{Graph ppm} \times \text{vol. of digested sample} \times \text{vol. made} \times 100}{\text{Weight of sample} \times \text{Aliquot taken} \times 10^6}$$

Results and Discussion

To study the biochemical basis of resistance among land races of rice against rice yellow stem borer, 18 landraces representing each resistance category were selected from the screening trial. The resistance categories include high resistance (0 percent damage; SES score 0), resistance (1 to 10 % damage; SES score 1), moderate resistance (11 to 20 % damage; SES score 3), moderately susceptible (21 to 30 % damage; SES score 5), susceptible (31 to 60 % damage; SES score 7) and highly susceptible (>61 % damage; SES score 9) **(Table 1)**.

Among the landraces, amount of total soluble sugars varied from 3.81 to 3.94 mg/g and was lower in highly resistant genotypes. However, in highly susceptible genotypes the amount of total soluble sugars was found significantly higher and varied from 8.11 to 8.59 mg/g. Similarly, among the genotypes, lower amount of total reducing sugars (6.79 to 7.44 mg/g) was observed in highly resistant genotypes, however in highly susceptible genotypes the amount of total reducing sugars (14.12 to 14.78 mg/g) was found significantly higher. Likewise, among the genotypes, lower amount of crude proteins was observed in highly resistant genotypes which varied from 2.89 to 3.10 mg/g. However in highly susceptible genotypes the amount of crude proteins was found significantly higher and varied from 6.70 to 6.80 mg/g. Also, among the genotypes, lower amount of total free amino acids was observed in highly resistant genotypes varied from 23.59 to 25.00 mg/g and in highly susceptible genotypes the amount of total free amino acids was found to be significantly higher which varied from 15.31 to 15.47 mg/g.

Among the genotypes, the lower amount of total soluble sugars was observed in highly resistant genotypes that varied from 3.81 to 3.94 mg/g, however in highly susceptible genotypes the amount of total soluble sugars was found significantly higher which varied from 8.11 to 8.59 mg/g. Among the genotypes, lower amount of nitrogen was observed in highly resistant genotypes which varied from 0.48 to 0.52 per cent. However, in highly susceptible genotypes the amount of nitrogen was found to be significantly higher that varied from 1.12 to 1.14. Similarly, among the genotypes, higher amount of phosphorus was observed in highly resistant genotypes which varied from 0.38 to 0.43 per cent. However, in highly susceptible genotypes the amount of phosphorous was found to be significantly lower and varied from 0.14 to 0.16. Among the genotypes, higher amount of potassium was observed in highly resistant genotypes which varied from 2.37 to 2.41 per cent. However, in highly susceptible genotypes the amount of potassium was found to be significantly lower which varied from 1.54 to 1.60. Also, among the genotypes, higher amount of tannins was observed in highly resistant genotypes that varied from 4.85 to 5.85 mg/g, however in highly susceptible genotypes the amount of tannins was found to be significantly higher and varied from 0.66 to 0.98 mg/g. Likewise, among the genotypes, higher amount of phenols was observed in highly resistant genotypes which varied from 0.54 to 0.69 mg/g.However in highly susceptible genotypes the amount of phenols was found to be significantly higher and which varied from 0.18 to 0.19 mg/g (**Table 1**) (**Figure 1**).

A significant, negatively correlation with the infestation of yellow stem borer and the contents of the total phenols (r= -0.85**), total free amino acids (r= -0.87**), tannins (r= -0.94**), total phosphorous (r= -0.96**) and total potassium (r= -0.95**) contents of the rice stem was recorded. Likewise, a significant positive correlation with the infestation of yellow stem borer, and the contents of total soluble sugars

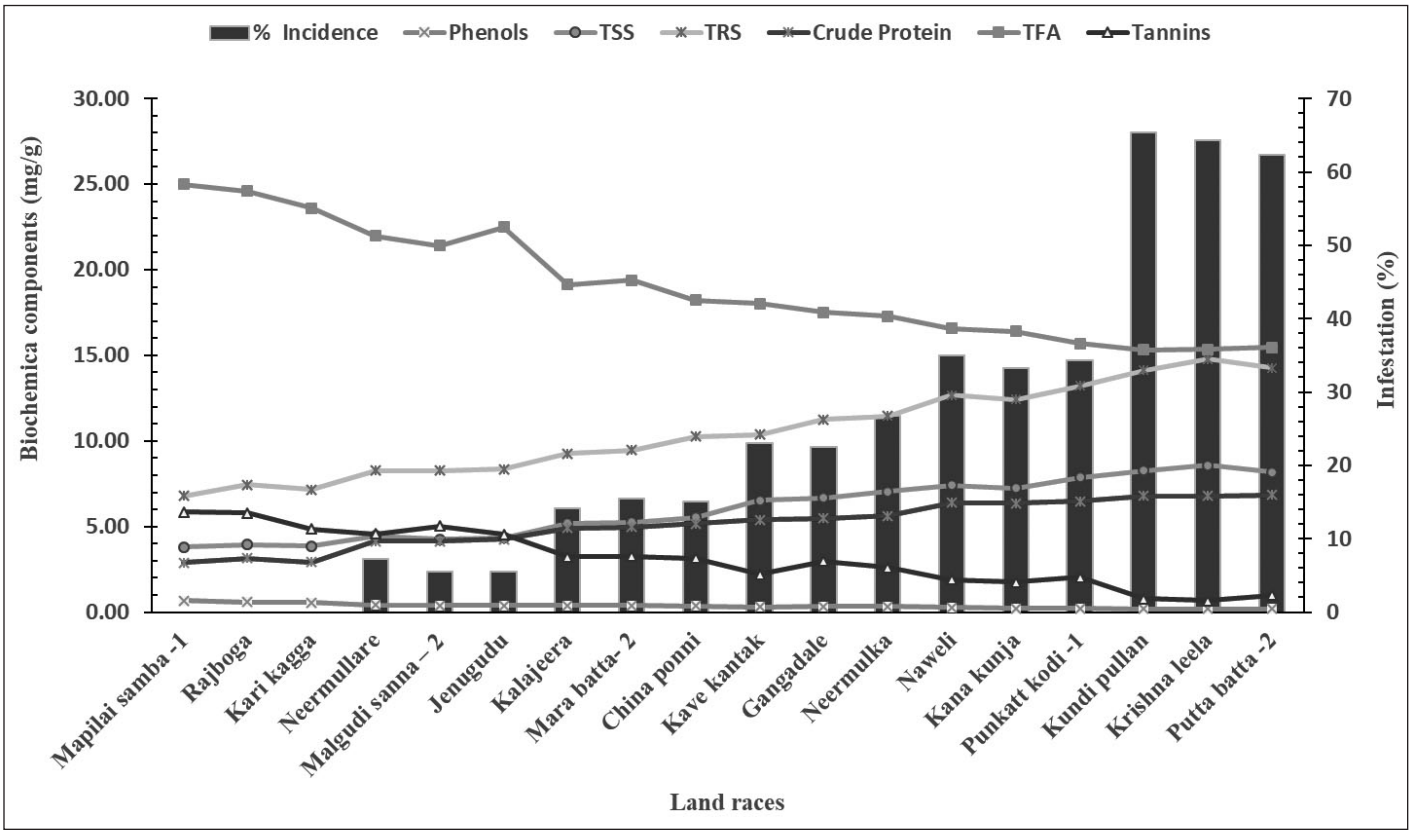


Figure 1. Relationship between biochemical components of rice stem and yellow stem borer infestation, *Kharif* 2019

Table 1. Dead heart damage by *S. incertulas* and biochemical constituents in rice stem samples of local land races

Sl. No.	Genotypes		Score	Incidence (% dead hearts)	Biochemical components (mg/g)						Minerals (%)		
					Phenols	TSS	TRS	CP	TFA	Tannins	N	P	K
1	HR	Malpali samba- 1	0	0.00 (0.00) [a]	0.68[a]	3.81[a]	6.79[a]	2.90[a]	25.00[a]	5.85[b]	0.48[a]	0.43[a]	2.41[a]
2		Rajboga	0	0.00 (0.00) [a]	0.59[b]	3.94[a]	7.44[b]	3.10[b]	24.60[a]	5.79[a]	0.52[a]	0.39[ab]	2.40[a]
3		Kari kagga	0	0.00 (0.00) [a]	0.54[b]	3.87[a]	7.17[ab]	2.89[a]	23.59[b]	4.85[ab]	0.48[a]	0.38[ab]	2.37[a]
4	R	Neermullare	1	7.27 (15.65) [b]	0.43[c]	4.46[c]	8.28[c]	4.10[c]	21.97[cd]	4.57[de]	0.68[b]	0.35[bc]	2.23[b]
5		Malgudisanna -2	1	5.47 (13.53) [b]	0.42[c]	4.25[b]	8.27[c]	4.10[c]	21.41[d]	5.02[c]	0.67[b]	0.34[bc]	2.24[b]
6		Jenugudu	1	5.50 (13.57) [b]	0.41[c]	4.33[c]	8.36[c]	4.15[c]	22.48[b]	4.54[cd]	0.70[b]	0.34[bc]	2.19[bc]
7	MR	Kalajeera	3	14.11 (22.07) [c]	0.38[cd]	5.19[d]	9.27[d]	4.87[d]	19.14[e]	3.23[f]	0.81[c]	0.31[cde]	2.16[bc]
8		Mara batta- 2	3	15.45 (23.16) [c]	0.37[cd]	5.25[d]	9.46[d]	4.90[de]	19.38[e]	3.25[g]	0.82[c]	0.32[cd]	2.15[bc]
9		China ponni	3	15.06 (22.85) [c]	0.36[de]	5.53[e]	10.27[e]	5.15[ef]	18.21[f]	3.16[e]	0.86[cd]	0.32[cd]	2.11[c]
10	MS	Kavekantak	5	23.08 (28.73) [d]	0.32[f]	6.54[f]	10.38[e]	5.39[fg]	18.01[fg]	2.21[gh]	0.90[de]	0.28[def]	1.92[d]
11		Gangadale	5	22.49 (28.32) [e]	0.33[fe]	6.67[f]	11.27[f]	5.46[fg]	17.52[fg]	2.97[h]	0.91[de]	0.26[efg]	1.87[d]
12		Neermulka	5	26.8 (31.19) [d]	0.32[fe]	7.05[g]	11.45[f]	5.52[g]	17.27[gh]	2.60[h]	0.92[e]	0.25[fg]	1.83[de]
13	S	Naweli	7	35.01 (36.30) [f]	0.27[g]	7.40[h]	12.67[g]	6.39[h]	16.56[hi]	1.89[i]	1.07[f]	0.21[gh]	1.74[e]
14		Kana kunja	7	33.2 (35.20) [f]	0.23[gh]	7.25[g]	12.43[g]	6.20[h]	16.41[hi]	1.74[j]	1.04[f]	0.20[h]	1.74[e]
15		Punkattkodi -1	7	34.28 (35.86) [f]	0.22[gh]	7.88[i]	13.20[h]	6.45[h]	15.70[ij]	2.03[i]	1.08[f]	0.21[gh]	1.71[ef]
16	HS	Kundipullan	9	65.35 (53.97) [g]	0.18[h]	8.27[j]	14.12[i]	6.77[i]	15.32[j]	0.78[j]	1.13[g]	0.16[hi]	1.60[fg]
17		Krishna leela	9	64.31 (53.34) [g]	0.19[h]	8.59[k]	14.78[j]	6.80[i]	15.36[j]	0.66[k]	1.14[g]	0.14[i]	1.54[g]
18		Puttabatta -2	9	62.34 (52.17) [g]	0.18[h]	8.18[j]	14.27[i]	6.70[i]	15.47[j]	0.98[k]	1.12[g]	0.14[i]	1.56[g]
SE m ±			-	**0.38**	**0.01**	**0.04**	**0.07**	**0.05**	**0.17**	**0.05**	**0.02**	**0.03**	**0.81**
CD @p=0.05				**1.12**	**0.04**	**0.11**	**0.23**	**0.13**	**0.50**	**0.16**	**0.08**	**0.10**	**2.47**

Values in the column followed by common letters are non-significant at p=0.05 as per Tukey's HSD (Tukey, 1953); TSS- Total soluble sugars; TRS- Total reducing sugars; CP- crude proteins; TFA- Total free amino acids; N- Nitrogen; P- Phosphorous; K- Potassium; Figures in the parentheses indicate arcsine transformed values.

(r= 0.94**), total reducing sugars (r= 0.94**), crude proteins (r= 0.91**) and total nitrogen (r= 0.93**) and these parameters which were significant was found influencing the infestation level to the extent of 92 per cent (R^2= 0.92) (**Table 2**).

The results of the present study corroborate with Vijaykumar *et al.,* (2012) where they reported higher amount of total sugars in all susceptible rice genotypes compared to resistant ones. Likewise, Rajdurai and Kumar (2017) also reported lower amount of sugars and soluble proteins in all resistant genotypes imparting resistance against the yellow stem borer. Similar observations on increased level of sugars were reported in all susceptible rice genotypes against Asian rice gall midge (Vijaykumar *et. al.,* 2009; 2012), rice leaf folder (Vanitha *et al.,*2015) and rice brown planthopper (Ashrith *et al.,* 2017). The present findings are in line with the results of Punithavalli *et al.,* (2013) who reported that lower protein content was evident invariably in all the infested rice genotypes. Further, Lokesh and Mehla (2017) reported that crude proteins were positive and significantly associated with growth, development and life cycle of maize stem borer, *Chilo partellus*. Similarly, from the correlation studies of Vijaykumar *et al.,* (2012) it was observed that the total free amino acids had significant negative influence on per cent incidence of rice gall midge.

The present findings are in close conformity with Punithavalli *et al.,* (2013) who reported that the contents of biochemicals such as phenol, orthodihydroxy phenol and tannins were negatively correlated with leaf folder damage indicating that these are defensive compounds contributing towards the rice yellow stem borer resistance. Further, Elanchezhyan *et al.,* (2017) reported that higher concentration of total phenols observed in the resistance group could be one of the factors contributing towards tolerance with antibiotic effect against yellow stem borer. Likewise, the study (Facknath and Lalljee, 2005) indicated that the phosphorus decreases the host suitability against various insect-pests by changing secondary metabolites such as phenolics, terpenes and accumulation of phenolics (tannins and lignin) acts as a barrier which has feeding deterrent and insecticidal effects on herbivores. Similarly, Bala *et al.,* (2018) reported higher levels of potassium in enhancing the secondary metabolites and reducing accumulation of carbohydrate during plant damage from various insect pests. The presently identified resistant genotypes are highly useful in breeding resistant varieties against rice yellow stem borer.

Table 2. Correlation matrix between the infestation of *S. incertulas* and biochemical constituents of rice stem at 60 DAT, *Kharif* 2019

Parameters	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	R^2 value
Y – Dead hearts (%) by YSB	-0.85**	0.94**	0.96**	0.91**	-0.87**	-0.94**	0.93**	-0.96**	-0.95**	
X_1 – Phenols	1.00	-0.91	-0.93	-0.97	0.96	-0.93	-0.96	0.95	0.94	
X_2 – TSS		1.00	0.99	0.97	-0.96	0.96	0.970	-0.97	-0.99	
X_3 – TRS			1.00	0.98	-0.95	0.97	0.98	-0.99	-0.99	
X_4 –Crude Proteins				1.00	-0.98	0.97	0.99	-0.97	-0.97	
X_5 – TFA					1.00	-0.93	-0.97	0.94	0.95	0.92
X_6 – Tannins						1.00	-0.97	00.96	0.96	
X_7– Nitrogen							1.00	-0.98	-0.98	
X_8– Phosphorous								1.00	0.99	
X_9– Potassium									1.00	

N = 18; ** Significant at P ≤ 0.01; YSB- yellow stem borer; TSS- Total soluble sugars; TRS- Total reducing sugars; TFA- Total free amino acids; YSB- Yellow stem borer

Acknowledgements

The authors are thankful to Science and Engineering Research Board (SERB), Department of Science and Technology, Ministry of Science and Technology, Government of India under the Grant No. EEQ/2017/000484/26/3/2018 for providing funding and laboratory facilities. The authors are also thankful to University of Agricultural Sciences, GKVK, Bangalore and B.Sc (Agri.) students of Agricultural College, V. C. Farm, Mandya for their help.

References

Amsagowri V, Muthukrishnan N, Muthiah C, Mini ML and Mohankumar S. 2018. Biochemical changes in rice yellow stem borer infested rice accessions. *Indian Journal of Entomology,* 80(3): 926-934.

Ashrith KN, Sreenivas AG, Guruprasad GS, Hanchinal SG and Krishnamurthy D. 2017. Biochemical basis of resistance in rice against brown and white backed plant hopper. *International Journal of Current Microbiology and Applied Sciences,* 6(10): 1699-1706.

Bala K, Sood AK, Pathania VS and Thakur S. 2018. Effect of plant nutrition in insect pest management: *A Review of Journal of Pharmacognosy and Phytochemicals*, 7(4): 2737-2742.

Bray HG, and Thorpe WV. 1954. Analysis of phenolic compounds of interest in metabolism, *Methods of Biochemical Analysis (Eds. David Glick),* Volume 1, pp 27-52.

Elanchezhyan K. 2017. Evaluation of promising rice cultures against yellow stem borer, *Scirpophaga incertulas* Walker (Crambidae: Lepidoptera). *Journal of Entomology and Zoology Studies*, 5(5): 1903-1905.

Facknath S and Lalljee B. 2005. Effect of soil-applied complex fertilizer on an insect–host plant relationship: *Liriomyza trifolii* on *Solanum tuberosum*. *Entomologia Experimentalis et Applicata*, 15(1): 67-77.

Kfir R, Overhalt WA, Khan ZR and Palaszek A. 2002. Biology and management of economically important lepidopteran cereal stem borers in Africa. *Annual Review of Entomology,* 47:701-731.

Khurana AD and Verma AN. 1983. Some biochemical plant characters in relation to susceptibility of sorghum to stem borer and shoofly. *Indian Journal of Entomology*, 45(1): 29-37.

Lokesh and Mehla JC. 2017. Study on morphological and biochemical features associated with maize (*Zea mays* L.) resistance against *Chilo partellus. International journal of pure and applied bioscience*, 5(4): 1011-1021.

Moore S and Stein WH. 1948. Photometric ninhydrin method for use in the chromatography of amino acids method. *Journal of Biological Chemistry*, 176(1): 367-388.

Prakash A, Rao J, Rath PC and Behera KS. 2006. Pests of rice. *Indian Farming*, 56(7): 4-9.

Punithavalli M, Muthukrishnan NM and BalajiRajkuma M. 2013. Defensive responses of rice genotypes for resistance against rice leaf folder *Cnaphalocrocis medinalis. Rice Science,* 20(5): 363-370.

Rajadurai G and Kumar K. 2017. Evaluation of 193 rice entries against yellow stem borer, *Scirpophaga incertulas. Journal of Entomological Research*, 41(2): 133-144.

Ramaswamy C, Shanmugam TR and Suresh D. 1996. Constraints to higher rice yields in different rice production environments and prioritization of rice research in south India. *Evanson, RE, Herdt, RW and Hussain, M. (Edt.). Rice Research in Asia: Progress and Priorities. International Rice Research Institute, Manila, Philippines*., 146-160.

Singh J and Dhaliwal GS. 1994. Insect pest management in rice: a perspective agriculture. In: *Trends in agricultural insect pest management* (Eds: Dhaliwal, G.S. and Ramesh Arora). Common Wealth Publishers, New Delhi, India, pp. 56-112.

Somogyi M. 1952. Estimation of sugars by colorimetric method. *Journal of Biological Chemistry*, 195(1):19-23.

Tukey JW. 1953. The problem of multiple comparison, Princeton, New Jersey, Princeton University, Department of Statistics. 396.

Vanitha BK, Kumar CTA, Kumar LV and Prashantha C. 2015. Association of biochemical factors in rice against infestation of rice leaf folder, *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae). *Journal of Experimental Zoology* (India),18(1): 373-376.

Vijaykumar L, Chakravarthy AK, Patil SU and Rajanna D. 2009. Resistance mechanism in rice to the midge *Orseolia oryzae* (Diptera: Cecidomyiidae). *Journal of Economic Entomology*, 102(4), 1628-1639.

Vijaykumar L, Patil SU and Chakravarthy AK. 2012. Biochemical basis of resistance in rice against Asian rice gall midge, *Orseolia oryzae* (Wood-Mason) (Diptera :Cecidomyiidae). *Current Biotica*, 6(2): 163-170.

Widowsky DA and O'toole JC. 1996. Prioritization of rice research agenda for eastern India, In: *Rice research in Asia: Progress and Priorities* (*Eds*: Evanson, R. E., Herdt, R.W. and Hussain, M.)., *International Rice Research Institute, Manila Philippines*., 109-129.

RESEARCH COMMUNICATION https://doi.org/10.58297/MJYG3539

Hypersensitive response and induced resistance in rice gene differentials against biotype 1 of Asian rice gall midge, *Orseolia oryzae* at Mandya, Karnataka

VijayKumar L[1*], Patil SU[2], Shivanna B[3], Kitturmath MS[3]

[1]Department of Agricultural Entomology, College of Agriculture, V. C. Farm, Mandya - 571405, University of Agricultural Sciences, Gandhi Krishi Vigyan Kendra (GKVK), Bangalore, Karnataka, India

[2]Department of Agricultural Entomology, Zonal Agricultural and Horticultural Research Station, Brahmavara - 576213, University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India

[3]Department of Agricultural Entomology, College of Agriculture, University of Agricultural Sciences, Gandhi Krishi Vigyan Kendra (GKVK), Bangalore - 560065, Karnataka, India.

[4] AICRP on rice, Zonal Agricultural Research Station, V. C. Farm, Mandya - 571405

[1]Corresponding author email: vkumaruasb@gmail.com

Received: 28th April 2022; Accepted: 6th June 2022

Abstract

Hypersensitive reaction (HR) and induced resistance were noticed in resistant rice genotypes infested by gall midge. Detailed observations on Phalguna (*Gm2* gene), Abhaya (*Gm4* gene), ARC 5984 (*Gm5* gene) infested with gall midge biotype 1 revealed that the infestation triggered HR in the plant, leading to extensive tissue necrosis at the apical meristem and browning of central leaf. This was followed by maggot mortality and premature tillering. In susceptible genotypes this phenomenon was not evident. HR leading to necrosis is fatal to host plant but premature tillering was observed. Further, the secondary tillers were infested subsequently with the gall midge biotype 1 eggs at 7, 14, 21 and 28 days after primary infestation, and maggots failed to establish and cause silver shoot. However, HR was observed 6 days after secondary tiller infestation, when the primary tillers were infested 28 days after. But cent per cent maggot mortality was observed, regardless of the time interval between infesting primary and secondary tillers in all the HR + plants. Thus, the HR is not confined to the tillers of primary infestation but it also triggers systemic acquired resistance in other tillers in Phalguna, Abhaya and ARC 5984, whereas, in W1263 (*Gm1* gene), HR+ was not evident but antibiotic effects were observed along with maggot mortality.

Key words: *Orseolia oryzae*, rice genotypes, hypersensitivity, induced resistance.

The Asian rice gall midge, *Orseolia oryzae* (Wood-Mason) (Diptera: Cecidomyiidae) is a major insect pest of rice in several Asian countries (Bentur *et al.,* 2003). In India, gall midge has been reported from almost all the rice growing states except the Western Uttar Pradesh, Uttaranchal, Punjab, Haryana and Hill states of Himachal Pradesh and Jammu and Kashmir (Bentur *et al.,* 1992). The insect being endoparasitic, use of resistant varieties is the most economical and feasible tool for its control (Heinrichs and Pathak, 1981; Mathur *et al*., 1999; Khush, 1997). But the emergence of new virulent biotypes of gall midge in popular rice varieties is capable of overcoming resistance and this is a cause for concern. So far 7 biotypes of gall midge were identified and characterized in India (Vijayalakshmi, 2006). Widespread cultivation of high yielding varieties made a radical change in the pest status of rice gall midge in coastal Karnataka.

Early studies tried to correlate morphological difference in attributes such as color and hairiness of the leaf or compactness of the leaf sheath with resistance (Rao *et al.,* 1971). Similarly, the density and length of trichomes were negatively correlated with gall midge incidence (Joshi, 1982; Devaiah, 1984) while tillering pattern, leaf sheath compactness and inter space had

no influence. Soon it was noted that resistant varieties offered no mechanical barrier since maggots reached the apical meristem in all the varieties (Shastry *et al.,* 1972; Sain 1988). No distinct oviposition preferences were noted among resistant and susceptible varieties (Hidaka, 1974; Kalode, 1980; Kalode *et al.,* 1983; Sain and Kalode, 1994). Hidaka and Vungsilabutr (1971) observed failure of moulting of first instar maggot in W1263. A predominant antibiosis component leading to mortality of first instar larvae has been also observed by many workers (Pathak and Heinrichs, 1982; Mathur and Rajamani, 1984). Since the beginning of 19th century, hypersensitivity has been recognized as an important defense mechanism (Fernandes, 1990) and is usually controlled by an individual gene or, rarely by a few genes. Detailed observations on rice variety Phalguna, a derivative of Siam 29 with *Gm2* gene for resistance, and gall midge biotype 1 (avirulent) and biotype 4 (virulent) revealed that infestation with the former biotype leads to premature tillering and triggers a hyper sensitive reaction (HR) within 5 days (Bentur and Kalode, 1996). So, a study on the hypersensitivity and induced resistance in 6 rice genotypes against local gall midge biotype 1 (Vijaykumar *et al.,* 2008) was initiated in 2020.

Gall midge culture: Fertile soil was collected from the field and fertilizers were mixed thoroughly. Twice in a week, seeds of susceptible variety TN 1 were soaked for germination and were sown in the plastic pots of 8 diameter and 10-inch height, 2 days later at the rate of 50-75 seeds/ pot. The potted plants were kept in the greenhouse with adequate light for 10-15 days after sowing. Eight potted plants were kept inside the oviposition cage covered by polythene cover. During evening 7.00 to 10.00 p.m. the adults of rice gall midge were collected near light source using aspirator developed by ICAR-IIRR (formerly Directorate of Rice Research (DRR)), Hyderabad and were released inside the oviposition cage for infestation. Twenty-five females and 25 males were released inside the oviposition cage containing 8-10 potted plants of 15-20 days old seedlings. Two cages were daily infested for routing rearing during the study period. Adults were provided with fresh 15-20 days old potted plants daily for oviposition. Two days after adult release, the potted plants were sprayed with water periodically at 2-3 h intervals to moisten the plants for egg hatching and for better movement of newly hatched maggot to reach the apical meristem region for better establishment and development. The potted plants were transferred to shallow water tray and water level of 2-3 cm above the basal part of the plant was maintained to create optimum humidity and to prevent predation of maggot. After gall formation, the potted plants were shifted from water tray to the adult emergence cage. The adults were collected every morning between 6.00 to 9.00 AM carefully with an aspirator. Then the collected adults were used for varietal screening, to study the mechanism of resistance and also for routine culture maintenance.

Hypersensitive reaction and Induced resistance: The studies on HR and induced resistance in gall midge resistant genotypes *viz.,* W 1263 (*Gm1* gene for resistance), Phalguna (*Gm2*), Abhaya (*Gm4*), ARC 5984 (*Gm5*), Jaya and TN 1 were undertaken at V.C. Farm, Mandya following Bentur and Kalode (1996). The local Asian rice gall midge biotype 1 population cultures were collected from the field under light source in the evening and maintained in the greenhouse.

Seedlings of W 1263 (*Gm1* gene for resistance), Phalguna (*Gm2*), Abhaya (*Gm4*), ARC 5984 (*Gm5*), Jaya and TN 1 were raised separately in plastic pots (10x8 inch) containing 5 hills groups of 2 seedlings. Such 10 seedlings in a pot represent replication and such 5 replications were maintained. When the seedlings attained 10-12 days, they were artificially infested with two fertile eggs of biotype 1 and an uninfested TN1 was maintained as control. Such 3 sets were maintained. The infested plants were observed for secondary tillers at 1, 7, 14 and 21 days after infestation and total numbers of tillers in each plant were recorded in first set. In second set, the infested plants were dissected to note HR and maggot mortality. In third set, the plants were infested with 2 fertile eggs of gall midge and the observations on total numbers of tillers in each genotypes at 1, 7, 14 and 21 days after infestation was taken.

Artificial infestation: Mated females were collected from the stock culture individually and held overnight for oviposition in air-tight plastic cups lined with moist filter paper at 25-28^{0}C. Fertile eggs could be observed after 3 days. Before maggot hatching, the gall midge eggs were collected using ordinary syringe along with tiny tissue paper under binocular microscope (Nikon SMZ 800N). the collected egg was placed between the leaf sheaths of central shoot. Such infested pots were carefully maintained till the establishment of maggots and gall. For 2 to 3 days the water was sprayed at 2 h intervals using hand atomizers to create high relative humidity for egg hutching and larval establishment. Egg hatching was verified by retrieving the filter paper bit one day after infestation and observing the empty eggshell. Plants were successfully infested using this technique with a fertile egg (Bentur and Kalode 1996) (**Figure 1).**

Figure 1: Artificial infestation of rice gall midge eggs for studying hypersensitive reaction

The observations on HR, maggot mortality and tiller bearing capacity were recorded on Phalguna, Abhaya and ARC5984. The presence of necrotic tissue in one of the tillers of the plant was considered enough to classify it as hypersensitive reaction (HR) and plants with two dead larvae in primary tiller was considered to show antibiosis. The plants were observed for HR reaction and maggot mortality at 2, 3, 4, 5 and 6 days after infestation. In third set, the tillers were infested with 2 fertile eggs of gall midge biotype 1 and the observations on total numbers of tillers in each genotypes at 1, 7, 14 and 21 days after infestation was taken. Further, in each genotype, the secondary tillers of Phalguna, Abhaya and ARC5984 were again infested by two more fertile eggs of gall midge biotype 1, at 7, 14, 21 and 28 days after primary tiller infestation. In each week of secondary infestation, the observations on HR and living larvae was taken (7, 14, 21 and 28) at 3, 4, 5, 6 and 7 days after secondary tiller infestation on 50 plants. Further, the collected data were subjected to Analysis of Variance (ANOVA) and means were separated by Tukey's HSD test (Tukey, 1953) for interpretation.

The resistant donors, Phalguna, Abhaya and ARC 5984 showed varying degrees of central leaf browning, including at times death of the entire leaf of young seedlings. The dissection of the apical region of the seedlings revealed extensive necrosis of meristem tissue. Dead first instar larvae were observed at the vicinity of the necrotic tissue. This response resembled a typical hypersensitive reaction (HR) (Fernandes, 1990) and this was observed in Phalguna, Abhaya and ARC 5984 resistant donors. In all the three resistant genotypes (Phalguna, Abhaya and ARC 5984) at 3, 4, 5, 6 and 7 days after secondary tiller infestation in each week after primary infestation, there was no expression of hypersensitive reaction but 100 cent per cent maggot mortality was observed. In W 1263 genotypes this phenomenon (HR) was not observed but antibiotic effects were seen with maggot mortality, while in susceptible check Jaya and TN 1 the insect completed life cycle successfully.

Studies indicated both extensive tissue necrosis at the apical meristem of the young seedlings, succeeded by maggot mortality and premature tillering. There was no significant difference among the resistant and susceptible donors with respect to number of tillers after a day of infestation. But, at 7 days after infestation, pre mature tillering was observed in all the resistant donors, except W1263. Significantly higher number of tillers were observed in Phalguna (1.42±0.20) followed by Abhaya (1.38±0.10) and ARC 5984 (1.34±0.00), while, it was not observed on susceptible TN 1, Jaya and also un-infested TN 1. Similar results were observed at 14 and 21 days after infestation indicating significant level of premature tillering on these resistant donors (**Table 1**).

Table 1. Effect of gall midge infestation on tiller bearing capacity in resistant and susceptible rice genotypes

Genotypes	No. tested*	Number of tillers/plant at DAI			
		1	7	14	21
Phalguna (*Gm2*)	50	1.00±0.00^{a}	1.42±0.20^{a}	1.72±0.40^{a}	2.41±0.12^{a}
Abhaya (*Gm4*)	50	1.00±0.00^{a}	1.38±0.10^{b}	1.64±0.20^{b}	2.09±0.10^{b}
ARC 5984 (*Gm5*)	50	1.00±0.00^{a}	1.34±0.00^{c}	1.61±0.24^{b}	1.98±0.20^{b}
Jaya (S)	50	1.00±0.00^{a}	1.00±0.00^{d}	1.00±0.00^{c}	1.10±0.22^{c}
TN 1 (S)	50	1.00±0.00^{a}	1.00±0.00^{d}	1.00±0.00^{c}	1.20±0.20^{c}
TN 1 (un-infested)	50	1.00±0.00^{a}	1.00±0.00^{d}	1.00±0.00^{c}	1.00±0.00^{c}

DAI-Days after infestation; *-@ 2eggs/seedling; *Gm2*, *Gm4*, *Gm5* are genes for resistance; S-Susceptible; Means in a column followed by different letter are significantly different as per Tukey's HSD (Tukey, 1953).

The detailed studies on HR and expression of maggot mortality in relation to gall midge infestation in Phalguna indicated that the HR expression started on third day of infestation (8.33 %) and 75 per cent of the plants infested with gall midge showed HR on the fifth day, and 100 per cent larval mortality within the same period (fifth day) (**Figure 2)**. The necrosis of the young apical meristem of the seedling when infested with gall midge is fatal to the plant (host plant) for further growth. The premature tillering was observed in resistant donor Phalguna after tissue necrosis. Further, the secondary tillers were again infested with the gall midge biotype 1 eggs at 7, 14, 21 and 28 days after primary infestation. However, HR was observed 6 days after secondary tiller infestation, when secondary tiller infestation followed by primary

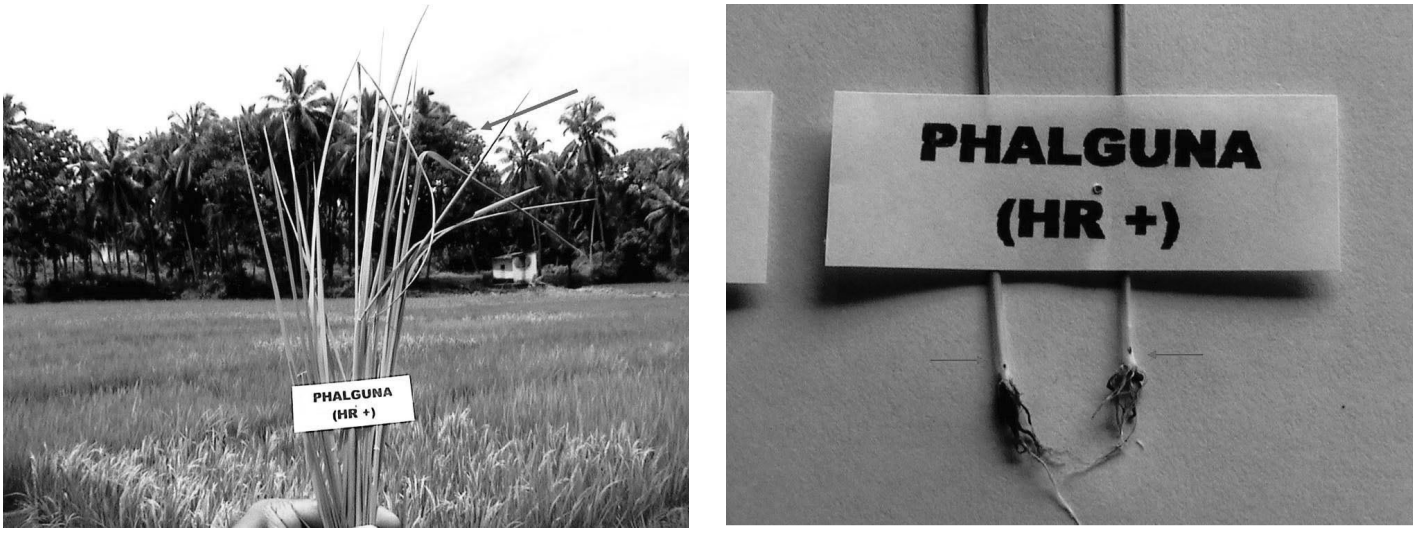


Figure 2: Expression of hypersensitive reaction (HR +) in Phalguna (*Gm2 gene*) in response to gall midge infestation

tiller infestation by 28 days. But cent per cent maggot mortality was observed, regardless of the time interval between infesting primary and secondary tillers. Mortality in secondary tillers without the expression of HR suggested that HR is not a prerequisite for maggot mortality. Further, a systemic induced resistance by primary infestation was evident which probably prevented other tillers from expressing HR. Thus, this study indicated that the HR was confined to the tillers of primary infestation and it also triggered systemic acquired resistance in other tillers (secondary tillers), and the duration and stability of the systemic acquired resistance in secondary tillers needs to be studied.

Despite many examples of HR of plants against pathogens such as fungi (De Wilt, 1992), bacteria (Jakobek *et al.,* 1993), and viruses (Ponz and Brueing, 1986; Zaitlin and Hull, 1987), there are only few examples of HR having any importance against insect herbivores (Fernandes, 1990). The known examples of plant hypersensitivity against insect herbivores come from gall forming aphids and tephritid flies (Anderson *et al.,* 1989). The present study may be the third instance of cecidomyiid gall formers after the reports of Bentur and Kalode (1990 and 1996). Tissue necrosis has also been described in wheat cultivars following attack by avirulent biotypes of the Hessian fly, *Mayetiola destructor* Say (Shukle *et al.,* 1992). Systemic acquired resistance is very well documented in pathogen-plant interactions (Bell, 1981). Necrosis of the apical meristem in all of the infested tillers would be fatal to the host plant. Hence, systematic acquired resistance rendering all other tillers resistant without necrosis has distinct survival value for the host plant. In pathogen-plant interactions the phenomenon of hypersensitivity is noted only against race specific defense and not in pathogen and non-host plant interactions (Bell, 1981). Likewise, the weed gall midge *Oreseolia fluvialis,* which can survive on *Paspalidium geminatum* and *Echinochloa crusgalli* but not on rice (Sain, 1988), did not elicit HR either in Phalguna or TN1 varieties of rice (Bentur and Kalode, 1996). Genetic diversity with (Phalguna, Abhaya and ARC 5984) or without (W1263) the expression of HR against rice gall midge was noted in the present study. Such diversity is known against pathogen (Dixon and Lamb, 1990).

These results on the HR and induced resistance by artificial infestation of biotype 1 indicated varied degrees of central leaf browning in Phalguna, Abhaya and ARC 5984 resistant genotypes. The dissection of the apical region of these seedlings revealed extensive necrosis of meristematic tissue. This response resembled a typical HR (Fernandes, 1990; Bentur and Kalode, 1990, 1996). While in W1263, this phenomenon was not observed but antibiotic effect was seen with maggot mortality. These results corroborate with the study made by Bentur and Kalode (1996). The detailed studies on HR in Phalguna, Abhaya and ARC 5984 indicated both extensive tissue necrosis at the apical meristem of the young seedlings, succeeded by maggot mortality and premature tillering. Significantly higher number of tillers was noticed in Phalguna followed by Abhaya and ARC 5984 at 7, 14 and 21 days after infestation compared to susceptible TN1 and un-infested TN1. Further studies indicated that the HR expression started on third day after infestation (8.33%) and 75 per cent of the infested plants showed HR on the fifth day along with 100 per cent maggot mortality in Phalguna (**Figure 3)**.

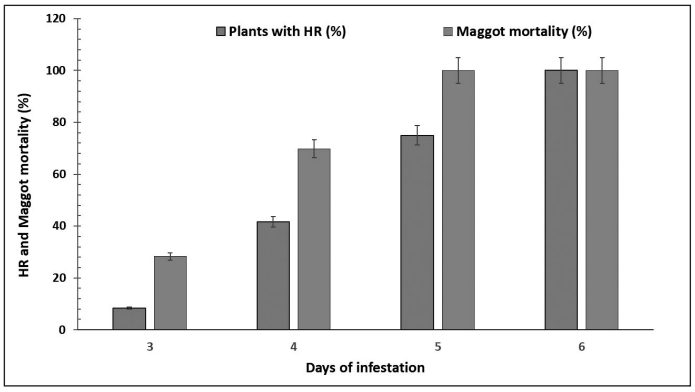


Figure 3: Hypersensitive reaction expression and maggot mortality in primary tillers of var Phalguna in response to gall midge infestation

Bentur and Kalode (1990, 1996) observed plants with cent per cent HR, results in maggot mortality on 5th day after infestation in Phalguna. But premature tillering was observed in resistant donors Phalguna, Abhaya and ARC 5984 subsequent to the tissue necrosis. Further studies, by infesting secondary tillers with gall midge eggs at 7, 14, 21 and 28 days after primary

infestation indicated 100 per cent maggot mortality, regardless of the time interval between infesting primary and secondary tillers.

Thus, the mortality in secondary tillers without the expression of HR suggested that HR is not a prerequisite for maggot mortality as reported by Bentur and Kalode (1996). Furthermore; a systemic induced resistance by primary infestation was evident which probably prevented other tillers (secondary tillers) from expressing HR. The present study revealed that the HR was confined to the tillers of primary infestation and it also triggered systemic acquired resistance in other tillers. Identification, field evaluation and utilization of such potential genotypes in rice breeding will lead to the suppression of virulent gall midge populations and stabilize the yields.

Acknowledgements

This work was supported and completely funded by Science and Engineering Research Board (SERB), Department of Science and Technology, Ministry of Science and Technology, Government of India under the Grant No. EEQ/2017/000484/26/3/2018. We thank Dr. J. S. Bentur, Principal Scientist (Rtd.) and Dr. Gururaj Katti, Principal Scientist, ICAR-Indian Institute of Rice Research, Hyderabad, India for continuous support and technical advice. The first author V.K.L acknowledges ICAR-Indian Institute of Rice Research, Hyderabad, India and AICRP on rice, V. C. Farm, Mandya for providing rice differentials and Post-graduate students of Department of Entomology, College of Agriculture, V. C. Farm, Mandya.

References

Anderson SS, Mccrea KD, Abrahamson WG and Hartzel LM. 1989. Host choice by the ball gall maker, *Eurosta solidaginis* (Diptera: Tephritidae). *Ecology*, 70: 1048-1054.

Bell AA. 1981. Biochemical mechanisms of disease resistance. *Annual Review of Plant Physiology,* 32: 21-81.

Bentur JS and Kalode MB. 1990. Hypersensitive reaction and induced resistance in rice against gall midge (*Orseolia oryzae* Wood-Mason). *Proceedings of International Symposium on Rice Research: New frontiers, November 15-18*. Directorate of Rice Research, Hyderabad, pp. 180-181.

Bentur JS and Kalode MB. 1996. Hypersensitive reaction and induced resistance in rice against Asian rice gall midge, *Orseolia oryzae*. *Entomologia Experimentalis et Applicata,* 78: 77-81.

Bentur JS, Pasalu IC and Kalode MB. 1992. Inheritance of virulence in rice-gall midge (*Orseolia oryzae*). *Indian Journal of Agricultural Sciences,* 62: 492-493.

Bentur JS, Pasalu IC, Sharma NP, Prasada Rao U and Mishra B. 2003. Gall midge resistance in rice: Current status in India and future strategies. *DRR Research Paper Series 01/2003*. Directorate of Rice Research, Rajendranagar, Hyderabad, pp. 20.

De Wilt PJGM. 1992. Molecular characterization for gene-for-gene system in plant-fungus interactions and in the application of Avirulent genes in control of plant pathogens. *Annual Review of Phytopathology*, 30: 391-418.

Devaiah MM. 1984. Further studies on the gall midge, *Orseolia oryzae* (Wood-Mason) (Cecidomyiidae: Diptera). *M. Sc. (Agri.) thesis*, Tamil Nadu Agricultural University, Coimbatore, pp. 107.

Dixon RA and Lamb CJ. 1990. Molecular communication in plant: microbial pathogen interactions. *Annual Review of Plant Physiology and Molecular Biology*, 41: 339-367.

Fernandes WG. 1990. Hypersensitivity: A neglected plant resistance mechanism against insect herbivores. *Environmental Entomology*, 19(5): 1173-1182.

Heinrichs EA and Pathak PK. 1981. Resistance to the gall midge, *Orseolia oryzae* (Wood-Mason) in rice. *Insect Science and its Application*, 1: 123-132.

Hidaka T and Vungsilabutr P. 1971. Study on larval development of rice gall midge on susceptible and resistant rice varieties. Annual Report, 1971, Rice

Department, Ministry of Agriculture, Bangkok, Thailand, pp. 728.

Hidaka T. 1974. Recent studies on the rice gall midge, *Orseolia oryzae* (Wood-Mason) (Cecidomyiidae: Diptera). *Review of Plant Protection Research,* **7**: 99-143.

Jakobek JL, Smith JA and Lindgreen PB. 1993. Suppression of bean defense responses by *Pseudomonas syringae*. *Plant Cell,* **5**: 57-63.

Joshi RC. 1982. Recent studies on rice gall midge, *Orseolia oryzae* (Wood-Mason) (Cecidomyiidae: Diptera). *M. Sc. (Agri.) thesis*, Tamil Nadu Agricultural University, Coimbatore, pp. 91.

Kalode MB. 1980. The rice gall midge: varietal resistance and chemical control. In: *Rice improvement in China and other Asian countries*. Manila (Philippines): International Rice Research Institute, pp. 173-193.

Kalode MB, Bentur JS and Rao UP. 1993. Rice cultures resistant to rice gall midge biotypes 1 and 4 under artificial infestation in greenhouse. *International Rice Research Notes*, 18(20): 17-18.

Khush GS. 1997. Breeding for resistance in rice. *Annals of the New York Academy of Science***,** 287: 296-308.

Mathur KC and Rajamani S. 1984. *Orseolia* and rice: Cecidogenous interactions. *Proceedings of Indian Academy of Sciences (Animal science),* 93: 283-292.

Mathur KC, Reddy PR, Rajamani S and Moorthi BTS. 1999. Integrated pest management in rice to improve productivity and sustainability. *Oryza*, 36(3): 195-207.

Pathak PK and Heinrichs EA. 1982. A bibliography of varietal resistance to the rice gall midge, *Orseolia oryzae* (Wood-Mason). London (UK): Commonwealth Agricultural Bureaux, pp. 45.

Ponz F and Brueing G. 1986. Mechanism of resistance to plant viruses. *Annual Review of Phytopathology*, 24: 355-381.

Sain M and Kalode MB. 1988. Production of unisexual progeny in rice gall midge, *Orseolia oryzae* (Wood-Mason). *Current Science,* 57: 860-861.

Sain M and Kalode MB. 1994. Greenhouse evaluation of rice cultivars for resistance to gall midge, *Orseolia oryzae* (Wood-Mason) and studies on mechanism of resistance. *Insect Science and its Application,* 15: 67-74.

Sastry MVS, Kalode MB, Prasada Rao U and Pophaly DJ. 1976. inheritance and interrelationships of genes governing resistance to rice gall midge, *Orseolia oryzae*. *Journal of Biological Science,* 19: 25-27.

Shukle RH, Grover PB and Mocelin G. 1992. Response of susceptible and resistant wheat associated with Hessian fly (Diptera: Cecidomyiidae) infestation. *Environmental Entomology*, 21: 845-853.

Tukey JW. 1953. The problem of multiple comparison, Princeton, New Jersey, Princeton University, Department of Statistics. 396

Vijaykumar L, Chakravarthy AK and Thyagaraj NE. 2008. Detection of Asian rice gall midge, *Orseolia oryzae* (Wood-Mason) biotype 1 in the new locations of Karnataka, South India. *Bull. Insectology* 61: 277-281.

Vijaya Lakshmi P, Amushan S, Hima Bindu K, Cheralu C and Bentur JS. 2006. A new biotype of the Asian rice gall midge *Orseolia oryzae* (Diptera: Cecidomyiidae) characterized from the Warangal population in Andhra Pradesh, India. *International Journal of Tropical Insect Science* 26: 207 – 211.

Zaitlin M and Hull R. 1987. Plant virus-host interactions. *Annual Review of Plant Physiology,* 38: 291-315.

JRR Best Paper Award

Guidelines for 2022

Purpose

The purpose of the Best Paper Award is to recognize and promote quality contributions to the Journal of Rice Research (JRR) and encourage young scientists, scholars and students who publish papers.

Eligibility

All the authors of one Research article or Review article or Short Communication published in the JRR during June and December of every year (as a regular or special issue submission) are eligible for this award. There are no limits in the number of authors involved.

Criteria

The best paper will be selected by a committee (Outside experts & Editorial Board members). Special attention will be given to the originality and novelty of the paper content, and to its utility. Additional criteria will be fixed by the committee. Award will start from June 2022 issue onwards.

How to Apply

All the published papers are automatically included in the list of papers for evaluation. No action is required.

Award

The "JRR Best paper award" includes:

- A certificate
- A Memento and
- A Cash prize

Journal of Rice Research - Authors Guidelines

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

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

General Requirement:

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

Submission of Manuscript:

Manuscripts should be sent by email to the chief editor (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.

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

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

Research papers

1. Durvasula V. Seshu**.** 2017. Networking a Pivotal Strategy for Rice Genetic Improvement. *Journal of Rice Research*, 10(1): 1-8.
2. Kemparaju KB, MS Ramesha, K Sruti, AS Hari Prasad, RM Sundaram, P Senguttuvel and P Revathi**.** 2018. Breeding strategy for improvement of rice maintainer lines through composite population for short term diversity. *Journal of Rice Research*, 11(2): 27-30
3. Paul M and Keegstra K. 2008. Cell-wall carbohydrates and their modification as a resource for biofuels. *Plant Journal,* 54: 559-568.

Thesis

Bhuiyan MDAR. 2010. Phenotypic and genotypic evaluation of selected transgressive variants derived from *Oryza rufipogon* Griff. x *Oryza sativa* L. cv. MR219. Ph D. Thesis. University Kebaangsaan Malaysia, Malaysia, 150 p.

Book chapter

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

Book

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

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

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

Published by :

Dr. RM Sundaram, President, Society for Advancement of Rice Research, Hyderabad.

Printed at :

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

Society for Advancement of Rice Research

ICAR-Indian Institute of Rice Research (ICAR-IIRR) Rajendranagar

Hyderabad – 500 030 (India), Phone No: 091 40 24591218
Fax: +91 40 24591217, www.sarr.co.in