ISSN 2319-3670

Journal of Rice Research

Volume 12, No. 2

December 2019

Society for Advancement of Rice Research

Society For Advancement of Rice Research

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

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

Aims and Objectives

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

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

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ISSN 2319-3670

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Dr. SVS Shastry Memorial Lecture

Designing future rice for enhanced profitability and nutritional security

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At the outset I wish to express my heartfelt gratitude to the Organizing Committee, ICAR-Indian Institute of Rice Research (ICAR-IIRR) and Society for Advancement of Rice Research (SARR), Hyderabad for giving me an opportunity to deliver the first Dr. S.V.S. Shastry Memorial Lecture 2019. It is indeed a great honour and am humbled with this noble association with Dr. Sishta Venkata Seetharama Shastry, with whom my association dates back to *Kharif* 1969 when he himself came to monitor the AICRIP trials. The trials were conducted at IARI Regional Station, Karnal. We travelled together and he knew the details of each and every trial being conducted at Karnal. Thus, through his exceptional vision and meticulous planning as the Project Coordinator, he spearheaded the All India Coordinated Rice Improvement Project (AICRIP), the first of its kind in the world for rice varietal evaluation and release.

The vision of Dr. Shastry was farfetched with respect to crop improvement in India, especially rice improvement. As a rice cytogeneticist, he published the seminal report on the pachytene analysis in rice for the first time (Shastry *et al.*, 1960). As an evolutionary biologist, his extensive research on biosystematics of *Oryza* complex helped in delineating the taxonomic identity of two new species of *Oryza* namely, *Oryza nivara* and *O. collina*. Based on their studies, they proposed that *O. nivara* is the progenitor of cultivated rice (Shastry and Sharma, 1973). One of the collections of *O. nivara* was found to be resistant to grassy stunt virus disease of rice at International Rice Research Institute (IRRI), Philippines which helped the development of grassy stunt virus resistant rice varieties. As a pioneer in rice varietal evaluation system in the country, he extended his interest on crop adaptation traits, extensively searching the gene pool for resistance to pests and diseases, tolerance to nutrient deficiency over and above the major focus of his research on yield improvement. Dr. Shastry was an ardent fighter for poverty alleviation worldwide, particularly in India, and he was one of the early researchers to realise the importance of improving rice yield in alleviating hunger in the country. He was conscious of rice grain quality and with his futuristic vision was instrumental in constituting the first slender grain varietal trial in 1969, which was later renamed as Basmati derivative trial. This trial later on evolved as National Basmati Trials. He has developed the premium quality slender grain rice variety, "Sona", which was widely used as one of the parents for developing fine grain rice varieties such as Haryana Basmati 1 (Sona/ Basmati 370).

Dr. Shastry recorded his futuristic views on rice breeding in India in an article published in the journal Crop Science, which I quote "This shift in priority from production to productivity, and then on to profitability is an index of progress with technology. It is also the continuing challenge for the future" (Shastry 2006). He was referring to the transformation of rice varietal evaluation system from production improvement as happened in the early green revolution period, to improved productivity as a function of grain output per unit land area, as it was realized later towards the end of last millennia. The transformation from the days of 'hands to mouth' to the 'enough in hands' has been a reality mainly because of the continuous crop improvement that has happened during the active period of Dr. Shastry as the founder leader of AICRIP system in India. Now, in order to metamorphose from the 'handful', I believe that there is a need to change the paradigm from "productivity" to "profitability" which needs to be addressed by the rice improvement programmes across the country. This has prompted me to address this important issue of "continuing challenge for the future of rice" in this maiden lecture commemorating Dr. Shastry titled "Designing future rice for enhanced profitability and nutritional security".

Present Status of Rice in India

India produced 112 million tonnes of rice from an area of 43 mha at an average productivity of 2.6 t/ ha. Out of the total area, 24 mha of rice area in Punjab, Haryana, Uttar Pradesh, West Bengal, Bihar, Odisha, and parts of Andhra

Pradesh, Tamil Nadu is irrigated. About 17.2 mha is under rainfed area, out of which more than 70% of which is in eastern India. Around 85% of the total rice production is contributed by the favourable ecologies of irrigated and shallow lowland. Rice is the most resource intensive crop among all the agricultural crops in India and annually it uses about 200 km3 of irrigation water, 6.5 mt of fertilizers, 17% pesticides used in Indian agriculture and emits 3.5 mt of methane (Pathak *et al.,* 2019).

Rice in India is at a critical juncture. The monopolistic development in rice area and production has almost stalled and the climb in the graph that has been witnessed since mid-sixty's has reached a plateau. The primary reason for this is the increase in human population which always outperformed the rate of agrarian development. This trend could be worrisome, because, if this level continues without significant improvement in food availability, we might be returning back to the dreaded olden days where hunger dominated our society. However, future is still bright, thanks to the explosive development in science especially genetics, that has already proven its role in securing food security by the way of green revolution. If one or few genes such as the dwarfing gene could produce a green revolution there are about 55,986 genes on rice genome that can be relied upon.

Achievement in Rice Improvement

Since 1950s, India has witnessed release of more than 1500 rice varieties, suitable for different agro-ecological zones. Comparing the yield levels of these varieties, one cannot witness any perceptible yield increase in the varieties that were released later than from the earlier ones. For instance, the popular variety, "Jaya" bred by Dr Shastry released in 1969 has an estimated yield level about 55-60 q/ha. Still, yield of Jaya can be regarded comparable to that of a variety released in 2000's. This implies that we are lacking somewhere in our assessment especially the yardstick for promoting a variety, which needs introspection. There have been a large number of landmark varieties evolved and released over time in India, which have ruled the rice production scenario in India and other rice growing countries during last few decades. The mega-varieties of rice such as Jaya, Swarna (MTU7029), Savithri (CR1009), Samba Mahsuri (BPT5204), IR36, IR64, Pusa Basmati 1, Cotton Dora Sannalu (MTU1010), Pusa Basmati 1121 and some new ones like Pusa Basmati 1509 slowly catching up with its popularity.

The green revolution could help in bringing a major leap in rice production with the milled grain production of 34.6 million tonnes in 1960 increasing to 115.0 million tonnes in 2019. The growth has been almost steady although few falls in the trend has been witnessed in some of the years. Furthermore, the overall rice production trend in India still pointed upwards trend in spite of a reduction in total area. The modern Indian rice varieties have been made smart, from the high resource hungriness to resource efficient. Thanks to the use of streamlined breeding efforts using precise tools of genomics, the modern-day rice varieties are tolerant to drought making it more water use efficient, resistant to disease and pest, and other abiotic stresses such as submergence, salinity and sodicity and as I understand efforts to develop rice varieties with improved nutrient use efficiencies and enhanced tolerance to low nutrient are at advanced stages especially for phosphorus (P) with the introgression of PSTOL locus which provides tolerance to low P. A beginning has been made and it has traverse long to meet the needs of the future. Our hope, lies with more than 55,986 genes on the rice genome and with more than 40,000 rice genotypes, in which these genes are being ingrained in different forms (alleles) together with an additional pool of more than 90,000 related wild and cultivated lines.

Challenges in Rice Research

India currently grows rice in about 43 million ha, almost a million hectare has been lost for rice production during this decade so far, owing to several factors such as shrinking water availability, nutrient depletion, uncertain rains, soil salinization, urbanisation, cropping system changes and poor economic returns. Comparing the global scenario, the loss is significant, because worldwide trend in rice area still remains upwards except China, although the slope has declined. Beginning from the days of green revolution, after the introduction of famous sd1 gene into the breeding lines from the Taiwanese variety, Dee-Geo-Woo-Gen, the average rice yield worldwide has doubled from 2.0 to 4.0 tonnes/ha (www.ricepedia.org). The increase corresponding to this period in India is however not in tune to the world figure, a meagre increase of 2.2 tonnes/ha from 0.7 tonnes/ha. The rice production, although indispensable, has been always a challenge to Indian farmers. Alternatively, the introduction of high yielding varieties has witnessed another dramatic climb in the area under the modern varieties. The area of less than 100 ha under HYVs in 1965, has risen to more than 40 million ha by the late 2010. The corresponding increase in total rice area was from 30 million ha to 40 million ha. Therefore, it is a fact that during green revolution, modern varieties not only replaced most of the 30 million ha occupied by traditional cultivars, but also brought in another 10 million ha under rice cultivation. Most of these areas came from non-traditional regions such as Punjab, Haryana and western Uttar Pradesh, where once the cultivation of rice was very limited. Long term consequence of this shift in cultivation aimed at higher earnings from farm holdings with support in the form of minimum support price (MSP) has led to significant damages to the environment and depletion of groundwater. The dwindling water resources has forced the ban on rice planting until mid-June, in states like Punjab and Haryana. The repercussions of pushing rice towards the fringes of wheat cultivation window, is now seen as air pollution by indiscriminate burning of stubbles with the aim to clear the lands in short period of time.

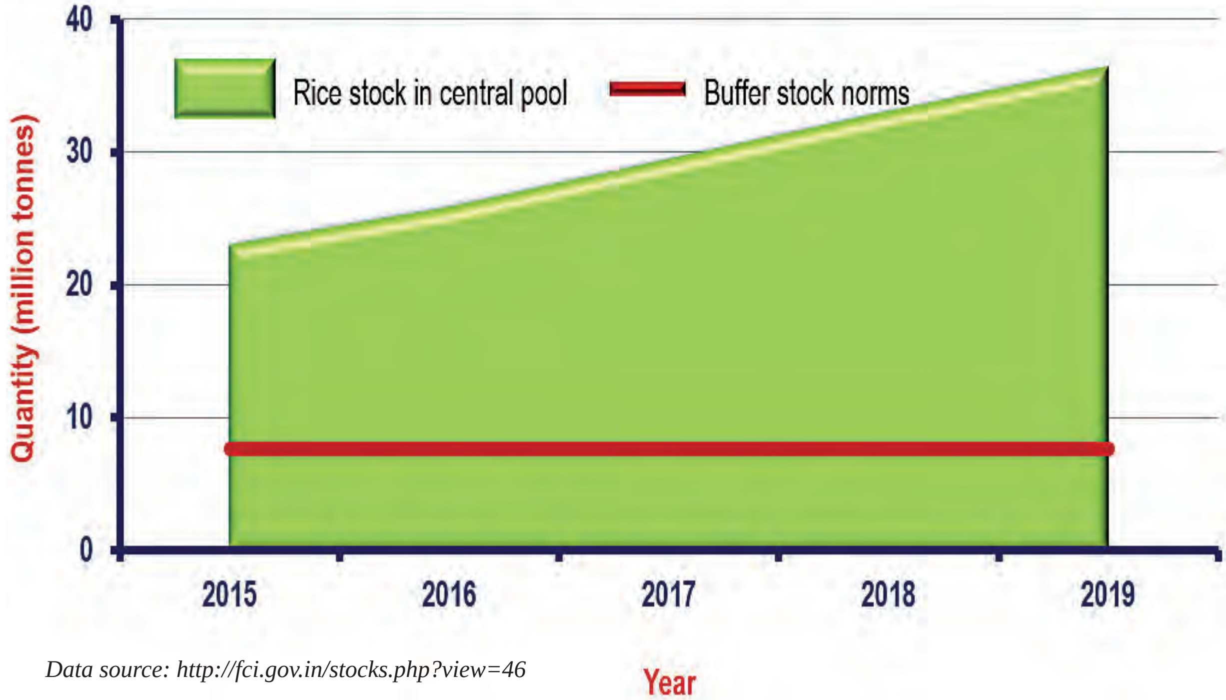
There was a concern raised by the critics that due to introduction of high yielding varieties there has been a severe loss of the wealth of traditional rice varieties in India. Taking a critical look, it is not hard to realise that most of these traditional cultivars are conserved in our National Gene Bank and valuable alleles, if any can be brought back into the modern-day cultivars using precision tools available to modern day breeders. In contrary, the high yielding modern varieties have in fact amassed a great proportion of alleles for yield and resource efficiency. Later, emphasis on quality was introduced, especially in the 1980s, which had brought out several commercially important varieties particularly known for grain quality. However, in spite of the boom in high yielding varieties, there has been several traditional cultivars conserved in the farm holds for special purposes that are even grown today. These landraces could be treasure trove for alleles governing specialty traits in rice.

Rice is consumed by 0.8 billion people across the country. In order to meet our commitments of the Sustainable Development Goals especially goal 2 (Zero hunger), we need to produce safe nutritious and sufficient food, which would be around 135 mt of rice by 2030. All along, we need to ensure doubling both agricultural productivity and incomes of small farmers. Add to this the limitations imposed by climate change and our commitment to maintain ecosystems and the genetic diversity of seeds, there is a need for introspection and act accordingly.

The severity in incidence of the major biotic and abiotic stresses, changing scenario of hitherto minor diseases, insect-pests and weeds causing major economic losses due to climate change are new challenges for rice improvement. For example, the increase in incidence of bakanae disease in Basmati rice, panicle blight in rice across northern India, false smut across rice growing regions of India especially in eastern India, brown plant hopper in rice, flash floods due to skewed rainfall distribution in eastern India and salt accumulation in coastal regions due to ingression of sea water needs to be addressed through strategies involving transdisciplinary research.

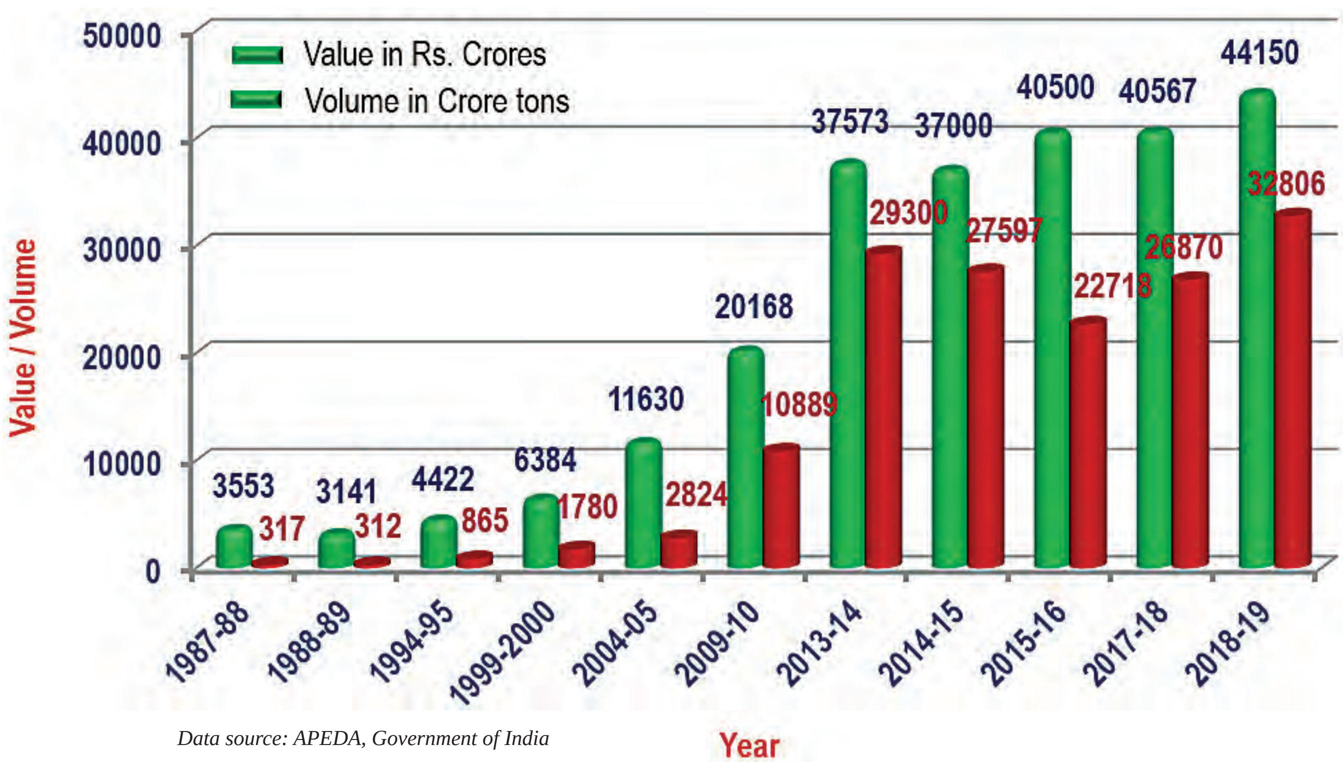
About 4.5 mha of the irrigated rice is cultivated in Punjab and Haryana, out of which about 3.3 mha is under non-Basmati rice which is primarily meant for the public distribution system (PDS). A cursory glance at the buffers stock norms and the actual stocks of rice in the central pool during the last five years (Fig. 1) indicates that there is around 4 times more rice stock in the central pool on an average over this period, which goes unattended in open storage resulting in huge losses if there is untimely rains after procurement.

Based on the basic principles of availability, accessibility and absorption, the problems of fast depleting ground water, unbalanced fertilizer use, indiscriminate use of agro-chemicals and other issues associated such as paddy straw burning for fast disposal resulting in air pollution, growing non-Basmati rice for PDS in these states are moving towards unsustainability in the long-term. With abundant water and well suited agro-climate the rice cultivation and schemes such as Bringing Green Revolution to Eastern India (BGREI), it will not take long for the sleeping giant in the eastern India to produce rice for not only meeting the needs of the PDS of the entire country but also for the potential export markets. In fact, there is a need to reduce the area under rice to around 35 mha, where there is a potential to increase the productivity to 5.0 t/ha, so that the 8 mha from the currently cultivated area with comparatively less potential for rice cultivation can be diverted for other crops.



Data source: http://fci.gov.in/stocks.php?view=46

Fig. 1: The buffer stock norms and the actual stocks of rice in the central pool during the last five years



Data source: APEDA, Government of India

Fig. 2: Trends in Basmati rice exports and foreign exchange earned.

Need for a Change in Paradigm

There is a need for paradigm shift in rice research, wherein it is time to focus from enhancing productivity to profitability enhancement which needs to take into account several factors including (a) economizing the cost of cultivation through development of rice varieties with resistance to major insect-pests, diseases and weeds thereby minimizing the potential risk of pesticide residues, (b) improving the water productivity by developing varieties suited for aerobic/ limited water environments, (c) making sure the timely availability of quality seeds and other agro-inputs in reasonable price, and (d) enacting policy measures for easy marketing and timely payment for the rice produce.

India is endowed with rich diversity of specialty rice including Anterved, Atmashital, Banspatri, Gangaprsad, Kadamphool, Kapursar, Loktimachhi, Shri Kamal, Tilkasturi, Gandhagasala, Keoni, Dudheswar, Dudda Dhan, Devbhog, Kavuni, Navara, Naa maa lhaa lay, Chakhao and Vishnubhog from different parts of the country. India is renowned across the globe for Basmati rice. Systematic research initiated by none other than the father of green revolution, Prof. M. S. Swaminathan, we have made huge strides in combining exceptional grain quality of Basmati with productivity improvement over the last eight and half decades. Scientific studies and proper follow up has resulted in development of exquisite Basmati rice varieties such as Pusa Basmati 1, Pusa Basmati 1121, Pusa Basmati 6 (Pusa 1401) and Pusa Basmati 1509, which has significantly improved the foreign exchange through the export (Fig. 2) resulting in not only improving the balance of trade for India but also created a Basmati revolution in the country by brining in prosperity to different stake holders including the farmers, seed producers, millers, exporters and consumers.

Noteworthy is the profit realisation by the Basmati growing farmers to more than four times since the release of Basmati 370. There is a need to emulate the Basmati rice model for creating the value chain for other specialty rice. Although there has been efforts made to develop nutritionally rich rice varieties such as low glycemic index rice variety, “Madhuraj 55” from the land race Chapati Gurmatiya, high zinc rice variety, “DRR Dhan 45”, high protein rice variety, “CR Dhan 10”, there is a need to strengthen our efforts towards nutrition rich high quality rice varieties. This can be possible through (a) validation of the medicinal properties of the specialty rice through metabolic profiling, (b) coordinated efforts among the national rice researchers in partnership to strengthen the efforts on improvement and designing appropriate package of practices, (c) more emphasis on the grain quality characters such as high density grain, higher brown rice per cent (> 80%), high head rice recovery, non-chalky grains, better mouthfeel, taste, palatability, faster cooking, longer shelf life and staleness of the rice entries being tested (d) constituting an independent body equipped with modern digital tools for organizing the coordinated trials to ease the burden on the rice researchers across the country, (e) time to time interaction with various stakeholders including farmers, millers, exporters and policy makers, (e) modernizing the rice breeding programmes by equipping them with modern technologies including artificial intelligence and modelling for assessing the rice genotypes, growing environments and testing facilities.

Conclusion

In conclusion, there is a need for coordinated efforts involving all stakeholders concerned including consumers, millers, farmers, exporters and scientists to realise the slogan “Dhan-Kisan-Vigyan-Udhyog”. As scientific researchers we need to gear up ourselves with modern scientific tools to address the emerging challenges in rice research. Finally, based on my own experience, I would like to emphasise to the young researchers that with all the enablers in place, the motivation to find joy in the testimony by the stakeholders and to work as a team is very important in shaping a better rice for the future.

Dr. Vijai Pal Singh

Born on January 17, 1945, Dr. Vijay Pal Singh received his M.Sc.(Ag) in Agricultural Botany in 1966 and Ph.D. in Agricultural Botany in 1977 from Agra University, Agra. Dr. Singh joined as Seed Production Assistant in the National Seeds Corporation in 1967 and later joined as Research Assistant in the Division of Genetics, Indian Agricultural Research Institute in 1968. Since then, he has been actively engaged in basic and applied aspects of rice research and retired as Project Leader (Rice) in January, 2007. Dr. Singh has served Agricultural and Processed Food Export Development Agency as Advisor in Basmati Development Fund and then a key member in establishing the state of art facilities at the Basmati Export Development Foundation at Modipuram, Meerut, Uttar Pradesh. Dr. Singh has done commendable work in understanding the genetics of quality traits in rice in general and Basmati rice in particular. He has been instrumental in developing the minimum quality standards for Basmati rice, which serves as the benchmark for the rice breeding programs across the world. Apart from application oriented basic research, he has been actively engaged in the development of a large number of high-yielding aromatic and non-aromatic rice varieties including Pusa 2-21 and Pusa 44, which brought the revolution in basmati productivity. Dr. Singh's significant contribution in Basmati rice improvement began with the development and release of the first high-yielding, semi-dwarf, non-lodging variety with Basmati quality characteristics, Pusa Basmati 1 in 1989, which has brought about Rs. 14,000 crores of foreign exchange earning to India and prosperity to millions of Basmati farmers of north-western India. His landmark contribution came in the form of phenomenal popularity of Pusa Basmati 1121, owing to its overwhelming acceptance in both national and global rice consumers, which led to the new era of prosperity for different stakeholders in Basmati rice supply chain including farmers, traders, millers and exporters. This is considered as one of the best models for improving the profitability and sustaining the rice supply chain across the world. Besides this, he has been instrumental in conceptualization and development of the country's first product of molecular breeding, Improved Pusa Basmati 1, by introgressing two genes, xa13 and Xa21 governing resistance to bacterial blight and another exquisite Basmati rice cultivar, Pusa Basmati 6 with excellent cooking and eating quality. He has guided four M.Sc. and seven Ph.D. students of which two have been awarded the Jawahar Lal Nehru Outstanding Doctoral Thesis Award by ICAR. He has published more than 30 research papers in reputed national and international journals, written 6 book chapters and has been member of the PG School faculty, IARI, New Delhi, from 1986-2007. Recognising the impact made through his outstanding contribution in Basmati rice improvement, he was honoured by conferred Padma Shri by the President of India in 2012. He has been awarded Hari Om Ashram Trust Award (1974-75), Jawahar Lal Nehru Award for outstanding doctoral thesis (1977), Dr. B.P. Pal Memorial Award (2005), ICAR Team Award for Crop Improvement (2007) and Sh. O.P. Bhasin Award (2012). He is fellow of the National Academy of Agricultural Sciences, New Delhi; Indian Society of Genetics and Plant Breeding, New Delhi and Association of Rice Research Workers, Cuttack.

Society for Advancement of Rice Research congratulates Dr Vijai pal Singh, first recipient of Dr SVS Shastry Award - 2019

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Rice production in India-varietal dynamics and diversity: Insights from empirical analysis of breeder seed indents

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Received: 18 September 2019; Accepted: 16 December 2019

Abstract

In the present study an attempt has been made to analyse rice varietal dynamics and diversity in India, using breeder seed indents data of selected years. An attempt has also been made to identify underlying factors of varietal dynamics and measures to improve varietal turnover by reviewing literature. It is observed that in recent years there is increase in rice varietal diversity at All India level. However higher weighted average age of top 10 varieties is indicating that varietal replacement is taking place with substitution by older varieties. In case of Basmati rice varieties also varietal diversity increased over the years in terms of number of varieties and decreasing share of top 3 varieties. In indent for *Kharif* 2015 and *Kharif* 2020, Seed Association of India share was 20 and 31 per cent, respectively. Multiple factors are influencing rice varietal dynamics. Hence for promoting adoption of improved rice varieties with reduced adoption lag there is a need for multi-pronged strategy. Targeted extension interventions based on share of farmers of different types of learning patterns, nudging varietal adoption behaviour by leveraging policies of subsidy and competition in seed sector, encouraging private sector participation in research and varietal commercialization, facilitating marketing of output of rice varieties of different durations by synchronizing marketing periods with crop harvesting period and participatory plant breeding are some of the suggested components in the multi-pronged strategy. In future different mechanisms for indenting for varieties and price fixation for breeder seed based on whether a variety is protected or not under PPV&FR Act, may also influence varietal dynamics.

Keywords: Varietal dynamics, Varietal diversity, Varietal concentration, Paddy, Rice, Weighted average age

Introduction

Rice is the main staple crop in India. In 2017-18 rice was cultivated in 43.79 million hectares in India, resulting in rice production of 112.91 million tonnes (DES, 2019). In 2016-17, Gross Value of Output (GVO) of rice crop (1.76 lakh crore rupees at 2011-12 prices) constituted 13.8 per cent of total GVO of crops and 50.8 per cent of GVO of cereals in India in monetary terms (GOI, 2019). In spite of its importance, rice yield in India is much below global average level. In 2017-18, rice yield across important rice cultivating states in India ranged from 1256 Kg per hectare (Chhattisgarh) to 4366 Kg/ha (Punjab), resulting in average yield of 2578 Kg per hectare at all India level. In 2016, average paddy yields in India stood at 3790 Kg/ha against world average of 4577 Kg/ha (DES, 2019).

Using simulation models, some studies reported heterogeneous impact of changes in various climatic factors on autumn, winter and summer rice in various ecosystems in India (Dabi and Khannan, 2018) and Asia (Matthews *et al*., 1997). One third of rice area in India is affected by drought (Birthal *et al*., 2015). Hence, efforts are being made to develop rice varieties/hybrids with higher productivity, climate resilience and biotic stress tolerance, and suitable for different ecosystems in India, through All India Coordinated Rice Improvement Project (AICRIP). So far 1329 high yielding rice varieties, which include 107 rice hybrids were released under AICRIP (Rao *et al*., 2019). These varieties are developed not only by public sector but also by private sector. More specifically, in hybrid rice development, private sector is playing important role (Senguttuvel *et al*., 2019) and its share in total rice hybrids released stands at 66 per cent. The aggregate effect of these crop improvement efforts in rice economy will depend on spread and adoption extent of these varieties across various ecosystems in the country. Further rice varietal diversity in a given region/ecosystem can contribute to risk reduction in production besides influencing yield directly. Duncan *et al*. (2017) reported that at All India level rice crop yield sensitivity to year to year fluctuations in climate shocks (drought and extreme heat exposure) has not decreased over time (during 1980 to 2009).

Studies on varietal adoption dynamics and varietal diversity in different crops including rice, used diverse data sources *viz.*, field level data (Byerlee and Heisey.,1990; Joshi *et al.*, 2018a), data collected through expert elicitation (Pandey *et al.*, 2012 & 2015; Pavithra *et al.*, 2017; Witcombe *et al.*, 2017; Pavithra *et al.*, 2018), certified seed distribution (Praveen *et al.*, 2017) and breeders seed indent/production data (Virk *et al.*, 1995, Witcombe *et al.*, 1998). Tsusaka et al. (2015) and Pandey et al. (2015) employed household surveys to validate the estimates obtained from expert elicitation in the context of South Asian countries. Singh and Kalra (2002) used crop cutting experiments data for analysing rice varietal adoption pattern in Punjab state of India.

Virk *et al.* (1995) and Witcombe *et al.* (1998) reported that at All India level weighted average age of rice varieties was 11.5 years during 1993. But the age of oldest cultivar was 25 years and its share in total breeder seed indent was 3 per cent. Further they reported that during 1993, there was breeder seed indent for 20 varieties of rice and most popular 3 varieties share in indent was 38 per cent during 1986-88 to 1990-93. Witcombe *et al.* (1998) observed that during 1986-88 to 1990-93, share of top 10 rice varieties in breeder seed indent was 60.4 per cent. Virk *et al.* (1995) argued that one of the major reasons for low adoption and replacement was lack of quick and wider dissemination of information about new rice varieties released and poor popularization.

Singh and Kalra (2002) reported that in Indian Punjab, there had been about 10 to 12 varieties in the field in any year during 1984-85 to 1998-99 and share of basmati variety ranged between 3 to 7 per cent in different years (despite of its lower yield compared to other high yielding varieties and not being covered under price support program) due to price advantage associated with it. They reported that rice variety PR-106 remained dominant for a decade and covered 63 per cent area in 1991-92 and was relegated to third position in 1998-99 by Pusa 44 and PR 111 varieties. They observed that aggregate level varietal diversity index in rice was not able to explain yield levels but average age of variety positively affected the yield of rice. They inferred that varieties with more stable yield stayed longer in field. Singh (2010) viewed that though Pusa 44 variety was not recommended for Punjab (in view of its high water requirement and susceptibility to bacterial leaf blight) it became popular with farmers due to higher yield and better quality. Manan *et al.* (2018) in the context of Kapurtala district of Indian Punjab (where farmers grow three crops per year), observed that farmers experience of problem in marketing of Paddy variety PR-126 (because of its early harvesting time than that notified by the Government) led to their preference for PR-121 variety.

Joshi *et al.*, (2018a) reported that in Punjab, “varietal stickiness” *i.e.* inertia to change from long duration Pusa-44 variety rice- (which was released in 1994) was due to combination of three factors *viz.*; higher yields of the variety, assured procurement and tariff free electricity. On the contrary Joshi *et al.*, (2018b) observed that the average age of basmati rice varieties cultivated in Punjab and Haryana was six years. This indicates that varietal replacement was faster in basmati rice. *Joshi et al.*, (2018b) also observed higher varietal diversity in Haryana compared to Punjab. They inferred that public private partnership, farmers’ beliefs regarding attributes (pest resistance, more yield of fodder, premium price for grain) and influence of early adopters (peer effect) have played role in faster diffusion of Pusa Basmati-1121 rice variety in Punjab and Haryana. Singh *et al.* (2018) reported that compared to traditional basmati rice, Pusa Basmati-1121 was of shorter duration and with double yield. These attributes also led to wider adoption of variety with a share of 63% of area under basmati rice in India in the year 2015. However, Pusa Basmati-1121 became susceptible to pests and diseases and hence being used as a parent in developing biotic stress resistant basmati varieties.

Gauchan and Pandey (2012) reported that a handful of older vintage improved rice varieties dominated in South Asia and the average varietal age was found to be in excess of 19 years in all locations studied. They reported that in India, rice variety Lalat was dominant in drought prone areas in Odisha, Mahsuri in submergence prone environment in Assam, Ranjit in submergence, prone environment in Assam and West Bengal, Swarna in all environments. Walker *et al.* (2015) observed average age of modern varieties of rice as 15.8 years and adoption rate of 38 per cent in Sub Saharan Africa. Malabayabas *et al.* (2012) reported weighted average age of paddy varieties in eastern India as 31 years indicating lower adoption of modern varieties. Singh (2015) reported that 50% varieties released during 2001-2013 were in active seed chain in India. However, during 2012-2014, only 24 varieties in breeder seed indent (out of indent for 226-275 varieties) constituted approximately 60 per cent share. Out of these 24 varieties, 13 were new varieties (released after 2001). Pandey *et al*., (2015) reported the average rice varietal age in Nepal as 20 years based on expert elicitation method and 24 years based on a household survey. Tsusaka *et al*. (2015) observed that average varietal age (as of year 2010) in rice ranged between 15 (Bhutan) to 23 years (West Bengal, India) in the case of different south Asian countries. They further observed that average lag in adoption ranged from 11-15 years, with the exception of Bhutan (7 years). Further, they observed that area under rice varieties of more than 10 years, constituted more than 60 per cent of area planted. Witcombe *et al*., (2017) in the context of Nepal, analysing household data pertaining to 18 districts at two points of time (*i.e*., 2008 and 2011) reported increase in rice varietal diversity. They observed that 13 varieties made up 75 per cent of the area in 2011 instead of nine in 2008. They also observed spatial variation in varietal distribution. Further, the average varietal age of varieties covering 75 per cent of area was 21.8 years in both the years and only two varieties were of less than 15 years age. Weighted average age of predominant varieties was 23 and 22.5 years in 2008 and 2011, respectively. Pandey *et al*., (2017) in the context of Odisha state in India, observed that in 2014, top 10 rice varieties seeds in public system accounted for 95 per cent of total quantity produced, top three varieties constituted 70 per cent of total seed production. Further all the three top varieties were released prior to year 2000. Atlin *et al*. (2017) reported that in India, the weighted average age of rainfed rice in the year 2014 was 28 years.

Veettil *et al*. (2018) in a study focusing on Bihar, West Bengal and Odisha states (based on farm level data) observed that average age of varieties was highest in the case of Bihar (38.37 years) followed by 32.28 years in case of West Bengal and 23.66 years in case of Odisha. On an average, the varieties were replaced in every 7.30 years and the seeds were replaced in every 2.75 years. Bihar was the state with fastest varietal replacement (5.31 years) followed by Odisha and West Bengal (8.35 years). Bihar was the state with fastest seed replacement (1.57 years) followed by West Bengal and Odisha (3.09 years). They also observed that varietal replacement is done with older varieties instead of new varieties leading to a very high average age of rice varieties.

From the literature it is clear that, so far the metrics used for assessing crop dynamics in case of paddy were (i) total number of varieties at two points of time (ii) weighted average age/average age of all varieties at two points of time (iii) age of oldest variety and its share (iv) number of varieties contributing 75% area and their average age at two points of time (v) number of varieties of below 10/15 years age and their share (vi) number of years for replacing a variety with another variety (at farm level) (vii) number of states/regions in which a particular variety is grown and (viii) average rice area per one improved variety of rice (Janaiah and Hossain, 2004). In some other crops, additional metrics were used *viz*., (i) proportion of area sown to varieties not sown in earlier period (Johnson and Gustafson,1963) (ii) proportion of the area that is sown to varieties released in the previous 5-10 years (Auer, 1963) or proportion of the area that is sown under varieties of less than 10 years old (Latha *et al*., 2018) **(**iii)) number of varieties which were sown on more than 5 per cent of total area individually (Brennan, 1984) and (iv) spatial indices like Herfindahl index calculated by squaring share of each variety in crop area or seed distributed and then summing these values across varieties, Margalef indices (ratio of number of varieties of a particular crop to logarithm of total area under the crop in a given locality in a given year) (Praveen *et al*., 2017).

In this backdrop, in the present study, an attempt has been made to analyse rice varietal dynamics and diversity in India using breeder seed indents data of selected years. An effort was also made to identify measures to improve varietal turnover by reviewing literature. Some additional metrics of varietal dynamics in paddy are used *viz*., (i) Number of varieties common in two selected years and their share and (ii) Number of varieties common in both years, with increased quantity indented in the second year.

Methodology

Data: This study is based on breeder seed indent data for selected years *viz*., 1997, 2007, 2015 and 2020. More specifically the current study was focused on rice varieties only (excluding rice hybrids, as hybrid rice adoption is limited to only 6 per cent of rice area in India confining to specific geographic areas). For the years 1997 (oldest year for which data was available in public domain) and 2007 data were collected from Directorate of Rice Research (DRR) Annual report and AICRIP report respectively, which gave variety wise indent only but not indenter-wise varietal demand. For the years 2015 and 2020 indenter-wise data for different rice varieties was collected from seednet website (https://seednet.gov.in).

Methodology: Simple tabular approach was used for analysing the data collected. Indenter-wise number of varieties indented was used as a simple measure of varietal diversity. Share of top 1 variety, top 3/5/10 varieties and number of varieties constituting 75 per cent of total indent were used as other measures of varietal diversity/ concentration at all India level.

Weighted average age of top 10 varieties was used as a measure of temporal diversity *i.e.*, cultivar replacement frequency. Varietal age was calculated as duration from the year of notification to a reference year. Weighted average age of top 10 varieties was calculated by dividing the sum of quantity weighted age of top 10 varieties with sum of quantity of top 10 varieties. That is, if age of top 10 varieties is represented as A1,A2,A3,A4,A5,A6,A7,A8,A9, A10 and quantity of seed indented of top 10 varieties is represented as Q1,Q2,Q3. Q4,Q5,Q6,Q7,Q8,Q9,Q10, then weighted average of top 10 varieties is calculated as

(A1 x Q1) + (A2 x Q2) + (A3xQ3) +..........+ (A10 x Q10) / (Q1 + Q2 + Q3+........+ Q10).

Hence Weighted Average Age (WAA) of top 10 varieties = $\sum_{i=1}^{10} AQ \div \sum_{i=}^{10} Q$ where i ranges from 1 to 10 (*i.e* top 10 varieties).

In this study, varietal newness was measured as age below 10 years. Some metrics of varietal diversity were calculated at indenter level. In this paper words 'paddy' and 'rice are used interchangeably in all portions except in introduction section where reference is made to paddy yields and rice yields with distinction.

Results and Discussion

Rice varietal dynamics and diversity

Some details regarding rice varietal dynamics at all India level are presented in Table1. In 1997 there were breeder seed indents for 56 varieties. It increased to 304 varieties in year 2020. There were only 34 varieties that were common in the indents for the years 1997 and 2007 contributing 77 and 53 per cent of total quantity of seed indented in the respective years. Out of the 34 varieties common in both the years, only in case of 15 rice varieties, quantity of seed indented in 2007 was more than that indented in 1997, indicating that in the case of other 19 common varieties indented quantity of seed decreased. Thus, in varieties common in both the selected years, some were in rising phase and some were in declining phase in accordance with established literature on varietal adoption pattern (initiation, increase and declining phase). Similar dynamics were observed between different years.

It is evident from Table 1 that when higher is the time period between selected years, lower is the number of varieties common in selected years. Further though total share of common varieties was always lower in second year (among selected years), there were some common varieties with increased seed quantity indent in second year. The results clearly indicate varietal dynamics with some deletions, some additions, and in common varieties some in increasing demand phase and some in decreasing demand phase in selected years.

Table1: Rice varietal dynamics in India

Period	Total number of varieties		Number of varieties common in both the years	Share of common varieties in total quantity of seed indented (%)		Number of common varieties in which quantity of seed indent increased in period 2 compared to period 1
	Year 1	Year 2		Year 1	Year 2	
1997-2007	56	113	34	77	53	15
2007-2015	113	218	70	86	52	30
2015-2020	218	304	133	89	65	55
1997-2020	56	304	24	61	14	8
2007-2020	113	304	55	77	27	17

Source: Computed from breeder seed indent data from (i) DRR Annual Report 1996-97, (ii) DRR Progress Report Varietal Improvement Vol 1, 2006 and (iii) https://seednet.gov.in

Rice varietal dynamics at state level was analysed using data on breeder seed indents for the years 2015 and 2020 and the results are presented in Table 2. In the year 2015, the number varieties for which indents were received ranged from 2 (Rajasthan) to 50 (Odisha). In the year 2020, the number of varieties for which indents were received ranged from 1 (Rajasthan) to 66 (West Bengal).

As it is evident from Table 2, the number of varieties common in indent for both the years (2015 and 2020) ranged from zero (Rajasthan) to 25 (West Bengal). The number of common varieties with respect to which indented quantity of seed increased in 2020 compared to 2015 ranged from zero (Assam, Haryana, Maharashtra, and Rajasthan) to 15 (West Bengal). In the case of indents from two organizations *i.e.*, Seed Association of India (SAI) and National Seed Corporation (NSC), the number of varieties for which indents were received in 2020 were more (134 and 60, respectively) compared to number of varieties in 2015 (106 and 33, respectively). But only in case of some states, the number of varieties for which indents were received in 2020 was more compared to number of varieties in 2015.

Table 2: Rice varietal dynamics across different indenters in selected years

Indenter	Number of varieties		Number of varieties common in both years	Share of common varieties in total indented breeder seed quantity (%)		Number of common varieties in which quantity of breeder seed indent increased in 2020 compared to 2015	Top three common varieties in which breeder seed indent increased in 2020 compared to 2015
	2015	2020		2015	2020		
Undivided Andhra Pradesh	22	37	17	90	61	8	MTU-1075, Jyothi, MTU-1061/RGL-2537
Assam	11	10	1	16	7	0	
Bihar	13	14	4	48	42	2	Sahabhagidhan, Sampada
Chhattisgarh	21	27	14	79	83	9	Swarna, Swarna Sub-1,IGKVR-1
Haryana	7	10	3	75	30	0	
Himachal Pradesh	5	4	3	58	70	3	HPR-2143, HPR-1156, Basmati Kasturi
Jammu & Kashmir	6	4	3	36	88	1	Basmati-370
Jharkhand	9	15	7	72	45	4	Rajendra Mahsuri-1, Sahabhagidhan, Naveen
Karnataka	18	22	16	99	84	9	Jyothi, Tunga, MTU-1010
Madhya Pradesh	8	35	7	99	36	4	Pusa Sugandh-5, Mtu-1010, MTU-1001
Maharashtra	26	26	14	81	77	0	
Odisha	50	46	18	87	63	1	Mrunalini
Punjab	9	13	4	81	42	2	Pusa Basmati-1121, PR-114
Rajasthan	2	1	0	0	0	0	
Tamil Nadu	5	5	2	54	76	2	Nellore Mahsuri, Swarna Sub-1
Tripura	10	12	8	90	83	4	Narendra Dhan-97, Naveen, Sahabhagidhan
Uttar Pradesh	28	20	4	11	40	4	Pusa Basmati-1509, NDR-2065, Pusa Basmati-1
Uttarakhand	33	13	8	40	23	1	HKR-127
West Bengal	34	66	25	90	63	15	Shatabdi, Swarna, Rajendra Bhagavathi
SAI	106	134	55	92	63	21	Pusa Basmati-1509, Puas-44, Swarna
NSC	33	60	18	77	59	3	Jyothi, Jaya, Gontra Bidhan-1

Source: Computed from breeder seed indent data from https://seednet.gov.in

Table 3: Rice varietal diversity concentration at all India level in selected years

Particulars	Unit	Year			
		1997	2007	2015	2020
Total quantity seed indented	Quintals	816.70	2100.41	4279.64	4805.32
Top 1 variety seed quantity indented and name of the variety	Quintals Name	73.70 IR-64	217.03 IR-64	498.63 MTU-1010	351.73 MTU-1010
Top 1 variety share (%) in total indent	Per cent	9	10	12	7
Age of top most variety	Years	6	16	15	20
Top 5 varieties seed quantity indented	Quintals	291.65	841.32	1509.04	1196.13
Top 5 varieties seed share (%) in total indent	Per cent	36.0	40	35	25
Top 10 varieties seed quantity indented	Quintals	462.65	1223.6	2221.25	1779.14
Top 10 varieties share (%) in total indent	Per cent	57	58	52	37
Weighted average age of top 10 varieties	Years	13	17	15	19
Average age of top 10 varieties	Years	13	17	16	16
Maximum age (top 10 varieties)	Years	28	27	35	40
Minimum age (top 10 varieties)	Years	6	7	4	4
Age range of top 10 varieties	Years	22	20	31	36
Number of varieties of below 10 years age in top 10 varieties	Number	5	2	3	4
Quantity of varieties of below 10 years age in top 10 varieties in total quantity seed	Quintals	209.30	237.17	704.7	530.6
Share of varieties of below 10 years age in top 10 varieties, in total quantity seed	Per cent	26	11	16	11

Source: Computed from breeder seed indent data from (i) DRR Annual Report 1996-97, (ii) DRR Progress Report Varietal Improvement Vol 1, 2006 and (iii) https://seednet.gov.in

Details of rice varietal diversity/concentration at all India level in selected years are presented in Table 3. At all India level indents for breeder seed quantity increased by around 6 times for *Kharif* 2020 compared to 1997. During the same years, share of top 5 varieties and top 10 varieties in total quantity of seed indented was decreased for 2020 compared to 1997, indicating increasing varietal diversity. But average age as well as weighted average age of top 10 varieties increased in indent for *Kharif* 2020 compared to *Kharif* 1997 indent. In indent for the year 1997 age range of top ten varieties was between 6 to 28 years while, the same in indent for 2020 ranged from 4 to 40 years. This indicates decrease in lower age limit of varieties indented but increase in upper age limit. This can be due to targeted policy intervention of introducing new varieties coupled with stable performance of older varieties. Among top 10 varieties, only 5 varieties were of age lower than 10 years in 1997. Corresponding figure in the case of indent for 2020 stood at 4. Share of these varieties of below 10 years age (in top 10 varieties) in total quantity of seed indent for 1997 and 2020 stood at 26 and 11 per cent, respectively. This lower share of new varieties (of below 10 years age) has led to higher weighted average age of top 10 varieties in indent for *Kharif* 2020.

Results of another way of looking at varietal dynamics/diversity at all India level are presented in Table 4. The number of varieties constituting 75 per cent to total breeder seed was 18 in 1997 increased to 21 in 2007, 27 in 2015 and 52 in 2020, once again indicating decreasing varietal concentration. But both average age as well as weighted average age of these varieties has not followed any consistent pattern. Share of varieties of below 10 years age (in varieties constituting 75 per cent of total seed indent) in total seed indent also did not follow any consistent pattern, stood at 35 per cent in 1997 and at 30 per cent in indent for *Kharif* 2020.

Table 4: Another approach to look at Rice varietal dynamics and concentration in India

Particulars	Unit	1997	2007	2015	2020
Total indented quantity of seed	Quintals	816.7	2100.41	4279.64	4805.32
Number of varieties indented	Number	56	113	218	304
Number of varieties constituting 75% of total quantity of breeder seed indent	Number	18	21	27	52
Average age of varieties	Years	13	17	14	13
Weighted average age	Years	13	17	15	16
Maximum age	Years	28	38	35	51
Minimum age	Years	3	3	3	2
Age range	Years	25	35	32	49
Number of varieties of below 10 years age in varieties constituting 75% of total quantity of breeder seed indent	Number	9	5	11	27
Seed quantity of below 10 years age varieties in varieties constituting 75% of total quantity of breeder seed indent	Quintals	282.3	331.92	1091.28	1417.6
	Share (%)	35	16	25	30

Source: Computed from breeder seed indent data from (i) DRR Annual Report 1996-97, (ii) DRR Progress Report Varietal Improvement Vol 1,2006 and (iii) https://seednet.gov.in

A weighted average age of less than 10 years and adoption rates of improved varieties to the extent of 35 per cent are generally considered as indicators of good progress in plant breeding (Walker *et al*. 2015). From this perspective weighted average age of more than 10 years of varieties constituting 75 per cent of breeder seed indent (Table 4) and lower share of varieties of below 10 years age are some indicators of Indian rice seed system that needs attention. However, literature indicates that optimum period for varietal replacement in a crop will vary over time and is dependent on (i) the yearly genetic gain in yield or improvement in other desirable characteristics (ii) the rate of varietal decay caused by breakdown in disease/pest resistance (iii) genetic diversity for disease resistance in varieties that are currently grown (iv) cost of breeding and multiplying seeds of new varieties (v) cost of providing extension to substitute new varieties (vi) farmers' seed purchase and learning costs (Heisy, 1990) and (vii) cost of seed and capital, margin required to encourage farmers to replace seed (Heisy and Brennan,1991). Hence, there can be regional variation in rice varietal dynamics. This aspect is examined and results are presented in Table 5.

In indent for *Kharif* 2015 and *Kharif* 2020, Seed Association of India (SAI) share was 20 and 31 per cent, respectively. Among states, Odisha was the topmost indenter (25%) with topmost variety age of 16 years in 2015 and 11 years for 2020 indent; Chhattisgarh was the topper with 21 per cent of total all India breeder seed indent with topmost variety age of 20 years. Consequently at all India level age of topmost rice variety indented was 15 and 20 years in 2015 and 2020 indents, respectively. States together with SAI indents constituted 98 and 99 per cent of total quantity of seed indented at all India level in 2015 and 2020, respectively.

In indent for *Kharif* 2015, across states, top most variety share ranged between 11 per cent (Odisha, Uttarakhand) to 94 per cent (Rajasthan). In indent for *Kharif* 2020 top most variety share ranged between 11 per cent (Odisha) to 100 per cent (Rajasthan). Age of top most rice variety ranged between 2 years (Haryana, Punjab) to 35 years (Tripura and Uttarakhand) in indent for *Kharif* 2015. Age of top one rice variety ranged between 2 years (Assam) to 47 years (Jammu and Kashmir) in indent for *Kharif* 2020. In 2015, in case of 7 states (Bihar, Haryana, Himachal Pradesh, Punjab, Tamil Nadu, Uttar Pradesh and West Bengal) top most variety age was below 10 years. In 2020 indent, in case of 7 states (Undivided Andhra Pradesh, Assam, Bihar, Punjab, Rajasthan, Uttar Pradesh and Uttarakhand) top most variety age was below 10 years. Thus, only in case of Bihar, Punjab and Uttar Pradesh, age of topmost variety was below 10 years in both 2015 and 2020 indents.

Table 5: Selected Indenter wise varietal concentration and varietal age

Indenter	Total quantity seed (Quintals)		Top most variety share (%)		Age of top most variety (years)		Top 10 varieties share (%)		Weighted average age of top 10 varieties (years)		Top 10 varieties age range in indent for 2015 (years)			Top 10 varieties age range in indent for 2020 (years)		
	2015	2020	2015	2020	2015	2020	2015	2020	2015	2020	Max	Min	Range	Max.	Min.	Range
Undivided Andhra Pradesh	317.5	237.06	31	13	15	4	87	70	19	15	35	5	30	31	2	29
Assam	204	270	34	15	21	2	98	100	21	6	41	6	35	14	2	12
Bihar	312	330.1	22	22	6	9	91	98	13	7	37	4	33	12	3	9
Chhattisgarh	739.44	988.4	19	18	15	20	74	82	12	19	19	3	16	40	5	35
Haryana	1.28	0.8	63	10	2	13	100	100	6	13	21	2	19	31	3	28
Himachal Pradesh	24	40	33	35	9	14	100	100	11	14	26	9	17	31	7	24
Jammu &Kashmir	9.6	4.3	52	58	10	47	100	100	19	38	42	2	40	47	19	28
Jharkhand	87	116.81	23	20	24	40	100	89	16	20	26	4	22	40	4	36
Karnataka	57.75	71.9	20	18	24	29	88	83	20	26	46	7	39	51	4	47
Madhya Pradesh	106	447.3	42	15	15	20	100	66	20	13	33	5	28	23	2	21
Maharashtra	129.05	51.2	20	29	22	27	76	82	17	26	46	5	41	51	4	47
Odisha	1067.6	274.7	11	11	16	11	80	63	14	19	35	4	31	40	5	35
Punjab	2.02	2.28	50	18	2	9	100	95	8	9	20	2	18	19	2	17
Rajasthan	0.8	0.1	94	100	10	7	100	100	10	7	10	8	2	7	7	0
Tamil Nadu	13	15.5	38	58	5	10	100	100	12	9	26	4	22	11	2	9
Tripura	2.05	2.6	29	19	35	27	100	96	20	20	35	4	31	40	5	35
Uttar Pradesh	122.6	153.3	15	20	6	7	69	81	13	6	41	4	37	9	1	8
Uttarakhand	17.86	15.2	11	53	35	6	61	94	18	8	35	2	33	40	5	35
West Bengal	89	211	17	13	9	20	79	57	16	19	35	4	31	40	5	35
SAI	872.89	1503.72	16	9	10	26	62	54	16	21	35	6	29	51	3	48
All India	4279.64	4805.32	12	7	15	20	52	37	15	19	35	4	31	40	4	36

Source: Computed from breeder seed indent data from https://seednet.gov.in

In indent for *Kharif* 2015, across states top 10 varieties share ranged between 61 per cent (Uttarakhand) to 100 per cent (Tripura) and their weighted average age ranged between 6 years (Haryana) to 21 years (Assam). In indent for *Kharif* 2020, across states top 10 varieties share ranged between 57 per cent (West Bengal) to 100 per cent (Chhattisgarh) and their weighted average age ranged between 6 years (Assam and Uttar Pradesh) to 38 years (Jammu and Kashmir). In 2015 indent, only with respect to two states (Haryana and Punjab) weighted average age of top 10 varieties was below 10 years. In 2020 indent, weighted average age of top 10 varieties was below 10 years in case of 7 states. Punjab was the only state indenter with weighted average age of top 10 varieties of below 10 years in both 2015 and 2020. In 2015, top 10 rice varieties with maximum diversified age range of 41 years (maximum 46 years and minimum 5 years) was observed in case of Maharashtra. In 2020 indent, top 10 rice varieties with maximum diversified age range of 47 years (maximum 51 years and minimum 4 years) was observed in case of Karnataka.

Details of top 10 varieties at all India level in terms of breeder seed indent (quantity) in selected years are presented in Table 6. Both in year 1997 and 2007, top 3 varieties were IR-64, MTU-7029 and IR-36 in that order. MTU-1010 (Cottondora Sannalu) variety which was in fourth place in 2007, became top most variety in 2015 and maintained its position in 2020 indent also. Jaya rice variety, was in fifth place in 1997, moved to 15th place in 2020 indent (with an age of 51 years) contributing 1.4 per cent of total breeder seed indent. In 2020 indent, major indenters for Jaya variety were Seed Association of India, Karnataka and Maharashtra. In 2020 indent, age of top two varieties *i.e* MTU-1010 and MTU-7029 varieties were 20 years and 40 years, respectively and their individual shares in breeder seed indent were 7.32 and 7.26 per cent, respectively. In 1997, 2015 indents, among top 10 rice varieties, one variety was Basmati variety. In 2020 breeder seed indent, among top 10 varieties, 2 were Basmati rice varieties.

Table 6: Top 10 varieties at all India level based on quantity of breeder seed indented

Rank	Year			
	1997	2007	2015	2020
1	IR-64	IR-64	MTU-1010	MTU-1010
2	MTU-7029 (Swarna)	MTU-7029 (Swarna)	Swarna-Sub 1	MTU-7029 (Swarna)
3	IR-36	IR-36	MTU-1001	Swarna-Sub 1
4	Kalinga-III	MTU-1010	MTU-7029 (Swarna)	Sahabhagi Dhan
5	Jaya	Samba Mahsuri	Sahabhagi Dhan	Pusa Basmati-1509
6	Heera	MTU-1001	Samba Mahsuri	Pusa-44
7	Samba Mahsuri	Kranti	Pusa Basmati-1121	DRR Dhan-42
8	Rasi	Shatabdi	IR-64	Sri Dhruthi
9	Pusa Basmati-1	NLR-145	Pratikshya	MTU-1001
10	Annada	Lalat	Pooja	Pusa Basmati-1121

Source: Computed from breeder seed indent data from (i) DRR Annual Report 1996-97, (ii) DRR Progress Report Varietal Improvement Vol 1,2006 and (iii) https://seednet.gov.in

Details of top 10 varieties at all India level in terms of number of indenters are presented in Table 7. MTU-1010 was the variety with highest number of indenters followed by Swarna-Sub1 in 2015 indent as well as in indent for 2020.

Basmati rice is a special rice type with IPR protection under Geographical Indications (GI) in India. Basmati rice is significant contributor in earnings from exports of rice from India. In 2018-19, earnings from basmati rice exports was 4.72 billion US$ against export earnings of 3.05 billion US$ from non-basmati rice exports (APEDA, 2020). Hence Basmati rice varietal dynamics was analysed separately and the results are presented in Table 8. So far 32 Basmati rice varieties were notified (AIREA, 2019). Number of Basmati varieties with respect to which breeder seed indent received in 1997 was three, it gradually increased to 14 in 2020. In 2020, share of Basmati indents in total quantity of rice breeder seed indent stood at 8.27 per cent. Age of top most Basmati rice variety indented was higher than age of topmost (all) rice variety seed indented in 1997 and 2007, but was lower in 2015 and 2020. Top 3 Basmati rice varieties share in total Basmati varieties breeder seed was 100 per cent in 1997, gradually declined over years and was 70.87 per cent in 2020 indent, indicating decrease in varietal concentration. Pusa Basmati-1 was the topmost variety in 1997 and was relegated to second position in 2015. Pusa Basmati-1121 which was in third position in 2007 became topmost variety in 2015, but was relegated to second position in 2020. Pusa Basmati -1509 variety which was in third position in 2015, became topmost variety in 2020 indent.

Table 7: Top 10 varieties at all India level based on number of indenters

	Year				
2015			2020		
Rank	**Variety**	**Number of indenters**	**Rank**	**Variety**	**Number of indenters**
1	MTU-1010	16	1	MTU-1010	12
2	Swarna Sub-1	14	2	Swarna Sub-1	11
3	MTU-7029 (Swarna)	13	3	DRR Dhan-42	10
4	Sahabaghi Dhan	12	4	MTU-7029(Swarna)	10
5	Samba Mahsuri	12	5	Sahabaghi Dhan	9
6	MTU-1001	12	6	MTU-1001	9
7	Pusa Basmati-1121	11	7	DRR Dhan-44	8
8	IR-64	10	8	CO-51	7
9	Pusa Basmati-1509	8	9	DRR Dhan-45	6
10	Shatabdi	7	10	Sambha Sub-1	6

Source: Computed from breeder seed indent data from https://seednet.gov.in

Table 8: Varietal dynamics in Basmati rice varieties

	Unit	1997	2007	2015	2020
Total number of varieties	Number	56	113	218	304
Number of Basmati varieties	Number	3	7	12	14
Total indented quantity of all varieties	Quintals	816.7	2100.41	4279.64	4805.32
Basmati varieties quantity indented	Quintals	41.70	43.93	274.50	397.41
Share of Basmati varieties in total indented quantity	Per centage	5.11	2.09	6.41	8.27
Maximum Age of Basmati variety	Years	24	34	42	47
Minimum Age of Basmati variety	Years	8	2	2	2
Age of topmost Basmati variety	Years	8	18	10	7
		Top 3 Basmati Varieties and their share in total Basmati seed indent			
		Pusa Basmati-1 (71.94)	Pusa Basmati-1 (58.11)	Pusa Basmati - 1121 (58.94)	Pusa Basmati-1509 (33.95)
		Kasturi (16.79)	Vasumathi (18.10)	Pusa Basmati-1 (18.21)	Pusa Basmati - 1121 (24.47)
		Basmati-370 (11.27)	Pusa Basmati - 1121 (9.86)	Pusa Basmati-1509 (8.16)	Pusa Basmathi-1637 (12.45)
Share of top 3 Basmati varieties in total Basmati varieties	Per centage	100	86.07	85.31	70.87
Weighted average age of top 3 Basmati varieties	Years	10	13	13	9

*Figures in parentheses indicate per centages

Source: Computed from breeder seed indent data from (i) DRR Annual Report 1996-97,(ii) DRR Progress Report Varietal Improvement Vol 1, 2006 and (iii) https://seednet.gov.in

Details of state-wise top 5 rice varieties based on quantity of breeder seed indent are presented in Table 9. MTU-1010 was the topmost variety in the case of undivided Andhra Pradesh, Chhattisgarh and Madhya Pradesh in 2015 indent. However, in indents for the year 2020, MTU-1010 rice variety was the topmost variety only in case of Chhattisgarh and Madhya Pradesh. In case of undivided Andhra Pradesh, KNM-118 became the topmost variety indented for year 2020. MTU-7029 was the topmost variety in case of Tripura, Uttarakhand in 2015 indent, but was topmost variety in case of only Jharkhand in 2020.

Table 9: State wise top 5 varieties in 2015 and 2020 breeder seed indents

		Rank				
Indenter	**Year**	**1**	**2**	**3**	**4**	**5**
Undivided Andhra Pradesh	2015	MTU-1010	Samba Mahsuri	MTU-1001	MTU-7029	Swarna-Sub 1
	2020	KNM-118	Samba Mahsuri	MTU-1010	RNR-15048	MTU 1075
Assam	2015	Ranjeet	Swarna-Sub-1	IR-64	Masuri	Bahadur
	2020	Bahadur Sub-1	Ranjit Sub-1	CR Dhan-310	Gitesh	CR Dhan 505
Bihar	2015	Swarna-Sub 1	Sahabhagi Dhan	MTU-1010	Samba Mahsuri	MTU-1001
	2020	Sahabaghi Dhan	Rajendra Baghavathi	Sabour Ardhjal	Sabour Surbhit	Sabour Shree
Chhattisgarh	2015	MTU-1010	Mahamaya	Narendra-8002	MTU-1001	PKV HMT
	2020	MTU-1010	MTU-7029	Swarna-SUB 1	IGKVR-1	Mahamaya
Haryana	2015	Pusa Basmati-1509	HKR-127	HKR-47	PR-113	PR-114
	2020	Pusa Basmati-1718	Pusa Basmati 1637	Pusa Basmati 1728	Pusa 1592	Pusa Basmati-1509
Himachal Pradesh	2015	HPR 2143	HPR-1068	HPR-1156	RP-2421	Basmati Kasturi
	2020	HPR 2143	HPR-2612	HPR-1156	Basmati Kasturi	
Jammu and Kashmir	2015	Pusa Basmati-1121	Basmati-370	Chenab	Pusa Basmati-1509	Giza-14
	2020	Basmati-370	Chenab	PR-113	Giza-14	
Jharkhand	2015	IR-64	Lalat	Abhishek	Rajendra Mahsuri-1	Sahabaghi Dhan
	2020	MTU-7029	IR64 DRT 1	Sahabhagi Dhan	Rajendra Mahsuri-1	MTU-1010
Karnataka	2015	IR-64	Samba Mahsuri	MTU-1001	Uma	JGL-1798
	2020	IR-64	MTU-1001	RNR-15048	MTU-1010	Jaya
Madhya Pradesh	2015	MTU-1010	IR-64	MTU-1001	Kranti	IR-36
	2020	MTU-1010	Pusa Sugandh-5	IR64 DRT 1	MTU-1001	Sahabhagi Dhan
Maharashtra	2015	Indrayani	MTU-1010	PKV HMT	Jaya	Karjat-3
	2020	Indrayani	MTU-1010	Jaya	CO 51	RTN-5
Odisha	2015	Pooja	Sahabhagi Dhan	Swarna-Sub 1	MTU-1010	Pratikshya
	2020	Swarna-Sub 1	Pooja	MTU-1010	MTU-1001	MTU-7029
Punjab	2015	Pusa Basmati-1509	PR-114	PR-111	PR-113	Pusa Basmati - 1121
	2020	PR 121	PR-114	PR-126	Pusa Basmati -1121	PR-127
Rajasthan	2015	Pusa Basmati - 1121	Improved Pusa Basmati-1			
	2020	Pratap -1				
Tamil Nadu	2015	NLR-34449	Samba Mahsuri	Swarna - Sub 1	Sahabahagi Dhan	JGL-1798
	2020	NLR-34449	Swarna-Sub 1	DRR Dhan-45	Sambha Sub - 1	DRR Dhan 50
Tripura	2015	MTU-7029	Naveen	Sahabhagi Dhan	Narendra Dhan-97	Shatabdi
	2020	Naveen	MTU-7029	Sahabhagidhan	Narendra Dhan-97	CO 51

Uttar Pradesh	2015	Swarna-Sub 1	Improved Samba Mahsuri	Narendra Dhan -359	Samba Mahsuri	NDR 2065
	2020	Pusa Basmati-1509	Pant Dhan-24	NDR 2065	PR 121	Sambha Sub - 1
Uttarakhand	2015	MTU-7029	VL.Dhan 85	Sarjoo-52	Vivek Dhan-62	Swarna-Sub 1
	2020	VL Dhan 68	VL Dhan 157	PR 121	HKR-127	VL Dhan 85
West Bengal	2015	Pratikshya	Swarna-Sub 1	MTU-7029	MTU-1010	Gontra Bidhan-1
	2020	Shatabdi	MTU-7029	Pratikshya	Swarna-Sub 1	MTU-1010

Source: Computed from breeder seed indent data from https://seednet.gov.in

Table 10: Top 10 varieties in breeders seed indent of SAI

2015			2020		
Variety	**Share in total SAI indent (%)**	**SAI share in total All India indent (%)**	**Variety Name**	**Share in total SAI indent (%)**	**SAI share in total All India indent (%)**
Pusa Basmati - 1121	16	84	Pusa-44	9	100
Swarna-Sub 1	9	21	MTU-7029 (Swarna)	8	35
MTU-7029 (Swarna)	8	33	Pusa Basmati-1509	7	76
Pusa-44	6	99	Sri Dhruthi	7	89
Pusa Basmati-1	6	98	Pusa Basmati -1121	6	94
Gontra Bidhan-1	5	88	PR-113	4	99
IR-64	4	26	Jaya	4	85
MTU-1010	4	7	Indrayani	3	77
PR-118	3	98	Pusa Basmati -1718	3	99
Shatabdi	3	67	Pusa Basmati 1637	3	89
Top10 varieties total	62	34		54	73

Source: Computed from breeder seed indent data from https://seednet.gov.in

In 2015 indent, Swarna-Sub 1 was the topmost variety in case of Bihar and Uttar Pradesh. In 2020 indent, Swarna-Sub 1 was the topmost variety in case of Odisha. Only in case of 6 states, there was no change in topmost variety in 2015 and 2020 indents. They are Chhattisgarh (MTU-1010), Himachal Pradesh (HPR-2143), Karnataka (IR-64), Madhya Pradesh (MTU-1010), Maharashtra (Indrayani), and Tamil Nadu (NLR-34449). Only in case of three states, there was no change in second top variety. They are undivided Andhra Pradesh (Samba Mahsuri), Maharashtra (MTU-1010) and Punjab (PR-114).

Varietal dynamics in seed indent of SAI in 2015 and 2020 was analysed separately and results are presented in Table 10 and 11. Share of topmost rice variety in SAI indent was 16 and 9 per cent in indents for 2015 and 2020, respectively with topmost variety of age of 10 and 26 years. Share of top 10 varieties indents in SAI indent was 62 and 54 per cent in 2015 and 2020 respectively. Among top 10 varieties of SAI indents in 2015 and 2020 only 3 varieties were common indicating high varietal dynamics. A comparison of top ten varieties at all India level (Table 6) and top ten varieties in SAI indents (Table 10) yields some interesting insights. In 2015, there were 4 varieties common in both the tables and in 2020 the number of common varieties increased to 5. In 2015 common varieties, SAI share in total all India level indent ranged from 21 to 84 per cent and in 2020 the range varied 35 to 100 per cent. From the Table 10 it is evident that, indents for certain varieties like Pusa-44, PR-118, PR-113 which is cultivated in Punjab and Haryana, were reflected in SAI indent (constituting more than 90 per cent of indented quantity for these varieties), rather than direct indents from the states. Same is the situation with Basmati rice varieties also as more quantity is indented by SAI than states like Punjab and Haryana. In 2015 at all India level there were indents for 12 Basmati varieties, SAI indent was there for seven Basmati varieties accounting for 80 per cent of Basmati varieties, seed indent at all India level. In 2020 at all India level, there were indents for 14 Basmati varieties, SAI indent was there for 11 Basmati varieties accounting for 84 per cent of Basmati varieties seed indent at all India level.

Table 11: Frequency distribution of number of varieties in SAI indent based on their share in total all India breeder seed indent

Share of SAI indent in total indent (%)	Number of varieties	
	2015 indents	2020 indents
100	29	44
90-99	9	12
80-89	4	13
70-79	4	5
60-69	5	2
50-59	2	1
40-49	3	9
30-39	9	4
20-29	7	12
10-19	13	9
<9	21	23
Total number of varieties	106	134

Source: Computed from breeder seed indent data https://seednet.gov.in

In 2015, SAI indent was for 106 varieties, out of this only in case of 53 varieties, share of SAI in total quantity of breeder seed indent was below 50 per cent (Table 11). Similarly in 2020, SAI indent was for 134 varieties, out of this only in case of 57 varieties, share of SAI in total quantity of breeder seed indent was below 50 per cent. Thus SAI indent was above 50% of total breeder seed indent in case of 53 varieties in 2015 and in case of 77 varieties in 2020. This might have some extent masked the indent for these varieties from states. Totally in 2015 indent, 100 per cent indent was from SAI for 29 varieties and in 2020 for 44 varieties. In other words if SAI indent was not there for these varieties, total number of varieties for which breeder seed indents received would have decreased to 189 and 260 in place of 218 and 304 in 2015 and 2020, respectively.

In January 2019, the Protection of Plant Varieties and Farmers' Rights Authority (PPV&FRA) through its notice on plant breeder's rights stated that the procedure of compiling indents by Central and State Government organizations for seed production and fixing of breeder seed price under Seeds Act (1966) henceforth will be restricted only to varieties which are not registered under (Protection of Plant Varieties and Farmers' Rights Act) PPV&FR Act 2001 or those whose period of protection under the registration has expired on the date (PPV&FRA, 2019). Thus, in future there will be different mechanisms for indenting for varieties based on whether a variety is protected or not under PPV&FR Act. Accordingly mechanism of price fixation for breeder seed also will differ and in turn may influence varietal dynamics.

Measures for improving rice varietal turnover

In the case of wheat cultivation in Punjab of India, Smale *et al.*, (2008) observed that slow varietal change had offset the positive productivity effects of diversifying the genetic base in wheat breeding during post green revolution period. Hence nudging varietal turnover in a particular direction by discouraging breeder seed indent for older varieties or withdrawal of old varieties can be a option for promoting varietal turnover (Krishna *et al.* 2014, Atlin *et al.* 2017). They also suggested the measures of withdrawal of seed subsidy for obsolete varieties, setting targets for the average varietal age in foundation seed production and farmers' fields (below 10 years). Spielman and Smale (2017) suggested leveraging seed subsidy program by targeting subsidy on a variety-specific basis with the goal of removing older varieties. Some efforts in this direction were observed in the case of Telangana and Andhra Pradesh states in recent years, wherein subsidy rates for paddy seeds are different, more for varieties less than 10 years old and less for varieties of more than ten years old. In recent draft seed bill (2019) of India, proposal of a validity period of ten years for registered variety of annual crops like rice also can be considered as a targeted policy approach for improving varietal turnover. However, it is being viewed that it is difficult to decide regarding the resistance threshold at which a variety should be withdrawn from circulation.

Supply side constraint with respect to new varieties can act as hindrance in promoting varietal turnover (Krishna *et al.* 2014). Atlin *et al.* (2017) observed that competitive commercial seed industry in temperate regions was able to address this supply side constraint. Further, Atlin *et al.* (2017) argued that rapid release of varieties by utilizing breeding tools based on sound quantitative genetics principles thereby reducing breeding cycle length is a key to cropping system adaptation to climate change. Witcombe *et al.* (2013) opined that efficiency of breeding programs would be increased by making fewer crosses among more carefully chosen parents. Muralidharan *et al.* (2019) in the context of India, suggested focussed rice breeding in four mega environments namely rainfed unfavourable uplands, rainfed favourable uplands, irrigated areas and rainfed lowlands.

Spielman and Smale (2017) suggested some policy measures for accelerating varietal turnover. They are (i) accelerating varietal registration and release (like recent agreement between India, Nepal, Bangladesh, Srilanka and Cambodia (IRRI, 2017) and harmonizing rice varietal registration procedure and mutual recognition of registration among countries) (ii) improving quality assurance systems (iii) increasing access to early generation seed (through licensing) (iv) leveraging seed enterprises marketing capabilities and (v) leveraging competition policy and antitrust regulation. But they also indicate that there can be trade-off between spatial and temporal diversification, making it complex to decide about appropriate policy choices. Some researchers also suggested leveraging competition and fast tracking release of varieties resistant or tolerant to biotic and abiotic stress for accelerating varietal turnover (Das *et al*., 2019, Berhanu *et al*., 2019).

Joshi *et al*., (2001) reported that in Nepal spread of rice varieties from a participatory plant breeding commenced five to six years earlier than would have been the case in a conventional system. Witcombe and Yadavendra (2014) reported that in India, Ashoka rice varieties developed through Client Oriented Breeding (COB) replaced landraces or varieties adopted from conventional breeding. Ashoka 200F rice variety was notified in 2005 (for Jharkhand, though it was identified for release in 2002) and 2006 (for Gujarat, Rajasthan and Madhya Pradesh) and in 2013, breeders seed indent for it was 16th highest of 225 notified rice varieties. Pray *et al*., (2011) reported that Gramin Vikas Trust (GVT) the developer of Asoka varieties was unsuccessful in getting approval for the varieties as there was no provision for release of new varieties by NGO under Indian seed regulation. Later GVT collaborated with Birsa Agricultural University (BAU) and Asoka rice varieties were released as BAU varieties.

Singh *et al*. (2014) reported that a salt tolerant rice variety CSR-43 developed through participatory variety selection (during 2001-2007) was widely adopted in sodic areas of Indo-Gangetic plains and was officially released in 2011. Conny *et al*. (2019) argued that many of currently used research methods are weak on capturing real-life context and provided fragmented snapshot nature understanding of farmer's preferences and demand for seeds. Carlo *et al*. (2019) suggested triadic comparison of varieties (involving repeated participatory evaluation under farm condition) can help in delivering the best seeds based on the actual climatic conditions of a particular village.

Narappa *et al*., (2018) reported that in Karnataka, seed village program helped in introduction of new rice variety "Gangavathisona" in 2011-12. They inferred that increase in salinity affected area in Tungabadra Project (TBP) Command area, susceptibility of rice variety samba mahsuri (BPT 5204) to pests and diseases, higher yields of Gangavathisona rice variety (compared to BPT-5204) under direct seeded rice practice and soil salinity led to spread of Gangavathisona rice variety to the extent of 15.1 per cent rice area in TBP area in 2014-15.

Nayak and Mosharaf (2019) observed in Odisha, in Evidence Hubs (EH), a new generation platform where in multiple varieties of rice were exhibited for performance evaluation by different stakeholders under different management or ecological condition, led to (a) selection of varieties such as CR 1009 Sub1, Swarna-Sub 1, Bina Dhan 11 and other climate resilient varieties in coastal areas and (b) selection of varieties like Sahabhagi Dhan, DRR Dhan-44 and Bina Dhan11 in upland areas. They further observed that though Bina Dhan 11 was the best variety in terms of yield, Sahabhagi Dhan was rated highest based on multi-attributes. Some unreleased varieties which were performing high in terms of stakeholders rating, triggered policy dialogue for release of those varieties.

Gars and Ward (2019) indicated that differences in learning behaviour of farmers can also lead to different pattern of technology adoption. They distinguished four types of learning processes. They are (i) Bayesian learning (belief updating as additional information becomes available) (ii) impressionable learning (first impression based) (iii) reactionary learning (give importance to recent information only) and (iv) myopic learning (not only give more weight to recent information but also do not consider the probabilities over which they are making their choices). They suggested pilot surveys for assessing share of farmers of different learning types in a location, for planning future interventions based on nature of technology.

Conclusions and Way forward

From the results of current study it can be concluded that rice varietal diversity in India in recent years is increasing in terms of metrics (i) number of varieties for which breeder seed indents received (ii) number of varieties contributing 75 per cent of total indented quantity and (iii) lower share of top 10 varieties in total breeder seed indent. However, a higher weighted average age of top 10 varieties is indicating that varietal replacement is taking place with substitution by older varieties.

Literature indicates that multiple factors *viz.*, characteristics of new varieties, market potential for different varieties of rice, divergent perceptions and learning behaviour of farmers and divergent contextual factors (like hydrology, soil suitability etc) are influencing rice varietal dynamics. Hence, for promoting adoption of improved rice varieties with reduced adoption lag, a multi-pronged strategy need to be adopted. Reducing breeding cycle length, targeted extension interventions based on share of farmers of different types of learning patterns, nudging varietal adoption behaviour by leveraging policies of subsidy and competition in seed sector, encouraging private sector participation in research and varietal commercialization, facilitating marketing of output of rice varieties of different durations by synchronizing marketing periods with crop harvesting period are some of the suggested components in the multi-pronged strategy.

The current study is based on macro level breeder seed indents only with some limitations. Hence, in future more disaggregated studies can yield more insights on varietal dynamics in different rice ecosystems and associated factors.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Genetic diversity analysis of rice germplasm in gujarat state of india using simple sequence repeat markers

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Received: 10 August 2019; Accepted: 12 December 2019

Abstract

The present investigation was undertaken to detect the polymorphism among blast resistant and susceptible rice genotypes employing twenty three simple sequence repeat (SSR) markers,which targeted nine blast resistance genes. The clustering patterns of 2D and 3D of principal component analysis (PCA) were in accordance with the dendrogram clustering pattern. The dendrogram obtained from the pooled SSR analysis showed significant differences between all genotypes. The cophenetic correlation analysis revealed that the dendrograms generated by pooled SSR data were good to fit. The highest similarity index value of 0.71 was found in Ajaya and IET-20006, while the least similarity index value of 0.14 was found in GR-7 and IET-21094, GR-7 and IET-21070. The average similarity coefficient among genotypes was 0.46. The SSR markers linked to the blast resistance genes showed a high level of polymorphism among the genotypes.

Keywords: *Oryza sativa*, Blast resistance, *Magnaporthe grisea*, simple sequence repeat marker

Introduction

Rice is the major staple food of nearly half of the world population (Khush *et al.*, 2005). It is planted on about 165 million hectares or on about 12% of the world's cultivated land with an annual production of 744 million tons (FAO, 2014) and 43.5 million hectares land is under rice cultivation in India. The demand for production is increasing steadily for meeting the requirement of a growing population in India. For achieving this increasing demand, rice production will have to increase by at least 40% by the year 2030. To meet this challenge, there is an imminent need to develop high yielding rice varieties with durable resistance to both biotic and abiotic stresses (Leung *et al.*, 2004). The rice blast disease caused by fungus *Magnaporthe grisea* (anamorph *Pyricularia oryzae*), is one of the most devastating diseases affecting the rice crop throughout the world and has been responsible for 35-50% world yield loss in rice (Variar *et al.*, 2007). Till now more than 100 blast rice genes have been identified (Khanna *et al.*, 2015) and 26 genes have been cloned. Most of the known blast-resistance genes are located on chromosomes 6, 11 and 12 (Sallaud *et al.*, 2003). Among the blast resistance genes, *Pi54*, *Pi1*, *Pi2*, *Pi9* and *Pizt*, etc. are considered to be highly useful under Indian conditions. It is possible to increase rice production without expansion of cultivation area by using molecular markers and breeding methodology. Molecular markers play an important role in plant genome analysis and crop improvement. Of the many types of molecular markers, simple sequence repeats (SSR) or microsatellite markers are widely used due to their abundance in the genome. SSR markers are co-dominant, easily assayed by PCR, and exhibit variation based on the motif repeats. Due to these features, SSRs are being widely used for the estimation of genetic diversity (Freeg *et al.*, 2016), establishment of varietal identity, analysis of genetic structure with in the cultivated rice (Garris *et al.*, 2005), construction of molecular genetic maps and marker assisted selection.

Genetic diversity is a pre-requisite for any crop improvement program as it helps in development of superior recombinants. It is a source of variation, which is raw material for any improvement work. Genetic diversity analysis is very useful for estimating and establishing genetic relationship in collection of different germplasm, for identifying the different parental combinations to create segregating progenies with maximum genetic variability for further selection and introgression of desirable genes into elite genotypes (Islam *et al*., 2012). Analysis of genetic diversity also helps breeder to enhance the progress of breeding program.

Therefore, the current study was carried out for the assessment of genetic variability at molecular level among 35 rice genotypes differing in their resistance to blast. Twenty three SSR markers were used for developing unique finger print for each genotype. This may also be used to identify the best genotype as donor for blast resistance in breeding program for development of new blast resistance varieties.

Materials and methods

Plant materials

The seeds of 35 rice genotypes were collected from the Main Rice Research Station, Nawagam, Gujarat. Names of these genotypes and their response against blast disease are mentioned in Table 1.

Table 1: List of genotypes

Sr. No.	Genotypes	Susceptibility/Resistance to Blast disease	Sr. No.	Genotypes	Susceptibility/Resistance to Blast disease
1	IR-64	Resistant	19	IET-20669	Resistant
2	GR-11	Susceptible	20	IET-20862	Resistant
3	Pankhali-203	Susceptible	21	IET-20866	Resistant
4	GR-7	Resistant	22	IET-20868	Resistant
5	GAR-1	Susceptible	23	IET-20872	Resistant
6	GR-12	Resistant	24	IET-20874	Resistant
7	GAR-13	Resistant	25	IET-20892	Resistant
8	Gurjari	Resistant	26	IET-20894	Resistant
9	NWGR-2006	Resistant	27	IET-20929	Resistant
10	Azucena	Susceptible	28	IET-21000	Resistant
11	Ajaya	Susceptible	29	IET-21070	Resistant
12	IET-20006	Resistant	30	IET-21094	Resistant
13	IET-20082	Resistant	31	IET-21190	Resistant
14	IET-20214	Resistant	32	IET-21200	Resistant
15	IET-20235	Resistant	33	IET-21216	Resistant
16	IET-20375	Resistant	34	IET-21299	Resistant
17	IET-20667	Resistant	35	IET-21649	Resistant
18	IET-20668	Resistant			

DNA isolation

A modified mini preparation procedure (Zaidani *et al*., 2005) for extraction of total genomic DNA from leaf samples of 35 genotypes was used. The quality and quantity of the isolated DNA samples were checked by spectrophotometer (Thermo electronic corporation, UV1) and 0.8% agarose gel electrophoresis with lambda (λ) *Hind* III restriction digested DNA as molecular weight standard. The concentration and quality of DNA in individual samples were determined based on the intensity and thickness of the genomic DNA bands, compared to *Hind* III digested lambda (λ) DNA.

Simple sequence repeats (SSR) amplification

A total number of 23 SSR markers which are related to nine blast resistance traits/QTLs were selected for the genetic analysis based on the Gramene Marker database (http:// www. gramene.org/markers) and previously published reports of Temnykh *et al*., 2001 and McCouch*et al*., 2002 (Table 2). The PCR reaction mixture contained 50ng template DNA,5 pM of each of the forward and reverse primers, 200µM dNTPs, 1X PCR buffer (10mM Tris-HCl, pH 8.3, 50mM KCl, 1.5mM of $MgCl_2$ and 0.01mg/ml gelatin) and 0.5U of Taq DNA polymerase (JONAKI) in a volume of 10µl. Amplification cycling was performed in a gradient 96 well programmable master cycler (Veriti™, Applied Biosystems). The PCR was carried out with one cycle of denaturation at 95°C for 5 min, followed by 35 cycles at 95°C for 45s, 55°C for 45s and 72°C for 1 min, with a final extension of 72°C for 10 minutes. The final PCR products were then mixed with Bromophenol blue and run in 3% agarose gels along with 50-bp ladder for an hour in 0.5x Tris-Acetic and EDTA (TAE) buffer. The resolved PCR bands were documented using gel documentation system (BIO-RAD Molecular Imager Gel Doc XR system) and images were stored for further scoring and permanent records.

Data analysis: The amplicons were scored individually as (1) and (0) for the presence or absence of an allele, respectively. Data entry was done in a binary data matrix as discrete variables PIC values were calculated by using the formula PIC=1 -∑ pi2, where pi indicates frequency of the allele of each locus (Botstein *et al*., 1980). The matrices were used to calculate pair wise genetic similarity based on Jaccard's coefficient. Dendrogram displaying relationships among 35 rice genotypes was constructed using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) by NTSYSpc version 2.02 (Rohlf, 1994).The allele frequency was determined by the use of GenAlEx version 6.3 (Peakall and Smouse 2006). The expected heterozygocity and observed heterozygocity were also determined as described in the following formula by the use of same software.

Ho = Observed Heterozygocity = No. of Hets / N

He = Expected Heterozygocity = 1 - Sum pi^2 Where pi is the frequency of the i^{th} allele for the population and Sum pi^2 is the sum of the squared population allele frequencies.

Results and Discussion

Allelic diversity of microsatellite markers

For the present study, the seeds of 35 rice genotypes were analyzed (Table 1). Among them, IR-64 was selected as the resistant check. The susceptible checks used for the present study were GR-11 and Pankhali-203. The remaining 32 genotypes were used for comparison with the above mentioned checks for blast resistance and for analysis with SSR markers. A total of 81alleles were detected of which 71 (87.65%) were polymorphic (Table 3). The Polymorphic Information Content (PIC) values ranged from 0.28 (RM339) to 1.00 (RM 28050) with an average of 0.59. The number of alleles per SSR locus detected in this study corresponded well with Sajib *et al*., (2012) and Singh *et al*., (2016) and was lower than the average number of alleles (11.9) reported by Zhou *et al*., (2004) and Lin *et al*., (2007). The wide variation in the number of alleles detected was due to the different sets of germplasm, number of genotypes, number of selective SSR markers and method of gel electrophoresis detection in different studies. The low number of alleles was usually obtained from closely related cultivars (IR64, IET 20667-IET20669 and Ajay and Azucena). High number of alleles was expected when a large number of landraces were chosen from different geographical origins (Masuduzzaman *et al*., 2016).

Table 2: List of blast resistance genes, markers and the chromosome details

Sr. No.	Gene	Marker	Chromosome		Primer sequences (5'-3')	Reference
1.1	*Pi25*	RM110	2	F	TCGAAGCCATCCACCAACGAAG	Temnykh *et al*., 2001
				R	TCCGTACGCCGACGAGGTCGAG	
1.2		RM485	2	F	CACACTTTCCAGTCCTCTCC	Temnykh *et al*., 2001
				R	CATCTTCCTCTCTTCGGCAC	
2.1	*Pi27*	RM549	6	F	ACGAACTGATCATATCCGCC	Temnykh *et al*., 2001
				R	CTGTGGTTGATCCCTGAACC	
2.2		RM538	6	F	GGTCGTTGAAGCTTACCAGC	Temnykh *et al*., 2001
				R	ACAAGCTCTCAAAACTCGCC	
2.3		RM276	6	F	CTCAACGTTGACACCTCGTG	Lopez-Gerena *et al*., 2004
				R	TCCTCCATCGAGCAGTATCA	
3.1	*Pi29*	RM339	8	F	GTAATCGATGCTGTGGGAAG	Lopez-Gerena *et al*., 2004
				R	GAGTCATGTGATAGCCGATATG	
3.2		RM325	8	F	GACGATGAATCAGGAGAACG	Temnykh *et al*., 2000
				R	GGCATGCATCTGAGTAATGG	
3.3		RM483	8	F	CTTCCACCATAAAACCGGAG	Temnykh *et al*., 2001
				R	ACACCGGTGATCTTGTAGCC	
4.1	*Pi30*	RM441	11	F	ACACCAGAGAGAGAGAGAGAGAG	Zeng *et al*., 2011
				R	TCTGCAACGGCTGATAGATG	
4.2		RM120	11	F	CACACAAGCCCTGTCTCACGACC	Lopez-Gerena *et al*., 2004
				R	CGCTGCGTCATGAGTATGTA	
5.1	*Pi31*	RM83	12	F	ACTCGATGACAAGTTGAGG	Chen *et al*., 1997
				R	CACCTAGACACGATCGAG	
5.2		RM277	12	F	CGGTCAAATCATCACCTGAC	Temnykh *et al*., 2000
				R	CAAGGCTTGCAAGGGAAG	
5.3		RM463	12	F	TTCCCCTCCTTTTATGGTGC	Temnykh *et al*., 2001
				R	TGTTCTCCTCAGTCACTGCG	
5.4		RM260	12	F	ACTCCACTATGACCCAGAG	Lopez-Gerena *et al*., 2004
				R	GAACAATCCCTTCTACGATCG	
6.1	*Pi33*	Pi33-53	8	F	ACAGGATCTTTACCGGCATTTA	Raboin *et al*., 2016
				R	ACGCAAGGAGATTGTTGAGATT	
6.2		RM5647	8	F	ACTCCGACTGCAGTTTTTGC	McCouch *et al*., 2002
				R	AACTTGGTCGTGGACAGTGC	
6.3		RM3507	8	F	ACCCCTATCGATCAACCCTC	McCouch *et al*., 2002
				R	TTCGTTTGGTGTTAGGGGC	
6.4		RM3374	8	F	ATGAACTAGTGAACCCCCCC	McCouch *et al*., 2002
				R	GTAGCGGTAGCTGCAAAAGC	
6.5		RM44	8	F	CGGGCAATCCGAACAACC	Lopez-Gerena *et al*., 2004
				R	TCGGGAAAACCTACCCTACC	
7.1	*Pita*	YL100-102	12	F	CAATGCCGAGTGTGCAAAGG	Eizenga *et al*., 2002
				R	TCAGGTTGAAGATGCATAGC	
7.2		YL183-87	12	F	AGCAGGTTATAAGCTAGCTAT	Eizenga *et al*., 2002
				R	CTACCAACAAGTTCATCAAA	
8.1	*Pib*	RM535	2	F	ACTACATACACGGCCCTTGC	Kim *et al*., 2005
				R	CTACGTGGACACCGTCACAC	
9.1	*Pi20*	RM28050	12	F	GATAAGACTTGGGTGGACATCACG	Li *et al*., 2008
				R	CTTCTATGGTCGCAATTCAGATGC	

Nine genes were used for analysis in the present study *viz.Pi25*, *Pi27*, *Pi29*, *Pi30*,*Pi31*, *Pi33*, *Pita*, *Pib* and *Pi20*. These genes are located on different chromosomes. The *Pi27* is located on chromosome No. 6, *Pi29* and *Pi33* on chromosome number 8, *Pita, Pi20 and Pi31* on chromosome 12, *Pib* and *Pi25* on chromosome 2 and *Pi30* on chromosome 11. The markers linked to these genes were selected (Table 2) for the study.

The nine different resistance imparting genes were targeted for the SSR study in which twenty three SSR markers were used. The twenty three SSR primers amplified a total of 81 alleles with an average of 3.52 alleles per marker. Based on the SSR data, cluster analysis was performed using genetic similarity values and a dendrogram was generated showing genetic relationships among these genotypes. The highest similarity index value of 0.71 was found between Ajaya and IET-20006, while the least similarity index value of 0.14 was found between GR-7 and IET-21094 and also between GR-7 and IET-21070. The average similarity coefficient among genotypes was 0.46. The SSR marker RM83, specific for *Pi31* gene amplified one allele, while RM463 for the same gene amplified5 alleles. The SSR marker RM441 for *Pi30* gene produced maximum number of alleles (7) and RM538 for *Pi27* gene, RM83 for *Pi31* gene, YL183-87 for *Pita* gene and RM28050 for *Pi20* gene produced only single allele. The average number of alleles amplified by 23 markers was 3.52. The highest PIC (Polymorphic Information Content) value obtained was 1.0 in RM538 for *Pi27* gene, YL183-87 for *Pita* gene, and RM28050 for *Pi20* gene and lowest PIC value was 0.12 for YL100-102 for *Pita* gene. The allele frequency, expected hetero zygosity and observed hetero zygosity of each marker were also determined in the present study through GenAl Ex software (version 6.3) (Peakall and Smouse., 2006). The SSR gel results of Pi-33-53 marker for gene *Pi33*is shown in Figure 1. The details of amplification products are given in Table 3.

Table 3: Results of candidate genes and respective molecular markers in SSR analysis

Sr. No.	Gene Name	Marker	Chromosome location	No. of alleles amplified	Molecular Weight Range	Total No. of bands	PIC value
1.1	*Pi25*	RM110	2	5	74-200	5	0.69
1.2		RM485	2	6	270-393	5	0.78
2.1	*Pi27*	RM549	6	3	145-813	3	0.40
2.2		RM538	6	1	290	1	1.0
2.3		RM276	6	4	98-146	4	0.59
3.1	*Pi29*	RM339	8	4	73-155	4	0.28
3.2		RM325	8	2	62-189	2	0.35
3.3		RM483	8	3	263-316	3	0.53
4.1	*Pi30*	RM441	11	7	49-194	7	0.78
4.2		RM120	11	4	144-658	3	0.50
5.1	*Pi31*	RM83	12	1	307	2	1.0
5.2		RM277	12	3	114-128	2	0.41
5.3		RM463	12	5	176-214	5	0.65
5.4		RM260	12	5	70-165	5	0.58
6.1	*Pi33*	Pi33-53	8	3	252-304	6	0.41
6.2		RM5647	8	6	61-144	3	0.70
6.3		RM3507	8	4	86-124	2	0.64
6.4		RM3374	8	3	63-115	1	0.53
6.5		RM44	8	5	104-138	2	0.58
7.1	*Pita*	YL100-102	12	2	366-419	3	0.12
7.2		YL183-87	12	1	308	2	1.0
8.1	*Pib*	RM535	2	3	132-429	3	0.54
9.1	*Pi20*	RM28050	12	1	174	1	1.0
Total	-	-	-	**81**	-	**74**	-
Average	-	-	-	**3.52**	-	**3.22**	**0.59**

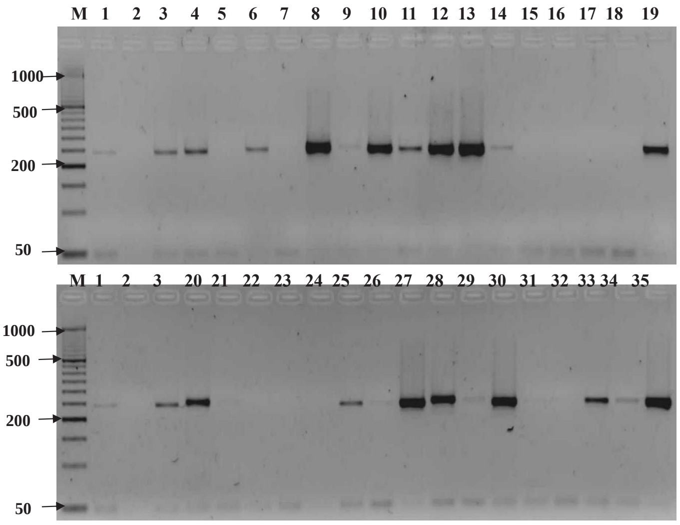

Figure 1: SSR Profile of Pi-33-53 marker for gene *Pi33*, M = 50 bp DNA ladder

1. IR-64	8. Gurjari	15. IET-20235	22. IET-20868	29. IET-21070
2. GR-11	9. NWGR-2006	16. IET-20275	23. IET-20872	30. IET-21094
3. Pankhali- 203	10. Azucena	17. IET-20667	24. IET-20874	31. IET-21190
4. GR-7	11. Ajay	18. IET-20668	25. IET-20892	32. IET-21200
5. GAR-1	12. IET-20006	19. IET-20669	26. IET-20894	33. IET-21216
6. GR-12	13. IET-20082	20. IET-20862	27. IET-20929	34. IET-21299
7. GAR-13	14. IET-20214	21. IET-20866	28. IET-21000	35. IET-21649

Clustering pattern of dendrogram generated by pooled molecular data of 23 markers of nine genes produced two main clusters namely A and B (Figure 1). Cluster A included IR64 and GR7. Cluster B was divided into two sub clusters B1 and B2. Sub cluster B1was again divided into two clusters *viz*. B1a and B1b. The cluster B1a consisted of GR-11, NWGR-2006, IET-20375, IET-20667, IET-20668 and IET-20669 and B1b comprised of only single genotype *i.e.* GAR-13. Sub cluster B2 was divided into B2a and B2b. The cluster B2b had only two genotypes namely IET-20235 and IET-21000. Subcluster B2a was again divided into B2ac and B2ad. The cluster B2ac consisted of Pankhali-203, IET-20892, IET-20868, IET-21190, IET-20894, GAR-1, IET-20866, IET-20872, IET-20874, IET-21070 and IET-21200 whereas cluster B2ad comprised of GR-12, Gurjari, Ajay, IET-20006, Azucena, IET-20862, IET-20082, IET-20214, IET-20929, IET-21216, IET-21299, IET-21094 and IET-21649.

The genotype IR-64 was selected as a resistant genotype in the present study which showed entirely different cluster *i.e.* Cluster A along with GR-7. The genotypes Ajay and Azucena came into the same cluster of B2ad. The genotype IET-20667, IET-20668 and IET-20669 were the pyramided lines of IR64 and were present in the same cluster B1a. (Figure 2) The principle component analysis (PCA) carried out with 35 genotypes almost coincided with the results of cluster analysis. The clustering pattern of 2D and 3D of PCA analysis were in accordance with the dendrogram clustering pattern. The 2D (Figure 3) plot showed that the genotypes IET-20375, IET-20667, IET-20668 and IET-20669 were nearer to each other. Similarly, genotypes IET-20892, IET-20868, IET-21190, IET-20894, GAR-1, IET-20866, IET-20872, IET-20874, IET-21070 and IET-21200 were found to be nearer to each other. In the 2D plot, GR-12, Gurjari, Ajay, Azucena, IET-20006, IET-20862 and IET-20214 were found in close proximity to each other. The genotypes IET-20082, IET-20929, IET-21216, IET-21299, IET-21094 and IET-21649 were also located nearer. The genotypes, Azucena and Ajay overlapped on one another. The principle component analysis (PCA) of 3D (Figure 4) plot coincided with the results of cluster analysis and 2D plot analysis. In 3D plot also genotypes IET-20892, IET-20868, IET-21190, IET-20894, GAR-1 came together. However, IET-20866, IET-20872, IET-20874, IET-21070 were found nearer but slightly away from it. The Ajaya and Azucena were nearer to each other. All other genotypes came in same group like in 2D and cluster analysis.

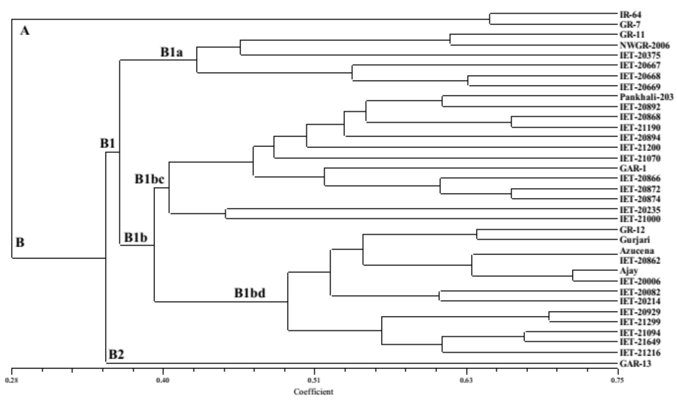

Figure 2: Dendrogram showing clustering of 35 rice genotypes for blast resistance genes constructed using UPGMA based on Jaccard's coefficient obtained from SSR analysis

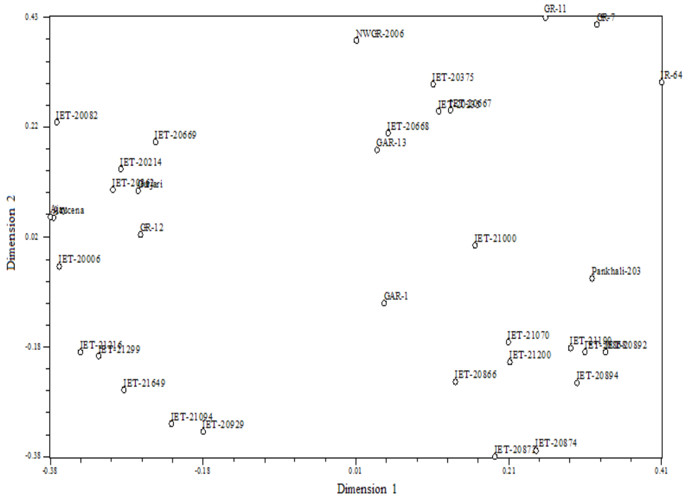

Figure 3: Two dimensional plot by PCA using SSR primers

Correlation study was carried out to compare the correlation of original similarity matrix of SSR results with the dendrogram clustering pattern. Using the COPH module of NTSYSpc version 2.02, ‘r’ value was calculated and results were also expressed graphically. High correlation between the similarity matrix and dendrogram pattern was justified by the ‘r’ value which was found to be 0.77 and was found good to fit. The genotypes IR 64 and GR-7 were found to be in the same cluster. The genotypes IET-20667, IET-20668 and IET-20669 showed similar allelic pattern in most of the markers. The Azucena and Ajaya were found to be closely related to each other as they showed similar banding pattern in maximum number of the markers studied. The diversity of alleles in blast resistant genotypes using 32 improved varieties with three micro-satellite marker including RM483 and a similarity matrix based on pair wise comparison of pooled data showed 60% similarity and three allelic conditions of the plants were produced (Lang *et al*., 2009). In the present study, 46% of similarity was observed and up to seven allelic conditions were also found using 23 markers including RM483. This variation may be due to the difference in number of markers and genotypes used in the present study and also may be due to the difference in varieties used.

The productivity and yield of cultivated rice, is severely affected by many diseases. Among them blast is the most destructive. Rice blast and gray leaf spot of grasses are caused by *Magnaporthe oryzae*. The disease often causes a significant yield loss, as high as 70-80% during an epidemic. Rice blast has been found in over 85 countries across the world. The disease has never been eradicated from the regions of rice cultivation.

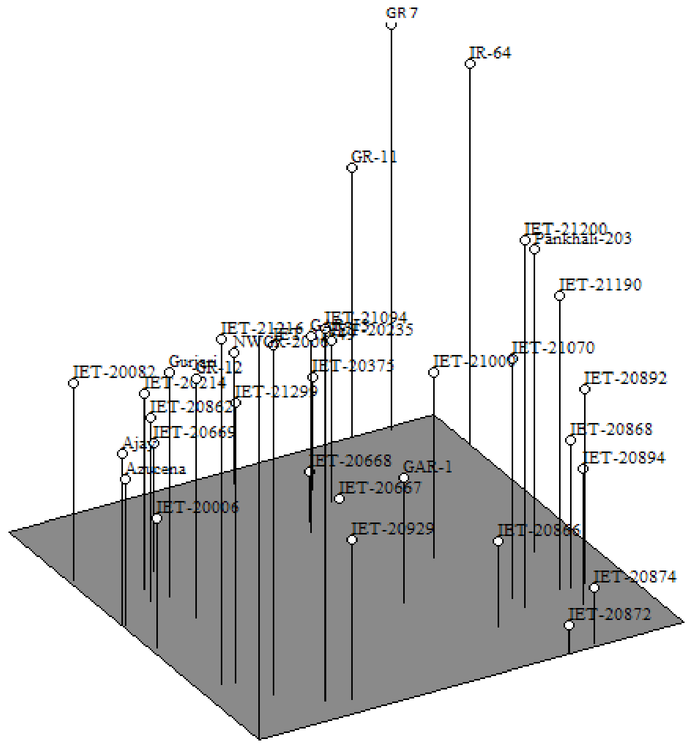

Figure 4: Three dimensional plot by PCA using SSR primers

To negate the effect of blast disease to some extent some has resorted to the use of fungicides and others to development of resistant cultivars. Utilization of genetic resistance is the most effective and economical way of controlling blast disease. In the past few decades, more than 40 major blast resistance genes have been mapped through molecular marker technology. However, because of either the rapid evolution of new pathogen races, or the selection of a rare component of the pathogen population that is already virulent the resistance has been short lived.

Molecular analysis of genotypes prior to crosses can increase genetic diversity among parental genotypes. This helps in maximizing genetic variation and minimizing the efforts in the screening, for direct selection in traditional breeding. Hence, the present investigation was undertaken to detect the polymorphism among blast resistant and susceptible rice genotypes. With the aid of information obtained from microsatellite marker study, assessment of linkage between specific marker and its gene could be done which will help in breeding and development of pyramided lines harboring different blast resistant genes leading to the development of much efficient genotypes for fighting the malady of blast.

Acknowledgments

Authors are thankful to the Department of Agricultural Botany, Department of Agricultural Biotechnology, Anand Agricultural University, Anand, Gujarat for providing basic laboratory facilities for the molecular research. We are also grateful to Late Dr. Atul Mehta, Main Rice Research Station, Nawagam, Gujarat,for valuable suggestions and providing seeds of rice genotypes. We are grateful to Dr. G.C. Jadeja, Former Principal, B.A. College of Agriculture,AnandAgricultural University, Anand, for his consistent guidance with constructive suggestions, deliberative discussions and active persuasion.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Effect of organic and inorganic substances on the growth and yield of rice

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Received: 28 July 2019; Accepted: 28 November 2019

Abstract

In order to enhance the yield of rice by foliar application of organic and inorganic substances, a field experiment was conducted in a Randomized Block Design with three replications at Annamalai University Experimental farm, Annamalai Nagar during Late *Kuruvai* season (July - September) with CO - 47 rice cultivar. Among the different treatments tried, application of 100% NPK + humic acid granules @ 12.5 kg/ha along with foliar application of Panchagavya @ 3% + Fish meal extract @ 3% + Auxin Gold sea weed extract @ 0.35% on 20, 35 and 50 DAT performed better compared to other treatments and ranked first in terms of plant height, number of productive tillers/m^2 (353), number of spikelets /panicle (119), sterility per centage (11.4), grain yield (5.6 t/ha) and straw yield (8.5 t/ha).

Keywords: Fish meal extract, Panchagavya, Seaweed extract, Potassium nitrate, Productive tillers and Yield

Introduction

In the world, rice is the second most widely consumed cereal next to wheat and it has occupied an area of 163.47 million hectares with production and productivity of 501.56 million tonnes and 4.58 t/ha, respectively (USDA, 2019). In Asia, rice and it derived products provide 60 to 70 per cent of energy requirement to more than 2 billion people. In Tamil Nadu state of India, rice is grown in an area of 17.80 lakh hectares with production and productivity of 60 lakh metric tonnes and 3.37 t/ha, respectively. In Cuddalore district rice is grown in an area of 134677 hectares with production of 641958 metric tonnes and productivity of 4.1 t/ha. Continuous use of chemical fertilizers causes long term imbalance in pH and fertility of the soil. Moreover, applied nitrogen (N), phosphorus (P) and potassium (K) fertilizer undergoes various transformation processes in soil. Application of organic nutrient is more appropriate because it contributes to the improvement and sustainability of natural resources and environment. Application of humic acid improves the plant growth and yield by increasing the soil organic matter. Panchagavya is used in different means in agriculture such as foliar spray, soil application along with irrigation water, seed or seedling treatments etc. Panchagavya contains several vitamins, macro nutrients, micro nutrients, amino acid, growth regulators like auxin, gibberellins and useful microorganisms which is required for the growth and development of crops (Ajay Alias Mangtu Ram, 2017). Fish amino acid, a potential source of auxin and promoting the vegetative growth through active cell division, cell enlargement and cell elongation. Sea weed extract application on plants resulted in wide benefits like early seed germination , establishment, improved crop performance and yield, improved resistance to biotic and abiotic stress, enhanced postharvest shelf life of perishable products. This study was onducted mainly to get higher yields, sustain soil health and to reduce the cost of fertilizers through the integration of foliar application of different organic sources with recommended NPK tried.

Materials and Methods

A field experiment was conducted at the Experimental Farm, Department of Agronomy, Annamalai University, Annamalai Nagar which is situated at 11°24’ N latitude and 79°44’ E longitude and at an altitude of +5.79 m above mean sea level during Late *Kuruvai* (July to September 2018) season with rice variety Co-47 to study the effect of organic and inorganic substances on rice along with 100 and 75% recommended dose of NPK individually and as combined spray. The experimental field soil was clayey loam in texture, low in available nitrogen (210 Kg/ha), medium in available phosphorus (19.6 Kg/ha) and high in available potassium (319.5 Kg/ha). The experiment was laid out in a Randomized Block Design with 12 treatments and replicated thrice. The twelve treatments were T_1- 100% NPK + Humic acid granules @ 12.5 kg/ha, T_2 - T_1 + Panchagavya spray @ 3% at 20, 35 and 50 DAT, T_3 - T_1 + Fish meal extract spray @ 3% at 20, 35 and 50 DAT, T_4 - T_1 + Auxin Gold sea weed extract spray @ 0.35% at 20, 35 and 50 DAT, T_5 - T_1 + Potassium nitrate spray @ 0.5% at 20, 35 and 50 DAT, T_6 - T_1 + Panchagavya + Fish meal extract + Auxin Gold sea weed extract spray at 20, 35 and 50 DAT, T_7 - 75% NPK + Humic acid granules @ 12.5 kg/ha, T_8 – T_7 + Panchagavya spray @ 3% at 20, 35 and 50 DAT, T_9 – T_7 + Fish meal extract spray @ 3% at 20, 35 and 50 DAT, T_{10} – T_7 + Auxin Gold sea weed extract spray @ 0.35% at 20, 35 and 50 DAT, T_{11} – T_7 + Potassium nitrate spray @ 0.5% at 20, 35 and 50 DAT, T_{12} – T_7 + Panchagavya + Fish meal extract + Auxin Gold sea weed extract spray at 20, 35 and 50 DAT. 21 days old seedlings were transplanted @ 2/hill with a spacing of 15x10 cm. The recommended dose of 120:40:40 kg/ha was adopted for 100 per cent and from that 75 per cent NPK was calculated and were applied to plots as per treatment schedule. Half dose of N, K and full dose of P were applied as basal and the remaining N and K were top dressed in two equal split at active tillering and panicle initiation stages. The various organic and inorganic substances were sprayed at respective concentrations at 20, 35 and 50 DAT as per treatment schedule.

Results and Discussion

Among the treatments tested, T_6–100% NPK + Humic acid granules @ 12.5 Kg/ha + Panchagavya + Fish meal extract + sea weed extract spray on 20,35 and 50 DAT excelled rest of the treatments and resulted in the tallest plant (Table 1) on 60 DAT and at harvest (89.10 and 99.73 cm, respectively). This might be due to the optimum level of nutrients available in the rhizo - ecosystem of soil applied with basal application of fertilizer resulting in better growth and development. Application of nutrients at critical stages would have resulted in better vegetative growth as observed by taller plants. In this treatment panchagavya spray would have provided growth promoting substances, encouraging quick growth and increased the plant height. This result of increased shoot length is in line with the report of Mallavarapu Geethav *et al.* (2015). Among the foliar spraying of organic substances individually with 75 or 100% NPK, spraying of fish meal extract was found to be better. Application of 75% NPK along with humic acid granules application @ 12.5 kg/ha resulted in reduced plant height to the tune of 8.3 and 9.2 cm compared to 100% NPK + humic acid granules application 12.5 kg/ha at 20, 35 and 50 DAT respectively. Within the treatments tested, T_6 - 100% NPK + Humic acid granules @ 12.5 Kg/ha + Panchagavya @ 3%+ Fish meal extract @ 3%+ sea weed extract spray @ 0.35% on 20, 35 and 50 DAT along with application of humic acid granules @ 12.5 kg/ha ranked first with a value of 353 productive tillers/m^2. This may be due to the presence of certain plant growth regulators, trace elements, vitamins and micronutrients in organic substances which might have enhanced the growth and yield contributing characters. Combined application of each 3% of Panchagavya + Fish meal extract + sea weed extract spray along with 100% NPK + humic acid granules application @ 12.5 kg/ha increased the number of productive tillers/m^2 to the tune of 7.29 per cent over T_1- 100% NPK + Humic acid granules application @ 12.5 kg/ha. Application of 75% NPK along with humic acid granules application @ 12.5 kg/ha resulted in reduced total number of productive tillers/m^2 to the tune of 6.8 per cent compared to 100% NPK + humic acid granules application @ 12.5 kg/ha.

Among the treatments tested, T_6 - 100% NPK + Humic acid granules @ 12.5 Kg/ha+ Panchagavya + Fish meal extract + sea weed extract spray on 20, 35 and 50 DAT surpassed all other treatments and produced the highest number of spikelets/panicle (119) and with least (11.41) sterility per centage. The greater availability of nutrients in soil, improvement of soil environment resulting in higher root proliferation leading to better absorption of moisture and nutrients which ultimately resulted in higher yield parameters and yield. In rice, increased grains/panicle, grain yield due to spraying of *Kappaphycus alvarezii* @ 10% + recommended NPK result is in agreement with the report of Singh *et al*. (2018). The second in order was T_3- 100% NPK + Humic acid granules @ 12.5 Kg/ha + Fish meal extract spray @ 3% on 20, 35 and 50 DAT and it was comparable with T_2 - T_1 + Panchagavya spray @ 3%, T_4-T_1 + Auxin Gold Sea weed extract spray @ 0.35% and T_5 - T_1 + Potassium nitrate spray @ 0.5% on 20, 35 and 50 DAT. Application of 75% NPK along with humic acid granules application @ 12.5 kg/ha resulted in reduced number of spikelets/panicle to the tune of 7.6 per cent compared to 100% NPK + humic acid granules @ 12.5 kg/ha. Foliar application of organic substances or inorganic substances individually with 75% NPK + humic acid @ 12.5 kg/ha resulted in similar effect.

Among the treatments tried out, T_6 - 100% NPK + Humic acid granules @ 12.5 Kg/ha + Panchagavya + Fish meal extract + sea weed extract spray on 20,35 and 50 DAT surpassed rest of the treatments with a grain yield of 5660 kg/ha which is 12.52 per cent higher than T_1- 100% NPK + Humic acid granules @ 12.5 kg/ha. That may be due to the highest number of productive tillers/m^2, number of spikelets/panicle and number of filled grains/panicle. Yield increase was due to quick absorption and assimilation of more nitrogen, phosphorus, potassium and micro nutrients present in inorganic fertilizers and organic substances. This lead to physiological and morphological character improvement and finally reflected in higher yield. Similar result of increased growth attributes and yield due to the combined application was earlier reported by Carol Lyngdoh *et al*., (2017). The second best was T_3 - T_1 + Fish meal extract spray @ 3% on 20, 35 and 50 DAT and it was on par with T_2 - T_1 + Panchagavya spray @ 3% on 20, 35 and 50 DAT and T_4 - T_1 + Auxin Gold Sea weed extract spray @ 0.35% on 20, 35 and 50 DAT. Foliar application of panchagavya @ 3% or fish meal extract 3% or sea weed extract 0.35% increased the grain yield to the tune of 372, 439 and 327 kg/ha respectively over T_1- 100% NPK + Humic acid granules @ 12.5 kg/ha. Application of 25% less NPK compared to 100% recommended NPK + humic acid granules @ 12.5 kg/ha reduced the grain yield to the extent of 790 kg/ha. This might be due to the unavailability of required quantity of nutrients present in soil during the crop period which resulted in reduced yield components. Similar results of reduced grain yield to the tune of 1.58 t/ha were obtained by Ali *et al.,* (2009). The combined application of Panchagavya + Fish meal extract + sea weed extract along with 75% NPK + humic acid granules @ 12.5 kg/ha gave 16.29 per cent additional yield over T_7 - 75% NPK + Humic acid granules @ 12.5 kg/ha. Application of

Table 1: Effect of various organic and inorganic substances on growth and yield of rice

Treatments	Plant height at 60 DAT (cm)	Plant height at harvest (cm)	No. of productive tillers/ m^2	No. of spikelets/ panicle	Sterility (%)	Grain yield (Kg/ha)	Straw yield (Kg/ha)
T_1- 100% NPK + Humic acid granules @ 12.5 kg/ha	77.7	89.73	329	108.45	18.03	5030	7599
T_2 - T_1 + Panchagavya spray @ 3% on 20, 35 and 50 DAT	82.49	93.66	341	111.18	16.03	5402	8242
T_3 - T_1 + Fish meal extract spray @ 3% on 20, 35 and 50 DAT	83.62	95.23	342	111.86	13.96	5469	8281
T_4 - T_1 + Auxin Gold Sea weed extract spray @ 0.35% on 20, 35, 50 DAT	82.13	92.87	339	110.3	16.54	5357	8157
T_5 - T_1 + Potassium nitrate spray @ 0.5% on 20, 35 and 50 DAT	81.1	91.82	334	108.44	17.5	5296	7803
T_6 - T_1 + Panchagavya, Fish meal extract and sea weed extract spray on 20, 35 and 50 DAT (T_2+T_3+T_4)	89.1	99.73	353	119	11.41	5660	8569
T_7 - 75% NPK + Humic acid granules @ 12.5 kg/ha	69.4	80.53	308	100.8	25.94	4240	6431
T_8 - T_7 + Panchagavya spray @ 3% on 20, 35 and 50 DAT	76.28	86.29	322	102.47	21.36	4627	7166
T_9 - T_7 + Fish meal extract spray @ 3% on 20, 35 and 50 DAT	77.37	88.45	322	103.72	20.61	4627	7008
T_{10} - T_7 + Auxin Gold Sea weed extract spray @ 0.35% on 20, 35 and 50 DAT	75.84	84.36	316	101.65	21.81	4638	6820
T_{11} - T_7 + Potassium nitrate spray @ 0.5% on 20, 35 and 50 DAT	72.61	81.94	310	101.94	25.34	4326	6920
T_{12} – T_7 + Panchagavya, Fish meal extract and sea weed extract spray on 20, 35 and 50 DAT (T_8+T_9+ T_{10})	79.83	90.25	329	106.29	19.44	4931	7445
S. Ed	1.42	1.62	6	1.95	0.41	90	137
CD (p = 0.05)	2.95	3.37	12	4.06	0.86	186	285

potassium nitrate either with 75% NPK or 100% NPK with humic acid granules @ 12.5 Kg/ha improved the grain yield to the tune of 86 and 266 Kg/ha, respectively.

Among the treatments tested, T_6 - 100% NPK + Humic acid granules @ 12.5 Kg/ha + Panchagavya + Fish meal extract + sea weed extract spray on 20, 35 and 50 DAT surpassed all other treatments and resulted in the highest straw yield (8569 kg/ha). This might be due to increased growth parameters like plant height, more number of tillers, leaf area index and dry matter production. Better performance of combined use of organic substances with chemical fertilizer might be due to synergistic effect of inorganic fertilizers and organic substances as well as the slow release of nutrients throughout the crop growth, thus helping to produce more photosynthates and also the immediate release of N and improved soil physical properties due to application of humic acid granules. The Application of 25% less NPK resulted in 18.16% less straw yield compared to 100% NPK application. Foliar application of panchagavya or fish meal extract or sea weed extract increased the straw yield to the tune of 735, 577 and 389 kg/ha over 75% NPK + humic acid granules @ 12.5 kg/ha, respectively.

Conclusion

Application of 100% NPK + Humic acid granules @ 12.5 Kg/ha + Panchagavya @ 3% + Fish meal extract @ 3% + sea weed extract spray @ 0.35% on 20, 35 and 50 DAT is recommended for low land rice to get higher yields.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Isolation, identification and characterization of efficient free-living nitrogen-fixing bacteria from rice rhizosphere ecosystem

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Received: 22 April 2019; Accepted: 28 August 2019

Abstract

Biological nitrogen fixation (BNF) is a natural bacterial process of utmost importance due to its potential to supply alternative source of plant available nitrogen for the crops to enable sustainable and economical production. The aim of the present study is to isolate efficient nitrogen fixing bacteria with a potential to be developed into bio-fertilizer for supplying nitrogen and for improving growth and yield of rice. With this background, rice (*Oryza sativa L.*) rhizosphere soil samples were collected from different locations and 197 nitrogen-fixing bacterial strains were isolated. Among these, eight promising bacterial strains that showed highest nitrogen fixing ability based on Acetylene Reduction Assay **(**ARA) were isolated and identified as *Stenotrophomonas sp.*, *Paenibacillus sp.* (isolate 1), *Ochrobactrum sp.*, *Paenibacillus sp.* (isolate 2), *Burkholderia cepacia* (isolate 1), *Burkholderia cepacia*, *Xanthomonas sacchari* and *Rhizobium sp.* The reduction rates of the eight potential isolates indicating the nitrogen-fixing ability ranged from 115 to 490 nm of C_2H_4 produced h^{-1} mg^{-1} protein. These bacterial isolates were further characterized for their morphological features and for their plant growth-promoting (PGP) activity like indole acetic acid (IAA) production and insoluble mineral (P, K, Zn) solubilization. All isolates exhibited IAA production ranging from 11.90 to 47.40 µg/ml while two isolates possessed phosphate and zinc solubilization activity.

Keywords: Nitrogen-fixing bacteria, IAA, ARA, Phosphate-solubilizing bacteria, rice ecosystem

Introduction

Microbes are considered as engineers of soils, playing a vital role in maintaining soil fertility by enriching the availability of nitrates, phosphorous and other nutrients (Rajendhran and Gunasekaran, 2008). Rice (*Oryza sativa)* is a primary food source for half of the world's population. Rice cultivated in different ecologies supports a wide diversity of soil microbes and soil fauna in aqua terrestrial ecosystems. Biologically fixed nitrogen is a potential alternative source of nitrogen for sustainable cereal crops production (Rogers and Oldroyd, 2014). Diazotrophic free-living bacteria is known to contribute 20 kilograms per hectare per year to the long-term nitrogen needs of cereals rotation cropping system (30-50% of the total needs) (Vadakattu and Paterson, 2006). Diazotrophs are found among most of the bacterial groups like alphaproteobacteria, gammaproteobacteria, firmicutes, beta proteobacteria and cyanobacteria, but are not the most abundant (dominant) bacteria in plant rhizospheres, so there is scope for increasing nitrogen-fixation by favouring their populations in rice rhizosphere. Many rhizospheric bacteria also contribute to plant growth promotion by producing plant hormones. Indole acetic acid (IAA) producing bacteria promotes the growth and root architecture of the host plant and is also thought to be a trait required for better rhizosphere colonization and competence (De Salamone *et al.*, 2005). IAA from plant growth-promoting rhizobacteria (PGPR) can loosen cell walls of the plant, which leads to enhancing level of root exudation that provides nutrients for the growth and development of bacteria in rhizosphere (Chi *et al.*, 2005).

Phosphate-solubilizing bacteria (PSB) enhance phosphorus availability to plant which leads to improved root architecture, uptake of mineral nutrients and water from the soil (Rodriguez *et al.*, 2006). Phosphate solubilizing microorganism (PSM) includes a diverse group of microorganisms including bacteria, fungi and actinomycetes with plant growth-promoting abilities like, biological nitrogen fixation, phytohormones production, biocontrol activities etc. and bacteria hold foremost position as PSM than fungi and actinomycetes with a population of 1-50% among total soil microbial populations (Alam *et al.*, 2002). Plant growth-promoting, zinc solubilizing rhizobacteria have also been found to enhance the plant growth and development by colonizing the rhizosphere and making zinc available to the plants (Rodriguez *et al.*, 2006). The aim of the present study was to isolate, identify and characterise nitrogen-fixing bacteria from different rice ecosystems.

Materials and methods

Soil samples: Rice rhizosphere soil samples were collected from farmers fields in Ranga Reddy, Vikarabad (Telangana) and Mandya districts (Karnataka) where rice was grown under irrigated and aerobic ecologies. Soil samples were collected at the rice reproductive stage (10-15 cm depth) and immediately stored in sterile polythene bags at 4^{o}C until further analysis.

Isolation of nitrogen-fixing bacteria: Ten grams of rhizosphere soil samples were suspended in Erlenmeyer flask containing 90 ml of sterile distilled water and mixed thoroughly in an incubated shaker (120 rpm) at 28 ^{o}C for 1 hr. Appropriate serial dilutions were prepared and 0.1 ml (10^{-3} to 10^{-6} dilutions) aliquots were plated on N (Nitrogen) free Rennie's media (Atlas, 2004). The petri plates were incubated at 28 ± 2 ºC in an incubator for 3-7 days. Bacterial isolates showing visually differential growth were picked up and purified on the same media. The purified bacterial cultures were maintained on N-free Rennie's slants and stored on 50% glycerol stock at 4 ºC and -80 ºC. Sub culturing of purified bacterial cultures were carried out as and when required.

Acetylene Reduction Assay (ARA): Nitrogenase activity *i.e.* acetylene reduction assay of isolated and purified culture was determined by using Gas Chromatography (GC) method (Hardy *et al.*, 1971). Purified cultures were inoculated on Rennie's slant and incubated at 28 ± 2 ºC for 3 days in an incubator. Acetylene gas (10% V/V) was injected in the tube and incubated for 24 hrs. Appropriate uninoculated controls were maintained. After incubation, 1 ml air sample was removed from the tubes and injected into Gas chromatograph for analysis. The ethylene produced by reduction of acetylene was assayed using a Gas chromatograph (Thermo Scientific, GC1110 model) with FID detector having Porapak N column using N_2 as a carrier gas. After completion of ARA, to determine protein content, cells were suspended in 2 ml of 0.2 N NaOH, incubated for 10 min at 65 ºC, followed by neutralization using 2 ml of 0.2 N HCl. Protein concentration in resultant mixture was determined by using Bradford method (Bradford, 1976). ARA was expressed in terms of hmoles of ethylene produced per mg protein per hr. The observations were recorded in three replications per treatment.

Genomic DNA Extraction: Eight cultures with high ARA activity were selected and grown in 5 ml of N- free Rennin's broth at 28 ± 2 ºC for 24 hrs. Log phase cultures were used for isolation of total genomic DNA using CTAB /NaCl method (William *et al.*, 2012). Precipitated DNA was pelleted down by centrifuging at 8000 rpm for 10 min, washed with 70% ethanol, air-dried, dissolved in the TE buffer and stored at 4 ^{o}C for further use.

Amplification of 16S rDNA and identification of isolates: The 16S rRNA gene from the bacterial genomic DNA was amplified using FGPS6 (forward primer) 5'GGAGAGTTAGATCTTGGCTCAG3' and FGPS1509 (reverse primer) 5'AAGGAGGGGATCCAGCCGCA3 (Normand *et al.*, 1992). In a reaction mixture, 50 ng of template DNA, primer (10 pmol each), dNTP (200 µM each) and 5 U Taq DNA polymerase (Takara) were used. The final volume of the reaction mixture was adjusted to 50 µl. Amplification was carried out under standard conditions (initial denaturation at 95 ºC for 5 min, 30 cycles of denaturation at 95 ºC for 1 min, 30 cycles of annealing at 60 ºC for 45 sec, extension at 72 ºC for 1 min and a final extension at 72 ºC for 7 min). Amplified PCR product was resolved on 1.2% of agarose gel in 1xTAE buffer. The amplified product was purified by using PCR cleanup kit (Favorgen) according to manufacturer instructions. Amplified 16S rRNA gene of the selected isolates was sequenced by Sanger sequencing (Eurofins, India). The sequences were compared using BLAST search tool with already known and identified microbial databases of National Center for Biotechnology Information (NCBI) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for identification of the isolates.

Indole acetic acid (IAA) production: Indole Acetic Acid (IAA) production ability of the identified cultures was carried out by using the Hartmann method by growing cultures in Tryptic soya broth amended filter sterilized tryptophan (100 µg/ml broth) for 7 days at 28^{o}C (Hartmann *et al.*, 1983). After incubation, an aliquot of the culture supernatant was mixed with equal volumes of Salkowski reagent solution and incubated for 30 minutes to allow the colour to develop before taking reading absorbance at 530 nm using Spectrophotometer (model Shimadzu, UV-1800). The IAA production by the cultures was expressed as µg IAA produced/ ml. Appropriate uninoculated controls were maintained. Three replications for each treatment were maintained and the experiment repeated twice.

Phosphorous-solubilization: The ability of the isolates to solubilize inorganic phosphate was determined by spotting 5 µl (10^8 CFU/ ml) of fresh bacterial culture on Pikovskaya media (Pikovskaya, 1948). Plates were observed for the formation of transparent halos around each bacterial colony and formation of a clear zone around the colony indicates solubilization.

Zinc solubilisation: Cultures were inoculated as spot on respective zinc medium plates for studying the zinc solubilization (Sharma *et al*., 2012). These plates were covered with aluminium foil and incubated in dark at 28 ^{o}C for 14 days and further observed for the zinc solubilization which appears as a halo around the colony.

Potassium solubilisation: A loopful of culture of each bacteria were inoculated into 25 ml Aleksandrov medium and incubated at 28 ±2 °C for 10 days. The growth suspension was centrifuged at 8,000 rpm for 10 min to separate the supernatant from the cell growth and insoluble potassium. One ml of the supernatant was diluted to 50 ml with distilled water, mixed thoroughly and the resultant solution was used to determine K content with atomic absorption spectrometer. (Alexander, 1985).

Results and Discussion

In the present investigation, a total of 197 nitrogen-fixing bacterial cultures were isolated from the rice fields. Among the 197 isolates, eight potential bacterial cultures were selected and further processed for molecular identification (16S rRNA gene sequencing), morphological and plant growth-promoting activities.

Acetylene reduction by the promising isolates

Nitrogenase, the enzyme that is responsible for biological nitrogen fixing, reduces N_2 to NH_3. It also has the capacity of reducing acetylene to ethylene. So, measuring the quantity of ethylene that is produced in the medium provides a way of estimating nitrogenase activity. Acetylene Reducing Assay (ARA) was carried out to examine the nitrogen-fixing ability of isolated cultures through gas chromatographic (GC) analysis. Out of 197 cultures, eight cultures showed a high ARA activity ranging from 115 to 490 nmoles ethylene/h/mg protein (Table 1).

Table 1: Nitrogenase (ARA) activity of promising isolates

S. No.	Selected isolates	Acetylene reduction assay (ARA)
		ηM of C_2H_4 produced h^{-1} mg^{-1} protein
1	IIRR A3	193
2	IIRR C4	314
3	IIRR E3	149
4	IIRR F4	490
5	IIRR J3	140
6	IIRR J4	115
7	IIRR M4	123
8	IIRR N	410

Identification of selected isolates

The molecular characterization of eight promising isolates (based on higher ARA activity) was carried out using PCR based 16S rRNA gene amplification and Sanger sequencing. After PCR based amplification of 16S rRNA gene (1,450 bp) and Sanger sequencing a BLASTN search of the amplified 16S rRNA gene sequence database was performed by the BLAST search tool and identified based on the similarity to the closest type strain affiliated to each of the isolated strains. The 16S rRNA gene nucleotide sequences of IIRR E3 isolate was showed the high similarity (100%) to the 16S rRNA gene sequences of strain *Ochrobactrum sp*. JF313266.1, while the other cultures *viz.*, IIRR A3 (92%), IIRR C4 (82%), IIRR J3 (97.49), IIRR F4 (96.57%), IIRR J4 (97.98%), IIRR M4 (90.65%) and IIRR N (95.7%) were showed the similarity to the 16S rRNA gene nucleotide sequences with *Stenotrophomonas sp., Paenibacillus sp., Burkholderia cepacia, Paenibacillus sp.* and *Burkholderia cepacia*, respectively (Table 2). Based on the identification, highest ARA activity among the promising isolates was recorded in *Rhizobium sp.* (490 nmoles ethylene/h/mg protein) and lowest activity was seen in *Burkholderia cepacia* (115 nmoles ethylene/h/ mg protein). All eight promising nitrogen-fixing cultures were stored at 4 °C and -20 °C in glycerol stock for further studies.

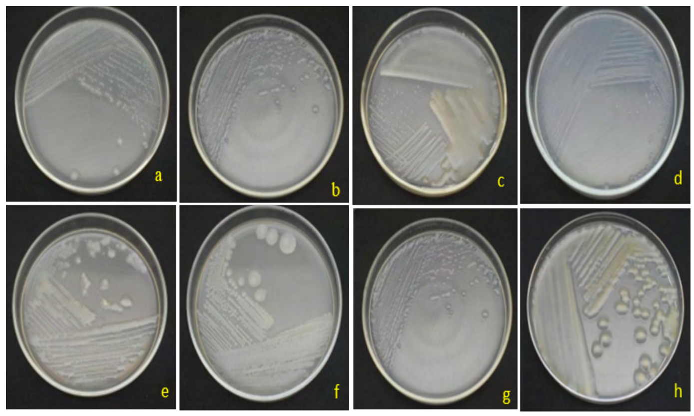
Table 2: Promising N-fixing bacteria identified from different rice ecosystems

S. No.	Selected isolates	Identified promising N-fixing bacteria
1	IIRR A3	*Stenotrophomonas sp.*
2	IIRR C4	*Paenibacillus sp.* (isolate 1)
3	IIRR E3	*Ochrobactrum sp.*
4	IIRR F4	*Paenibacillus sp.* (isolate 2)
5	IIRR J3	*Burkholderia cepacia* (isolate 1)
6	IIRR J4	*Burkholderia cepacia* (isolate 2)
7	IIRR M4	*Xanthomonas sacchari*
8	IIRR N	*Rhizobium sp.*

Our results are similar to earlier reports on the identification of nitrogen-fixing bacteria in various crops. Majeed *et al.* (2015) demonstrated that diazotrophic strains of *Stenotrophomonas sp.* exhibited multiple plant growth promotion activities and colonized the wheat rhizosphere as well as in the root interiors. They also identified five *Paenibacillus sp.* exhibiting IAA production and nitrogenase activities (*Paenibacillus sp.* SZ-10, *Paenibacillus sp.* JS-4, *Paenibacillus sp.* SZ-14, *Paenibacillus sp.* SZ-15 and *Paenibacillus sp.* BJ-4), which significantly enhanced the growth and dry weight of wheat (Liu *et al*. 2019). Also, *Paenibacillus polymyxa* M1 isolated from root tissues of wheat was reported to promote plant growth (Yao *et al.*, 2008; Niu *et al.*, 2011). In relation with our results, *Ochrobactrum sp.* has been earlier reported in some legumes showed the complete symbiotic relationship and also establishing the biological nitrogen fixation with host plant (Trujillo *et al.*, 2005, Meng *et al.*, 2014). Dobereiner (1997) identified microaerophilic bacteria *viz. Burkholderia sp.* that colonize shoots, leaves and roots of rice, wheat and maize leading to enhanced growth.

Morphological characterization

The identified eight bacterial cultures were characterized morphologically based on their size (which varied from very small to small, moderate and large) and apparent differences in slime production. Among these, four bacterial cultures (*Stenotrophomonas sp.*, *Paenibacillus sp.* isolate 1, *Xanthomonas sacchari* and *Rhizobium sp.*) were fast growers and the other four cultures (*Ochrobactrum sp.*, *Paenibacillus sp.* isolate 2, *Burkholderia cepacia.* isolate 1 and *Burkholderia cepacia.* isolate 2) showed slow growth on N-free medium. Pigmentation of colonies was less and varied from non-pigmentation, to creamy yellow (*Xanthomonas sacchari*) pigmentation. (Figure 1, Table 3).



a- *Stenotrophomonas* sp, b- *Paenibacillus* sp, c- *Burkholderia cepacia*, d- *Burkholderia cepacia*, e- *Onchrobactrum* sp, f- *Rhizobium* sp, g- *Paenibacillus* sp, h- *Xanthomonas sacchari*

Figure 1: Colony morphology of identified eight promising nitrogen-fixing bacteria

Table 3: Morphological characterization of identified nitrogen-fixing bacteria

S. No.	Selected isolates	Growth	Size	Slime Production	Pigmentation
1	*Stenotrophomonas sp.*	Active	Very small	++	Creamy - Non-pigmentation
2	*Paenibacillus sp.* (isolate 1)	Active	Small	++	Creamy - Non-pigmentation
3	*Ochrobactrum sp.*	Slow	Small	+	Creamy- Yellow Pigmentation
4	*Paenibacillus sp.* (isolate 2)	Slow	Moderate	++	Non-pigmentation
5	*Burkholderia cepacia* (isolate 1)	Slow	Large	+	Creamy Non-pigmentation
6	*Burkholderia cepacia* (isolate 2)	Slow	Large	++	Cream - Non-pigmentation
7	*Xanthomonas sacchari*	Active	Moderate	++++	Cream - Non-pigmentation
8	*Rhizobium sp.*	Active	Large	+++	Yellow - Pigmentation

Media: NA - Nutrient Agar (NA) and Rennie, ++++ More slime production, + Less slime production

Characterization for Indole acetic acid (IAA) production and Mineral solubilization

The eight isolates were further tested for their ability to produce IAA and to solubilize insoluble potassium, phosphorous and zinc solubilization. All eight isolates grown in the minimal medium supplemented with tryptophan exhibited considerable production of IAA with amounts ranging from 11.90 to 47.40 µg/ml. Among these, *Orthobacterium sp*. showed the greatest IAA production (47.40 µg/ml) and *Burkholderia cepacia* had lower IAA production of 11.90 µg/ml. (Table 4). In addition to the nitrogen-fixing ability of isolates, the two isolates *viz*. *Ochrobacterum sp*. and *Xanthomonas sacchari* displayed solubilization of phosphate and zinc. None of the isolates demonstrated potassium solubilizing activity (Table 4).

Table 4: IAA production and mineral solubilizing capacity of promising isolates

S. No.	Selected isolates	Indole acetic acid (IAA) assay (µg/ml)	Insoluble minerals solubilization assay		
			Potassium	Phosphorous	Zinc
1	*Stenotrophomonas sp.*	28.70	-	-	-
2	*Paenibacillus sp.* (isolate 1)	15.60	-	-	-
3	*Ochrobactrum sp.*	47.40	-	+	+
4	*Paenibacillus sp.* (isolate 2)	30.75	-	-	-
5	*Burkholderia cepacia* (isolate 1)	14.80	-	-	-
6	*Burkholderia cepacia* (isolate 2)	11.90	-	-	-
7	*Xanthomonas sacchari*	38.50	-	+	+
8	*Rhizobium sp.*	19.91	-	-	-

+ (Positive) - Showing solubilization, - (Negative) - Not showing solubilization

Leinhos (1994) reported bacterial biosynthesis of IAA in many rhizobacteria and it has been assumed that approximately 80% of rhizospheric bacteria can secrete IAA. However, Joseph *et al.* (2007) reported that the ability of bacteria to produce IAA in the rhizosphere depends on the availability of precursors and uptake of microbial IAA by the plant. In the present study, all the eight promising N-fixing bacteria are capable of producing the IAA similar to earlier studied reports (Reetha *et al*., 2014, Oteino *et al*., 2015). The capacity to produce IAA is widespread among soil plant growth-promoting bacteria such as *Azospirillum sp.*, *Enterobacter cloacae* and *Klebsiella sp.* (Spaepen *et al*., 2007). Furthermore, IAA production has been reported in *P. stutzeri* strain A15 group of bacteria (Mehnaz *et al*., 2009) wherein the strain A15 was re-isolated from inoculated rice roots (Lalucat *et al*., 2006). Also, *Pseudomonas sp.* have been reported to enhance the growth of onion crop (Reetha *et al*., 2014, Oteino *et al*., 2015). *Pseudomonas fluorescens* has shown indole acetic acid (IAA) production resulting in enhanced growth of onion as well as phosphate solubilization (Reetha *et al.*, 2014, Oteino *et al*., 2015).

Phosphate solubilizing bacteria (PSB) provides available form of phosphorus (P) to the plants and is hence a viable substitute to chemical phosphatic fertilizers (Khan *et al*., 2009). Our results are similar to the previous reports on phosphate solubilizing bacterial genera *viz. Azotobacter, Bacillus, Burkholderia, Erwinia, Enterobacter, Pseudomonas* and *Rhizobium* (Bhattacharyya and Jha, 2012). Current results in relation to phosphate solubilization also correlate with earlier studies on plant growth-promoting abilities of *Enterobacter* species (Ahemad and Khan, 2010, Khalifa *et al*., 2016). *Enterobacter asburiae* has shown phosphate solubilization, IAA and ammonia as well as siderophore production (Ahemad and Khan, 2010). Similarly, *Rhizobium sp.* and *E. cloacae* have been found to possess phosphate solubilization and ability for phytohormone production like acetoin, which leads to significant growth and development of the pea (*Pisum sativum*) (Khalifa *et al*., 2016).

Our results on zinc solubilization are correlated with the earlier reports on identified zinc solubilizing bacterial genera belonging to *Pseudomonas*, *Bacillus*, *Enterobacter, Xanthomonas*, *Stenotrophomonas* and *Acinetobacter* (Saravanan *et al.,* 2003, Gandhi and Muralidharan 2016; Sunithakumari *et al.,* 2016). Zinc solubilizing ability has been proven for *Bacillus sp*. and *Pseudomonas sp*. which was observed as production of visible halo zones on the zinc oxide-amended media (Saravanan *et al.,* 2003).

In summary, from our investigation, we have isolated and identified eight promising N-fixing bacteria *viz. Stenotrophomonas sp.* (IIRR A3), *Paenibacillus sp.* (isolate 1) (IIRR C4), *Burkholderia cepacian.* (isolate 1) (IIRR J3), *Burkholderia cepacian* (isolate 2) (IIRR J4), *Ochrobactrum sp.* (IIRR E3), *Paenibacillus sp.* (isolate 2) (IIRR F4), *Xanthomonas sacchari* (IIRR M4) and *Rhizobium sp*. (IIRR N) with additional plant growth promoting traits with a potential for enhancing rice plant growth.

Acknowledgements

The authors are thankful to ICAR (Plan Scheme network project entitled-Incentivizing Research Project III- "Genetic modifications to improve biological nitrogen fixation for augmenting nitrogen needs of rice") and Director, ICAR -IIRR for providing financial assistance and necessary facilities for research.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Effect of seaweed extract as biostimulant on crop growth and yield in rice (*oryza sativa* l.) under transplanted condition

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Received: 10 October 2019; Accepted: 21 December 2019

Abstract

Enhancing productivity through integrated nutrient management is pertinent to sustainable intensification of agricultural ecosystems. Field experiments were conducted during *kharif* and *rabi* 2016 at the research farm of Indian Institute of Rice Research, Hyderabad, Telangana in a clay loam soil to study the effect of sea weed extract (Liquid Bio-stimulant6, LBS6_S, LBS8, LBS9 and LBS10) with various concentrations on growth, yield of rice under transplanted condition. Positive effects and significant variation was observed on the plant height (98.9 cm) at harvest stage of rice when LBS6_S bio-stimulant (T_3) was sprayed when compared to other bio-stimulant application while least plant height was recorded with recommended dose of fertilizer (120:60:40 NPK Kg/ha). Grain yield due to seaweed bio-stimulants application varied from 5.31 to 5.58 t/ha and significantly increased over recommended dose of fertilizer alone (T_7). Per centage increase of grain yield was 4.15 to 9.14 per cent over recommended dose of fertilizer. Hence, bio-stimulant LBS6_S can be applied for improving growth and grain yield of transplanted rice.

Keywords: Liquid Biostimulant, Seaweed Extracts, transplanted rice

Introduction

Rice is the most important staple food crop for more than half of the global population and is grown extensively in the world. India occupies first position in area (44.2 M ha) and second position in production after China (140.8 million tonnes) in the world. In India, rice production has increased by fivefold from 20.51 mt during 1950 – 1951 to more than 108.86 mt in 2016 -17 and this increase in rice production can be attributed to the development of fertilizer responsive high yielding rice varieties which made nearly 50 per cent contribution in tapping the high yield potential of varietal technology (Anonymous, 2017). Indiscriminate use of fertilizers had resulted in imbalanced use which gradually decreased the productivity of the soil over the years and balanced fertilizer use is a pre-requisite for sustaining rice production. Seaweeds are one of the most important marine resources of the world and derived products have been widely used as amendments in crop production system due to the presence of number of plant growth- stimulating compounds such as cytokinin, auxins, gibberellins, betaines, as well as presence of macronutrients such as Ca, K, P, and micronutrients like Fe, Cu, Zn, B, Mn, Co and Mo, which are necessary for plant growth and development (Mahima Begum, 2018). Seaweeds, particularly brown algae, have been used in agriculture since the twelveth century with much success (Temple and Bomke, 1988). They were added either directly to the soil as seaweed compost or dried and ground and added to the soil as seaweed meal. Both the seaweed compost and meal serve as a slow release fertilizer and act as soil conditioner, improving aeration and aggregate stability. However, the bio-stimulatory potential of many of these products has not been fully exploited due to the lack of scientific data on growth factors present in sea weeds and their mode of action in affecting plant growth. The seaweed bio-product is a bio-stimulant formulation that is a mixture of micro and macro nutrients, amino acids, carbohydrates, plant growth regulators and other plant growth elicitors emerging as commercial formulations for use as plant growth-promoting factors and a method to improve tolerance to salinity, heat, and drought due to presence of plant hormones (Kavipriya *et al*, 2011). Modern agriculture is searching for new technologies that would allow for a reduction in the use of chemical inputs without negatively affecting crop yield or the farmers' income. In recent years, the use of natural seaweed as fertilizer has allowed for partial substitution of conventional inorganic fertilizer (Zodape *et al*., 2010). One of the most consistent effects of sea weed concentrate (SWC) application is the development of a vigorous root system if applied during the early vegetative growth phase (Metting *et al*., 1990).

In this connection it is essential to utilize the different bio-stimulants which indirectly enhances the plant to assimilate more nutrients from the soil and also translates into increase yield. Hence, the present investigation was conducted to study the response of different concentration seaweed bio-stimulant LBS6, LBS6_S, LBS8, LBS9 and LBS10 as foliar application in addition to recommended fertilizer dose on growth, yield and productivity of the system.

Materials and Methods

A field experiment was conducted during *kharif and rabi* 2016 with rice variety RP Bio-226 (Improved Samba Mahsuri) of 135 days duration (latitude - 17°23´ N, longitude - 78°25´ E and altitude – 508 M) at the research farm of Indian Institute of Rice Research Rajendranagar Hyderabad. The soil of the experimental field was clay loam in texture with a pH 7.9, organic carbon (0.37%), low in available nitrogen (206 kg / ha), medium in available phosphorous (22.3 kg / ha) and high in available potassium (304 kg / ha). Entire dose of phosphorus and potassium (60 and 40 K Kg / ha) and one third dose of nitrogen (40 Kg /ha) was applied as basal. The remaining nitrogen (80 Kg / ha) was top dressed equally at tillering and panicle initiation stage. The experiment consisted of seven treatments *viz*.,T_1 – LBS6 @ 1ml / Litre of water – one spray during nursery transplanting + two sprays (30 and 60 days after transplanting) T_2 – LBS6 @ 1.0 ml / Litre of water – one spray during nursery transplanting + two sprays during vegetative stage (30 and 60 days after transplanting) (20% Less NPK fertilizer dose) T_3- LBS6_S @ 1.0 ml / Litre of water – one spray during nursery transplanting + two sprays during vegetative stage (30 and 60 days after transplanting) T_4- LBS8 @ 1.0 ml / Litre of water – one spray during nursery transplanting + two sprays during vegetative stage (30 and 60 days after transplanting), T_5- LBS9 @ 1.0 ml / Litre of water – one spray during nursery transplanting + two sprays during vegetative stage (30 and 60 days after transplanting) T_6- LBS10 @ 1.0 ml / Litre of water - one spray during nursery transplanting + two sprays during vegetative stage (30 and 60 days after transplanting) T_7- Recommended NPK (120: 60: 40 NPK kg /ha) (Nitrogen -1/3 basal 1/3 tillering and 1/3 panicle initiation stage). The experiment was laid out in randomized block design with three replications. Twenty five days seedlings of rice were transplanted manually with a spacing of 20 x 15 cm. Recommended agronomic practices such as gap filling, weeding and pest and disease control were carried out uniformly as and when needed. Two hand weeding at 25 and 45 DAT were done for weed control. The growth attributes like plant height and dry matter production were recorded from randomly selected five plants in each plot (30, 60, 90 DAT and at harvest). The yield parameters (panicles hill^{-1}, effective grains panicle^{-1} and test weight) were measured at maturity from randomly selected five hills in each plot. The post harvest data on grain and straw yields and harvest index (HI) were recorded from the net plot yield 6 x 2.0 m. and data were statistically analyzed using variance for randomized block design (Gomez and Gomez, 1984).

Results and Discussion

Growth and physiological attributes in rice

Crop growth attributes showed significant improvement with application of various sea weed liquid bio-stimulant formulations over recommended dose of fertilizer. All the growth and yield parameters such as plant height, number of leaves per hill, number of tillers / m^2 and SPAD reading recorded at 30 DAT and 60 DAT increased with the application of various seaweed bio-stimulants as compared to recommended dose of fertilizer (Table 1 & 2). The growth parameters were higher in Seaweed bio-stimulants (LBS6, LBS6_S, LBS-8, LBS-9 and LBS-10) over recommended dose of fertilizer (T_7) at 90 days after transplanting (Table 3). SPAD meter reading was highest in all the seaweed liquid bio-stimulant treatment compared to recommended dose of fertilizer (T_7) at 30, 60 and 90 days after transplanting. The organic constituents of seaweed extract include plant hormones which elicit strong physiological responses in low doses. The seaweed extracts helps in meristematic growth, translocation of photosynthates, enzyme activation, cell elongation and cell stability (Pramanick *et al*., 2013).

Positive effects and significant variation was observed on the plant height of rice when LBS6_S bio-stimulant (T_3) (81.3 cm and 80.3 cm, respectively) was sprayed when compared to other bio-stimulant application and the least plant height was recorded in recommended dose of fertilizer (72.1 cm and 71.0 cm) (Table 3). More number of tillers was obtained from LBS6_S (T_3) (562 tillers / m^2) followed by LBS6 (T_1) (554 tillers/ m^2) (Pooled data). Days to 50 per cent flowering was minimum in LBS6_S (91.7 days) and maximum in recommended dose of fertilizer (99.1 days) (T_7) (Figure 1). Layek *et al* (2018) reported that foliar spray sea weed sap of *Kappaphycus* and *Gracilara* species at 5 per cent and above concentration increased plant height, dry matter accumulation, chlorophyll index and crop growth rate as compared to recommended dose of fertilizer.

Yield attributes

All the yield parameters such as number of panicles/ m^2, panicle weight, grains per panicle, test weight, grain yield (t/ha) and harvest index increased with the application of various seaweed bio-stimulants as compared to recommended dose of fertilizer (Figure 1 & 2). Highest number of panicles was recorded in LBS6_S (T_3) (508 and 478 number of panicles / m^2) followed by LBS10 (T_6) (489 and 449 panicles / m^2) and LBS6 (T_1) (484 and 454 panicles / m^2) during *kharif* and *rabi* season. Number of grains per panicle was highest in LBS6_S (197 and 193 grains/ panicle *kharif* and *rabi* season, respectively) when compared to all treatments The increase in yield attributes in rice crop is due to efficient utilization of native as well as applied nutrients through roots and foliar application of seaweed bio-stimulants. The spraying of seaweed extract at 30 and 60 days interval after planting recorded higher tuber yield, improved nitrogen, total soluble solids and protein contents of the potato tubers (Haider *et al.* 2012).

Table 1: Effect of Sea6 – Bio-stimulant on the plant growth of rice under puddled condition at 30 days after transplanting

Treatment	Plant Height (cm)			Number of leaves / hill			Tillers/ m^2			SPAD Meter Reading		
	Kharif	*Rabi*	Pooled	*Kharif*	*Rabi*	Pooled	*Kharif*	*Rabi*	*Pooled*	*Kharif*	*Rabi*	Pooled
T_1	33.6	31.3	32.5	16.0	15.5	15.8	187	179	183	32.30	30.40	31.35
T_2	31.0	30.7	30.8	13.9	13.2	13.55	189	173	181	32.60	30.26	31.43
T_3	34.9	33.8	34.4	19.3	17.9	18.6	201	190	196	33.93	32.49	33.21
T_4	31.6	31.9	31.8	17.4	16.6	17.0	197	177	187	33.17	31.20	32.19
T_5	33.9	32.6	33.3	15.1	14.4	14.75	181	169	175	32.63	30.60	31.62
T_6	34.5	33.1	33.8	15.8	15.2	15.5	183	171	177	33.59	31.92	32.76
T_7	27.7	28.5	28.1	13.7	12.1	12.9	167	159	163	31.23	30.12	30.68
CD (0.05)	1.86	1.82	1.84	2.81	2.70	2.78	28.13	26.34	27.25	2.09	2.01	2.06
CV	3.41	3.36	3.38	10.53	10.41	10.46	8.93	8.43	8.69	4.67	4.45	4.59

Table 2: Effect of Sea6 - Bio-stimulant on the plant growth of rice under puddle condition at 60 days after transplanting

Treatment	Plant Height (cm)			Number of leaves / hill			Tillers/ m^2			SPAD Meter Reading		
	Kharif	*Rabi*	Pooled	*Kharif*	*Rabi*	Pooled	*Kharif*	*Rabi*	Mean	*Kharif*	*Rabi*	Mean
T_1	63.1	61.9	62.5	34.1	33.3	33.7	289	284	286.5	37.8	36.07	36.93
T_2	60.8	61.7	61.3	36.3	33.7	35.0	321	310	315.5	38.5	35.63	37.07
T_3	63.9	63.5	63.7	38.1	37.1	37.6	324	304	314	38.9	36.65	37.78
T_4	61.6	60.8	61.2	36.0	35.6	35.8	302	288	295	37.1	37.18	37.14
T_5	62.9	61.4	62.1	34.3	33.5	33.9	309	300	304.5	37.4	35.49	36.45
T_6	63.5	61.1	62.3	32.4	31.9	32.1	293	281	287	36.3	35.90	36.1
T_7	57.7	52.9	55.3	30.3	29.1	29.7	230	252	241	35.2	33.47	34.34
CD (0.05)	4.39	3.85	4.12	2.91	2.83	2.87	41.01	38.03	39.52	3.38	3.04	3.21
CV	3.41	4.31	3.86	7.92	8.7	8.31	8.89	8.22	8.56	5.3	5.12	5.21

T_1- LBS6@ 1ml/lts (1spray at seedling transplantation +2 spray (30 and 60 DAS) + Recommended Dose of Fertilizer); T_2 - LBS6@ 1ml/lts (1 spray transplantation + 2 spray (30 and 60DAS 20% lower Recommended Dose of Fertilizer); T_3 - LBS6_S ml/lts (1 spray at seedling transplantation+ 2 spray (30 and 60DAT); T_4 - LBS8 @ 1ml/ lts 1 spray at seedling transplantation+ 2 spray (30 and 60 DAT); T_5 - LBS9 1Ml/lts (1 Spay at seedling transplantation + 2 spray (30 and 60 DAT); T_6 - LBS10 1ml/lts (1 spray at seedling transplantation + 2 spray (30 and 60 DAT); T_7 - Recommended Dose of Fertilizer (100 per cent).

Table 3: Effect of Sea6 – Bio-stimulant on the plant growth of rice under puddle condition at 90 days after transplanting

Treatments	Plant height (cm)			No of leaves / hill			Tillers / m²			SPAD Meter Reading		
	Kharif	*Rabi*	Pooled	*Kharif*	*Rabi*	Pooled	*Kharif*	*Rabi*	Pooled	*Kharif*	*Rabi*	Pooled
T_1	75.8	74.1	74.9	42.3	43.0	42.7	557	551	554	39.77	39.9	39.83
T_2	76.3	75.2	75.8	43.1	41.3	42.2	509	503	506	35.97	35.8	35.9
T_3	81.3	80.3	80.8	46.3	45.8	46.1	565	560	562	37.50	37.6	37.6
T_4	76.5	75.5	76.0	42.3	42.1	42.2	513	506	509	38.03	38.5	38.27
T_5	77.4	76.3	76.9	40.3	42.6	41.5	545	540	542	40.13	40.7	40.42
T_6	77.5	75.0	76.3	39.0	39.3	39.1	547	541	544	39.23	39.3	39.27
T_7	72.1	71.0	71.6	35.7	37.0	36.4	503	499	501	37.67	37.1	37.39
CD (0.05)	4.49	4.52	4.50	4.89	4.92	4.90	65.51	65.65	65.57	5.03	5.06	5.04
CV	3.43	3.47	3.45	6.78	6.83	6.80	7.32	7.38	7.35	7.74	7.79	7.76

T_1- LBS6@ 1ml/lts (1spray at seedling transplantation +2 spray (30 and 60 DAS) + Recommended Dose of Fertilizer); T_2 - LBS6@ 1ml/lts (1 spray transplantation + 2 spray (30 and 60DAS 20% lower Recommended Dose of Fertilizer); T_3 - LBS6_S ml/lts (1 spray at seedling transplantation+ 2 spray (30 and 60DAT); T_4 - LBS8 @ 1ml/ lts 1 spray at seedling transplantation+ 2 spray (30 and 60 DAT); T_5 - LBS9 1Ml/lts (1 Spay at seedling transplantation + 2 spray (30 and 60 DAT); T_6 - LBS10 1ml/lts (1 spray at seedling transplantation + 2 spray (30 and 60 DAT); T_7 - Recommended Dose of Fertilizer (100 per cent).

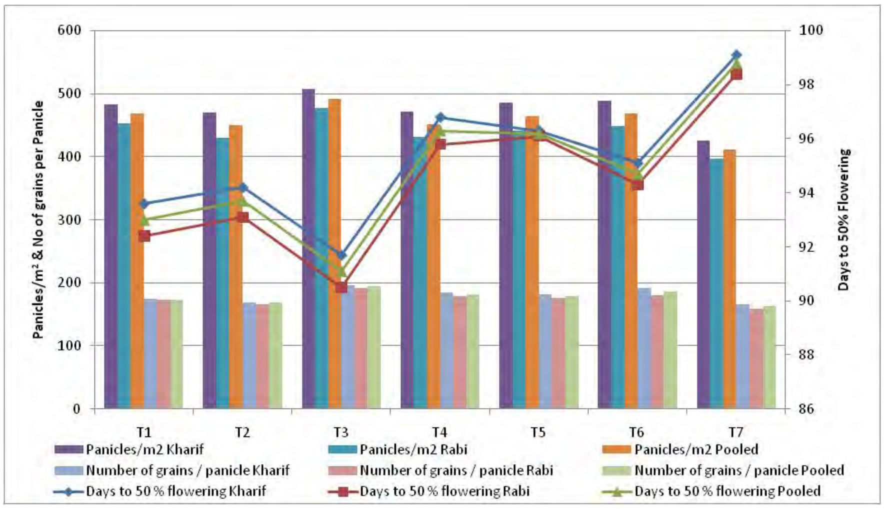

Figure 1: Effect of Sea6 – Bio-stimulant on number of panicles /m², number of grains / panicle and days to 50 per cent flowering in rice

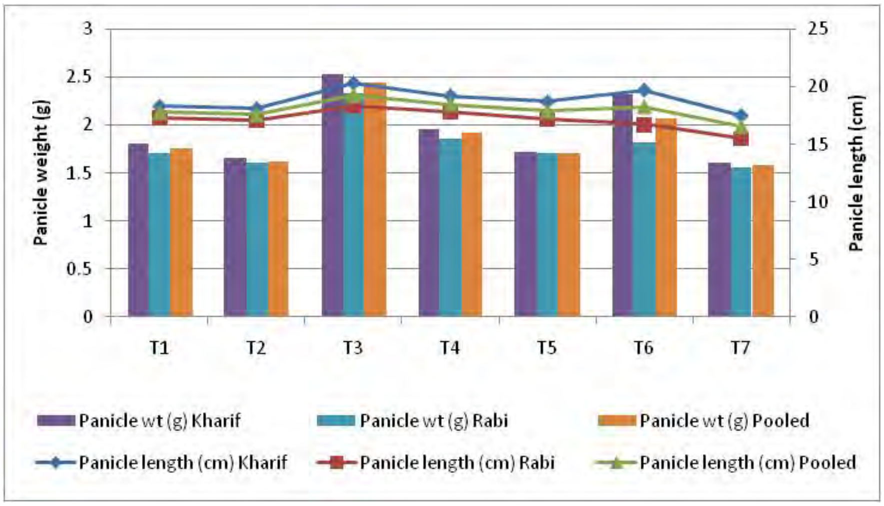

Figure 2: Effect of Sea6 – Bio-stimulant on panicle weight and panicle length in rice crop

Test Weight and Grain Yield (Kg /ha)

The highest test weight was recorded in LBS6_S (T_3) (21.68 g) followed by LBS6 (21.4 g) (pooled data). Grain yield due to seaweed bio-stimulants application varied from 5.31 t/ha to 5.58 t/ha (pooled) and significantly increased over recommended dose of fertilizer alone (T_7). Per centage increase of grain yield was 3.51 to 11.62 per cent in *kharif* and 2.05 per cent to 6.65 per cent during *rabi* season over recommended dose of fertilizer (Fig. 3). Favourable response to yield might be attributed to the better availability of plant nutrients throughout the growth period and especially in critical growth period of rice crop which resulted into better plant vigour and superior yield attributes. This is due to rapid growth caused by adequate nutrient supply to the rice crop which resulted in increase in various metabolic processes and better mobilization of synthesized carbohydrates in amino acids and protein which in turn stimulated the rapid cell division and cell elongation thus allowed the plant to grow faster (Anil *et al.*, 2014). The positive effect of seaweed extracts application on soybean yield was also investigated. Foliar application of extracts in different concentrations resulted in higher yield, more intensive growth and better nutrients absorption of soybean (Rathore *et al.*, 2009). Seaweed extracts can act by increasing plant vigor and vitality due to the presence of several bioactive substances that are important for plants and also they can improve nutrient uptake from soil. There are many advantages of using seaweed extracts as stimulants of plant growth, including higher germination rates, root-system development, increased leaf area, fruit quality and plant vigor. Besides this, plants treated with seaweed extracts have a higher content of biochemical constituents such as chlorophyll, carotenoids, protein, and amylases (Rosalba Mireya Hernández-Herrera *et al.*, 2018). Layek *et al* (2018) reported that foliar spray of sea weed sap of *Kappaphycus* and *Gracilara* species at 5 per cent and above concentration increased yield attributes and yield of rice 5.4 to 18.4 per cent higher as compared to control.

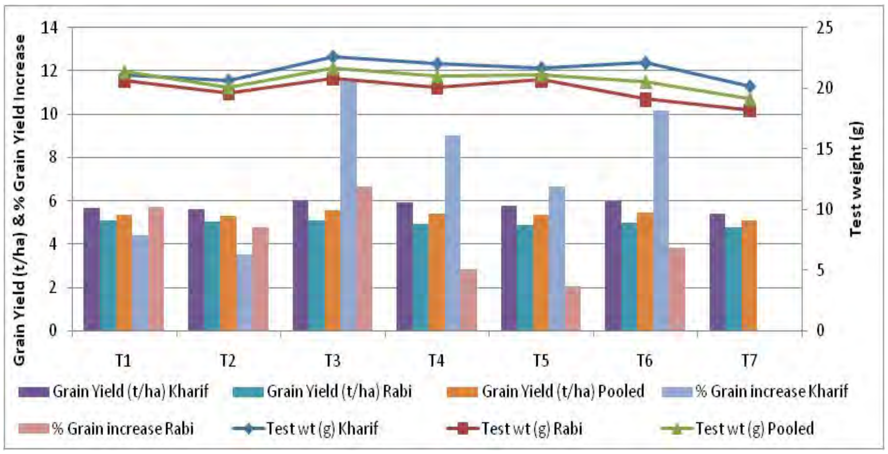

Fig. 3: Effect of Sea6 – Bio-stimulant on Test weight, Grain yield and per centage grain yield in rice

Conclusion

Seaweeds provide an abundant source of natural growth substances that can be used to enhance plant growth. Seaweed liquid bio-stimulants are complex and diverse in nature and yet have great potential for enhancing crop productivity and offer novel biological mechanisms to exploit to ensure farmers for improving yield. It improves plant growth and yield with increased crop resistance to certain pests allowing for lower amounts of synthetic fertilizers and pesticides to be used. Escalating costs and increased awareness of the negative effects of using synthetic agrochemicals makes the use of sea weed extracts an attractive alternative. The results of the present study showed that the application of liquid bio-stimulant LBS6_S was found superior to all other bio-stimulants along with recommended dose of fertilizer which helped in the balance availability of nutrients to the plant and maintenance of organic and inorganic nutrient in soil and hence increased rice productivity by 3.51 to 11.62 per cent in *kharif* and 2.05 to 6.65 per cent during *rabi* season. In places where inorganic fertilizer effects are limited, liquid bio-stimulant may provide a powerful and environmentally friendly approach to nutrient management and enhance the sustainability of rice productivity.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Efficacy of azoxystrobin 25 SC against rice sheath blight and glume discoloration diseases of rice

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Received: 15 October 2019; Accepted: 12 December 2019

Abstract

Field experiments were conducted at Rice Research Station, Kerala Agricultural University, Moncompu during *Kharif* 2012, *Kharif* 2013 and *Rabi* 2013-14 to evaluate the fungicides against sheath blight and glume discoloration. The six fungicides evaluated were trifloxystrobin25%+tebuconazole 50% 75 WG (Nativo), kresoxim methyl (Ergon 44.3 SC), azoxystrobin 25 SC (Amistar), tricyclazole 75 WP (Beam), carbendazim 50 WP (Bavistin) and propiconazole 25 EC (Tilt). The pooled analysis of three seasons data showed that trifloxystrobin 25%+tebuconazole 50% 75 WG @ 0.4 g/l and azoxystrobin 25 SC @ 1 ml/l were found equally effective against the sheath blight and glume discoloration than other molecules. It was promoted as farm trials at 7 different farmers field during *Rabi* 2014-15, and proved that the azoxystrobin 25 SC @ 1 ml/l and trifloxystrobin 25%+tebuconazole 50% 75 WG @ 0.4 g/l restricted the incidence of sheath blight (18.12 and 21.52 %) and glume discoloration panicles (12.16 and 12.01 %) and spikelets (8.97 and 9.56%) than standard check fungicide carbendazim @ 1g/l (28.26, 12.69 and 9.76 %). Highest yield (6930 kg/ha) was recorded by the azoxystrobin 25 SC followed by trifloxystrobin 25%+tebuconazole 50% 75 WG (6732 Kg/ha) and standard check fungicide (6464 Kg/ha).

Key words: Rice, sheath blight, glume discoloration, fungicide

Introduction

Rice (*Oryza sativa L.*) is the most widely cultivated food crop in the world. It is the staple food grain for the people living in humid and sub-humid regions Asia. Ever growing population in India is further demanding more rice production and productivity. Under field condition, the productivity of rice is affected by many biotic and abiotic factors. Sheath blight of rice (*Rhizoctonia solani* – AGA1-IA) is an important location specific disease in Kuttanad region of Kerala causing 35 to 50 per cent yield loss. The weather and soil conditions such as high relative humidity, temperature and extremely acidic soil pH prevailing during *Rabi* season in Kuttanad region are conducive for the occurrence of sheath blight and glume discoloration diseases. Sheath blight is a destructive disease worldwide that causes significant yield loss and quality degradation (Savary *et al*., 2006). Grain discoloration is caused by complex of fungal species such as *Sarocladium oryzae, Bipolaris oryzae (Cochliobolus miyabeanus), Pyricularia grisea (Magnaporthe grisea) Curvularia lunata, Phoma sp., Microdochium sp., Nigrospora sp., and Fusarium sp.* It is an important constraint for lowland and upland rice production and becoming serious under changing climatic conditions. Of late, the disease was found to be very severe in all over the Kerala causing 5 to 10 per cent yield loss (Surendran *et al*., 2016). If most of the pathogens appear simultaneously, the total devastation of the rice crop may take place. Earlier, it was reported that the grain discoloration pathogens increased due to low soil amendments (Kaur and Padmanadhan, 1974; Datnoff *et al.*, 1991 and Dallagnol *et al.*, 2009). Use of suitable fungicide is the primary one for the effective management of the rice diseases. In the present study, considering the severity of diseases and its economic importance, the field experiments were conducted using different fungicides available in the market for the control of sheath blight and glume discoloration of rice.

Materials and Methods

Field experiments were conducted at Rice Research Station, Moncompu, Alappuzha during *Kharif* 2012, *Kharif* 2013 and *Rabi* 2013-14 under AICRIP programme for evaluating the fungicides against location specific rice disease management *viz.*, sheath blight and glume discoloration. Three different fungicides *viz.*, trifloxystrobin 25% + tebuconazole 50% (Nativo 75 WG) @ 0.4g/l, kresoxim methyl (Ergon 44.3 SC)@ 1.0 ml/l and azoxystrobin 25 SC(Amistar) @ 1ml/l with three standard fungicides were tested against sheath blight and glume discoloration. The standard check fungicides were tricyclazole 75 WP (Beam), carbendazim 50 WP (Bavistin) and propiconazole 25 EC (Tilt). The experiments were laid out in randomized block design with 4 replications in 5x2 m^2 plots using the locally popular susceptible variety, Uma (MO 16). The NPK fertilizers were applied as per the recommendations (90:45:45 kg/ha) of Kerala Agricultural University. The chemicals were applied as foliar spray at the time of appearance of the above diseases under natural conditions. Three sampling units of 1 m^2 area were fixed in each plot at random. A single spray was given at the time of panicle emergence. Observations on sheath blight disease severity were recorded just before the spray and 15-20 days after the spray. Degree of severity was graded based on height of the plant portions affected by the disease and expressed as percentage of the total area as per the SES scale of rice (IRRI, 2013). Glume discoloration was measured based on the percentage of panicles and spikelet's infection at 15 days before harvest. The per cent panicle infection was calculated based on the number of panicles affected from the total number of panicles present in the sampling area. The spikelet infection per cent was recorded by counting the infected grains from each panicle. Grain yield of the each plot was recorded and expressed in Kg/ha at 14 per cent moisture. Data on percentages were transformed and analysis of variance was performed with transformed values. Significance among mean treatments was determined according to Duncan's multiple range tests (Gomez and Gomez, 1984).

The confirmatory farm trials were conducted for testing the effective molecules during *Rabi* 2014-15 at seven locations *viz.*, Kavalam, E-block 24000 kayal, Venattukadu, Peringara, Nedumudy, Veeyapuram and Punnapra area of Kuttanad region. The four treatments were trifloxystrobin 25% + tebuconazole 50% 75 WG @ 0.4g/l, azoxystrobin 25 SC @ 1.0 ml/l, standard Kerala Agricultural University Package of Practice (KAU POP) recommended fungicide, carbendazim 50 WP @ 1.0 g/l and untreated check. The seven locations were considered as replication of the farm trial. The farm trial was laid out in a randomized complete block design (RBD), using MO 16 (Uma) as the test variety in the farmers field. Pregerminated seeds were used for direct sowing with the plot size of 20x10 m^2. Fertilizers were applied @ 90:45:45 NPK Kg/ha as per the KAU POP. The sheath blight severity was recorded at 15-20 days after spray. Grain discoloration observations on panicles and spikelets infection were recorded at 25-30 days after spray or 15 days before harvest. Percentage of panicles and spikelets affected was calculated on 25 plants per sampling unit, by counting the number of infected panicles/spikelets.

Results and Discussion

The results of station trial at Rice Research Station, Moncompu during KHARIF 2012 (Table 1) revealed that the plots treated with fungicide azoxystrobin 25 SC recorded lower sheath blight severity (15.17%). This was followed by tebuconazole 50% +trifloxystrobin 25% 75 WG (16.48%) and check fungicides, tricyclazole 75 WP (18.13 %) and propiconazole 25 EC (19.05%). The maximum yield was obtained from kresoxim methyl 44.3 SC (5820 Kg ha^{-1}) followed by azoxystrobin 25 SC (5780 Kg ha^{-1}) and trifloxystrobin 25%+tebuconazole 50% 75 WG (5630 Kg ha^{-1}). The control plot recorded lowest yield of 4660 Kg ha^{-1}.

During *Kharif* 2013, the systemic fungicide, azoxystrobin 25 SC was found superior in restricting sheath blight disease severity (17.28%) followed by tricyclazole 75WP (18.46%) and carbendazim 50 WP (19.10 %). The systemic standard check fungicide tricyclazole 75 WP was found most effective against sheath blight disease during *Rabi 2013-14* and followed by azoxystrobin 25 SC, carbendazim 50 WP and kresoxim methyl 44.3 SC. There were significant differences in the grain yield among the treatments. The pooled data of station trial results showed that the azoxystrobin 25 SC gave the maximum reduction in disease severity (19.10 %) followed by tricyclazole 75 WP (19.42 %), carbendazim 50 WP (20.11%), kresoxim methyl 44.3 SC (23.31 %) and tebuconazole +trifloxystrobin 75 WG (23.49) (Table 1).

Table 1: Influence of different fungicides on sheath blight disease severity

S. No.	Fungicides	Dose/ Liter water	Sheath blight Disease severity (%)			
			Kharif 2012	*Kharif 2013*	*Rabi 13-14*	Mean
1	Trifloxystrobin25%+Tebuconazole 50% 75 WG (Nativo)	0.4g	16.48 (23.89)	29.46 (32.83)	24.52 (29.67)	23.48 (28.79)
2	Kresoxim methyl (Ergon 44.3 SC)	1.0ml	19.44 (26.13)	29.01 (32.65)	21.47 (27.56)	23.30 (28.78)
3	Azoxystrobin 25 SC (Amistar)	1.0ml	15.17 (23.34)	24.50 (29.67)	17.63 (24.80)	19.10 (25.93)
4	Tricyclazole 75 WP (Beam)	0.6g	18.13 (25.33)	25.42 (30.26)	14.71 (22.54)	19.42 (26.04)
5	Carbendazim 50 WP (Bavistin)	1.0g	19.48 (26.13)	25.91 (30.59)	14.95 (22.71)	20.11 (26.47)
6	Propiconazole 25 EC (Tilt)	1.0ml	19.05 (26.21)	30.28 (33.34)	28.92 (32.52)	26.08 (30.69)
7	Control		22.32 (28.18)	45.11 (42.19)	31.76 (34.27)	33.06 (34.88)
	LSD (*P*= 0.05)		**NS**	**3.22**	**3.21**	
	CV (%)		**7.12**	**6.20**	**6.79**	

**Figures given in parentheses are arcsine transformed values*

The fungicide azoxystrobin 25 SC gave maximum yield of 5588 kg/ha and on par with standard fungicide propiconazole 25 EC(5598 kg/ha), kresoxim methyl 44.3 SC (5572 kg/ha), tricyclazole 75 WP (5510 kg/ha) and tebuconazole + trifloxystrobin 75 WG (5468 kg/ha) (Table 2 and Figure1). The present findings are in corroboration with several workers who also reported on the possibility of controlling sheath blight disease by application of fungicides like carbendazim (Bavistin), mancozeb (Dithane M-45) and validamycin A (Dev and Mary 1986).

Table 2: Influence of different fungicides on grain yield

S. No.	Fungicides	Dose/ Liter water	Grain Yield (Kg/ha)			
			Kharif 2012	*Kharif 2013*	*Rabi 13-14*	Mean
1	Trifloxystrobin 25% + tebuconazole 50% 75 WG (Nativo)	0.4g	5630	5461	5913	5668
2	Kresoxim methyl (Ergon 44.3 SC)	1.0ml	5820	5354	5543	5572
3	Azoxystrobin 25 SC (Amistar)	1.0ml	5780	5289	5695	5588
4	Tricyclazole 75 WP (Beam)	0.6g	5440	5655	5435	5510
5	Carbendazim 50 WP (Bavistin)	1.0g	4860	5311	5050	5074
6	Propiconazole 25 EC (Tilt)	1.0ml	5400	5999	5395	5598
7	Control		4660	3806	4445	4304
	LSD (*P*= 0.05)		**NS**	**884.77**	**610.7**	
	CV(%)		**10.57**	**11.30**	**7.67**	

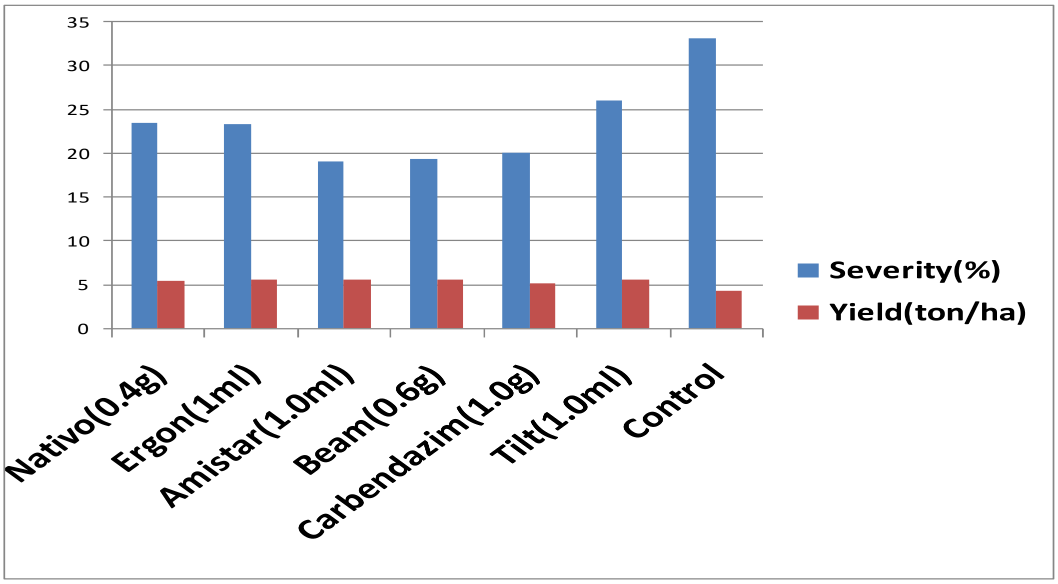

Figure 1: Effectiveness of different fungicides on sheath blight disease severity and grain yield

Grain discoloration

The data on grain discoloration, panicles and spikelets infection indicated that standard fungicide carbendazim 50 WP reduced disease effectively (1.96 and 1.01 %) when compared with fungicides *viz.*, azoxystrobin 25 SC (2.00 and 1.12 %), trifloxystrobin 25% + tebuconazole 50% 75 WG (2.22 and 1.15 %) and kresoxim methyl 44.3 SC (2.19 and 1.16 %) during *Kharif 2012*. During *Kharif 2013*, carbendazim @ 1g/l was found to be effective in limiting the glume discoloration and the maximum yield was obtained from carbendazim 50 WP treated plot (2645 kg ha^{-1}) followed by kresoxim methyl 44.3 SC (2623 kg ha^{-1}) and azoxystrobin 25 SC 2430 kg ha^{-1}. Out of six commercially available fungicides tested, the standard check fungicide carbendazim 50 WP was found superior against the glume discoloration followed by azoxystrobin 25 SC and trifloxystrobin 25%+tebuconazole 50% 75 WG during *Rabi* 2013-14. The highest yield was recorded in the trifloxystrobin 25%+ tebuconazole 50% 75 WG (5913 kg ha^{-1}) treated plot followed by azoxystrobin 25 SC(5695 kg ha^{-1}). The pooled data of three season station trials showed that the standard check fungicide carbendazim 50 WP was very effective in restricting glume discolored panicle and spikelets incidence followed by azoxystrobin 25 SC and trifloxystrobin 25%+tebuconazole 50% 75 WG. The analysis of pooled data on panicle percentage affected showed that carbendazim 50 WP (3.09 %) and azoxystrobin 25 SC (3.26 %) were found equally effective than other fungicides (Table 3).

Table 3: Influence of different fungicides on glume discoloration panicles

S. No.	Fungicides	Dose/ Liter water	Panicle affected (%)			
			Kharif 2012	*Kharif 2013*	*Rabi 13-14*	Mean
1	Trifloxystrobin 25% + tebuconazole 50% 75 WG (Nativo)	0.4g	2.22	5.14	6.07	4.47
2	Kresoxim methyl (Ergon 44.3 SC)	1.0ml	2.19	5.19	7.36	4.91
3	Azoxystrobin 25 SC (Amistar)	1.0ml	2.00	5.14	2.63	3.25
4	Tricyclazole 75 WP (Beam)	0.6g	2.37	5.24	5.60	4.40
5	Carbendazim 50 WP (Bavistin)	1.0g	1.96	4.95	2.37	3.09
6	Propiconazole 25 EC (Tilt)	1.0ml	2.19	5.13	5.45	4.25
7	Control		2.90	7.94	8.92	6.58
	LSD (*P*= 0.05)		**NS**	**0.34**	**0.04**	
	CV (%)		**16.52**	**9.78**	**1.23**	

**Figures given in parentheses are square root transformed values*

The data on spikelets affected indicated that carbendazim 50 WP (2.36 %), trifloxystrobin 25% + tebuconazole 50% 75 WG (2.43 %) and azoxystrobin 25 SC (2.44 %), were significantly superior to all other fungicides tried (Table 4).

Table 4: Influence of different fungicides on glume discoloration spikelets

S. No.	Fungicides	Dose/ Liter water	Spikelets affected (%)			
			Kharif 2012	*Kharif 2013*	*Rabi 13-14*	Mean
1	Trifloxystrobin25% + tebuconazole 50% 75 WG (Nativo)	0.4g	1.15	2.35	3.80	2.43
2	Kresoxim methyl (Ergon 44.3 SC)	1.0ml	1.16	2.41	4.26	2.61
3	Azoxystrobin 25 SC (Amistar)	1.0ml	1.12	2.23	3.98	2.44
4	Tricyclazole 75 WP (Beam)	0.6g	1.20	2.22	4.01	2.47
5	Carbendazim 50 WP (Bavistin)	1.0g	1.01	2.40	3.68	2.36
6	Propiconazole 25 EC (Tilt)	1.0ml	1.30	2.48	3.74	2.50
7	Control		1.82	2.48	4.66	2.98
	LSD (*P*= 0.05)		**0.07**	**NS**	**0.01**	
	CV (%)		**4.73**	**4.16**	**0.48**	

**Figures given in parentheses are square root transformed values*

Table 5: Influence of different fungicides on grain yield (Kg/ha)

S. No.	Fungicides	Dose/ Liter water	Grain Yield (Kg/ha)			
			Kharif 2012	*Kharif 2013*	*Rabi 13-14*	Mean
1	Trifloxystrobin25%+tebuconazole 50% 75 WG (Nativo)	0.4g	5890	1978	5913	4594
2	Kresoxim methyl (Ergon 44.3 SC)	1.0ml	4710	2623	5543	4292
3	Azoxystrobin 25 SC (Amistar)	1.0ml	5610	2430	5695	4578
4	Tricyclazole 75 WP (Beam)	0.6g	5570	1892	5435	4299
5	Carbendazim 50 WP (Bavistin)	1.0g	5810	2645	5050	4502
6	Propiconazole 25 EC (Tilt)	1.0ml	5050	2288	5395	4244
7	Control		4320	1657	4445	3474
	LSD (*P*= 0.05)		**942.47**	**594.78**	**262.05**	
	CV (%)		**12.02**	**18.07**	**15.17**	

The maximum yield was obtained from trifloxystrobin 25% + tebuconazole 50% 75 WG (4593 kg ha^{-1}) treated plot followed by azoxystrobin 25 SC (4578 kg ha^{-1}). The control plot recorded with lowest yield of 3474 kg ha^{-1}(Table 5 and Figure 2). Several workers have reported on the scope for controlling grain discoloration disease by application of fungicides like edifenphos and copper oxychloride (Govindarajan and Kannaiyan,1982), propiconazole (Lore *et al.*, 2007) and captan 70% + hexaconazole 5% (Kumar and Kumar, 2011).

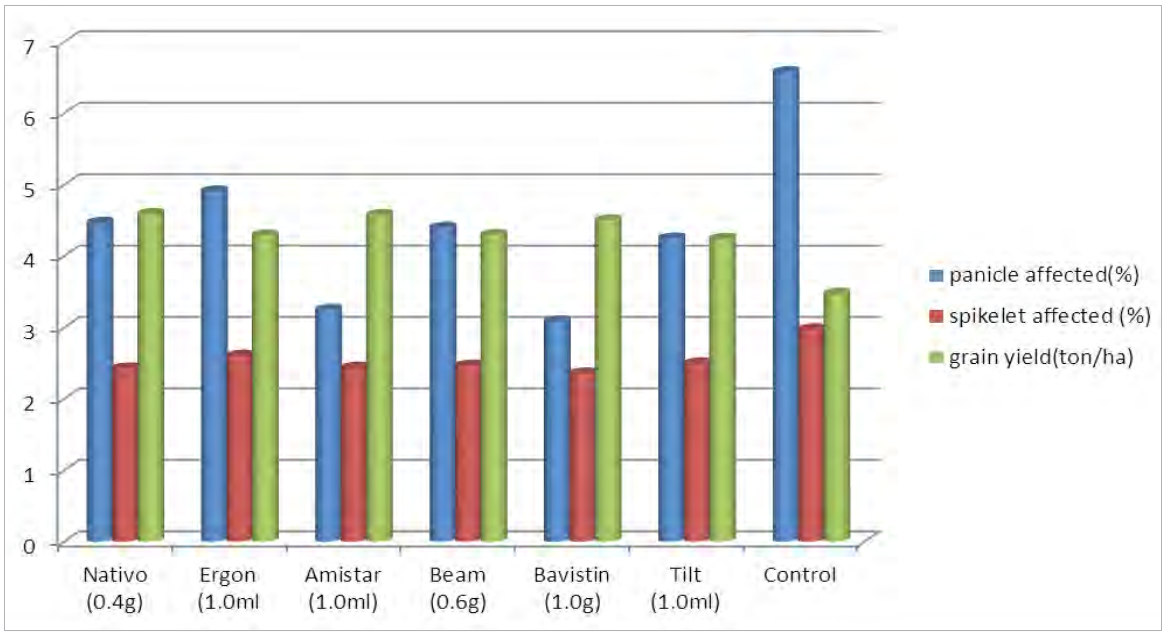

Figure 2: Effectiveness of fungicides on glume discoloration

Farmers field trials

The farm trial results showed that the azoxystrobin 25 SC @ 1 ml/l gave the minimum of sheath blight (18.12%), glume discoloration panicles (12.16%) and spikelets (8.97 %) incidence, followed by combination molecule of trifloxystrobin 25% + tebuconazole 50% 75 WG @ 0.4 g/lit (21.52, 12.01 and 9.56 %, respectively) (Table 6, 7 and 8). Similar results were reported by Surendran *et al.***,** (2019) who reported that the application of trifloxystrobin 25% + tebuconazole 50% WG effectively controlled the sheath blight disease.

Table 6: Efficacy of trifloxystrobin 25 % + tebuconazole 50 % 75 WG and azoxystrobin 25 SC on sheath blight severity in rice

S. No.	Fungicides	Rate g/l	Locations							Mean (%)
			1	2	3	4	5	6	7	
1	Trifloxystrobin 25 % + tebuconazole 50 % 75 WG	0.4 g	24.35	18.24	19.55	17.66	18.15	24.95	27.76	21.52
2	Azoxystrobin 25 SC	1.0 ml	19.09	14.06	15.68	15.00	15.89	24.58	22.54	18.12
3	Carbendazim 50 WP	1.0 g	42.30	26.64	20.44	32.90	25.84	22.71	26.99	28.26
4	Control		29.40	28.39	36.03	32.58	33.02	35.24	31.24	32.27
	LSD (*P*= 0.05)		**5.34**							
	CV(%)		**10.28**							

**Figures given in parentheses are arcsine transformed values*

Table 7: Effect of trifloxystrobin 25% + tebuconazole 50 % 75 WG and azoxystrobin 25 SC on glume discoloration of panicles

S. No.	Fungicides	Rate g/l	Locations							Mean (%)
			1	2	3	4	5	6	7	
1	Trifloxystrobin 25 % +tebuconazole 50 % 75 WG	0.4g	13.18	11.97	7.49	13.31	14.06	11.97	12.11	12.01
2	Azoxystrobin 25 SC	1.0ml	10.63	12.11	11.24	13.31	16.64	9.97	11.24	12.16
3	Carbendazim 50 WP	1.0g	10.94	12.79	12.38	14.89	12.52	12.79	12.52	12.69
4	Control		13.44	15.68	15.23	14.77	14.06	14.42	14.65	14.61
	LSD (*P*= 0.05)		**1.75**							
	CV(%)		**6.35**							

**Figures given in parentheses are square root transformed values*

Table 8: Efficiency of trifloxystrobin 25 % + tebuconazole 50 % 75 WG and azoxystrobin 25 SC on glume discoloration of spikelets

S. No.	Fungicides	Rate g/l	Locations							Mean (%)
			1	2	3	4	5	6	7	
1	Trifloxystrobin 25 % +tebuconazole 50 % 75 WG	0.4g	7.92	9.97	9.80	9.80	9.80	9.80	9.80	9.56
2	Azoxystrobin 25 SC	1.0ml	7.49	9.46	9.10	9.10	8.91	9.28	9.46	8.97
3	Carbendazim 50 WP	1.0g	7.71	10.30	10.47	9.46	9.63	10.30	10.47	9.76
4	Control		8.13	11.24	11.24	11.39	11.97	11.54	11.54	11.01
	LSD (*P*= 0.05)		**0.43**							
	CV (%)		**1.84**							

**Figures given in parentheses are square root transformed values*

The grain yield data indicated that the highest yield (6930 kg/ha) was recorded by the treatment azoxystrobin 25 SC (Table 9) and on par with combination fungicide trifloxystrobin 25% + tebuconazole 50% 75 WG (6732 kg/ha) and standard check carbendazim 50 WP (6464 kg/ha). The lowest yield was recorded in the untreated control plot (3679 kg ha^{-1}). Surendran et al., (2016) proved that the contact and systemic action fungicide carbendazim 12 %+mancozeb 63 % was promising molecule against glume discoloration when compared with individual molecule. Hossain et al., (2011) reported that the systemic fungicides azoxystrobin and propiconazole were found to be effective against rice glume discoloration and increase the yield. The present study also proved the effectiveness of azoxystrobin 25 SC and trifloxystrobin 25%+tebuconazole 50% 75 WG were best molecules for the sheath blight and grain discoloration than other systemic fungicides.

Table 9: Influence of trifloxystrobin 25 %+tebuconazole 50 % 75 WG and azoxystrobin 25 SC on grain yield

S. No.	Fungicides	Rate g/l	Locations							Mean (%)
			1	2	3	4	5	6	7	
1	Trifloxystrobin 25 % +tebuconazole 50 % 75 WG	0.4g	7250	4000	5500	3500	8125	10000	8750	6732
2	Azoxystrobin 25 SC	1.0ml	8125	8250	7250	6125	6188	6325	6250	6930
3	Carbendazim 50 WP	1.0g	4750	8000	7250	8000	3000	6250	8000	6464
4	Control		4750	4000	3500	4000	3250	3250	3000	3679
	LSD @5% (*P*= 0.05)		**2053**							
	CV(%)		**30.72**							

Conclusion

Systemic fungicide azoxystrobin 25 SC @ 1 ml/l was found most effective against the sheath blight and glume discoloration and trifloxystrobin 25% + tebuconazole 50% 75 WG @ 0.4 g/l treatment was on par. The strobilurin fungicide azoxystrobin 25 SC @ 1 ml/l can be recommended for the management of sheath blight and grain discoloration and improve the quality of seeds in Kuttanad region.

Acknowledgement

The authors are thankful to the Director of Research, Kerala Agricultural University, Vellanikkara and Professor and Head, Rice Research Station, Moncompu for their support and encouragement during the course of this study. Authors are also thankful to The Director, ICAR-IIRR, Hyderabad and Principal Investigator, AICRIP-Rice Pathology, for their help during the formulation and execution of the trial.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

A bio-intensive insect pest management module for samba organic rice cultivation in new cauvery delta zone

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Received: 25 October 2019; Accepted: 20 December 2019

Abstract

Bio-intensive organic insect pest management module (BOIPM) and Farmers organic module (FOM) were compared for the pest intensity, natural enemy abundance and grain yield. In BOIPM, the interventions were incorporation of leaves of *Calotropis* + Neem into the nursery soil. Freshly prepared neem seed kernel extract 5% / *Pseudomonas fluorescens* 0.2% was used to manage insect pest / diseases respectively in nursery. While in the main field, *Daincha* was raised in situ; basal application of *Azospirillum* + Phosphobacteria + silica solubilizing bacteria + *Pseudomonas fluorescens* was done. During the last ploughing, neem cake was added to the soil. Seed treatment done with *Pseudomonas fluorescens* @ 10g/ kg of seed. Solar light trap was installed to monitor the insect pest activity. *Trichogramma japonicum* and *T. chilonis* were released at weekly interval thrice. Bird perches were set up at 2-3 ft height in vegetative stage. To enhance natural enemies' activity floral plants (cowpea, gingelly, sunflower, maize, black gram) were raised in the bunds all around the field. The FOM practices included incorporation of *Tephrosia purpurea* / *Calotropis* in nursery. Neem cake or crushed neem seed was added to the soil during the last ploughing. Panchagavya 3% was sprayed thrice during maximum tillering, panicle initiation and booting stage. The insect pest load was 2.05 to 2.15 times higher in the FOM as compared to the BOIPM. Population of the natural enemies was 1.96 to 2.88 times higher in the BOIPM as compared to the FOM. The mean pest defender ratio in BOIPM was 1: 2.46 compared to 1: 0.75 in the FOM. The profit earned from the BOIPM ranged from Rs. 70109/- to Rs. 77389/- per ha. There was a 10.38 per cent increased yield in *Samba*, 2018 over *Samba*, 2017 trial in BOIPM. Conversely, the profit earned form -FOM ranged from Rs. 68849/- to Rs. 56529/- per ha. There was a decreased yield trend of -17.89 per cent in *Samba*, 2018 over *Samba*, 2017 trial.

Key words: Bio-intensive organic insect pest management module, Farmers organic module, pest intensity, natural enemy abundance, pest defender ratio

Introduction

FAO/WHO defined "organic agriculture" as holistic food production management system, which promoted and enhanced agro-ecosystem health, including biodiversity, biological cycles and soil biological activity". The use of pesticides had led to enormous levels of chemical buildup in our environment, soil, water, air, animals and even in our own bodies. Fertilizers have a short-term effect on productivity but a long-term negative effect on the environment where they remain for years after leaching and run off, contaminating ground water and water bodies. The principal interventions included crop rotation, addition of green manures and compost, biological pest management and mechanical cultivation. These measures use the natural environment to enhance agricultural productivity; legumes were planted to fix nitrogen into the soil, natural insect predators were encouraged, crops were rotated to confuse pests and renew soil.

Owing to the water shrinkage in the New Cauvery Delta zone in the double cropped paddy area the alternative cropping system proposed particularly for organic rice cultivators was to go for green manure crop (*Dhaincha* / *Sunnhemp*) with the summer showers and in situ incorporation before the Samba paddy crop. Organic matter enrichment enhanced the association of above and below ground food chains and augmented natural plant defense mechanism. Strengthening of the food webs and natural plant defense mechanism would naturally sustain the biological insect pest control and increase rice productivity over years. In this context, the present study was taken up "To develop a bio-intensive insect pest management module for Samba organic rice cultivation in New Cauvery Delta Zone" with a long term goal to enhance the pest defender ratio and sustain the productivity.

Materials and Methods

Field experiments were taken up in the organically maintained block for 10 years at Soil and Water Management Research Institute, Thanjavur, Tamil Nadu, India during Samba season (2017 and 2018) with cultivar Improved White Ponni. Two treatments viz., Bio-intensive organic insect pest management module and Farmers organic module were compared for the insect pest intensity, natural enemy abundance and grain yield.

In Bio-intensive organic insect pest management module, leaves of *Calotropis* @ 200 kg + Neem 300 kg/ 800 m^2 were incorporated into the nursery soil. Freshly prepared neem seed kernel extract 5% / *Pseudomonas fluorescens* 0.2% was used to manage insect pest / diseases respectively in nursery. In the main field, green manure *Daincha* @ 50 kg seed/ha was raised in situ and incorporated to a depth of 15 cm. Basal application of *Azospirillum* + Phosphobacteria + silica solubilizing bacteria @ each 2 kg/ha + *Pseudomonas fluorescens* (Pf 1) at 2.5 kg/ha was done. Neem cake @ 1500 kg/ha was added to the soil during the last ploughing. Wet seed treatment with *Pseudomonas fluorescens* @ 10 g/kg of seed. Solar light trap was installed to monitor the insect pest activity. When the stem borer and leaf folder moth activity was noticed, *Trichogramma japonicum* and *Trichogramma chilonis* @ 1,00,000 (5 cc) /ha each, were released thrice. Bird perches were set up at 2-3 ft height in vegetative stage @ 50 /ha. To enhance natural enemies' activity floral plants (cowpea, gingelly, sunflower, maize, black gram) were raised in the bunds all around the field. With regard to water management, alternate wetting and drying was maintained and submergence recommended during critical periods only. Need based application of freshly prepared Neem Seed Kernel Extract 5% as well as foliar spray of *Pseudomonas fluorescens* 0.2% if insect and disease incidence crossed ETL.

The farmers' organic module practices included incorporation of green leaf manure, Wild indigo (*Tephrosia purpurea*) / *Calotropis* in nursery @ 50 kg / 800 m^2. Neem cake or crushed neem seed @ 100 kg/ha was added to the soil during the last ploughing. Panchagavya 3 per cent was sprayed thrice during maximum tillering, panicle initiation and booting stage. Need based application of neem commercial formulation (Neem Azal/Achook) was done when insect and disease incidence crossed ETL. Each trial was taken up in one acre plot with Exploded block design (Rothamsted, 1974; Ramachander *et al.*, 1989). In each one acre plot, five micro-plots were maintained and observations were made at this point.

Profitability and sustainability of Bio-intensive organic insect pest management module and farmers organic module were determined in terms of net profit per ha and cost benefit ratio. A t-test carried out to know whether there was a significant difference between the Bio-intensive organic insect pest management module and farmer's organic module.

Results

Herbivore population in the rice field

During Samba the herbivores that affected the rice crop included yellow stem borer (YSB), *Scirpophaga incertulas* (Walker), Crambidae, Lepidoptera; green leafhopper (GLH), *Nephotettix* spp., Cicadellidae, Hemiptera; Short horned grasshopper, *Acrida exaltata* (Walker), Acrididae, Orthoptera; Stink bug, *Menida versicolor* (Gmelin) and Black bug, *Scotinophora lurida*, Pentatomidae, Hemiptera; Earhead bug, *Leptocorisa* spp., Alydidae, Hemiptera.

In *Samba* 2017 field trial, the yellow stem borer damage in bio-intensive organic insect pest management module averaged 0.83 per cent as against 3.18 per cent in the farmers' organic module. The damage level was 3.83 times higher in the farmers' organic module as compared to the bio-intensive organic insect pest management module. With regard to leaf folder damage, the bio-intensive organic insect pest management module had 1.13 times lesser leaf damage compared to farmer's organic module (Table 1). In *Samba* 2017 field trial, the total insect pests in bio-intensive organic insect pest management module averaged 1.78 nos./ 5 sweeps as against 3.83 nos./ 5 sweeps in the farmers organic module. The insect pest load was 2.15 times higher in the farmers' organic module as compared to the Bio-intensive organic insect pest management module.

In the *Samba* 2018 trial the total insect pests in bio-intensive organic insect pest management module averaged 3.37 nos./ 5 sweeps as against 6.91 nos./ 5 sweeps in the farmers organic module. In the second year trial also the insect pest load was 2.05 times higher in the farmers' organic module as compared to the Bio-intensive organic insect pest management module (Table 2).

Natural enemies' population in the rice field

Assassin bug, *Polytoxus* sp., Reduviidae, Hemiptera; Long horned grasshopper, *Conocephalus* sp., Tettigoniidae, Orthoptera; Tachinid fly, *Argyrophylax* sp., Tachinidae, Diptera; Rove beetle, *Paederus fuscipes*, Staphylinidae, Coleoptera; Ground beetle, *Ophionea nigrofasciata*, Carabidae, Coleoptera; Coccinellids, *Micraspis* sp., and *Menochilus sexmaculatus*, Coccinellidae Coleoptera; Damselfly, *Agriocnemis* sp., Coenagrionidae, Odonata; Dragonfly, *Diplacodes* sp., Libellulidae, Anisoptera, Odonata; Owl fly, Ascalaphidae, Neuroptera; Braconid wasps, *Stenobracon nicevillei* and *Macrocentrus* sp., Braconidae, Hymenoptera and Ichneumonids, *Trichomma cnaphalocrocis* and Xanthopimpla sp. lchneumonidae, Hymenoptera. Of the spiders, *Oxyopes* sp., Oxyopidae; *Argiope* sp., Araneidae; *Araneus* sp., Araneidae; *Tetragnatha* sp., Tetragnathidae and *Lycosa*, Lycosidae, Araneae were recorded.

Table 1: Insect pest and natural enemies in bio-intensive organic insect pest management module compared to farmers organic module, Samba season 2017

Period	YSB (% DH/WE)	Leaf folder (% LD)	Insect pests (no./5 sweeps)				Natural enemies (no./5 sweeps)				
			YSB moth	GLH	Namavandu	Total	Coccinellids	Damselfly	Spiders	Total	P:D ratio
	T1 – Bio-intensive organic insect pest management module										
I Oct, 2017	1.54	1.61	0.00	1.67	0.00	**1.67**	3.00	2.67	3.33	**9.00**	1: 5.40
II Oct 2017	1.00	1.51	0.00	2.00	0.00	**2.00**	2.00	2.67	3.00	**7.67**	1: 3.83
I Nov,2017	0.92	1.30	0.33	1.00	0.00	**1.33**	2.67	3.00	4.33	**10.00**	1: 7.50
IINov,2017	0.48	1.05	0.00	0.00	1.33	**1.33**	2.00	2.00	2.00	**6.00**	1: 4.50
I Dec, 2017	0.51	0.82	0.00	0.00	2.33	**2.33**	2.00	1.33	2.67	**6.00**	1: 2.57
II Dec, 2017	0.44	1.21	0.33	0.00	1.67	**2.00**	0.33	0.00	0.33	**0.66**	1: 0.33
I Jan, 2017	0.95	0.86	0.00	0.00	2.33	**2.33**	0.00	0.00	0.00	**0.00**	1: 0.00
Mean	**0.83**	**1.19**	**0.09**	**0.67**	**1.09**	**1.86**	**1.71**	**1.67**	**2.24**	**5.62**	
	T2 – Farmers organic module										
I Oct., 2017	3.19	2.07	1.00	2.33	0.33	**2.66**	1.67	1.67	2.67	**6.01**	1: 1.64
II Oct 2017	4.70	1.79	0.33	4.00	0.00	**4.00**	1.33	0.67	1.00	**3.00**	1: 0.69
I Nov, 2017	3.70	1.13	0.67	2.33	0.00	**2.33**	0.00	1.67	1.33	**3.00**	1: 1.00
II Nov, 2017	4.05	1.07	0.33	1.33	2.33	**3.66**	0.00	0.67	0.67	**1.34**	1: 0.33
I Dec, 2017	2.81	1.18	0.33	1.00	3.67	**4.67**	0.33	0.00	0.00	**0.33**	1: 0.07
II Dec, 2017	2.26	0.83	0.67	0.33	5.33	**5.66**	0.00	0.00	0.00	**0.00**	1: 0.00
I Jan, 2017	1.54	1.39	0.00	0.00	4.67	**4.67**	0.00	0.00	0.00	**0.00**	1: 0.00
Mean	**3.18**	**1.35**	**0.48**	**1.62**	**2.33**	**3.95**	**0.48**	**0.67**	**0.81**	**1.95**	
t value	-5.60	-1.22	-3.37	-3.70	-2.42		3.42	3.55	3.23		

** Mean of five replications   t critical value (one-tail) - 1.94; t critical value (two-tail) - 2.45*

Table 2: Insect pests and natural enemies in bio-intensive organic insect pest management and farmer's organic module, Samba season 2018

Period	Insect pests (no./5 sweeps)						Natural enemies (no./5 sweeps)									P:D ratio
	Black bug	GLH	Earhead bug	SHG	Namavandu	Total	Coccinellids	Damselfly	Dragonfly	Dipteran	Ground beetle	Wasp	LHG	Spiders	Total	
Bio-intensive organic insect pest management module																
I Oct., 2018	1.00	1.67	0.00	0.00	3.00	**5.67**	7.67	1.00	0.33	0.33	2.67	0.33	0.33	6.00	**18.67**	**1:3.29**
II Oct., 2018	0.33	4.33	0.00	0.33	2.00	**7.00**	6.00	1.33	0.00	0.67	1.67	0.00	0.33	8.67	**18.67**	**1:2.67**
I Nov., 2018	0.00	3.00	0.00	0.00	3.33	**6.33**	4.33	3.00	0.33	1.00	2.00	1.33	0.00	4.00	**16.00**	**1:2.53**
II Nov., 2018	1.00	0.00	0.0	0.00	1.00	**2.00**	0.33	0.00	0.00	0.33	0.00	0.00	0.00	2.67	**3.33**	**1:1.67**
I Dec., 2018	0.00	0.00	2.67	0.00	2.00	**4.67**	4.33	5.00	0.33	0.00	2.33	2.00	0.00	7.33	**11.67**	**1:2.50**
II Dec., 2018	0.00	0.00	2.33	0.00	3.33	**5.67**	2.67	5.00	1.00	0.00	3.67	2.67	0.00	5.67	**12.00**	**1:2.12**
Farmers organic module																
I Oct., 2018	4.67	3.33	0.00	0.00	1.33	**9.33**	3.00	1.00	0.33	0.33	0.67	0.33	0.00	4.00	**9.67**	**1:1.04**
II Oct., 2018	0.00	10.67	0.00	0.00	1.00	**11.67**	3.67	0.33	0.33	0.00	1.67	0.67	0.00	4.00	**10.67**	**1:0.91**
I Nov., 2018	0.00	5.00	1.00	0.00	5.67	**11.67**	2.33	1.67	0.00	1.00	1.00	0.33	0.00	3.67	**10.00**	**1:0.86**
II Nov., 2018	0.00	3.67	0	1.33	2.33	**7.33**	1.67	0.00	0.00	0.00	0.67	0.00	0.00	1.00	**3.33**	**1:0.45**
I Dec., 2018	0.00	0.00	0.33	6.33	5.33	**12.00**	2.67	4.00	0.00	0.00	2.67	0.33	0.00	4.00	**7.00**	**1:0.58**
II Dec., 2018	0.00	0.00	0.00	4.67	6.33	**11.00**	1.33	3.33	0.33	0.00	2.33	0.33	0.00	5.00	**7.67**	**1:0.70**
	-0.58	-2.31	1.08	-1.74	-1.42		2.25	2.95	1.17	1.46	1.29	1.55	1.58	3.15		

**Mean of five replications   t critical value (one-tail) – 2.02; t critical value (two-tail) – 2.57*

The mean natural enemies population in *Samba*, 2017 bio-intensive organic insect pest management module was 6.56 nos./ 5 sweeps as against 2.28 nos./ 5 sweeps in the farmers organic module. Population of the natural enemies was 2.88 times higher in the Bio-intensive organic insect pest management module as compared to the farmers' organic module. In *Samba* 2018, the mean natural enemies in Bio-intensive organic insect pest management module was 9.71 nos./ 5 sweeps as against 4.95 nos./ 5 sweeps in the farmers organic module. Population of the natural enemies was 1.96 times higher in the bio-intensive organic insect pest management module as compared to the farmers' organic module.

Pest defender ratio

In Bio-intensive organic insect pest management module, the pest-defender ratio was highest 1:7.50 during the first fortnight of November, 2017 as against 1:1.00 in the farmers' organic module during *Samba* 2017. The mean pest defender ratio in Bio-intensive organic insect pest management module was 1: 4.02 compared to 1: 1.24 in the farmers' organic module during *Samba*, 2017. In *Samba* 2018, Bio-intensive organic insect pest management module the highest pest-defender ratio was 1:3.29 during the first fortnight of October, 2018 compared to farmers' organic module (1:1.04). The mean pest defender ratio in Bio-intensive organic insect pest management module was 1: 2.46 compared to 1: 0.75 in the farmers' organic module during *Samba*, 2018.

Grain yield

In *Samba* 2017 field trial, the grain yield recorded was 4080 kg/ha in Bio-intensive organic insect pest management module as compared 3880 kg/ha in farmers' organic module. During Samba, 2018 grain yield recorded was 4340 kg/ha in Bio-intensive organic insect pest management module as compared 3440 kg/ha in farmers' organic module.

Profitability and sustainability

The total production cost for organic paddy accounted to Rs. 33041/- per ha in both Bio-intensive organic IPM module and farmers' organic module as uniformity was maintained in cultivation aspects. However with regard to the plant protection aspects it varied between Bio-intensive organic IPM module and farmers' organic module. The expenditure towards IPM (Rs.) was Rs. 11090/- per ha in bio-intensive organic IPM module as compared to Rs. 6750/- per ha in Farmers organic module. The profit earned form the bio-intensive organic IPM module was Rs. 70109/- during *Samba*, 2017 and Rs. 77389/- during *Samba*, 2018. There was a 10.38 per cent increased yield in *Samba*, 2018 over *Samba*, 2017 trial. Conversely, the profit earned form the Farmers organic module was Rs. 68849/- during *Samba*, 2017 and Rs. 56529/- during *Samba*, 2018. There was a decreased yield trend of -17.89 per cent in *Samba*, 2018 over *Samba*, 2017 trial.

Table 3: Profitability and sustainability of bio-intensive organic insect pest management module and farmers organic module

Parameter	Bio-intensive IPM module		Farmers organic module	
	2017-18	2018-19	2017-18	2018-19
Yield (kg/ha)	4080	4340	3880	3440
Net return (Rs./ha)	114240	121520	108640	96320
Total production cost (Rs./ha)	33041	33041	33041	33041
Expenditure towards Plant Protection (Rs./ha)	11090	11090	6750	6750
Profit (Rs./ha)	70109	77389	68849	56529
% increase yield over 2017 trial		10.38		-17.89
Cost benefit ratio	1:1.59	1:1.73	1:1.75	1:1.42

Discussion

Enhancing natural pest control in organic systems could help reduce costs, stabilize production, and increase the ability of organic practices to meet global demand. Decreased insect pests on long term organic farms have largely been attributed to practices that limit pest build-up, increase predator biodiversity, and increase the numbers of beneficial insects (Hole, 2005; Crowder *et al*., 2010, Garratt *et al*., 2011).

The green manure *Daincha* incorporated in the soil promoted soil microbes, the nutrient availability to plants and increased plant immunity against insect. Organic management strategies can increase microbial activity and biomass in soils, alter microbial communities, and in some cases enhance plant associations with beneficial microbes in the rhizosphere. Microorganisms that associate with plant roots play a critical role in resistance to abiotic and biotic stress (Vannette and Hunter, 2009).

In our study, the insect pest load was 2.15 times lesser in the bio-intensive organic insect pest management module as compared to the farmers' organic module. *Lyashenko et al*., 1982 reported that organic matter is mainly supplemented through Farm Yard Manure (FYM) in rice fields that increased levels of leucoanthocyanins, catechins, flavanol glycosides and phenol carboxylic acids in plants and this would be responsible for lesser incidence of many rice insect pests. Mohankumar *et al.* (1995) reported that organic manure application lowered gall midge incidence than inorganic fertilizer application. Basal application of *Azospirillum* + Phosphobacteria + silica solubilizing bacteria + *Pseudomonas fluorescens* (Pf 1) reduced the insect pest load. The total insect pests in Bio-intensive organic insect pest management module averaged 3.37 nos./ 5 sweeps as against 6.91 nos./ 5 sweeps in the farmers organic module. Earlier reports also revealed that incorporation of *Azospirillum* significantly decreased the incidence of BPH, GLH and leaf folder in rice (Anuradha, 1989). The combined application of *Azospirillum* with organic manure decreased the feeding rate of BPH and adversely affected its growth and development (Athisamy and Venugopal, 1995). Mohan *et al.* (1988) reported that *Azospirillum* would have favourably activated Phenyl Ammonia Lyase enzyme concerned in the biosynthesis of phenolics resulting in increased plant phenolics in plants which are responsible for lower damage. *Azospirillum* promotes growth of plant mainly by the production of phytohormornes viz., auxins, gibberlins and cytokinins in addition to biological nitrogen fixation (Cicciari *et al*., 1989). Neem cake soil application during the last ploughing increased the plant phenol contentwhich in turn had negative effect on the insect multiplication and development (Krishnaiah and Kalode,1985). The role of neem cake in reducing the incidence of planthoppers and leaf hoppers, gall midge, stem borer and leaf folder has been reported by Athisamy (1994) and Ambethgar (1996).

The mean natural enemies in Bio-intensive organic insect pest management module was 9.71 nos./ 5 sweeps as against 4.95 nos./ 5 sweeps in the farmers organic module. Population of the natural enemies was 1.96 times higher in the Bio-intensive organic insect pest management module as compared to the farmers' organic module. Ragini *et al*. (1995) observed that organic farming supported more *Ophionea*, *Paederus*, coccinellid beetles, mirids and spiders. In organic paddy ecosystem also the grain yield recorded was 4340 kg/ha in Bio-intensive organic insect pest management module as compared 3440 kg/ha in farmers' organic module.

In Bio-intensive organic insect pest management module there was a 10.38 per cent increased yield in *Samba*, 2018 over *Samba*, 2017 trial. Conversely, in farmers' organic module there was a decreased yield trend of -17.89 per cent in *Samba*, 2018 over *Samba*, 2017 trial. It could be concluded that organic matter enrichment and increased biocontrol agents activities in bio-intensive organic insect pest management module enhanced the association of above and below ground food chains and augmented natural plant defense mechanism furthermore would reflect in the increased rice productivity over years.

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SHORT COMMUNICATION OPEN ACCESS

Isolation of crude toxin, thin layer chromatography (TLC) and HPLC analysis of *Bipolaris oryzae,* inciting brown spot disease of rice

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Received: 22 April 2019; Accepted: 19 October 2019

Abstract

Brown spot disease (*Bipolaris oryzae*) has been associated with two major epidemics in India and one of the strongest yield reducers among rice diseases in recent years. The disease especially occurs in environment where water supply is scarce combined with nutritional imbalance particularly lack of nitrogen and hence often referred to as 'poor man's disease'. In the present study, six isolates of *B. oryzae* were used for extraction of toxin by solvent extraction procedure. Toxin profile of six isolates of *B. oryzae* on Thin-layer chromatography (TLC) plate showed a total of eight bands; five bands at Rf 0.93, 0.85, 0.77, 0.61 and 0.49 were distinct and all isolates produced a common dark band at Rf 0.61 in iodine chamber at 254 nm. The quantitative analysis of toxin produced by different isolates was carried out by High-performance liquid chromatography (HPLC) analysis. Six isolates of *B. oryzae* showed variation in their peak values at different retention time. In all isolates unique peak with retention time at 4.3 min was observed. This compound which was observed in all the isolates can further be characterized for its property of toxin by using distinct standards.

Keywords: Brown spot disease, *Bipolaris oryzae*, Thin-layer chromatography (TLC), High-performance liquid chromatography (HPLC)

Rice is one of the most important cereal crops and feeds more than one third of the world's population (Burgos *et al.*, 2013). Rice is susceptible to several leaf spot diseases like blast and brown spot, which cause significant yield losses across the globe. The yield loss was upto 90% in epiphytotic form at leaf spot phase during the year 1942-43 of Great Bengal Famine (Ghose *et al.*, 1960). When the disease was reported in 1919, extensive research has been carried due to which the disease was controlled. In recent years, because of the climate change and cultivation practices, disease was found to be severe in dry/ direct seeded rice in the states of Bihar, Chhattisgarh, Madhya Pradesh, Odisha, Assam, Jharkhand and West Bengal. It especially occurs in the environment where water is a scarce resource combined with nutritional imbalance particularly lack of nitrogen and hence often referred to as "Poor man's disease'' (Baranwal *et al.*, 2013).

The pathogen attacks the crop from seedling to milky stage. The symptoms appear as minute spots on the coleoptile, leaf blade, leaf sheath and glume, being most prominent on leaf blades and glumes. On leaves, typical spots are brown in colour with grey or whitish centre resembling sesame seed with typical yellow halo over the spot (Sunder *et al.*, 2005). Conidia are 5-10 septate with the oldest conidium towards base. Typically conidia are slightly curved and widest at the middle. The optimum temperature for growth and conidial germination has been found to be 27-30 °C and 25-30°C, respectively (Ou, 1985) wherein conidia are formed between 5-38 °C, optimum being 25 °C (Ou, 1985; Vinay Kumari *et al.*, 1997). Both light and dark periods were required for sporulation of *B. oryzae*. However, it was stimulated by near-ultra violet light and inhibited by blue light (Ou, 1985).

The pathogen is reported to produce phytotoxins in culture. Goto (1958) reported the toxic effect of the culture filtrate of *B. oryzae* on rice plants. Terashima *et al.* (1962) isolated ergosterol from the mycelium of the fungus. Cochliobolin extracted and purified from filtrate and ophiobolin detected in diseased leaves inhibited the growth of roots, coleoptiles and leaves (Nakamura and Oku, 1960; Ou, 1985). Narain and Simhachalam (1976) claimed that the toxin was completely inactivated by copper oxychloride. Beside these, the pathogen has also been observed to produce host specific toxin, which elicited the characteristic brown spot symptoms (Vidhyasekaran *et al.,* 1986) through suppression of defense mechanism by decreasing phenolic content and phenyalanine-ammonia lyase activity in rice leaves (Vidhyasekaran *et al.,* 1992). Hegazy *et al.* (1992) reported that partially purified toxin (s) from the pathogen inhibited seed germination and reduced root and shoot length in rice cultivars. Ophiobolin A from *Bipolaris oryzae* perturbs motility and membrane integrities of porcine sperm and induces cell death on mammalian somatic cell lines (Bencsik *et al*., 2014).

Morpho-pathological and molecular characterization of *B. oryzae* has been carried out for fifty isolates in India (Kumar *et al.,* 2011). Diversity and pathogenicity of the rice brown spot pathogen were investigated earlier by many workers using morphological characteristics as well as genetic fingerprint analysis in India as well as in other rice growing countries (Motlagh and Kaviani, 2008; Kamal and Mia, 2009; Motlagh and Anvari, 2010; Burgoss *et al.,* 2013, Archana *et al*., 2014, Kandan *et al*., 2014 and Nazari *et al*., 2015). Morphological, molecular characterization and grouping of 27 isolates of *Bipolaris oryzae* from India were carried out by Singh *et al.,* (2016). Morpho-molecular diversity for 116 isolates of *Bipolaris oryzae* from different rice growing areas of India was studied by Kumar *et al.,* (2016). Morphological, molecular characterization and grouping of 17 isolates of *Bipolaris oryzae* from India were carried out by Valarmathi and Ladhalakshmi (2018). This paper mainly dealt with the isolation of crude toxin followed by TLC and HPLC studies.

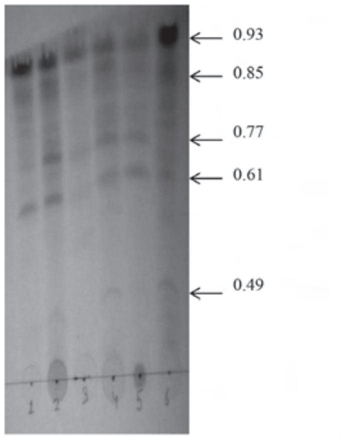
The toxin was extracted from the six *B. oryzae* isolates using solvent extraction procedure. Each isolate inoculated into three replicated flasks (1 L) containing 200 ml PDA medium were incubated at 25 ± 1° for 3 weeks. The culture filtrate was extracted with chloroform taken in a separating funnel. The collected chloroform layer was evaporated by rotary evaporator (55 ºC and 120 rpm) and the residue (crude toxin) dissolved in 1ml of methanol was stored at 4 ºC. 10 µl of partially purified toxin isolated from all isolates was loaded on Silica-gel TLC plates and developed in Benzene: Acetone (1:1) solvent system. Air dried plates were visualized by iodine vapours in iodine chamber and under UV light (254 and 365 nm). Rf values of various compounds resolved on TLC plate were measured (Jahani *et al.,* 2013). The quantitative analysis of toxin produced by different isolates was carried out by High-performance liquid chromatography (HPLC) as per the protocol given by Jahani *et al.,* (2013).

Toxin profile of six isolates of *B. oryzae* on TLC plate showed a total of eight; bands of which, five bands at Rf 0.93, 0.85, 0.77, 0.61 and 0.49 were distinct (Fig. 1). All the isolates produced a common dark band at Rf 0.61 in iodine chamber at 254 nm. HPLC analysis of six isolates of *B. oryzae* showed variation in their peak values at different retention time (Fig. 2a-2f). In the isolate BO 1, four peaks were observed at 4.3, 5.2, 6.1 and 12.2 min, in BO 2 three peaks at 4.2, 5.5 and 12.2 min and in BO 3 three peaks at 4.2, 5.4 and 13.1 min were observed respectively. In the isolate BO 1319 and BO 5326 two peaks at 4.3 and 12.1 min were observed. In the isolate BO 23, various peaks were observed at 4.3, 5.2, 5.5, 6.6, 11.1 and 12.8 min respectively. In all isolates unique peak with retention time at 4.3 min was observed (Table 1). The compound which was observed in all the isolates can further be characterized for its property of toxin.

Crude toxin extracted from the isolates of BO 1, 2, 3, 1319, 5326 and BO 23 were used for thin layer chromatography (TLC). In the TLC, five bands at Rf 0.93, 0.85, 0.77, 0.61 and 0.49 were distinct. Similar studies in *B.sorokiniana* by Jahani *et al.,* (2013) showed Rf values 0.90, 0.81, 0.76, 0.67 and 0.44 distinct wherein 0.44 produced common dark band in iodine chamber at 254 nm. In the HPLC analysis, single peak at Rt 4.3 min was observed in all the isolates used. Further this single peak can be characterized to know its toxin property. In the HPLC analysis of purified toxin *B.sorokiniana* showed a single peak at Rt 3.03 min with 96.16 purity and characterized as 'bipolaroxin' by H-NMR studies (Jahani *et al.,* 2013).

Table 1: HPLC analysis of *B. oryzae* isolates

Isolates	Rt	Area	% Area	Height
BO 1	4.34	2528620	4.59	97736
BO 2	4.15	6676871	13.34	102700
BO 3	4.21	64617	0.60	4580
BO 1319	4.26	512353	4.98	39601
BO 5326	4.34	1007467	9.42	26424
BO 23	4.22	766500	3.44	54070



Lane 1. BO 1; Lane 2. BO 2; Lane 3. BO 3; Lane 4. BO 5326; Lane 5. BO 1319 and Lane 6. BO 23

Fig 1. Thin layer chromatography (TLC)

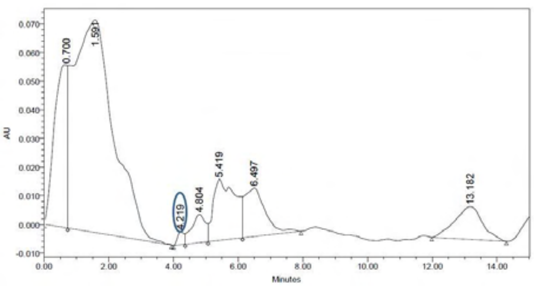


Fig 2a.HPLC analysis of *B. oryzae* isolates (BO 1)

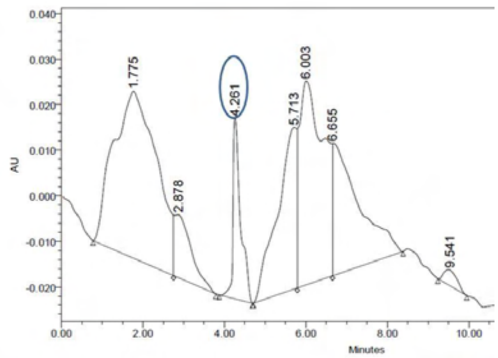


Fig 2d.HPLC analysis of *B. oryzae* isolates (BO 1319)

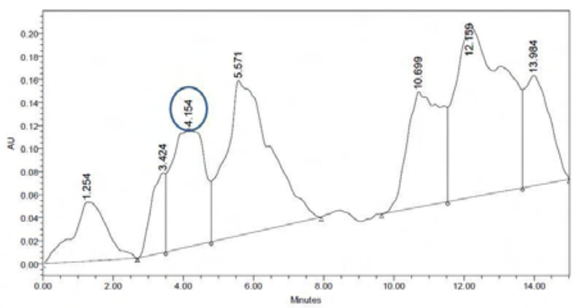


Fig 2b.HPLC analysis of *B. oryzae* isolates (BO 2)

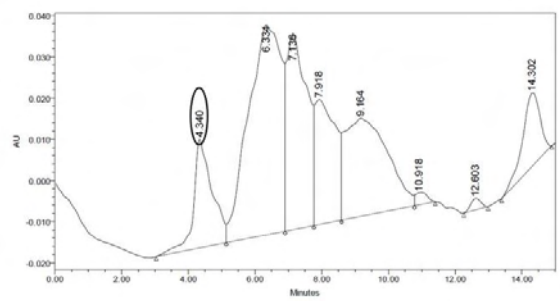


Fig 2e.HPLC analysis of *B. oryzae* isolates (BO 5326)

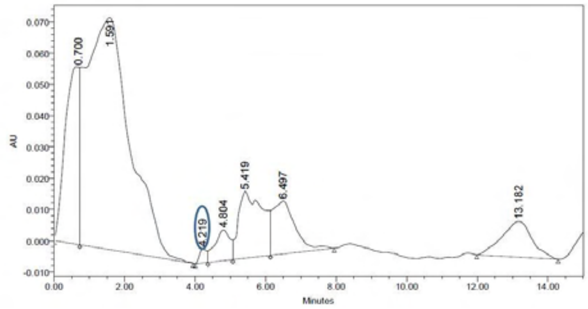


Fig 2c.HPLC analysis of *B. oryzae* isolates (BO 3)

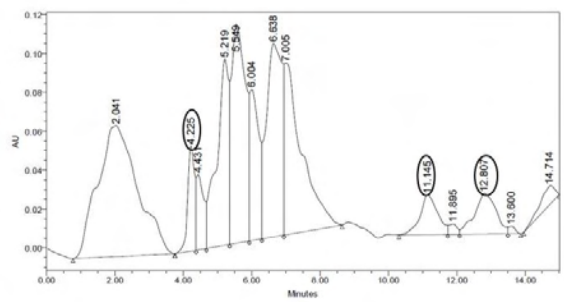


Fig 2f.HPLC analysis of *B. oryzae* isolates (BO 23)

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SHORT COMMUNICATION OPEN ACCESS

Reaction of rice cultivars to rice root-knot nematode *Meloidogyne graminicola*

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Received: 10 September 2019; Accepted: 25 November 2019

Abstract

The rice root knot nematode *Meloidogyne graminicola* is one of the important nematode problems in many rice-growing areas of the world. The development of nematode-resistant cultivars is the most cost-effective and sustainable method for nematode management in rice. The success of a breeding programme for resistance depends on the availability of diverse sources of resistance. The present study was conducted to screen fourteen rice cultivars for resistance to *M. graminicola* in search of strong resistant source. The rice cultivars were screened for resistance to *M. graminicola* by the germination paper screening method using the relative root-gall index (RRGI) and relative reproduction index (RRI) scores. Out of the fourteen rice cultivars tested, only two cultivars *viz*., Swarnadhan and Dhanrasi were found resistant to *M. graminicola*. These two rice cultivars can be studied further to use them as donors in resistance breeding against *M. graminicola*.

Keywords: Rice, *Meloidogyne graminicola*, resistance, screening

The rice root-knot nematode *Meloidogyne graminicola* is one of the important plant-parasitic nematodes infecting rice crop (Prasad *et al*., 2010; Ravindra *et al*., 2017). It is a pest of international importance to rice due to its presence in most of the rice-growing areas in the world (De Waele and Elsen, 2007; Dutta *et al*., 2012; Mantelin *et al*., 2017). It infects rice in almost all types of rice production systems, but the damage is more severe in rice nurseries and upland rice. *M. graminicola* infected rice plants show stunting and chlorosis due to the characteristic terminal swellings or hook-like galls on the roots, which ultimately results in severe reduction in growth and yield (Prasad *et al*., 2010; Pankaj *et al*., 2010). In India, *M. graminicola* is one of the major concerns in rice production in many states including Karnataka, West Bengal, Orissa, Uttar Pradesh, Himachal Pradesh, Assam (Jain *et al*., 2012). It is reported to cause 10-30% yield loss in different rice production systems (Jain *et al*., 2007).

In view of limited options for the management and poor awareness among the farmers about nematodes, the only effective management option for *M. graminicola* is the use of resistant cultivars. The development of nematode-resistant cultivars is the most cost-effective and sustainable method for nematode management for small as well as large-scale farmers in poor and developing countries (Pokharel *et al*., 2012). Several resistant to moderately resistant rice cultivars have been identified against this nematode, still there is a need for strong resistant source for breeding nematode resistant cultivars. Hence the present study was conducted to screen fourteen rice cultivars for resistance to *M. graminicola* in rice.

The rice cultivars tested in this experiment were reported to be resistant to multiple insect pests and diseases of rice (Padmavathi *et al*., 2013), but the information on the reaction of these cultivars to rice root-knot nematode *M. graminicola* is lacking. Hence these cultivars were selected for this study. The cultivars were screened for resistance to *M. graminicola* by the germination paper screening method (Somasekhar *et al*., 2019). Rice seeds were soaked in water for two days and sprouted seeds were transferred to the germination paper rolls. Ten seeds were placed in each germination paper roll. The germination paper rolls were placed in test tubes half filled with distilled water and incubated at 25-30 ^{o}C. Each seedling was inoculated with 50 second-stage infective juveniles (J_2) of *M. graminicola* 4-5 days after placing seeds in germination paper roll. Nematode susceptible cultivar TN 1 was used as a susceptible check and five replications were maintained for each cultivar. Observations on root galls and total nematode population including eggs and juveniles were recorded three weeks after nematode inoculation. The experiment was repeated twice and the data from all the individual plants of a test entry from both the experiments were combined to compute the mean. The cultivars were categorized as resistant or susceptible based on the relative root-gall index (RRGI) and relative reproduction index (RRI) scores (Jena and Rao, 1976). RRGI/RRI score followed: 0 (Highly resistant), 0.1-1.0 (Resistant), 1.1-2.0 (Moderately susceptible), 2.1-3.0 (Susceptible) and >3.1 (Highly susceptible). The RRGI and RRI are computed as per the formula given below.

$$\text{Relative root-gall index} = \frac{\text{Number of galls in test entry x 4}}{\text{Number of galls in susceptible check}}$$

$$\text{Relative reproduction index} = \frac{\text{Total nematode population in test entry x 4}}{\text{Total nematode population in susceptible check}}$$

A large variation in the number of galls and total nematode population per root system was observed among the cultivars tested. The reaction of test entries was rated based on the relative root-gall index and relative reproduction index. Out of the fourteen rice cultivars tested, only two cultivars *viz*., Swarnadhan and Dhanrasi were found resistant to *M. graminicola* while the cultivars Vasumati, Suraksha, Nidhi, Mansarovar and DRRH2 were found moderately susceptible. All other cultivars were either susceptible or highly susceptible (Table 1). The galls on resistant cultivars Swarnadhan and Dhanrasi were very small and contained less numbers of eggs as compared to the galls in susceptible check TN 1. Also, the size of females dissected from the galls of resistant cultivars Swarnadhan and Dhanrasi were relatively small as compared to the females from the galls of TN 1. It is evident from the results that though the rice cultivars were reported to be resistant to multiple insect pests and diseases of rice, most of them were susceptible to the rice root-knot nematode *M. graminicola*. The resistant rice cultivars Swarnadhan and Dhanrasi can be studied further to use them as donors in resistance breeding against *M. graminicola*.

Table 1: The reaction of rice cultivars to rice root-knot nematode *Meloidogyne graminicola*

Sr. No.	Cultivars	RRGI	RRI	Reaction
1	Triguna	3.82	3.79	HS
2	Vasumati	1.58	1.40	MS
3	Shanthi	5.81	3.70	HS
4	Aghani	1.89	1.30	MS
5	Suraksha	1.53	1.10	MS
6	Akshaydhan	3.74	2.57	HS
7	Swarnadhan	0.89	0.30	R
8	Nidhi	1.14	1.10	MS
9	Tulsi	3.44	2.67	HS
10	Mansarovar	1.26	1.11	MS
11	Dhanrasi	0.95	0.20	R
12	Vardhan	2.65	1.12	S
13	Vikramarya	4.84	4.59	HS
14	DRRH2	1.40	1.10	MS
15	TN 1 (Susceptible check)	4	4	HS

RRGI: Relative root-gall index, RRI: Relative reproduction index, R: resistant, MS: moderately susceptible, S: Susceptible, HS: Highly susceptible.

Acknowledgements

The authors are grateful to the Director, ICAR-IIRR, Hyderabad for his constant support and encouragement in carrying out this study.

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SHORT COMMUNICATION OPEN ACCESS

DRR Dhan 52 [IET 23354 (RP5125-12-5-3-B-IR84898-B-B)]

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Received: 10 November 2019; Accepted: 11 December 2019

The first heat tolerant variety DRR Dhan 52 was notified in 2019 for cultivation in Haryana, Gujarat and Odisha. It was developed from the cross IR78877-208-B-1-1/IR78878-53-2-2-2 under the IRRI-India collaborative project 'Stress Tolerant Rice for Asia and South Africa' (STRASA). Initial cross of DRR Dhan 52 was made at IRRI, Philippines based on the selection of donors for yield under vegetative/reproductive stage drought stress at multi-location trials (MLTs) across the centres in partnering countries (India, Nepal and Bangladesh) under STRASA. ICAR-IIRR, Hyderabad is part of MLT. Bulk seed with designation 'IR84898-B-B' in segregating generation (F_3) was received from IRRI and single plant selections for superior segregants from F_3 to F_5 generation, progeny rows evaluation and yield trials were done at ICAR-IIRR. Semi-dwarf plant height, earliness, grain type, drought tolerance and high yield are the traits considered in the selection and evaluation process. The line selected was designated as RP5125-12-5-3-B-IR84898-B-B, in which the former part of the designation (RP5125-12-5-3-B) indicates sequential selections from F_3 onwards at ICAR-IIRR and latter part of the designation indicates the initial cross made at IRRI. DRR Dhan 52 (IET No. 23354) was evaluated in All India Coordinated Rice Improvement Project (AICRIP) in Early Transplanted (ETP) trial during 2013-2015 and 2017 and yielded 5428 kg/ha with +9.71, +23.68 and +10.88% yield advantage over national, zonal and local checks, respectively on over all mean in the states released.

Significant increase of Chlorophyll a, Chlorophyll b, total chlorophyll and carotenoids during heat stress (42°C/36°C day/night for five days) at vegetative as well as reproductive stages were observed in DRR Dhan 52, as compared to well established high temperature tolerant rice cultivar N22. Further, gaseous exchange parameters and gene expression data suggests that DRR Dhan 52 has more robust physiological and molecular machinery to cope with heat stress as compared to N22. DRR Dhan 52 showed resistance to blast, moderate resistance to neck blast, brown spot, sheath rot and rice tungro disease.

Figure 1: Paddy, brown, polished and cooked rice of IET 23354 (DRR Dhan 52)

DRR Dhan 52 has high milling recovery (71 %) and head rice recovery (66%), long bold grain with 6.23 mm kernel length, 2.34 mm kernel breadth and 2.67 L/B ratio. It has intermediate alkali spreading value (4.0), amylose content (20%) and GC (55 mm) with very occasional chalkiness indicating good cooking quality (Fig.1). DRR Dhan 52 is distinguishable through morphological features like strong culm, anthocyanin coloration of basal leaf sheath, light green foliage, creamish septum, long white split ligules, apiculus non pigmented, white stigma, late senescence, erect and wide flag leaf, compact, heavy and well exerted panicles and 90 day flowering duration. DRR Dhan 52 would certainly increase and stabilize the production of rice in irrigated ecology in the states of Haryana, Gujarat and Odisha.

Dr. Sishta Venkata Seetharama Shastry

4 November 1928 - 5 February 2019

Dr. SVS Shastry, a pioneer of rice research in India, departed his soul on 5 February, 2019, at the age of 91 years. He obtained his Bachelor's degree from Agriculture College, Bapatla and his Master of Science and Doctoral degree from University of Wisconsin in 1958. He started his career as Assistant Professor (Cytogenetics) in 1958-61 and Botanist, 1961-1966 at the Indian Agricultural Research Institute (IARI), New Delhi. He took over as the Project Coordinator (Rice) in 1966 and served till 1977. He joined as Senior Agricultural Officer, Agricultural Production and Protection and then as Executive Secretary, International Rice Commission, Food and Agriculture Organisation (FAO), Rome from 1975-1977. He served as Director of Research, International Institute of Tropical Agriculture (IITA), Nigeria from 1977-1983. He also served as the Honorary Trustee and Vice-Chairman of the Programme Committee of International Rice Research Institute (IRRI), Philippines during 1970-73 and Chairman, Steering committee and Member, Scientific and Technical Committee, West Africa Rice Development Association (WARDA) from 1977-82.

Taking forward the expertise gained on pachytene analysis in Melilotus species at the University of Wisconsin, he published the seminal report on the pachytene stage of rice chromosomes (Shastry *et al.*, 1960). He published a series of papers on cytology of cultivated and wild species, their interspecific hybrids and re-interpretation of species relationships in the genus, *Oryza* (Shastry and Mishra, 1961; Shastry *et al.*, 1961; Shastry and Ranga Rao, 1961; Shastry 1964a, b). Through his exhaustive biosystematic analysis, he could delineate two new species of *Oryza* namely, *Oryza nivara* and *O. collina* (Sharma & Shastry 1965a, b). Based on their studies, they proposed that *O. nivara* is the progenitor of cultivated rice (Shastry and Sharma, 1973). One of the collections of *O. nivara* was found to be resistant to grassy stunt virus disease of rice at IRRI which helped in the development of grassy stunt virus resistant rice varieties. His research spanned wide range of subjects including Cytogenetics, Taxonomy, Genetics, Plant Breeding, Physiology, Crop Science, Agronomy, Extension and Development. He guided three Ph.D. students and developed four rice varieties namely Jaya, Phalguna, Prakash and Sona. All of these varieties were very popular among the farmers and noteworthy among these is Jaya, which is still popular with the farmers.

Through his futuristic vision, he has been instrumental in planning and establishment of the then All Indian Coordinated Rice Improvement Project (AICRIP) at Rajendranagar, Hyderabad which later evolved as Directorate of Rice Research (DRR). The state-of-the-art rice quality lab and glass house facilities developed by him at the present day ICAR-Indian Rice Research Institute (IIRR) is a standing testimony of his monumental contribution for the rice fraternity as outstanding researcher and administrator.

Recognising his monumental contributions in the area of science and technology especially in rice research, the President of India honored him with the Padma Shri award in 1971. He has also been recipient of Borlaug Award in 1974, Janna Reddy Venkatareddy Prize in 1974, Tonnage Club Medal, 1974; West Godavari Farmers' Trophy, 1975. He was elected as Fellow of two prestigious science academies of India *viz.*, Indian National Science Academy (INSA) and National Academy of Agricultural Sciences (NAAS). He had a pleasing personality and has been a role model for many rice workers.

Society for Advancement of Rice Research (SARR) pays its respects and deep sense of gratitude to the departed soul and prays the almighty that his soul rests in peace. We also convey our deep condolences to the bereaved family.

Dr S V S Shastry with International Scientists at IIRR Farm

Thank you....

Editorial Board of Journal of Rice Research extends their gratefulness to the following reviewers of 2019 Volume 12, Issues 1 & 2. We thank you for sparing your valuable time and expertise in reviewing each manuscript twice and providing valuable inputs.

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Research papers

1. Durvasula V. Seshu**.** 2017. Networking a Pivotal Strategy for Rice Genetic Improvement. *Journal of Rice Research*, 10(1): 1-8.
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. S R Voleti, President, Society for Advancement of Rice Research, Hyderabad.

Printed at :

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

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