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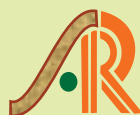
# Journal of Rice Research

Volume 13, No. 2

December 2020



**Society for Advancement  
of Rice Research**



# Society For Advancement of Rice Research

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

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

## Aims and Objectives

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

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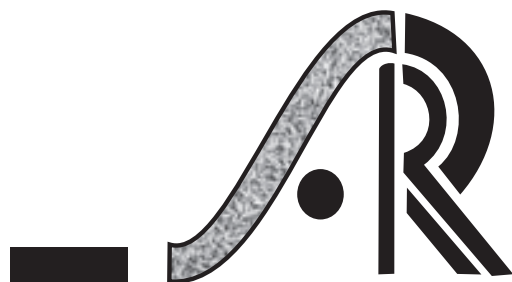
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## Strategic networking for global rice genetic advancement

Dr Durvasula V Seshu

Former Global Coordinator, INGER and Rice Breeder,  
International Rice Research Institute, Philippines

A network approach for boosting efficiency in agricultural research has been widely accepted by various international and national research organizations, because of the wide-ranging benefits, most important of which is the acceleration of the transfer of technology to the farmer.

Rice, the world's foremost food crop derived from a wild progenitor was born as a semi-aquatic plant in the hot humid tropics with a strong monsoon pattern. However, it has gradually forayed into a diversity of habitats, breaking the environmental, as well as geographical barriers, and encompassing agroecosystems that reflect a wide range of water and temperature regimes, altitude level, and edaphic properties. Its cultivation extends to latitudes that circumscribe the tropical and semi temperate environments, ranging from 40° south in central Argentina to 51° north in north-eastern China. Thus, rice is grown in more diverse environmental conditions than any other major crop. The flip side of such an ecological sprawl is its face-off with a plethora of biotic and abiotic stresses, posing a strong challenge for rice genetic improvement.

Prior to the Green Revolution era, rice scientists in the developing countries used to work in scientific isolation with limited experimental materials, the paucity of research facilities including literature, inadequate training, and lack off opportunities to interact with fellow rice scientists at other research centers. Moreover, the experimental stations in several instances were not quite representative of the ecosystems they were purported to serve. Progress in rice yields and thereby its production thus remained at a pace that allowed it to be overrun by the rate of population growth. That was the post-world war II scenario in several developing countries, where rice is the main staple, and that situation has raised concerns

and awareness at both national and international levels.

Because of the geographical and ecological diversity, structured networking of rice breeding programs across the world is strategically vital for global genetic improvement of rice for cultivation in different ecosystems and raising the world output of the grain. Such an approach is also effective within national programs with wide-ranging rice cultural systems. Networks are inexpensive and at the same time are effective catalysts for research. Collaborative networks help to spread useful research results among regions with similar agro-ecologies.

Some national programs that gained experience in rice research turned towards pooling up their resources for a nationwide cooperative crop improvement program. An excellent example is the All India Coordinated Rice Improvement Project (AICRIP), the largest national rice research network, established by the Indian Council of Agricultural Research (ICAR) in 1965. AICRIP has successfully brought together scientists working at over 100 research stations across different states, and through its exchange platform, forged national cooperation in research on genetic enhancement, nutrition management, and protection against major insects and pathogens.

Historically, at an international level, a limited and informal exchange of plant germplasm among scientists from a few countries with common interest took place before World War II. The international Wheat Stem Rust Nursery established by the United States Department of Agriculture (USDA) in 1950 was the first formal and systematic nursery to transcend the national borders. This was necessitated by a serious outbreak of a new race of stem rust in the 1950s. The Rice Blast Nursery organized by IRRI in 1963 and the Spring Wheat Yield Nursery organized by the



International Maize and Wheat Improvement Center (CIMMYT) in 1964 represents the first efforts by the International Agricultural Research Centers (IARCs) to work cooperatively with the National Agricultural Research & Education System (NARES).



The establishment of a series of IARCs under the aegis of the Consultative Group of International Agricultural Research Centers (CGIAR) was a quintessential response to the emerging food crisis in the early sixties of the last century in the developing world. The first among those was the International Rice Research Institute (IRRI), originally funded by the Rockefeller and Ford Foundations, and established in the Philippines in 1960. IRRI in its first decade primarily focused on research and related activities at its center, which resulted among other things in the development of a high-yielding semi-

dwarf variety, IR8 (IRRI's flagship); establishment of a gene bank; development of screening techniques for resistance to major diseases and insects; establishment of a comprehensive training program, setting up of a library with world's largest collection of rice literature, and so on. To buttress the varietal improvement research, a multidisciplinary 'Genetic Evaluation, and Utilization (GEU)' program was introduced in IRRI's second decade. Once equipped with the necessary research wherewithal, and having acquired the capacity to take a lead role, IRRI initiated the establishment of various research networks with the cooperation and commitment of the NARES. The International Rice Testing Program (IRTP), which was subsequently renamed as International Network for Genetic Evaluation of Rice (INGER) was the first among those networks. Initiation of the networks also reflects on the concern and realization of IRRI, that while it has a global mandate for rice improvement, its research facilities are located in but one of the several rice growing environments. For example, gall midge, a major insect pest in parts of South Asia does not occur in the Philippines, which limits IRRI's capabilities to carry research related to that pest without collaboration with scientists in the concerned national programs. Similar is the case with problems such as deepwater, low temperature, acid sulphate soils, etc. Thus, networking involving NARES has become imperative for global rice improvement.

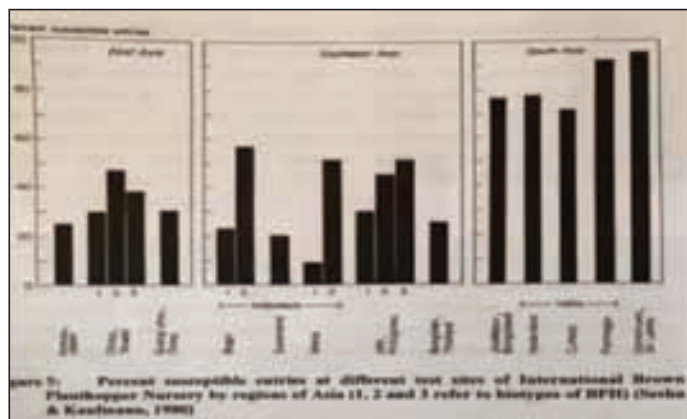
INGER established in 1975 is the world's largest agricultural research network participated by more than 75 countries in Asia, Africa, Latin America, the Caribbean, and Oceania. When the program started in 1975, over 80% of the test entries came from IRRI and the remaining from the NARES. In course of ten years after the start, the proportion of sources of entries has significantly changed, with over 65% originating from NARES and 35% from IARCs, primarily from IRRI. That reflects on the strength gained by the national breeding programs, as resulting from the active participation of its scientists in INGER, and in the network-sponsored joint site visits, workshops, and training programs, with an opportunity to interact with fellow rice scientists from other countries. Thus, with the strengthening of capabilities and institution building, the breeding researches of the NARES



progressed from dependency to interdependency. NARES materials get DNA fingerprinted to alleviate their concerns relating to intellectual property rights.

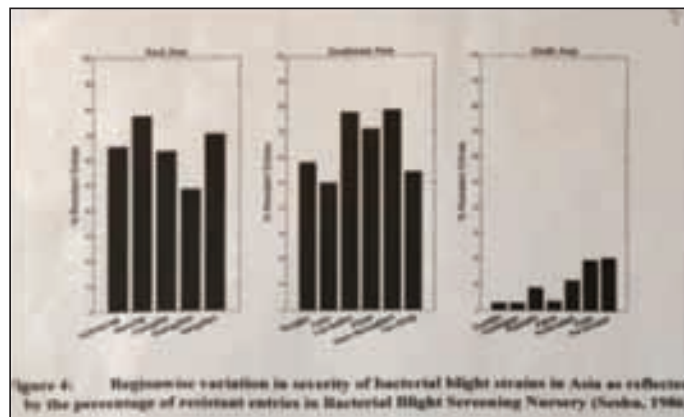


Through the cooperative exchange and evaluation of promising breeding lines, as of date, a little over 1200 INGER-tested lines were directly released as varieties to farmers in 74 countries. Further, several entries were used in crosses as genetic donors for important traits, and over 1000 elite lines from those progenies were released as varieties in 21 countries. Some entries have been successfully utilized as restorers in hybrid rice programs. For example, 36 hybrids released in India and 34 in China, owe their restorer source to INGER. The multilocation screening trials have provided valuable information on pathogenic variation in major disease-causing organisms, and biotype variation in severe crop-damaging insect pests. Various aspects of the interaction of rice with weather variables have



**Regional Variation in Response to BPH**

been elucidated through specially designed studies conducted at selected representative INGER test sites in different countries.



**Regional Variation in Response to Bacterial Blight**

From 1975, when INGER was established to date, the global rice production increased by about 30% (Source: Statista), whereas the acreage during that period increased by only 12%. The major contribution to the increase is from the improved varieties. INGER-tested and released varieties caused a significant part of that increase, as indicated by the number of varieties released through that mechanism. Two Yale University economists, Robert Evenson and Douglas Gollin studied 591 INGER-derived high-yielding and pest-resistant varieties released in 64 countries. They estimated that each released variety contributes annually USD 2.5 million to the global economy at 1990's costs. Using that old figure on current data, the 1,200 INGER-derived varieties contribute annually USD 2.8 billion to the world economy.

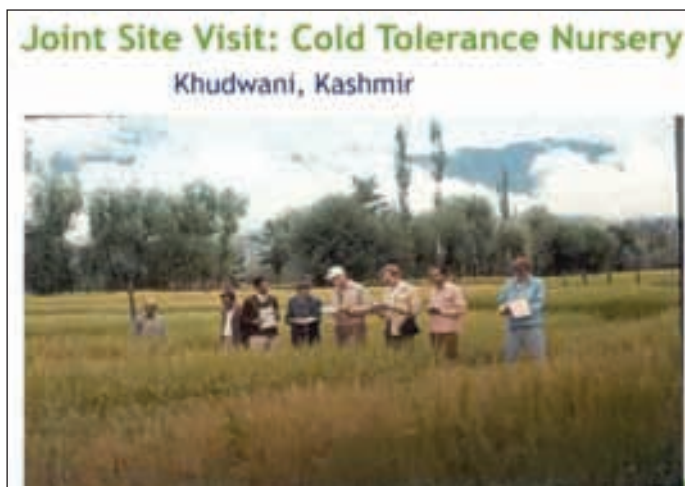
According to Gelia Castillo, a noted social scientist from the Philippines, rice seeds share a common food value and speak a common language that transcends politics, geography, and culture. In Africa, for instance, INGER helped break a barrier in rice science between English and French-speaking countries. She maintains that "INGER is a beautiful illustration of humanity working together for our common future in a world filled with social conflicts, tribal wars, and fierce competition over the control of natural resources" (Rice Today, 2015).

Past accomplishments, however, do not ensure the network to sustain its relevance to the national programs, unless it is kept dynamic, and the emerging



needs are addressed with a rational and pragmatic approach. In this context, I have summarized my thoughts for updating the program, as follows:

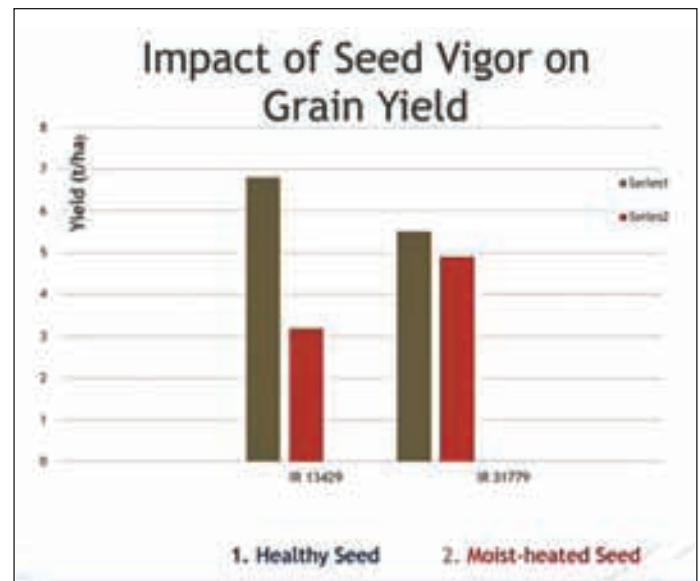
INGER has been established over a while as a strong cooperative platform for rice genetic improvement through the concerted efforts of the world community of rice scientists, breaking down social, cultural, and political barriers. Every effort should be made to maintain and nurture such a well-proven excellent mechanism through both technical prowess and financial sustainability to uphold and validate the prodigious efforts that have gone into its establishment. In the larger interests of the world's food security, the concerned research institutions and funding agencies should take cognizance of the need to enable INGER to maintain its dynamism in effectively addressing the changing needs of rice improvement. The cooperative structure so carefully crafted should be efficiently utilized for all future challenges.



Several national programs have gained adequate strength in terms of research capabilities and facilities, and thus are in a position now to share some of the financial and organizational responsibilities to carry forward the successful network program. Delegation and assumption of technical responsibilities should be based on the respective ecosystem advantages with attending stresses. A comprehensive discussion with the concerned NARES will help set the stage for an effective and unhampered continuation of the network to meet the needs arising out of the new challenges.

Rice breeding programs hitherto have mostly been focused on traits relating to the growth stages from seeding to harvest, assuming the post-harvest care to

be a function of crop husbandry. However, significant genetic variation exists in respect of important post-harvest traits, such as seed vigour and threshability. Thus, entries in observation and yield trials must be characterized for those traits and their values included in the data collection, as they have a recognizable influence on the ultimate yield. The yield figures as recorded now are not reflective of the realizable yields after postharvest operations, which may obliterate the varietal rankings.



Shortage of water is probably the single most significant challenge that will confront the world's farmers in the coming years. Rice is a water guzzler when compared to other crops. It uses up to two to three times more water than other food crops such as maize or wheat and consumes around 30% of the freshwater used for crops worldwide. Thus, there is an urgent need to regulate the water footprints contributed by rice culture. While scientists from relevant disciplines have been pursuing research toward this goal, INGER should do its part by capitalizing on its cooperative base to evaluate varieties at selected representative sites for their performance under a range of hydrological situations. Carefully planned testing should enable identifying varieties with good performance under an optimal input of irrigation water. On the other hand, efforts should be intensified to screen for tolerance to water stress. Also, systematic monitoring should be done to elucidate the utilization of several INGER entries identified in the past for drought tolerance, and

promising progenies from those breeding efforts have to be evaluated by the INGER system.

Yet another important area necessitating breeding inputs is to determine the genetic variation in the efficiency of utilization of the native and applied nutrients. This would significantly supplement the management methodologies recommended by the agronomists.

Issues like climate change must be taken up more systematically from the genetic angle, through the INGER mechanism.

Cropping-systems trials at a given test site need to be linked appropriately with INGER trials to derive maximum benefits from the combined information in choosing the more productive location- and system- based varieties, that would provide a holistic advantage to the farmer.

An important suggestion relates to the gene bank. Through painstaking and sustained efforts, substantial number of traditional varieties and landraces, possessing a repertoire of treasured genes have been fingerprinted and characterized at IRRI for morphological and physiological traits, and reactions to major diseases and insect pests. However, it may be noted that the data gathered pertains to the environment of IRRI's home base. The gene bank is meant for the benefit of the entire rice-growing world. INGER screening trials have brought out the differential reactions of varieties to major stresses in different environments. Thus, the data collected at IRRI may not have relevance to other countries. The gene bank's varietal characterization chart should reflect the differential reactions where they exist, for it to serve its global mandate. Therefore, it is essential to initiate a special screening nursery for the traditional germplasm for testing at selected hotspots identified by INGER. Since it involves a large-scale planning for screening in batches of the voluminous collection and analysing the data systematically, external funding is very essential to avoid financial burden on the cooperating centers.

Rice, as stated earlier, has evolved through very high levels of adaptation to various ecological habitats and has its cultivation spread across the continents. Genetic improvement remains a challenge when trying to maintain harmony between rice and its environment.

Thus, it is of paramount importance to have active cooperation of scientists within and among the rice-growing countries to facilitate pooling and sharing of research materials and expertise through a structured network mechanism. Valuable bonuses from such an approach are savings in time and monetary inputs, and more importantly, acceleration of the transfer of technology to the farmer. The pooling of materials from diverse sources also promotes the much-needed genetic diversity. While the agro-ecological diversity of rice crop poses a 'challenge' for varietal improvement, geographical diversity provides an 'opportunity' for cooperation. The rice genetic improvement network has been founded on this sanguine concept. Individual strengths of the national systems may vary, but their collective strength is formidable. A meaningful fusion of those complementary strengths has powered INGER to effectively serve the needs of the various participating countries in fostering location-specific genetic enhancement of rice. INGER thus has proved to be an epitome of veritable synergy and signifies the power of cooperation. The incontrovertible benefits such as efficacy in the use of resources, reduced costs, and saving on time, render the network concept continue to permeate various aspects of agricultural research for the betterment of the farmers and consumers. In this context, the funding agencies need to take cognizance of the fact that their financial support to cooperative networks such as INGER would yield very significant returns in terms of the world's food security, paying the value of their investments.

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*Let me express my sincere thanks to the Society for Advancement of Rice Research (SARR) and the Indian Institute of Rice Research (IIRR) for the excellent opportunity given to me to share with the rice research community, my viewpoints as cognizant of networking. It is indeed a great pleasure and privilege for me to deliver the 'SVS Shastry Memorial Lecture'. I have chosen a subject that is close to the heart of the departed rice scientist. In articulating the content of the above article, I have drawn upon my years of experience in networking at both national and international levels. I was with AICRIP (ICAR) network for 10 years; had helped organize and coordinate the INGER network program at IRRI*



for 20 years; had established and directed the Fish Genetic Improvement Network (INGA) for two years at World Fish Center; and served as a consultant to West Africa Rice Research and Development Network (WARDA), headquartered in Liberia, during its initial stage of establishment in the early seventies. Thus, I earnestly hope that my article will trigger enough interest in my rice research colleagues, and I would thankfully welcome any suggestions.

I now feel it an immense pleasure and duty to script a few lines about my erstwhile friend and colleague, Dr. Sishta Venkata Sitarama Shastry: I had a long professional association with Dr. Shastry, which dates back to 1955 when I joined the Botany Department at Central Rice Research Institute (CRRI), Cuttack, where he was already a staff member for three years. We had an overlap of one year before he proceeded to the USA for higher studies. Since then, our professional, as well as, social interactions gathered mileage and flourished all through his life. At CRRI (present National Rice Research Institute (NRRI), Cuttack), both of us were in the Japonica x indica Hybridization Scheme. The variety, Masoori was one of the well-known products of that program. After returning from the USA, Dr. Shastry joined IARI, where he pursued research on rice cytology. At the same time, I was involved in research on Cytogenetics of interspecific hybrids in *Oryza*. We had a good collection of wild relatives of rice, as well as, wild species of *Oryza* at CRRI, which formed the experimental material for my research. With the techniques then available, rice was relatively less amenable for distinct cytomorphological characterization of full-length pachytene chromosomes. Our parallel efforts, however, provided an opportunity for closer interactions through the exchange of ideas, visits, and materials, leading to useful results in an area of rice research that remained uncharted until that time. After CRRI, our professional paths crossed again, when we were brought together at AICRIP in 1966, where he directed the Project, and I was heading the breeding program. We spent ten years together with commitment to that ICAR's flagship coordinated research endeavour. The AICRIP chapter in my research career was most memorable because that coincided with the launch of the Green Revolution.

Recognition of the impact of physiologically efficient plant architecture on grain yields, opened up new vistas for genetic improvement of rice, through the development of a series of semidwarf cultivars. With no physical facilities and limited fiscal support, we initiated the coordination program. Dr. Shastry, Dr. Freeman, and I were sharing a single room in the Sorghum Center (PIRRCOM). The seed of important breeding lines was also stored in the same room. The paucity of facilities did not come in the way of our research efforts, having been engulfed by the excitement and determination driven by the promise of new technology. An excellent team spirit forged by Dr. Shastry galvanized our efforts. Here I must make a mention of the deep commitment and valuable inputs of Dr. Freeman to the program, which acted complementary to the able leadership provided by Dr. Shastry. When the first batch of HYV's resulting from our breeding efforts (Jaya, Sona, Rasi, etc.) made a mark in All-India testing, ICAR provided funds for physical facilities and research staff. Dr. Shastry, Dr. Freeman, and I, in our internal dialogues often used to refer to AICRIP as '66 spirit'. The Deputy Chairman of Planning Commission during his visit to AICRIP in early 1976, stated in the guest book, 'few staff members, yet very significant output'. That was a great tribute to the program driven by team spirit.

In 1975, Dr. Shastry left AICRIP on an FAO assignment, when I took charge of the Institute for some months. I also left AICRIP in 1976 on an invitation from IRRI to join its staff. I took up a position at IRRI as Rice Breeder and Global Coordinator of IRTP/INGER. After spending two years at FAO, Dr. Shastry joined IITA in Nigeria, as Director of Research, where he actively pursued the Rice research program. As Global Coordinator of INGER, I had a regional unit for West Africa, headquartered at IITA. Thus our professional interactions continued as staff members of different CGIAR centers, but with common involvement in rice. After his retirement, I had the privilege of having Dr. Shastry as a consultant for INGER on two or three occasions. Even after both of us retired, we had been periodically communicating with each other relative to various aspects of rice research. The keen interest he had evinced in rice in our most recent interactions just a few months before he passed away, was no less

*intense than those we used to have when we were actively working together at AICRIP. Our professional association is well reflected by the fact, that we had several joint research publications resonating with the Green Revolution era.*



*Dr. Shastry's research contributions to rice improvement are well known and were duly recognized with distinguished awards. However, I wish to add my impressions emanating from long years of active association with him. Dr. Shastry's scholarly pre-eminence reflects a combination of a high level of intelligence and an extraordinary memory. He never had room for nonchalance in his work aptitude. He had a unique personality that was well comprehensible to many of his colleagues, while remained abstruse for others. He had the habit of being forthright and candid in speaking his mind, without bringing into play the art of diplomacy. Even when facing an adverse comment,*

*he used to have an apt and measured response, rather than getting impulsive. I was always impressed with his quick grasp and critical analysis during our research deliberations, which trait used to bolster his efforts in formulating research plans that were both logical and scientific. His professional competence may well be described as an amalgam of scientific calibre and leadership acumen. It was that leverage of his, which proved pivotal for the success of AICRIP.*

*Dr. Shastry had an ardent passion for mythological stage-plays in Telugu. He was an excellent bridge player. One of his fascinating hobbies was cooking. Our family had the good fortune of enjoying his culinary skills, when he took command of the kitchen in our residence in Ithaca, NY, during his visit to Cornell University in 1984. I take great pride in being a close friend and associate of Dr. Shastry. May his soul rest in peace!!*

### **Dr. Durvasula Venkata Seshu**

Dr. Durvasula Venkata Seshu (Dr. D.V. Seshu) was born on 19 April 1933 in Jeypore, Odisha. He obtained his B.Sc. degree from Andhra University (1952), M.Sc. (1954) and Ph.D. in Agricultural Botany (1964) from Utkal University (now Ravenshaw University). He was awarded with Rockefeller Foundation Fellowship for Post-Doctoral Research on Rice breeding at IRRI during 1967-68. He underwent a Senior Research Management Training organized by O'Hare Association, USA.



Dr. Seshu had more than 40 years of research experience on Rice, and three years on Fish. He served with the Indian Council of Agricultural Research (CRRI & AICRIP) for over 20 years (1955-76) as Rice Breeder and Geneticist. He was invited by the International Rice Research Institute (IRRI) to join its staff as Rice Breeder in 1976. He established and served as Global Coordinator of the highly successful International Network on Genetic Evaluation of Rice (INGER) from 1976-95. Recognizing the success



of INGER, he was invited to establish and direct an International Network on Genetics of Aquaculture (INGA) by the World Fish Center. He was Adjunct Professor of Cornell University, USA, 1983-86 and Visiting Professor of University of Philippines, 1977-93.

As a Rice Scientist of the ICAR for over 20 years, Dr. Seshu made significant contributions to the Green Revolution in rice in India through providing inputs to development of early generation modern varieties (such as Jaya, Sona, Phalguna, Prakash, Rasi etc.), and through painstaking data analysis of nationwide coordinated trials and identification of location-specific elite varieties for different states. These varieties led to a three-fold increase in the rice production in India. Drought-tolerant Rasi was released in countries outside India also. Sona gave rise to the popular rice variety, Sona-Masoori. Genetic studies carried out by Dr. Seshu have greatly assisted in developing rice varieties resistant to major biotic and abiotic stresses.

As an International Scientist and Global Coordinator of INGER network, Dr. Seshu promoted international cooperation in agricultural research by bringing together scientists from more than 75 countries with different cultural, ethnic and political backgrounds, and encouraging them to exchange and evaluate their promising varieties and share their test results. The leadership provided by Dr. Seshu led to the release of more than thousand rice varieties for different ecosystems in 74 countries including India. As a result, the global rice production has increased by 30% from 1975 to date, while the area increased only by 12% during that period. Under the guidance of Dr. Seshu, INGER established itself as world's most successful and largest agricultural research network. Millions of farmers across the world were benefitted by the network through availability of improved rice varieties tolerant to various pathological and physiological stresses. INGER under his direction was given a CGIAR award.

Dr. Seshu established a very high quality Seed Processing and Research Laboratory at IRRI. His major contributions to Rice seed research include identification of biochemical markers for high protein content, and for salt and low temperature tolerance. When the prestigious 'Seed Science Research' journal was launched in UK in 1992, he was invited to present a paper on 'Mechanism of Rice Seed Dormancy' for publication in the inaugural journal.

Dr. Seshu was actively involved in climate change issues and served on several international panels on the subject. He was appointed as a Member of the Scientific Panel of the Secretary-General of the United Nations to study the climatic effects of nuclear war. He was a special invitee to International dialogue on Global Climatic Change sponsored by National Governors' Association, USA held at New York (Feb. 1989) and to the Global Forum on Environment and Development for Survival held at Moscow (Jan. 1990).

Dr. Seshu served as a consultant on various occasions to International organizations such as UNDP and FAO. He served as a member of the Research Advisory committees of DRR and NBPGR. He is a Fellow / Member of various international professional societies. He served as a Chairman of the organizing committee for three International Rice Conferences at IRRI. He was invited as a Keynote speaker at a major International conference organized by the Asian Vegetable Research Institute in Taiwan in 1982. He traveled to more than seventy countries in his professional capacity. Dr. Seshu served as a research guide for several students from various countries and universities for their Ph.D. and MS degrees. He published more than 150 research papers in reputed journals, authored nine Technical bulletins and three books. A rice variety has been named in as SESHU in recognition of his breeding contributions. He received citations of honor from China and South Korea.

## Brown planthopper: A genetic marvel\*

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### Abstract

Rice planthoppers have again attained a major pest status in this century causing enormous yield losses through direct feeding and transmission of pathogenic viruses. Consequently, there has been increased focus of research on their biology, genetics and molecular biology in search of alternative methods to manage them. This review highlights main findings of these studies to reveal how genetic plasticity of the brown planthopper (BPH) has equipped the species to fight back adversities and pose challenges for its effective management. Genome of BPH with size of 1141 Mb spread across 30 chromosomes is predicted to have 27,571 genes. Salivary glands, first line of offense, secrete proteins that trigger either susceptibility (ETS) or immunity (ETI) reaction in the host plant. Fat bodies, spread all over the body, are sites of primary lipid metabolism, endocrine regulation, systemic immunity, vitellogenesis, and housing of microbial symbionts. BPH harbours yeast like symbionts (YLS) in its fat bodies that play critical role in insect survival. BPH populations across Asia have acquired resistance against almost all classes of insecticides which has been attributed to neofunctionalization of duplicated P450 genes. So far about 40 major host plant resistance genes and 72 QTLs have been reported from cultivated rice and its wild relatives but BPH has ability to quickly evolve virulent populations. Role of cytochrome P450 enzymes and of symbiotic YLS in this ability has been shown. Several studies have focused on the reproductive physiology of BPH and identified key genes that can be target for RNAi mediated silencing as novel strategy for pest management. Several recent studies have also covered genetic and molecular basis of wing polymorphism and adaptation for long distance migration in this insect. Other recent studies on effect of climate change on BPH incidence and its genetic ability to adapt to the changes in the weather and climate have cautioned that this insect is likely to continue to be a major problem in days to come. On a larger perspective, this rapidly expanding knowledge is providing us with novel approaches and tools to contain the pest and stay a step ahead of it in the evolutionary race.

**Key words:** BPH, biology, host plant resistance, insecticide resistance, climate change

### Introduction

Rice brown planthopper (BPH), *Nilaparvata lugens* (Stal), along with the other two sympatric species whitebacked planthopper (WBPH), *Sogatella furcifera* (Horvath) and small brown planthopper (SBPH), *Laodelphax striatella* (Fallen), has again attained a major pest status in this century (Bentur and Viraktamath, 2008; Bottrell and Schoenly, 2012). Despite 46 species of planthoppers being reported to

feed on rice, these three species, especially BPH, have challenged rice production in intensive rice cultivation belt across Asia. It especially causes direct damage in all stages of life cycle, particularly nymph and adult stages, by direct feeding on phloem sap and by transmitting ragged stunt virus and grassy stunt virus diseases. Prior to 1980s BPH was not even reckoned as a pest of rice. This phenomenal evolutionary success, as it is now emerging through intensive studies, is

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attributed to its genetic plasticity and adaptability. Due to its high economic impact on rice production, a large number of researcher groups across the world have been engaged in intensive studies during the past five decades. This has resulted in explosion of over 770 research publications during last five decades (Figure 1). The major focus of these studies has been on insecticide resistance related (30%), insect-plant interactions (22%) and reproduction (19%). There have been excellent reviews published recently (Fujita *et al.*, 2013; Ling and Weillin 2016, Du *et al.*, 2020, Haliru *et al.*, 2020) covering specifically insect-plant interactions. However, no comprehensive review is available. An attempt is made here to briefly review the broad progress in our understanding of BPH biology, genetics and its interaction with host plant.

Genome of BPH has been sequenced (Xue *et al.*, 2014) and noted with size of 1141 Mb spread across 30 chromosomes. Relatively, BPH genome size is the largest in comparison with those of WBPH (720 Mb, Wang *et al.*, 2017) or SBPH (541 Mb, Zhu *et al.*, 2017). In all, 27,571 genes have been predicted; of which 10,245 have been assigned gene ontology while 16,330 genes are noted to be specific to BPH. BPH is monophagous with its feeding confined to rice (*Oryza*), having shifted its host from *Leersia* over the past 0.25 million years (Sezer and Butlin, 1998).

## Salivary Glands

Salivary glands are the first line offense of BPH while attempting to feed on rice plant. These produce two types of secretions: a coagulating gelling secretion that quickly hardens to form salivary sheath around the probing stylets consisting of polyphenol oxidase and peroxidase enzymes, and a watery secretion that contains digestive enzymes like alkaline phosphatase, esterase, amylase, beta glucosidase as well as other components mainly secretory salivary gland proteins (SSGPs). These SSGPs are main class of effectors that trigger susceptibility (ETS) or immunity (ETI) depending on the rice genotype BPH is attempting to feed upon (Huang *et al.*, 2017; Ji *et al.*, 2017; Ye *et al.*, 2017). Of the 352 reported genes encoding putative secreted proteins of salivary gland, 67 genes are differentially expressed in TN1 and Mudgo reared insects (Ji *et al.*, 2013). Rao *et al.*, (2019) characterized six of the effector proteins. A mucin-like protein is required for feeding by BPH but it also induces immunity response in plants acting like an effector (Shangguan *et al.*, 2018). Application of salivary gland extract to rice plants induces systemic host mRNA patterns associated with nutrient remobilization (Petrova and Smith, 2015). Thus, salivary gland of BPH has a repertoire of effector proteins which can evolve rapidly to overcome host mediated resistance.

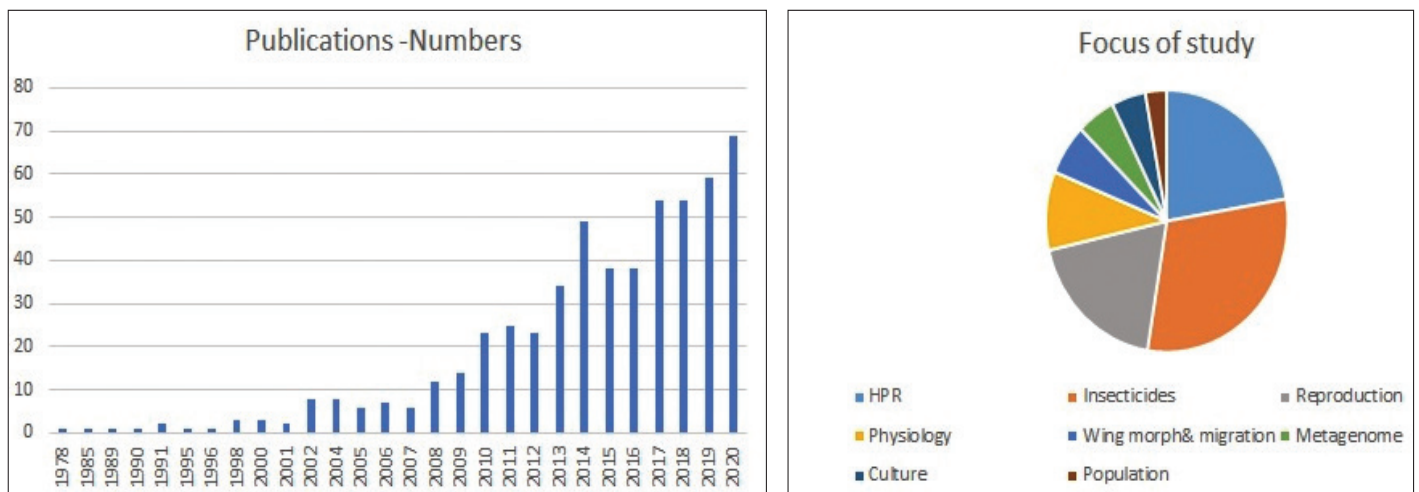


Figure 1: Number of publications on BPH appearing year-wise and the focus of the study (Source: Pubmed)



## Fat Bodies

Insect fat body is a highly dynamic tissue composed primarily of storage cells. It is distributed throughout the insect's internal body cavity; the haemocoel, near the epidermis, digestive organs and ovaries. Akin to the combination of liver and adipose tissue in mammals, fat bodies are site of primary lipid metabolism, endocrine regulation, systemic immunity, vitellogenesis, and housing of microbial symbionts. BPH harbours yeast like symbionts (YLS) in its fat bodies that play critical role in insect survival. While the insect can degrade seven of the amino acids independently, six of these are catabolised exclusively by YLS while seven more of these are degraded by both (Wan *et al.*, 2015). Impaired lysine degradation by YLS negatively affects the survival and development of *N. lugens*. Transcriptome analysis of fat bodies from two BPH populations with different virulence levels revealed that these are sites of intermediate metabolism, immunity and detoxification of xenobiotics (Yu *et al.*, 2014). Expression of several of the genes in BPH population reared on Mudgo rice differed significantly from those reared on TN1 rice. Lipid, amino acid and xenobiotic metabolism related genes were significantly upregulated in the Mudgo population. Protein transport related genes in YLS, and cell division and ribosome assembly related genes in another symbiont *Wolbachia* were upregulated in this population. These studies highlight the critical role of the symbionts in BPH virulence on resistant genotypes.

## Insecticide Resistance

BPH populations across Asia have acquired resistance against almost all classes of insecticides (Matsumura *et al.*, 2009; Garrood *et al.*, 2015; Wu *et al.*, 2018). There have been intensive studies on evolution and mechanism of insecticide resistance. Role of P450 genes, a family with 54 genes, in insecticide resistance in BPH has been extensively documented. Neofunctionalization of duplicated P450 genes drives the evolution of insecticide resistance in the insect (Zimmer *et al.*, 2018). Garrood *et al.*, (2015) studied field-evolved resistance to imidacloprid and ethiprole in populations of BPH collected from across South and East Asia. Association of overexpression

of CYP6ER1 gene with resistance to imidacloprid, thiamethoxam and buprofezin was noted. RNA interference of this CYP6ER1 gene resulted in susceptibility (Garrood *et al.*, 2015; Pang *et al.*, 2016). However, P450 CYP6AY1 was over expressed in one of the Indian populations. Synergistic and compensatory effects of two- point mutations in the insect GABA receptor RDL confer resistance to fipronil (Zhang *et al.*, 2015). Carboxyl esterase gene (*NlCarE*) plays an important role in chlorpyrifos detoxification and its overexpression may be involved in chlorpyrifos resistance in *N. lugens* (Lu *et al.*, 2017). RNA interference of NADPH-cytochrome P450 reductase (CPR) increases susceptibility to insecticides including buprofezin (Liu *et al.*, 2015).

## Host-plant Resistance

Major emphasis of BPH management is on development of resistant rice varieties exploiting host plant resistance (Brar *et al.*, 2009). So far about 40 major genes and 72 QTLs conferring resistance to BPH have been reported from cultivated rice and its wild relatives (Fujita *et al.*, 2013, Ling and Weillin 2016, Du *et al.*, 2020, Haliru *et al.*, 2020). A major limitation here is the ability of BPH to quickly evolve virulent populations, often referred to as biotypes, capable of overcoming host resistance. Recent studies have been aiming to understand genetic and molecular basis of such virulence in BPH. Role of cytochrome P450 enzymes in virulence was studied by Peng *et al.*, (2017) who noted that among the 21 Cyp genes CYP4C61 gene expressions was more in virulent biotype Y than in biotype 1. They suggest that duplication of P450 genes in BPH genome is likely contributing to adaptation to host plant resistance, as in case of insecticide resistance noted above. Role of symbiotic YLS in metabolic response of BPH while feeding on resistant rice has been studied (Liu *et al.*, 2017). The study showed significant metabolic differences between BPH nymphs feeding on the resistant NIL-BPH15 and susceptible TN1 rice. To survive on this resistant rice, BPH nymphs probably ingest more sap and/or get necessary nutrients from their yeast-like symbionts at later feeding stages. Jing *et al.*, (2014) and Kobayashi *et al.*, (2014) have attempted to map virulence loci that break down host



resistance on BPH genome using molecular markers. Over 860 markers including 125 gene specific markers have been reported and used in mapping three virulence loci Qhp7, Qgr5 & Qgr14 (Jing *et al.*, 2014). Kobayashi *et al.*, (2014) mapped the virulence gene *vBph1* located in the 1.8 cM genomic region flanked by SNP markers VLS01 and VLS05 of linkage group 10.

### Reproductive Physiology

Another feature of the brown planthopper that has enabled its evolutionary success is its reproductive potential. Several studies have focused on the reproductive physiology of BPH. Role of Broad-Complex (Br) and Krüppel homolog 1 (Kr-h1), two transcription factor genes downstream of juvenile hormone and ecdysone pathways in the ovary development was noted by Jiang *et al.*, (2017). An adenyl cyclase like-9 gene (NIAC9) influences growth and fecundity in BPH (Ge *et al.*, 2017a). PHF7, a novel male gene influences female fecundity and population growth through secretion of accessory gland and transferred to female through mating (Ge *et al.*, 2017b). This can be a target gene for RNAi mediated gene silencing to reduce population growth of the insect. Ge *et al.*, (2016) have also shown that suppressing male spermatogenesis-associated protein 5-like gene expression reduces vitellogenin gene expression and fecundity. Yu *et al.*, (2016) identified 94 seminal fluid proteins in the male accessory glands of BPH of which four were unique to this insect. Forkhead box transcription factor L2 activated follicle cell protein gene *NIFcp3C* to regulate insect chorion formation (Ye *et al.*, 2017). Role of juvenile hormone (JH) secreted by corpora allata has been studied intensively. TOR pathway-mediated JH synthesis regulates nutrient-dependent female reproduction (Lu *et al.*, 2016), while nutritional signaling also regulates vitellogenin synthesis and egg development through JH (Liu *et al.*, 2016). Silencing a sugar transporter gene *Nlst6* reduces growth and fecundity. Several agro-chemicals like antibiotics or even insecticides stimulate BPH reproduction. Adipose triglyceride lipase (Atgl) mediates the antibiotic jinggangmycin-stimulated reproduction (Jiang *et al.*, 2015). Sub-

lethal doses of triazophos and fenvelrate insecticides also induce reproduction (Bao *et al.*, 2008).

### Wing Polymorphism and Migration

A special trait of interest for BPH is its ability to migrate long distance being carried by the wind currents (Otuka *et al.*, 2008). To suit to this adaptation, the insect has wing dimorphism involving short winged brachypterous forms with high fecundity and long winged macropterous form suited to long distance migration. Several recent studies have covered genetic and molecular basis of wing polymorphism and adaptation for long distance migration. There is an ovarian diapause in macropterous females prior to migration and TOR (target of rapamycin) genes are shown to be involved in this diapause (Liu *et al.*, 2016). Zhou *et al.*, (2017) showed that transformer-2 (*NItra-2*) determines sex in nymph and wing shape of the progeny. They also suggested the important role of *NIJHEH* (juvenile hormone epoxide hydrolase) gene in determining the wing morph. Higher levels of JH in V instar nymph lead to development of short wings. Xu and Zhang (2017) explained the molecular basis by which two insulin receptors (InR1 and InR2) act as switches to determine alternative wing morphs in the BPH.

### Climate Change Adaptation

There have been several recent studies on effect of climate change on BPH incidence and genetic ability of the insect to adapt to the changes in the weather and climate. Pandi *et al.*, (2018) studied impact of elevated CO<sub>2</sub> and temperature on BPH in rice ecosystem. They observed that increased CO<sub>2</sub> and temperature resulted in escalated BPH multiplication through increase in both fecundity and number of adults, thus inflicting higher yield loss in rice. Similar study in Bangladesh by Ali *et al.*, (2014) showed months or areas characterized by a climate that is either cold and dry or hot and wet that are likely to experience higher levels of BPH due to climate change. At high temperature (37 °C), heat shock protein (HSP) genes were the most co-regulated (Huang *et al.*, 2017). Macropterous are more heat resistant than brachypterous adults. Up-regulation of *NIHsc70* gene provides more thermal tolerance/resistance in macropterous adults (Lu *et al.*, 2016a, b).

## Perspectives

This rapidly expanding knowledge on BPH and other related planthoppers of rice has not only given us an insight into its evolutionary superiority but also has revealed several ‘weak points’ that can be tweaked to our advantage from pest management point of view. RNAi based approach and the key target genes identified can be used for the purpose. Genome editing aiming at susceptibility genes is another promising alternative. Resequencing of scores of rice genotypes and the emerging identification of superior haplotypes of the known and unknown resistance genes can widen our choice in host-plant resistance deployment approach. It is thus probable that we may stay a step ahead of it in the evolutionary race.

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## Phenotyping and correlation studies of recombinant inbred lines derived from interspecific cross of *Oryza sativa* and *Oryza nivara*

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### Abstract

Recombinant Inbred Lines (RILs) derived from crosses between elite cultivars and wild introgression lines are important sources of new genetic variability. In this study 177 RILs derived from a cross of Swarna / 166s (Swarna / *Oryza nivara*), were characterized for yield and grain shape related traits for two seasons Rabi 2018-19 and Kharif 2019. Phenotypic correlation analysis showed that, single plant yield had a positive association with productive tiller number, total tiller number, panicle weight, biomass and harvest index in both the seasons. Significant and positive correlation was observed between grain length and grain perimeter followed by productive tiller number and total tiller number. A perfect negative linear relationship was observed between filled grain percentage and unfilled grain percentage. Across the seasons, biomass weight consistently showed significant positive correlations with the majority of yield contributing traits, followed by days to 50 percent flowering. Grain shape traits (grain length, grain width, length/width ratio, grain area and grain perimeter) were interrelated with each other significantly. Trait association study in this mapping population showed that though the yield performance has always been the main focus of breeding programmes, the complex interaction of the yield attributes is also equally significant to understand the genetic mechanisms for yield improvement. Selected RILs from this study are being used in rice improvement programmes to detect favorable genes from *O. nivara* for yield improvement.

**Key Words:** Rice, yield, grain shape, correlation coefficient, recombinant inbred lines

### Introduction

Rice (*Oryza sativa* L) is one of the most important staple food crops of the world providing nutritional and economic benefits to more than half of the world population (Roy and Shil, 2020). Rice yield needs to be continuously improved to fulfill the future demands of increasing population and to enhance living standards. However, it is the most challenging part in breeding programmes because yield is the most variable and complex trait influenced by several environmental and genetic factors (Pimsaen *et al.*, 2010; Sreedhar *et al.*, 2011; Christopher and Sangodoyin, 2011). The current practice of crossing between elite

lines of *Oryza sativa* is expected to reduce genetic variability in the working germplasm and slow down the discovery of novel traits to improve yield. Yield level in the released varieties has plateaued due to the narrow genetic base in parental lines used in crossing procedure (Tanksley and McCouch, 1997). One of the alternatives for increasing agriculture production is to identify the genes lost or weakened during the domestication process and use them in subsequent targeted breeding programmes (Shakiba and Eizenga, 2014). The wild species of the genus *Oryza* serve as a nearly untapped reservoir of genetic diversity that can be used to improve the world's most important food

crop, rice. The high diversity existing in wild rice species as they have not undergone rigorous human selection, makes them potential sources of important agronomic traits for exploitation by plant breeders for varietal improvement (Brar and Khush 1997; Gur and Zamir, 2004; Xie *et al.*, 2008; Yadav *et al.*, 2013; Sharma *et al.*, 2013).

The genus *Oryza* has 22 wild species, with tremendous variability and contains numerous valuable genes for improvement of several traits. This was demonstrated by Khush *et al.*, (1977), by introgressing the gene for resistance to grassy stunt virus from *O. nivara* to cultivated rice. Since then, wild species have been widely used for introgression of several agronomically important traits such as tolerance to biotic stresses like blast (Jeung *et al.*, 2007), brown planthopper (Rahman *et al.*, 2009; Jena and Kim, 2010; Sarao *et al.*, 2016), and bacterial leaf blight (Natrajkumar *et al.*, 2011). *O. nivara*, a cross compatible wild species of rice with AA genome is known as a source of important genes like cytoplasmic male sterility (Li *et al.*, 2005), resistance to grassy stunt virus (Brar and Khush, 1997), bacterial leaf blight (Cheema *et al.*, 2008) and BPH (brown planthopper) (Madurangi *et al.*, 2013; Sarao *et al.*, 2016). Introgression from non-AA genome species *Oryza minuta* (CCDD) was used in the successful transfer of blast resistance genes and *O. australiensis* (EE) for blast and BPH resistance genes into *O. sativa* (Jena and Khush, 2000; Fu *et al.*, 2008; Suh *et al.*, 2014).

Previous studies showed that introgression from wild rice species also enhances tolerance to abiotic stresses like drought, salinity and cold (Koseki *et al.*, 2010; Ndjiondjop *et al.*, 2010; Yang *et al.*, 2012), diversification of cytoplasmic male sterility sources (Li *et al.*, 2005), adaptability and yield-enhancing components for improving rice yield (Swamy and Sarla, 2008; Brar and Singh, 2011; Shakiba and Eizenga, 2014; Furuta *et al.*, 2014; Bhatia *et al.*, 2017; Haritha *et al.*, 2018; Kaur *et al.*, 2018; Zhu *et al.*, 2018) and grain quality (Yun *et al.*, 2016; Gaikwad *et al.*, 2018). It was reported that transferring yield-

enhancing segments/ QTLs into the genetic background of elite varieties (Kaladhar *et al.*, 2008; Fu *et al.*, 2010; Bai *et al.*, 2012; Thalapati *et al.*, 2012; Gaikwad *et al.*, 2014; Swamy *et al.*, 2014; Surapaneni *et al.*, 2017) helped in the identification of improved cultivars with wild introgressions (Sarla *et al.*, 2009). In India, Dhanarasi and Jarava are two rice varieties released for increased yield and they were derived from crosses with *O. rufipogon* (Ram *et al.*, 2007, 2010). DRR Dhan 40, a medium duration rice variety (BIL derived from Swarna × *O. nivara* IRGC81848) was released for Maharashtra, West Bengal and Tamil Nadu states of India (Sarla, 2014; Haritha *et al.*, 2016). Improving mega varieties which are well adapted, farmer accepted, popular and with good grain quality for any specific trait, is an important strategy in crop breeding programmes. In the present study, RILs derived from a cross between cultivar Swarna and BIL166s were used to understand the correlations among the yield and grain shape related traits.

## Materials and Methods

### Plant material

Swarna [(MTU 7029/ IET5656 (Vasistha/Mahsuri)] is a low-land high yielding *indica* type mega rice variety released in 1982 in India by RARS, Maruteru and it is a semi-dwarf variety maturing within 135-140 days with an average yield of 6.5 t/ha. 166s (male parent), a stable advanced backcross introgression line derived from a cross between Swarna and *Oryza nivara* IRGC81848 from IIRR, Hyderabad, is a late duration genotype, with strong culm strength and panicle weight. 166s has higher grain yield and yield stability than Swarna but with similar grain type to Swarna (Balakrishnan *et al.*, 2016). Both were used in crossing programmes (Kavitha *et al.*, 2020). In the present study, the F<sub>6</sub> and F<sub>7</sub> generations of the cross were raised during *Rabi* 2018/19 (dry) and *Kharif* 2019 (wet) seasons respectively, at research farm, ICAR-Indian Institute of Rice Research, Hyderabad (17°19' N, 78°29' E) at an altitude of 549 m above sea level in alkaline vertisol soil under irrigated field condition.



## Phenotypic evaluation of RILs

The 177 RILs obtained from ICAR-IIRR were planted in the field as three-row families in both seasons. One row consisted of ten plants and each replication had thirty plants. A randomized complete block design was followed with two replications. Parental lines Swarna and 166s were used as check varieties. The spacing between rows was 20 cm and 15 cm between plants. The crop was grown under irrigated condition and phenotypic observations were recorded for yield and grain shape-related traits from the randomly selected three plants in the middle row of each family. Twelve yield related traits *viz.*, days to 50% flowering (DFF), plant height (PH), number of productive tillers per plant (PTN), number of total tillers per plant (TTN), panicle length (PL), panicle weight (PW), 1000 grain weight (TGW), filled grain percentage (FG), unfilled grain percentage (UFG), single plant yield (SPY), biomass weight (BM) and harvest index (HI) were measured following Standard Evaluation System (IRRI, 2013). The days to 50 per cent flowering was taken as the number of days taken for 50 per cent heading in the family considering the 30 plants. Grain shape-related five traits were taken *viz.*, grain length (GL), grain width (GW), grain length/width ratio (LW), grain area (GA) and grain perimeter (GP). All the parameters were measured using Image J (image analysis software) and the mean of 10 seeds was taken in each replication.

## Statistical analysis

The mean values from each replication were subjected to statistical analysis. Correlation analysis was performed with Statistical Tool for Agricultural Research STAR (STAR 2.0.1, <http://bbi.irri.org>) and significant values were determined using Pearson coefficients at the 0.05 and 0.01 levels.

## Results and Discussion

As a complex quantitative character, estimation of grain yield depends on contribution from other traits which ultimately affects the overall yield potential of the genotype. The trait association which is either in positive or negative direction helps the breeder to select

traits to improve grain yield in breeding programmes. Hence, association analysis was undertaken to determine the direction of selection and to get an understanding of the characters to be considered in improving grain yield. The phenotypic correlation coefficient among seventeen characters was assessed related to yield and grain shape-related traits using RILs derived from Swarna / 166s, in *Rabi* 2018-19 and *Kharif* 2019 with two replications (Table 1).

The r-value for Karl Pearson's correlation coefficient helps in identification of an association between two distinct traits. Though it does not measure the magnitude of association it does give an idea of the relationship (Yusuff *et al.*, 2018). For the correlation coefficient interpretations, Ratner, (2009) gave a standard accepted guideline. The r-value of 0, +1, and -1 indicates no linear relationship, a perfect positive linear relationship, and negative linear relationship, respectively. The values that range from 0 to 0.3, 0.3 to 0.7, and 0.7 to 1 indicate a low, moderate, and strong positive linear relationships, respectively, while the values that range from 0 to -0.3, -0.3 to -0.7, and -0.7 to -1 indicate a low, moderate, and strong negative linear relationships, respectively.

In the present study, single plant yield showed association with one of the main yield components, panicle weight and a moderate positive association with tiller number in *Rabi* 2018/19 and *Kharif* 2019 seasons, respectively. Further single plant yield showed significant and positive association with panicle weight, biomass and harvest index. Panicle length had a positive association with days to fifty percent flowering, plant height, biomass and panicle weight. Panicle weight showed significant and positive association with days to fifty percent flowering, plant height, panicle length, single plant yield and thousand grain weight, while thousand grain weight showed significant and positive association with harvest index and panicle weight. As expected, percentage of filled and unfilled grain had a perfect negative linear relationship. Filled grain had significant and positive



**Table 1: Correlation coefficients among yield and grain shape-related traits in Swarna x 166s RILs in Rabi 2018/19 and Kharif 2019**

DFP	PH	PTN	TTN	PL	PW	TGW	FG	UFG	SPY	BM	HI	GL	GW	LW	GA	GP
1	0.407**	0.135*	0.084	0.357**	0.412**	-0.097	-0.112*	0.112*	0.318**	0.432**	-0.236**	-0.055	0.009	-0.046	-0.016	-0.068
0.392**	1	0.052	-0.019	0.443**	0.519**	0.057	-0.052	0.052	0.393**	0.528**	-0.302**	-0.077	-0.016	-0.008	-0.079	-0.078
0.072	-0.166**	1	0.952**	0.146**	0.074	-0.084	0.018	-0.018	0.561**	0.565**	-0.134*	-0.129*	-0.048	-0.031	-0.099	-0.134*
0.098	-0.177**	0.968**	1	0.097	-0.001	-0.09	0.042	-0.042	0.478**	0.533**	-0.188**	-0.155**	-0.024	-0.065	-0.094	-0.158**
0.294**	0.512**	-0.063	-0.068	1	0.677**	-0.079	-0.054	0.054	0.392**	0.389**	-0.099	0.002	0.0293	-0.027	0.025	0.005
0.315**	0.455**	-0.137**	-0.146**	0.601**	1	0.109*	0.054	-0.054	0.579**	0.387**	0.076	0.014	-0.07	0.064	-0.047	0.008
-0.221**	0.081	-0.229**	-0.247**	-0.061	0.134*	1	0.034	-0.034	0.097	-0.067	0.173**	0.011	0.061	-0.033	0.037	-0.006
-0.233**	-0.115*	0.071	0.054	-0.049	0.266**	0.095	1	-1.00**	0.036	-0.118*	0.184**	-0.119*	-0.039	-0.019	-0.078	-0.122*
0.233**	0.115*	-0.071	-0.054	0.049	-0.266**	-0.095	-1**	1	-0.036	0.118*	-0.184**	0.119*	0.039	0.019	0.078	0.122*
0.065	0.088	0.509**	0.517**	0.102	0.217**	-0.059	0.254**	-0.254**	1	0.689**	0.154**	-0.018	-0.057	0.042	-0.054	-0.035
0.226**	0.175**	0.523**	0.536**	0.110*	0.053	-0.178**	-0.066	0.066	0.602**	1	-0.574**	-0.088	0.044	-0.087	-0.016	-0.091
-0.153**	-0.045	0.081	0.067	0.032	0.267**	0.131*	0.393**	-0.393**	0.608**	-0.195**	1	0.095	-0.136**	0.171**	-0.051	0.074
-0.021	0.043	0.098	0.082	0.019	-0.048	0.001	-0.091	0.091	-0.091	-0.061	-0.063	1	0.064	0.483**	0.529**	0.953**
-0.026	-0.036	-0.061	-0.044	0.043	-0.029	0.006	-0.062	0.061	-0.08	-0.037	-0.095	-0.026	1	-0.821**	0.846**	0.198**
-0.022	0.047	0.068	0.055	-0.052	-0.028	-0.055	-0.006	0.006	-0.014	-0.054	0.025	0.477**	-0.118*	1	-0.458**	0.349**
-0.02	0.004	0.0004	-0.002	0.082	-0.032	0.071	-0.1	0.101	-0.111*	-0.035	-0.101	0.497**	0.08	-0.503**	1	0.648**
-0.021	0.042	0.069	0.057	0.049	-0.055	0.013	-0.109*	0.109*	-0.127*	-0.081	-0.088	0.955**	0.013	0.331**	0.627**	1

Significance levels: \* $P < 0.05$ , and \*\* $P < 0.01$ . Upper diagonal shows the phenotypic correlation of mean data in Rabi 2018/19 and lower diagonal shows phenotypic correlations of Kharif 2019. DFP-Days to 50% flowering, PH-plant height, PTN-number of productive tillers per plant, TTN-number of total tillers per plant, PL-panicle length, PW-panicle weight, TGW-1000 grain weight, FG-filled grain percentage, UFG-unfilled grain percentage, SPY-single plant yield, BM-biomass weight, HI-harvest index, GL-grain length, GW-grain width, LW-grain length/width ratio, GA-grain area and GP-grain perimeter



association with harvest index while it showed significant and negative association with grain perimeter and days to fifty percent flowering. Unfilled grain percentage showed significant and negative association with harvest index and significant, positive association with grain perimeter and days to fifty percent flowering. Biomass showed significant positive correlations with the majority of other traits such as days to fifty percent flowering, plant height, productive tiller number, panicle length, panicle weight, unfilled grains and single plant yield in *Rabi* 2018/19 followed by days to fifty percent flowering with plant height, productive tiller number, panicle length, panicle weight, unfilled grains, single plant yield and biomass.

Rice grain yield is contributed by many component traits measured mainly by four indices: panicles per unit area, number of spikelets per panicle, the weight of spikelets (1000 grain weight) and spikelet fertility or filled grain percentage (Fageria, 2007; Xing and Zhang 2010; Ikeda *et al.*, 2013). Also, shoot dry weight, grain harvest index, and nitrogen (N) harvest index are positively associated with grain yield (Fageria, 2007). Supportive to the idea of four main components of grain yield, Bagati *et al.*, (2016) using a RIL population derived from a cross between Pusa Basmati and Basmati 370, reported that tillering ability, spikelet fertility percentage, number of grains per panicle and 1000-grain weight had a significantly positive correlation with grain yield. A study carried out by Kar *et al.*, (2018) with low land rice varieties indicated that grain yield was positively correlated with fertile grain number, fertility percentage, days to flowering and plant height indicating the importance of such traits for the realization of high yield in rice. Haritha *et al.*, (2017) showed that yield per plant was significantly correlated with above-ground biomass, total dry matter and harvest index using ILs of a cross between *Oryza sativa* (KMR3) × *Oryza rufipogon* (WR120). Yusuff, *et al.*, (2018) reported that, the yield per hectare showed strong, positive and highly significant association with tillers per hill and panicles per hill. Sudhakar *et al.*, (2012) reported

that grain yield per plant (single plant yield) showed a significant positive association with grain weight per panicle (PW) using ILs of KMR3 × *O. rufipogon*.

In *Kharif* 2019, the same pattern of correlation of biomass with days to fifty percent flowering, plant height, productive tiller number, panicle length and single plant yield was observed; followed by days to fifty percent flowering with plant height, panicle length, panicle weight, unfilled grains and biomass with significant and positive correlation for the majority of the yield contributing traits. Biomass showed significant and negative association with harvest index. Haritha *et al.*, (2017) reported that above ground biomass was significantly correlated with total dry matter and harvest index. HI showed significant negative correlations with most of the contributing traits like days to fifty percent flowering, plant height, productive tiller number, total tiller number, unfilled grains and biomass; followed by filled and unfilled grains, biomass, grain length and grain perimeter in *Rabi* 2018/19. In *Kharif* 2019 days to fifty percent flowering (with thousand grain weight, filled grains and harvest index), plant height (with productive tiller number, total tiller number and filled grains), thousand grain weight (with days to fifty percent flowering, productive tiller number and total tiller number) and harvest index (with days to fifty percent flowering, unfilled grains and biomass) showed the significant and negative correlations with the majority of the traits under study. HI showed significant and positive association with thousand grain weight, number of filled grains, and single plant yield. It showed significant and negative association with days to fifty percent flowering, unfilled grains and biomass. Balakrishnan *et al.*, (2016) observed that single plant yield exhibited a highly significant association with panicle weight, biomass and harvest index in the introgression lines derived from *Oryza sativa* cv. Swarna /*Oryza nivara*.

Grain shape traits (GL, GW, LW, GA and GP) were interrelated with each other significantly across the seasons. Grain length had significant and positive

association with length: width, grain area and grain perimeter. The positive correlation between grain length and length by width ratio was also reported by Abdala *et al.*, (2016) and Golam *et al.*, (2014). Grain width had a significant and negative association with LW. Abdala *et al.*, (2016) also reported a similar association, but observed a strong correlation between brown rice shape and paddy rice width. In the present study, there was no correlation between grain length and grain width as reported by Abdala *et al.*, (2016). Length/ width ratio had a negative and significant association with grain width and grain area while it had a positive and strong association with grain perimeter and grain length. Grain area had a significant, moderate and positive association with grain length and grain perimeter, but significant negative association with grain length/width ratio.

In this study most of the trait associations were consistently significant in both *Rabi* 2018/19 and *Kharif* 2019 seasons. Days to fifty percent flowering had a significant positive association with plant height, panicle length, panicle weight, unfilled grains and biomass and had a significant negative association with harvest index and number of filled grains. Balakrishnan *et al.*, (2016) reported that days to fifty percent flowering showed a negative correlation with number of filled grains, harvest index and days to fifty percent flowering while highly significant association observed between panicle length and panicle weight. Following associations showed the significant correlation, in same direction across the seasons; single plant yield with biomass, harvest index, grain length and grain weight; grain length/width ratio with grain area and grain perimeter; grain length showed significant association with grain length/width ratio, grain area and grain perimeter in both the seasons. Plant height showed significant, positive relationship with days to fifty percent flowering, panicle length, panicle weight and biomass. This corroborates with the findings of Immanuel *et al.*, (2011) for panicle length. Productive tiller number and total tiller number had a strong and positive association with each other and moderate positive association with single plant yield and biomass. Immanuel *et al.*, (2011), and Sudhakar *et*

*al.*, (2012) also reported the significant and positive association of total tiller number with productive tiller number. Yusuff, *et al.*, (2018) revealed that tillers per hill and grain weight per hill possessed positive association with yield per hectare.

For some traits, significant associations were observed only in one season (Table 1). Following associations were significant and positive in *Rabi* 2018/19 season only. Days to fifty percent flowering with productive tiller number and single plant yield; plant height with single plant yield; productive tiller number with panicle length; panicle length with single plant yield; panicle weight with biomass; unfilled grains with biomass and grain length; harvest index with grain length/width ratio; grain weight with grain area and grain perimeter. Following associations were significant and negative, productive tiller number with harvest index and grain length; total tiller number with harvest index, grain length and grain perimeter; number of filled grains with biomass and grain length. In *Kharif* 2019 only association among plant height with unfilled grains; panicle weight with number of filled grains and harvest index; number of filled grains with single plant yield were significant and positive, while following associations were significant and negative, days to fifty percent flowering with thousand grain weight; plant height with productive tiller number, total tiller number and number of filled grains; productive tiller number with panicle weight, thousand grain weight; total tiller number with panicle weight, thousand grain weight; panicle weight with unfilled grains; thousand grain weight with biomass; unfilled grains with single plant yield; single plant yield with grain perimeter. Bagati *et al.*, (2016) reported panicle length exhibited significant positive and negative association with grain yield during 2014 and 2015, respectively. The major reason for this difference in trait association is mainly the varying environmental conditions. After transplanting, the 21 days old seedlings in the field were exposed to total submergence for few days and partially for about ten days due to heavy rains in *Kharif* 2019. In general, the observed disparity in cultivation conditions, the inconsistencies in individual genotype expression, are



reasonable causes for fluctuations in environmental indices (Adesola, 2013). Trait associations showed seasonal variation mainly due to the fact that genotypic performance depends on environmental conditions (Bose *et al.*, 2014; Balakrishnan *et al.*, 2016). Similar observations on GEI were made by Tariku *et al.*, (2013) and Akter *et al.*, (2014) on multi-environment studies using rice genotypes.

## Conclusion

In the present study, association analysis was done using 177 RILs derived from a cross between Swarna x 166s for seventeen yield and grain shape-related traits in *Rabi* 2018/19 and *Kharif* 2019 seasons. Biomass showed significant positive correlations with the majority of traits, followed by days to fifty percent flowering in both seasons. Among all the traits, highest significant and strong positive correlation was observed between grain length and grain perimeter followed by productive tiller number and total tiller number in *Rabi* season. When harvest index showed significant negative correlations with majority of the yield contributing traits followed by filled grains in *Rabi* season, days to fifty percent flowering, plant height, 1000 grain weight and harvest index showed the same correlations in *Kharif* season. Grain shape traits were interrelated with each other significantly in both seasons. Grain length had a significant, positive and moderate association with grain length/width ratio, grain area and a high association with grain perimeter. Single plant yield showed a moderate positive association with both productive and total tillers per plant, panicle weight, biomass and harvest index in both seasons. According to our results, the focus on productive tiller number and panicle weight will be most helpful while selecting the genotypes in the field for higher yield in this population.

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## The potential of wet rice cultivation in Mizoram, India: A case for attaining self sufficiency in food security

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### Abstract

A large number of rural people in Mizoram are fully dependent on agriculture for running their livelihoods. Paddy is the staple crop, grown both under shifting and wet rice cultivation whereas arable land under paddy is significantly low under wet rice cultivation. This paper examines the potential of wet rice cultivation to attain self-sufficiency in food security in Mizoram based on Time series data of 2009-10 and 2014-15 on area, production, and yield of wet rice cultivation. It has been suggested that if a sizeable portion of arable land is devoted to wet rice cultivation and adequate irrigation facilities are provided, then the production and yield of rice will increase multifold.

**Key Words:** WRC, food security, self-sufficiency, system rice intensification, Mizoram

### Introduction

Half of the world's population produces and relies on the consumption of paddy (rice), a staple diet, and important food crop, mainly in the tropical and sub-tropical climate zones (Ramakrishnan, 1992a; Swift *et al.*, 1996). Wet Rice Cultivation (WRC) is irrigated and practiced in tropical countries mainly in Asia, where about 90% of the world's rice is grown because of suitable geographical and climatic conditions. The yield of WRC as well as quality of rice varies significantly depending upon the socio-ecological conditions (Ramakrishnan, 1992b; Mitchell, 1979; Nguu and Palis, 1977; Dazhong and Pimental, 1984; Leach, 1976).

Rice is the staple food in India, grown in the vast wetland of the river valleys, mainly the Ganges and the Brahmaputra, which are considered suitable for the cultivation of paddy (Raghavan, 1964). Further, the coastal regions such as the states of Tamil Nadu, Andhra Pradesh, Odisha, and West Bengal grow rice in large areas (Subbalakshmi *et al.*, 2020).

The state of Mizoram, an eastern extension of the Himalaya, has a primitive economy. Agriculture is the major source of livelihoods, which is dominated by shifting cultivation with low production and yield. A huge number of rural people (>50%) are dependent on the output of traditionally grown crops. However, arable land is significantly less therefore, people of rural areas are very poor as about 19.63% of people living below the poverty line (BPL). WRC is practiced as a double-crop – both as *Kharif* and *Rabi* crops whereas few districts practice WRC only in *Rabi* season with a small proportion of arable land. WRC has a high potential to attain self-sufficiency in food grain and to reduce the number of people living in BPL. So far, no substantial studies have been carried out on WRC and literature is scanty. One of the major problems related to WRC is the identification of plots because of their tiny sizes, though there has been attempt to use Remote Sensing Data in an earlier study (Ravan *et al.*, 2004).



This study attempts to examine the potential of WRC in Mizoram. It aims to analyze the area, production, and yield of WRC compared to the potential of shifting cultivation, correlate households involved in WRC and BPL families, as well as irrigation and production of WRC. It also analyzes the change in the area, production and yield; WRC potential area and utilization of WRC potential area; district wise ranking of WRC and requirement of WRC for self-sufficiency.

### Study Area

Mizoram, an eastern extension of the Himalaya, has rich subtropical and montane agro-climate. Its total geographical area is 21,027 sq km of which, 97% is hilly and undulating terrain. The economy of the state mainly depends on the output from the traditional

subsistence agriculture. Total arable land in Mizoram is 51,033 ha of the total geographical area, of which, WRC area is 16,866 ha (33% of the total arable land), which is less than 0.8% of the total geographical area. Mizoram practices two types of agriculture – shifting and WRC. The arable area under WRC is low; however, its production and yield are substantial and quite higher than shifting cultivation. WRC is practiced in the flood plains, formed by the perennial river after deposition of alluvial soils, while arable patches are also found in the river valley. Mizoram receives 2,400 mm average annual rainfall and average annual temperature is 22.5°C, which is quite suitable for growing crops mainly for WRC. Figure 1 shows a small patch of WRC and a WRC field in the Met river valley, the Serchhip district.



Figure 1: Wet rice fields (a) small patch of WRC (b) wide WRC fields; both in the Met river valley, Serchhip district

### Methodology

A set of qualitative and quantitative methods were employed to conduct this study. Data were gathered mainly from secondary sources (Statistical abstracts – 2009-10 and 2014-15, Department of Agriculture, Aizawl) and through field observation. At the district level, the data were collected on area and production of WRC, irrigation, the number of households (HHs) involved in practicing WRC and people living below the poverty line (BPL). The collated data were subjected to analysis by SPSS using levels and indices,

correlation, regression, and rank-score methods. SPSS was also used to correlate irrigation, production and HHs involved in WRC including BPL HHs. Based on the indices of area, production, and yield, WRC districts were categorized into high, medium, and low levels. The districts were ranked and scored according to their potential in WRC and the final ranking was given to districts for their future potential of WRC. Maps were digitalized showing the district point levels of WRC and the future prospective of WRC. A regression model was used to correlated WRC HHs and BPL HHs, and irrigation and production of WRC

through a linear curb. Field visits were also made to understand the WRC potential in Mizoram.

## Results

### Area, production, and yield of paddy under both shifting and WRC

An analysis of the area, production, and yield of paddy under both shifting and WRC in Mizoram was carried out. Firstly, the status of WRC in total crops was compared in terms of area, production, and yield followed by the comparison of WRC with shifting cultivation of paddy. The total arable land in Mizoram was 64,900 ha, and WRC shared 15.1% area (9,774 ha), while, in terms of production, its share was 19.85%. The yield of WRC was just double

the average yield of total crops. In 2014-15, the total arable land decreased to 51,033 ha (21.37%), however WRC area share increased to 16,866 ha (33.04%). The production share of WRC increased by 29.71%, from 20,544 MT to 37,096 MT.

The paddy area under shifting cultivation in 2009-10 was 3.7 times higher than WRC whereas in 2014-15, WRC area increased very close to the area of paddy under shifting cultivation (Table 1). In terms of production, WRC yield which was half in 2009-10 increased to double than that of shifting cultivation in 2014-15. Hence, it was evident that the area, production, and yield of paddy increased under WRC, while shifting cultivation showed a decline.

**Table 1: Area, production, and yield of paddy under shifting and WRC**

Variable	2009-10		2014-15		Change (%)	
	Shifting	WRC	Shifting	WRC	Shifting	WRC
Area	36841	9774	20064	16866	-45.54	+72.56
Production	43985	20544	23583	37096	-46.38	+80.57
Yield	1.19	2.10	1.18	2.20	-0.84	+4.76

Source: Statistical Abstract, Department of Agriculture, Aizawl, Mizoram

### District-wise area of WRC

Mizoram had a total WRC area of 16,866 ha with production of 37,096 MT, and a productivity level of 2.20 MT/ha in 2014-15. The District-wise area of WRC was categorized into three levels – high, medium, and low (Table 2), with three indices - >2000 ha, 1000-2000 ha, and <1000 ha in 2009-10. Champhai and Kolasib belonged to high category with an area of >2000 ha, while two districts – Serchhip and Lawngtlai were of Medium level (1000-2000 ha). Four districts – Mamit, Lunglei, Saiha, and Aizawl showed low area (<1000 ha). In 2014-15, due to the increase in the area of WRC indices, levels varied from >4000 ha (high), 2000-4000 ha (medium) and <2000 ha (low). Champhai and Kolasib maintained the first position with >4000 ha area and Lawngtlai and Serchhip continued to be under the medium category. The remaining four districts – Lunglei, Mamit, Aizawl, and Saiha maintained a low level (<2000 ha). All the districts maintained their status except Saiha and Aizawl.

**Table 2: Area of WRC (ha)**

Levels	Indices	Districts
<b>Area (2009-10)</b>		
High	>2000	Champhai and Kolasib
Medium	1000-2000	Serchhip and Lawngtlai
Low	<1000	Mamit, Lunglei, Saiha, and Aizawl
<b>Area (2014-15)</b>		
High	>4000	Champhai and Kolasib
Medium	2000-4000	Lawngtlai and Serchhip
Low	<2000	Lunglei, Mamit, Aizawl, and Saiha



### District-wise production of WRC

Champhai and Kolasib districts showed high production under WRC (>5000 MT) in 2009-2010, whereas Lawngtlai, Mamit, and Serchhip districts had medium-level production (1000-5000 MT), and Lunglei, Aizawl, and Saiha districts recorded low level of production (<1000 MT). In 2014-15, production increased to >8000 MT in Kolasib and Champhai districts (Table 3). Kolasib district had higher production than the Champhai district. Medium level production (4000-8000 MT) was reported by Lawngtlai and Serchhip districts. Lunglei, Aizawl, and Saiha districts had low level production (<4000 MT).

**Table 3: Production of WRC (MT)**

Levels	Indices	Districts
<b>Production (2009-10)</b>		
High	>5000	Champhai and Kolasib
Medium	1000-5000	Lawngtlai, Mamit, and Serchhip
Low	<1000	Lunglei, Aizawl, and Saiha
<b>Production (2014-15)</b>		
High	>8000	Kolasib and Champhai
Medium	4000-8000	Lawngtlai and Serchhip
Low	<4000	Lunglei, Mamit, Aizawl, and Saiha

### District-wise yield of WRC

In 2009-10, the WRC productivity was high (>2.5 MT/ha) in Champhai and Mamit districts (2009-10), followed by the districts of Aizawl, Kolasib, Lunglei, Saiha, and Lawngtlai (Table 4) with medium level of productivity (1.5-2.5 MT/ha). Only Serchhip district reported low yield (<1.5 MT/ha). In 2014-15, WRC productivity in the Lawngtlai district jumped to a high level while, the yield of WRC decreased in the Champhai district, to a low level. Serchhip district was promoted to medium level category along with other districts – Aizawl, Lunglei, Saiha, and Kolasib.

**Table 4: Productivity (Yield/ha) of WRC**

Levels	Indices	Districts
<b>Yield (2009-10)</b>		
High	>2.5	Champhai, Mamit
Medium	1.5-2.5	Aizawl, Kolasib, Lunglei, Saiha, and Lawngtlai
Low	<1.5	Serchhip
<b>Yield (2014-15)</b>		
High	>2.5	Lawngtlai and Mamit (equal)
Medium	1.5-2.5	Aizawl, Lunglei, Serchhip, Saiha, and Kolasib
Low	<1.5	Champhai

### Change in area, production, and yield of WRC

Analysis of data on change in area, production, and yield between 2009-10 and 2014-15 (Figure 2) revealed an increase in the area under WRC in all the districts of Mizoram and it varied from 171.1% in the Aizawl district (highest) to 26.82% in the Saiha district (lowest). Two other districts viz., Lunglei and Lawngtlai showed >100% increase, whereas in Kolasib and Mamit there was an escalation of 73.67% and 64.35% area, respectively. Champhai and Serchhip districts registered less than a 50% increase in the WRC area, however Mizoram as a whole registered a 62.75% expansion in WRC. In terms of production, Serchhip district ranked first showing a 320.77% increase, followed by Lawngtlai (252.38%) and Lunglei (234.44%) districts while the increase in production was up to 189.21% in Aizawl. Champhai district registered a negative change with a 11.47% decrease. Other districts – Saiha, Kolasib, and Mamit districts registered 80.76%, 63.76%, and 51.34% increase in WRC production, respectively, while overall increase of production due to WRC in Mizoram was 67.51%. There was 2.8% increase in productivity due to WRC in Mizoram.

### Household level area and production of WRC

The Kolasib district had the highest area (1.46 ha) of WRC/HH in 2009-10 (Figure 3), followed by Saiha

(1.25 ha), Mamit (1.22 ha), and Champhai (1.08 ha) districts. Other districts had less than 1 ha area/HH. The state of Mizoram had an average of 1.06 ha area/HH. In 2014-15, the rice area increased to 1.28 ha/HH in Mizoram. Among the districts, Kolasib district

maintained the top position with 1.97 ha area/HH, while. Lawngtlai, Champhai, Serchhip, and Lunglei districts also registered an increase in rice area/HH. There was decrease in area/HH in Mamit, Aizawl, and Saiha districts.

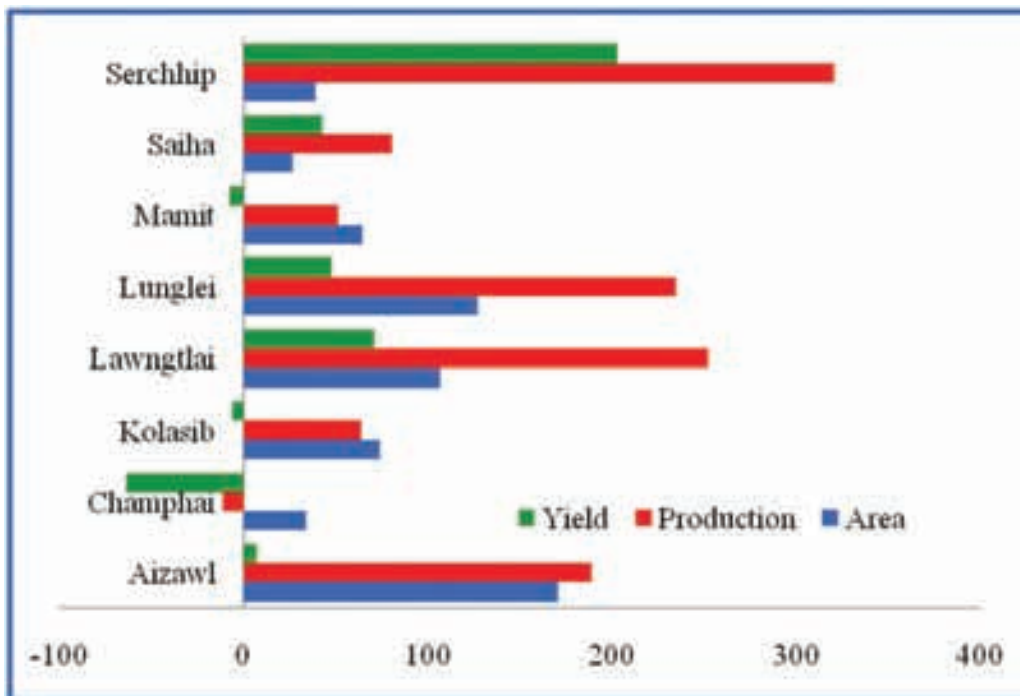


Figure 2: Change (%) in area, production, and yield of WRC 2009-10 and 2014-15

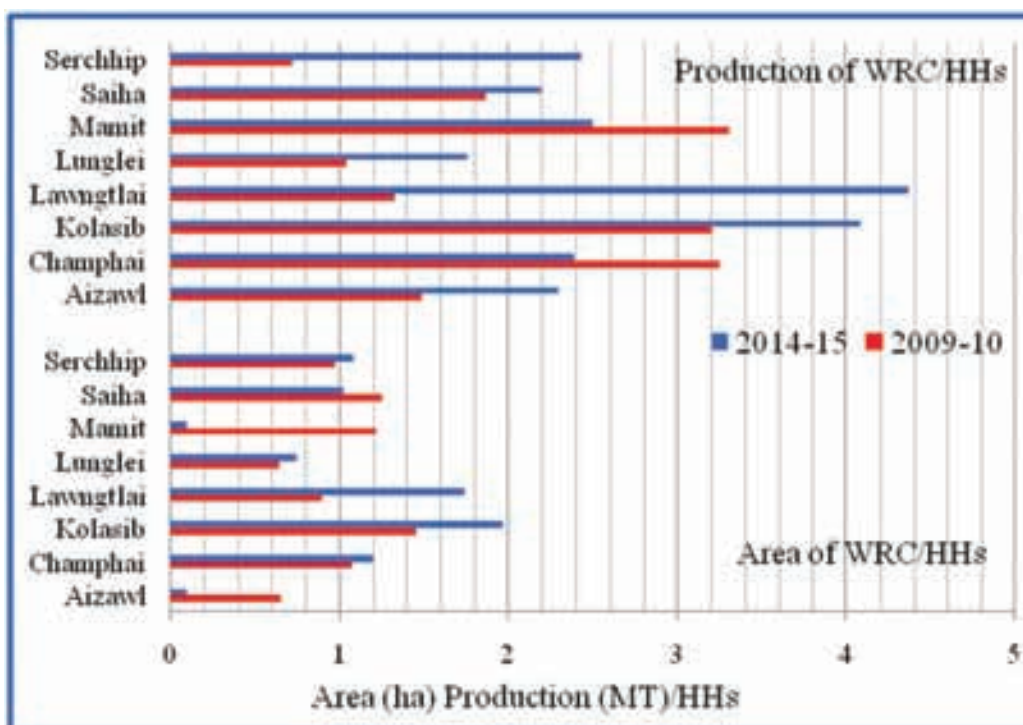


Figure 3: HHs level area and production of WRC



Mamit district topped in productivity per HH with 3.31 MT/ha/HH, followed by Champhai (3.25) and Kolasib (3.21) districts in 2009-10, while it was less than 1.5 MT/ha/HH. In 2014-15, the household productivity was the highest in Lawngtlai district (4.37 MT/ha/HH), followed by the Kolasib district (4.04). There was a decrease in two districts – Champhai (-0.86%) and Mamit (-0.81%). Overall, Mizoram state showed very little change in rice production/HH (2.80-2.81 MT/ha) during 2009-2015.

### Household involved in WRC and BPL families

The total number of households involved in WRC increased from 9748 in 2008-09 to 13,182 in 2014-15 (35.23%). It was the highest in Champhai district showing a 20.35% increase, followed by Kolasib with an increase of 28.85%. The number of households involved in WRC was lowest in Saiha, Aizawl, and Serchhip districts. However, all districts registered an increase in the number of households involved in WRC during this period.

The data on BPL revealed that Mizoram had 56,584 (19.63%) people living BPL. At the district level, the highest numbers BPL was recorded in Lawngtlai district (13,126), followed by Aizawl (12,668) and Lunglei (11,437). Serchhip district had the lowest number of people living BPL (1770), followed by Champhai (2715) and Kolasib (3401). Other two districts – Saiha and Mamit had 4245 people BPL.

### Correlation among irrigation, production, people living BPL and WRC HHs

There was a positive correlation between irrigation and production as evidenced by 0.725 r-value (Table 5, Figure 4), indicating that the districts with adequate irrigation resources showed a higher production. There was significantly negative correlation ( $r = -0.504$ ) between irrigation and BPL, hence the districts with irrigation facilities had less people living BPL.

Data also revealed that there was high involvement of HH in districts with adequate irrigation facilities ( $r = +0.458$ ). The productivity of WRC had a direct impact on people living BPL and the analysis showed less people living BPL in areas with higher WRC productivity ( $r = -0.377$ ). There was a significantly positive correlation between productivity and number of people involved in WRC ( $r = 0.835$ ).

### Utilization of WRC potential area

Mizoram has a total WRC potential area of 74,644 ha of which only 22.60% (16,866 ha area) has been utilized so far. WRC potential area is grouped into three levels – high (>10000 ha), medium (5000-10000 ha) and low (<5000 ha). The Serchhip district, followed by Champhai and Saiha districts are high WRC potential areas with >10000 ha area. The Lunglei and Lawngtlai districts have medium WRC potential while three districts – Kolasib, Aizawl, and Mamit have a low

**Table 5: Correlation among the variables related to WRC**

Variables	Correlation	Irrigation	Production	People living BPL	WRC HHs
Irrigation	Pearson Correlation	1	0.727*	0-.504	0.458
	Sig. (2-tailed)		0.041	0.203	0.254
Production	Pearson Correlation	0.727*	1	0-.377	0.853**
	Sig. (2-tailed)	0.041		0.357	0.007
People living BPL	Pearson Correlation	0-.504	0-.377	1	0-.468
	Sig. (2-tailed)	0.203	0.357		0.242
WRC HHs	Pearson Correlation	0.458	0.853**	0-.468	1
	Sig. (2-tailed)	0.254	0.007	0.242	

\*. Correlation is significant at the 0.05 level (2-tailed), \*\*. Correlation is significant at the 0.01 level (2-tailed).

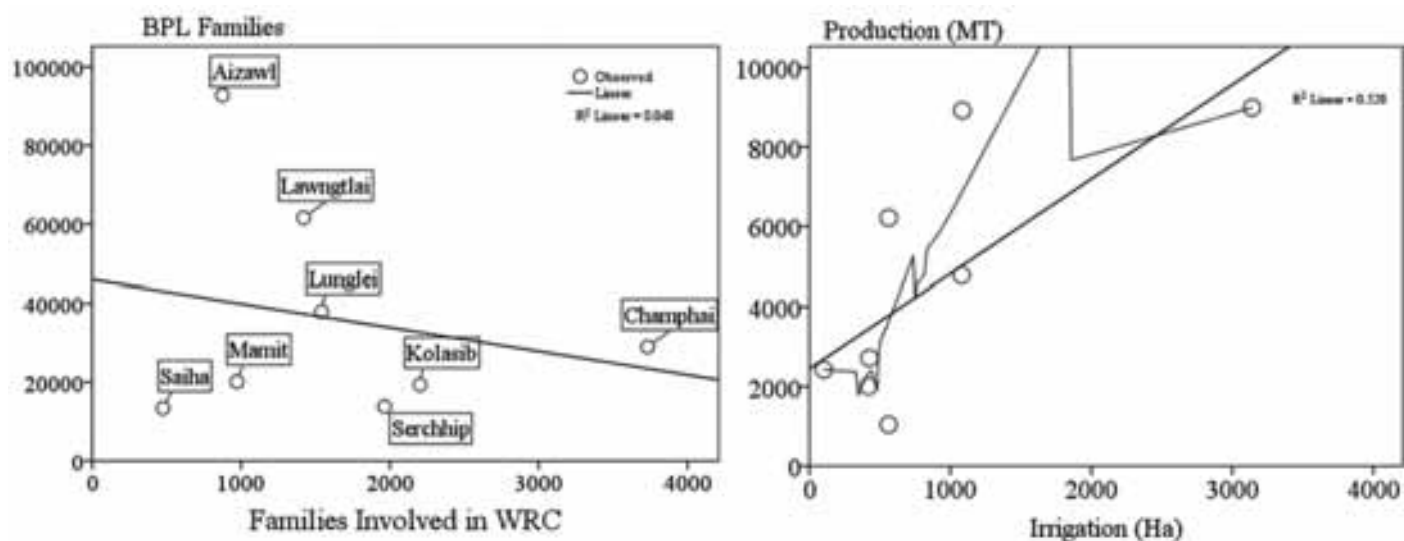


Figure 4: Correlation between families involved in WRC and BPL families and irrigation and production

potential for WRC (Table 6, Figure 5). On the other hand, the actual area under WRC at the district level is high (>2000 ha) in Champhai, followed by Lunglei, Lawngtlai, and Serchhip districts. The Kolasib district has a medium area of 1000-2000 ha, whereas Mamit, Aizawl, and Saiha districts possess low area (<2000 ha) under WRC.

The analysis of utilization of the WRC potential area revealed that Lunglei and Champhai districts were potential areas (>30%) while Lawngtlai, Kolasib, Mamit, and Aizawl districts had WRC areas of medium potential. (15-30%), Serchhip and Saiha districts showed low potential.

Table 6: Levels of WRC Potentials Area (ha), Area under WRC (ha), and Percentage of Utilization of WRC potential area

Levels	Indices	Districts
<b>WRC potential area (ha)</b>		
High	>10000	Serchhip, Champhai, and Saiha
Medium	5000-10000	Lunglei and Lawngtlai
Low	<5000	Kolasib, Aizawl, and Mamit
<b>The area under WRC (ha)</b>		
High	>2000	Champhai, Lunglei, Lawngtlai, and Serchhip
Medium	1000-2000	Kolasib
Low	<1000	Mamit, Aizawl, and Saiha
<b>Utilization of WRC potential area (%)</b>		
High	>30	Lunglei and Champhai
Medium	15-30	Lawngtlai, Kolasib, Mamit, and Aizawl
Low	<15	Serchhip and Saiha

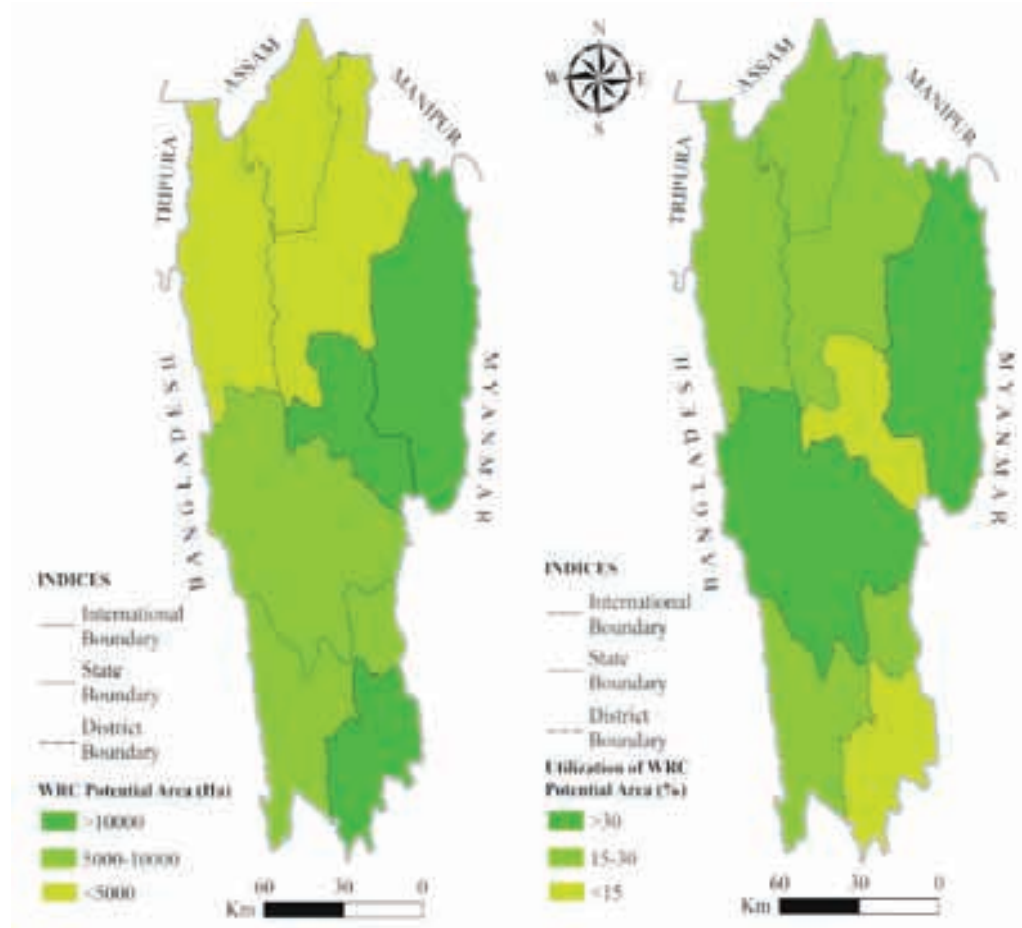


Figure 5: Change (%) in area, production, and yield of WRC from 2009-10 to 2014-15

### District-wise ranking of different components of WRC

An analysis of the area, production, yield, WRC potential, and irrigated area under WRC was carried out using the ranking method (Table 7). Ranks from 1 to 8 were assigned to districts based on their performance in a different component of WRC. The districts were further categorized into four potential groups based on area, production and productivity of WRC as very high, high, moderate, and low. Champhai and Kolasib districts showed very high potential, followed by Lawngtlai and Serchhip with high potential. Lunglei and Mamit districts belonged to moderate potential, whereas Aizawl and Saiha districts had low potential in area under WRC. WRC production was the highest in Kolasib and Champhai districts (very high), followed by Lawngtlai and Serchhip districts (high). The production was moderate in Lunglei and

Mamit districts and low in Aizawl and Saiha districts. Lawngtlai, Mamit, and Aizawl districts showed very high potential in productivity, followed by Lunglei and Serchhip districts (high). Saiha and Kolasib (Moderate). Champhai district had a low potential. WRC potential area was the highest in Serchhip and Champhai (very high) and the lowest in the Aizawl and Mamit districts (low). Saiha and Lunglei districts had high potential, while Lawngtlai and Kolasib had moderate potential. In terms of irrigation, 83.7% area is irrigated under WRC (2014-15). Kolasib and Champhai districts were ranked very high followed by Serchhip and Lawngtlai districts (high). Saiha and Lunglei districts showed moderate potential and Aizawl and Mamit districts had low potential.

The final ranking for future potential of WRC in Mizoram was given based on summation scores of rankings of different variables related to WRC.



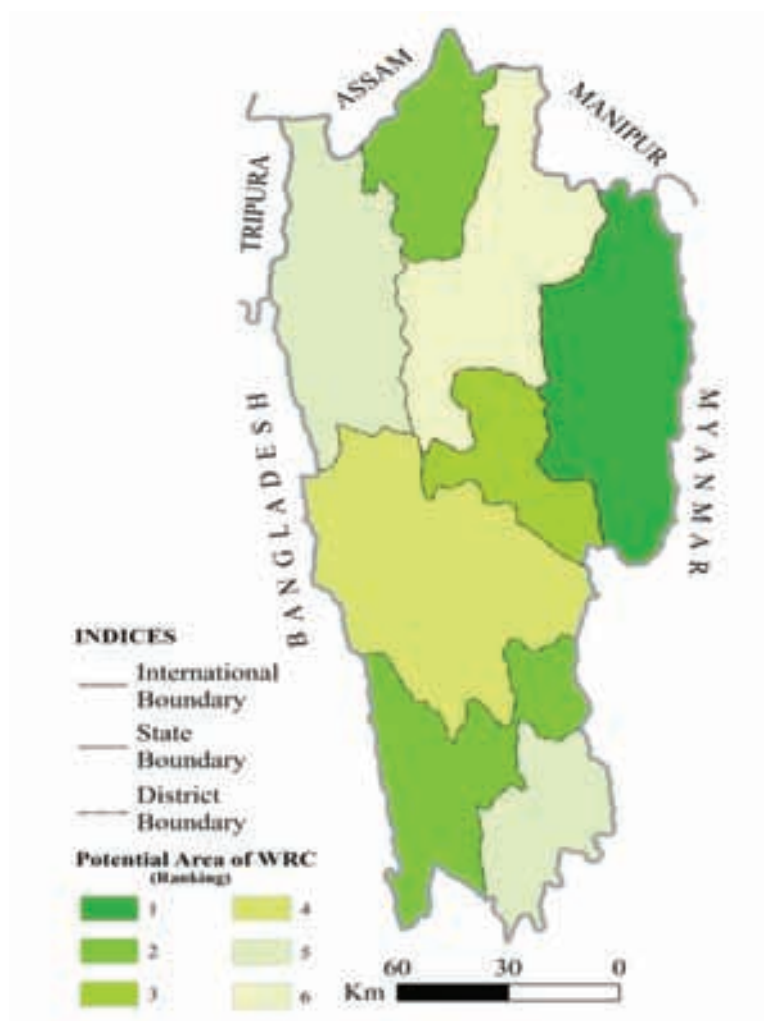
Champhai district showed highest rank for future potential in WRC followed by Kolasib and Lawngtlai districts. Serchhip district was ranked next followed by Lunglei, Mamit, Saiha and Aizawl (Figure 6).

**Table 7: District-wise ranking of different components of WRC (2015)**

District	Area	Production	Yield	WRC Potential	Irrigated area
Aizawl	8	7	1	7	7
Champhai	1	2	4	2	2
Kolasib	2	1	6	6	1
Lawngtlai	3	3	5	5	4
Lunglei	4	4	3	4	3
Mamit	5	5	2	8	8
Saiha	6	6	7	3	5
Serchhip	7	8	8	1	6

1	2	3	4	5	6	7	8
Very high potential		High potential		Moderate potential		Low potential	



**Figure 6: Final ranking for the districts based on WRC potential in different variables**



## A requirement of rice for self-sufficiency

The analysis of population, WRC production, and requirement of rice for self-sufficiency revealed substantial increase in self sufficiency in rice during 2009-2015 (Table 8). However, total production was only 26.82 % of total rice requirement, during this period, showing a deficiency of 73.18% in production in 2014-15. It was also estimated that an arable area of 105,876 ha was required to attain self-sufficiency in rice production.

## Discussion

Mizoram has an agricultural economy with majority of rice area and production under shifting cultivation while Wet Rice Cultivation areas are significantly low. It was also evident that though the area of WRC is less than that under shifting cultivation, the production and yield are higher and have been increasing. At the district level, it has been observed that the two districts Champhai and Kolasib had high areas under WRC both in 2009-10 and 2014-15, Serchhip and Lawngtlai districts registered medium areas and the remaining four districts had low area under WRC. The trends have also indicated there has been doubling of the area under WRC with variations at the district levels and there was also substantial increase in production. However, district-wise, the status of

production was almost the same in both periods with little variations. In terms of productivity of WRC, some districts showed significant increase but it was stagnant in other districts during 2009-2015. The area and production of WRC increased in all districts with changing values whereas a decrease in WRC productivity was noticed in Champhai, Kolasib, and Mamit districts though, these three districts showed high potential at HH level in WRC.

Higher production of WRC has a significant impact on attaining food security and lowering the numbers of BPL families. An analysis of levels of WRC potential area, actual area under WRC, and percent utilization of WRC has shown that Serchhip, Champhai, and Saiha districts have high potentials for WRC and the current area under WRC is also high in these districts along with Lawngtlai. Further, utilization of WRC potential area (%) is the highest in Lunglei and Champhai districts, as indicated by the rank-score analysis, however very high potential of WRC was observed in Champhai and Kolasib districts in terms of area and production. Aizawl and Mamit districts ranked highest in productivity. In the potential of WRC, Serchhip and Champhai districts had the highest rank while Champai district also has the best irrigation resources along with Kolasib. Overall, Champhai showed the first rank in WRC followed by

**Table 8: Requirement of rice as per population need in 2009-10 and 2014-15 and change**

Variables	2009-10	2014-15	Change (%)
Population (2011)	9,21,970	10,91,014	18.3
Average requirement of rice per person per year at the rate of 450 gm per day (Quintal)	1.64	1.64	Nil
Total requirement of rice for 1 year for human consumption only (Quintal)	15,12,030	17,89,263	18.3
Total annual requirement of rice for Mizoram (Quintal)	19,22,030	21,99,263	14.4
Total production of rice (Quintal)	4,62,924	5,89,940	27.4
% of the production of the total requirement	24.09	26.82	11.3
% Deficiency in production	75.91	73.18	-3.6
Land required for self-sufficiency in Mizoram (Ha)	148,888	105,876	-28.9

Source: Statistical Abstract, Department of Agriculture, Aizawl, Mizoram

Lawngtlai and Kolasib placed with second rank and Serchhip was third ranked place in practicing WRC. Other districts showed low potential.

Shifting cultivation has been reported to be economically unviable (Tawnenga *et al.*, 1997; MART 2011; Sati 2014, 2017, 2018, 2019). Therefore, the future of WRC is potentially very high. Efforts made so far by the state government to increase the area and production of WRC, have resulted in 28.4% increase in area and 10% increase in production, respectively (Zodinsanga, 2013). These efforts should be continued so that the state will attain self-sufficiency in food grain production.

## Conclusions

Mizoram has a high potential to attain self-sufficiency in food grain through practicing WRC because of its higher production and productivity than that of shifting cultivation. Data between 2009-10 and 2014-15 shows that there has been a large increase in the production of rice and substantial decrease in annual requirement for rice in the region. Since Mizoram has a conducive climate to practice WRC, bringing a sizable proportion of arable land under WRC can help in efforts to attain self-sufficiency in food grain. The second important component of WRC is adequate irrigation. So, if irrigation facilities can be extended to rain-fed areas, intensive irrigation can meet the water requirements under the situation of high climate variability and change. A system of rice intensification can also be a suitable approach to increase the production under WRC. Also, more arable land can be devoted to WRC under the *Rabi* crop in all the districts. A sustainable practice of WRC will reduce the number of people living in BPL and progress towards attaining self-sufficiency in food grain and food security.

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## Laboratory evaluation of aqueous plant extracts against rice yellow stem borer, *Scirpophaga incertulas* (Walker) (Lepidoptera:Crambidae) and its safety to natural enemies

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### Abstract

Laboratory studies were conducted to evaluate the effect of aqueous extracts of pungam, tulsi, periwinkle, notchi, sweet flag and neem seed kernels on egg, larva and adult of rice yellow stem borer. The effect of botanicals on egg masses revealed the maximum ovicidal effect of reduced hatching (5.58 %) by sweet flag rhizome extract followed by NSKE (21.35 %) and pungam leaf extract (34.08 %). The maximum antifeedant effect in terms of stem protection was observed in NSKE (74.55 %) followed by pungam leaf extract (67.24 %). All the botanicals were observed to exert equal anti-oviposition effect (100 %) on adult moths of yellow stem borer except tulsi (96.67 %). The botanicals were found to be safe to the egg parasitoids *Telenomus dignus* and *Tetrastichus schoenobii* with maximum adult emergence (> 90 %) from the treated egg masses of yellow stem borer compared to control exhibiting maximum natural parasitism (93.33 %), during *Kharif*, 2018 and *Rabi*, 2019.

**Keywords:** Yellow stem borer, botanicals, toxicity, egg parasitoids, safety

### Introduction

Rice (*Oryza sativa* L.) belonging to family Poaceae is an important grain crop and staple food for more than half of the human population (Zhang, 2019). Globally, it is the second most cultivated cereal crop next to wheat. India ranks first in area (43.79 m. ha) and second in production (109.70 MT) (Anonymous, 2018). Tamil Nadu is one of the major rice producing states in India. The rice crop is subjected to a considerable damage by nearly 300 species of insect pests; among them only 23 species are serious pests of rice (Pasalu and Katti, 2006). In India, out of the total loss incurred by different insect pests of paddy, 30 per cent damage is done by stem borer alone (Krishnaiah and Varma, 2012). The yellow stem borer (YSB), *Scirpophaga incertulas* (Walker) (Lepidoptera:

Crambidae) is widely distributed and the most predominant species of stem borer in rice ecosystem (Reuolin and Soundararajan, 2019).

Globally, rice stem borer management accounts for 50 per cent of the insecticides used in rice fields (Huesing and English, 2004). Overreliance on synthetic pesticides causes ecological adversity and health related problems (Carvalho, 2017). It has also led to an exponential increase in the number of insect species developing resistance to insecticides (Sparks and Nauen, 2015) and destruction of population of beneficial insects (Jafar *et al.*, 2013). Rice ecosystem harbours a wide range of natural enemy complex (Singh and Kumawat, 2020). Parasappa (2017) reported a significant positive correlation of YSB population with the hymenopteran parasitoids of rice



ecosystem. The extent of natural egg parasitism of yellow stem borer in rice ecosystem has been reported to a maximum of 95.00 per cent (Lakshmi *et al.*, 2010). However, a significant reduction in predators and adults of the egg parasitoids *Trichogramma sp.*, *Telenomus sp.* and *Tetrastichus sp.* has been reported in the insecticide treated rice plots compared to untreated plots (Rahaman and Stout, 2019). These reports suggest the potential role of indigenous parasitoids and the need for their conservation *in situ* for suppressing the population of YSB. The use of botanicals in pest management is the best alternative to insecticides as they are safe to environment and compatible with natural enemies and other beneficial organisms. With this in view, a preliminary study was conducted in the laboratory to evaluate the detrimental effect of botanicals on different stages of yellow stem borer and their safety to the egg parasitoids of YSB.

## Materials and Methods

### Field study

#### *Natural parasitism of yellow stem borer egg mass*

A field trial was conducted at the experimental farm of Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli District, Tamil Nadu, in a Randomised Block Design during *Kharif*, 2018 and *Rabi*, 2019 with rice cv. TRY 3. The standard agronomic practices recommended by Tamil Nadu Agricultural University were adopted except the plant protection measures. The egg masses of yellow stem borer (YSB) were collected thrice per month with 10 days interval (30 egg masses/replication) during *Kharif*, 2018 and *Rabi*, 2019 from the field and kept in the petri plates with moist filter paper to avoid drying of leaves and observed for the emergence of the adult parasitoids. Once emergence was completed, the emerged adult parasitoids were observed under the stereo-zoom microscope to identify the species. The extent of parasitism of egg masses of YSB was worked out as given under (Vennila *et al.*, 2018)

$$\text{Parasitism (\%)} = \frac{\text{No. of parasitized egg masses}}{\text{No. of sample egg masses}} \times 100$$

### Laboratory studies

#### *Mass rearing of yellow stem borer*

The YSB was reared following the methodology described by Subashrao (2009) with slight modifications. The YSB adults collected from the field using sweep net were sexed and caged by providing rice leaves for oviposition by female. The lower portion of rice leaves (cv. TN 1) were kept in small glass vials containing water, so as to keep the leaves fresh and turgid for longer period. A small piece of cotton, duly saturated with five per cent sugar solution was provided in the cage as food for adults. The egg masses laid on the leaves were taken out daily along with leaf and kept in the petri dishes with filter paper and stored at 15°C. All the egg masses collected and stored were taken out on the same day and placed in petri dishes with moist filter paper to obtain uniform hatching of the larvae.

The newly hatched larvae were transferred to the tender pieces of rice stem with the help of soft camel hair brush. The hollow stems of rice from the top portion of the plant with leaf sheaths were selected for the neonate larvae and the hollow stems from the bottom portion of the plant were given as the larva reached the third instar. The hollow stems were cut such that each contained a node on both the end. The bases of rice stems were wrapped with foam and placed horizontally in a tray with thin film of water to enable the development of roots from both the nodes to keep the stems fresh and turgid for longer period. Then the larvae (10 neonate/ cut stem) were introduced into the small slit made at the centre of the rice stem with the help of camel hair brush. The top of the tray was covered with muslin cloth and fastened with rubber band to prevent the larvae from escaping and to keep the stems fresh and turgid by maintaining the humid condition in the tray. The larvae were transferred to fresh rice stems at two days interval until they pupated. After pupation, each stem was split opened from the middle of the stem up to the site of pupation. This facilitates easy emergence of the adult from the pupa in the laboratory. The stems with pupae were kept inverted in a glass vial with water

and kept inside the adult emergence cage to keep the stems moist until the adults emerged from the pupae. The emerged adults were used for subsequent rearing. The reared eggs, larvae and adults were utilised for the laboratory experiments.

### ***Effect of botanicals on adult emergence of egg parasitoids of YSB***

The naturally parasitised egg masses of YSB were collected from the field and kept in Petri dishes lined with moist filter paper to avoid drying of leaves. The egg masses were then treated by dipping in the aqueous extract of botanicals for five seconds. Water was used as untreated check. The treatments were replicated thrice and the treated egg masses were shade dried for 15 min. and then kept in Petri dishes at the rate of three egg masses per treatment and sealed. The egg masses were observed for the emergence of the adult parasitoids. The experiment was terminated on completion of adult emergence in the control. The egg masses were later dipped in 70 per cent ethanol and the hairs were removed with needle. The eggs were then separated out with the help of a fine camel hair brush and needle and the number of hatched and unhatched eggs as well as emerged and unemerged adults were counted under a stereo zoom microscope. The emergence of each species of egg parasitoid from the egg masses was worked out by the formula given by Vennila *et al.*, (2018).

$$\text{Adult emergence (\%)} = \frac{a}{a+b} \times 100$$

Where, a- number of parasitoids emerged, b- number of parasitoids un-emerged

### ***Effect of botanicals on different stages of YSB***

#### ***Preparation of botanical extracts***

The aqueous extracts of botanicals were prepared following the standard procedure described. The leaves of pungam, *Millettia pinnata* (L.) Panigrahi; tulsi, *Ocimum sanctum* L.; periwinkle, *Catharanthus roseus* (L.) G. Don; notchi, *Vitex negundo* L.; rhizome of sweet flag, *Acorus calamus* L. and neem seed kernels were collected, washed and cut into small

pieces. The cut leaves (Jazzar and Hammad, 2003), rhizomes (Yasodha and Natarajan, 2007) and neem seed kernels (Venkat Reddy *et al.*, 2012) were ground separately and mixed with distilled water at 5 per cent concentration *i.e.* 50 g leaves/rhizomes/neem seed kernels per litre of water, soaked overnight, then filtered through muslin cloth. Tween 20 (0.5 %) was added as a surfactant.

#### ***Ovicidal activity of botanicals on YSB egg mass***

Rice leaves with egg masses (3 days old) from the laboratory rearing were collected and immersed for five seconds in each botanical (Temerak, 2006). The treated egg masses were allowed to shade dry for 15 minutes and placed in Petri dishes lined with moistened filter paper to avoid drying of leaves and was sealed and observed for seven days. Three replications were maintained per treatment. The experiment was terminated after hatching of all the eggs in the control and the egg masses were dipped in 70 per cent ethanol. After five minutes the egg masses were taken out and the hairs were removed with needle under microscope. The hatched and unhatched larvae/eggs were counted under a stereo zoom microscope. The ovicidal action was measured by calculating the hatching percentage (Heinrichs, 1981).

Larval hatching (%) = [(No. of larva hatched)/(No. of larva hatched + No. of eggs unhatched)] × 100.

#### ***Antifeedant effect of botanicals on YSB larvae***

The larval bioassay with botanicals was conducted by following the methodology suggested by Heinrichs (1981). Cut stalks of rice culms from the base of the plant at a length of about 75 mm with nodes at both the ends were chosen and placed in a glass vial/ test tube each, with water to prevent drying of the stalk and by the rooting of the node at the bottom of the stalk. The rice culms were dipped in the botanicals for five seconds for proper soaking. At one day after treatment, both the ends of the culm were sealed by dipping them in hot paraffin wax to seal the ends and were infested with a third instar larva. Each vial/ test tube served as a replication. Cotton soaked with water was placed at the top over the end of the tube to



prevent the drying of stalk. Two days after infestation, the cut stems were dissected and the length of stem feeding was measured (Islam *et al.*, 2013). The stem protection over control (%) was worked out by the formula,

$$A (\%) = [(B - C) / (100 - C)] \times 100$$

Where, A - stem length protection over control (%),

B- stem length protection in treatment (%), C- stem length protection in control (%)

#### *Oviposition deterrent effect of botanicals on YSB adults by free choice test*

Rice leaf tips (cv. TN1) were dipped in the botanicals for five seconds for uniform soaking and shade dried. The treated leaf tips were kept in glass vials with water to prevent the drying of leaves. Three replications were maintained. Water was used as untreated check. Food source of five per cent honey solution soaked in cotton wool was provided for the stem borer moths. Twenty adults of YSB (female) were released into the cage, 24 h after treatment. Oviposition was observed in the leaves after 24 h of adult release. The anti-ovipositional effect was calculated for each treatment by using the following formula:

$$\text{Oviposition deterrent effect (\%)} = [\text{No. of eggs laid on untreated surface} / \text{Total no. of eggs laid (treated + untreated surface)}] \times 100$$

When mortality (%) was observed in the control for the laboratory bioassays, the value was corrected by the following Abbot's formula (Abbott, 1925):

$$\text{Corrected mortality (\%)} = [(\text{Test mortality (\%)} - \text{Control mortality (\%)} / (100 - \text{Control mortality})) \times 100$$

## Results and Discussion

### Safety of botanicals to egg parasitoids of YSB

The natural parasitism of egg mass of YSB was maximum in January 2019 (93.33 %), which was on par with the parasitism in February 2019 (90.00 %) and in December 2018 (80.00 %) during *Rabi*, 2019 (Table 1). The parasitism of egg masses during *khariif* 2018 was minimum in August (20.00 %) which

subsequently increased in September 2018 (33.33 %) and October 2018 (40.00 %).

**Table 1: Natural parasitism of egg mass of yellow stem borer, *S. incertulas***

Season	Month	Egg mass (no.)*		Parasitism (%)*
		Sampled	Parasitised	
<i>Khariif</i> , 2018	July	0.00	0.00	0.00 (0.91) e
	August	30.00	6.00	20.00 (26.67) d
	September	30.00	10.00	33.33(35.26) cd
	October	30.00	12.00	40.00 (39.23) c
<i>Rabi</i> , 2019	November	30.00	13.00	43.33 (41.17) c
	December	30.00	24.00	80.00 (63.44) ab
	January	30.00	28.00	93.33 (75.03) a
	February	30.00	27.00	90.00 (71.57) ab
	March	30.00	22.00	73.33 (58.91) b
Total	-	240.00	141.00	63.08
SEd	-	-	-	8.07
CD (p=0.05)	-	-	-	16.96

\*Mean of three replications; Figures in parentheses are arcsine transformed values; In a column, means followed by similar letter(s) are not statistically different (p=0.05) by LSD

*Telenomus dignus* (Gahan) and *Tetrastichus schoenobii* (Ferriere) were the two parasitoids observed in the egg masses collected from the experimental plot. Earlier, Varma *et al.*, (2013) reported maximum parasitisation of YSB egg masses by *Telenomus sp.*, while, Manjunath (1990) observed that maximum parasitic potential in YSB egg mass parasitised by *T. schoenobii* alone, followed by *T. dignus* and *T. schoenobii* in combination.

Observations on safety to egg parasitoids revealed that, the botanicals *Millettia*, *Acorus*, *Vitex*, *Catharanthus*, *Ocimum* and NSKE at a concentration of five per cent were safe to egg parasitoids *T. dignus* and *T. schoenobii*, which was evident by above 90 per cent emergence of adult parasitoids from the treated egg masses (Table 2 & Figure 1). This is in alignment with the previous findings of the repellent effect of neem

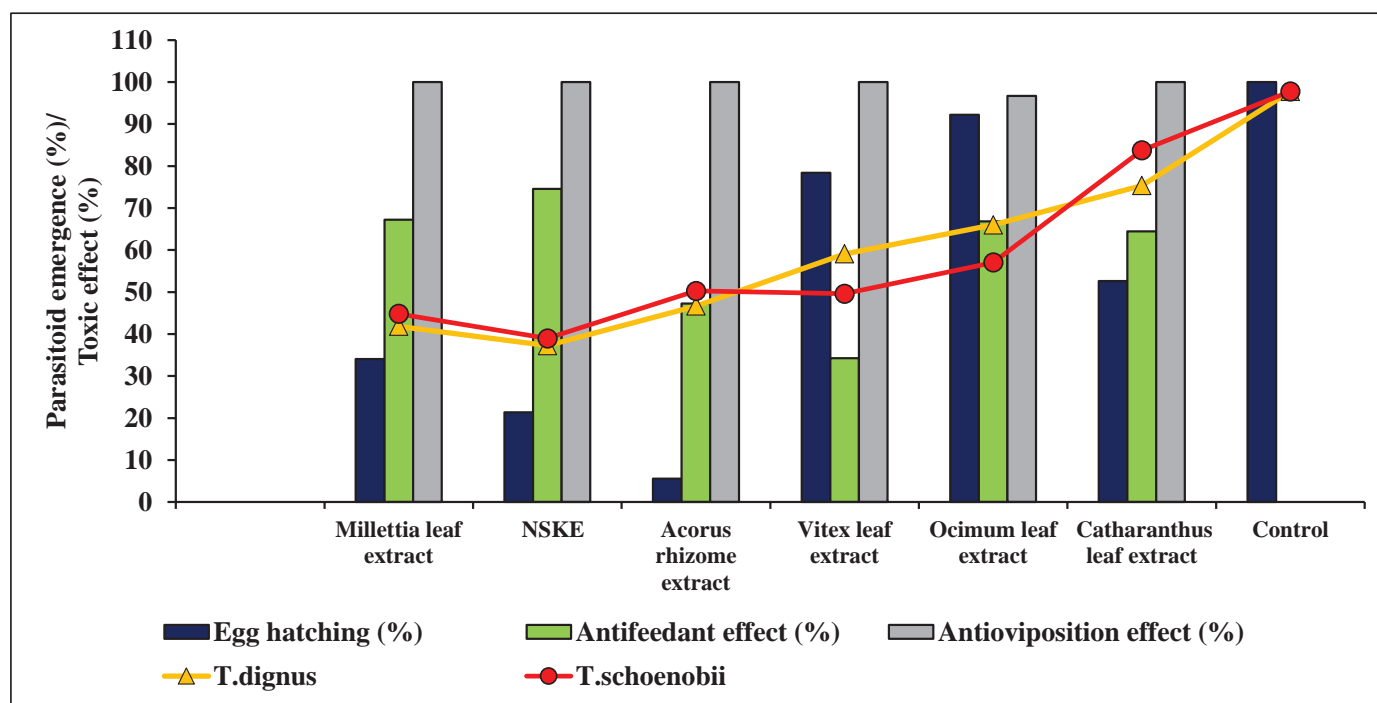


cake and neem oil on YSB moths with a significant reduction in oviposition but without reducing the extent of egg parasitism by the parasitoids *T. dignus* and *T. schoenobii* (Manju and David, 2004).

**Table 2: Laboratory evaluation on the safety of botanicals on egg parasitoids of yellow stem borer, *S. incertulas***

Treatments	Conc. (%)	Parasitoid emergence (%)	
		<i>T. dignus</i>	<i>T. schoenobii</i>
T1 - <i>Millettia</i> leaf extract	5	90.85 (72.39) d	91.33 (72.87) c
T2 – NSKE	5	90.09 (71.65) d	90.38 (71.93) c
T3 - <i>Acorus</i> rhizome extract	5	91.64 (73.20) cd	92.22 (73.81) bc
T4 - <i>Vitex</i> leaf extract	5	93.67 (75.43) bc	92.12 (73.70) bc
T5 - <i>Ocimum</i> leaf extract	5	94.80 (76.82) bc	93.33 (75.03) bc
T6 - <i>Catharanthus</i> leaf extract	5	96.33 (78.96) b	97.70 (81.28) ab
T7 – Control	-	100.00 (89.10) a	100.00 (89.10) a
SEd		1.59	4.93
CD (p=0.05)		3.41	10.57

\*Mean of three replications, Figures in parentheses are arcsine transformed values  
In a column, means followed by similar letter(s) are not different statistically (p=0.05) by LSD



**Figure 1: Effect of botanicals on the different stages of yellow stem borer and its safety to egg parasitoids**

**Evaluation of botanicals on different stages of YSB**

Among the botanicals evaluated, *Acorus* rhizome extract exerted maximum ovicidal action which was evident by significantly low percentage of larval

hatching of YSB (5.58 %), followed by NSKE (5 %) showing 21.35 per cent hatching and *Millettia* leaf extract (5 %) with 34.08 per cent hatching of YSB eggs (Table 3 & Figure 1). Aqueous extract of



*A. calamus* was found to exhibit maximum ovicidal action (38.89 % egg hatch) on *Plutella xylostella* (L.) (Matharu and Mehta, 2017) while Lall *et al.*, (2014) reported maximum reduction in egg hatching of egg masses of *Spodoptera litura* (Fabricius) treated with NSKE. *V. negundo* was reported to exhibit 52.02 per cent ovicidal activity against *S. litura* egg mass

(Arivoli and Tennyson, 2013), which is in alignment with the least effect of *Vitex* on YSB eggs (78.39 % YSB larval hatching). The ovicidal effect of *Millettia* on YSB is supported by the 80 per cent mortality by aqueous extract of *Pongamia glabra* Vent. on eggs of *Oligonychus coffeae* Nietner (Vasanthakumar *et al.*, 2012).

**Table 3: Laboratory evaluation of botanicals on different stages of rice yellow stem borer, *S. incertulas***

Treatments	Conc. (%)	Larval hatching (%)*	Antifeedant effect (%) *	Antioviposition effect (%)*
T <sub>1</sub> – <i>Millettia</i> (Pungam) leaf extract	5	34.08 (35.72)c	67.24 (55.09)b	100.00 (89.38)a
T <sub>2</sub> – Neem Seed Kernel Extract (NSKE)	5	21.35 (27.52)b	74.55 (59.70)a	100.00 (89.38)a
T <sub>3</sub> - <i>Acorus</i> rhizome extract	5	5.58 (13.66)a	47.25 (43.42)c	100.00 (89.38)a
T <sub>4</sub> - <i>Vitex</i> leaf extract	5	78.39 (62.30)e	34.22 (35.80)d	100.00 (89.38)a
T <sub>5</sub> - <i>Ocimum</i> leaf extract	5	92.20 (73.78)f	66.81 (54.82) b	96.67 (79.49)b
T <sub>6</sub> - <i>Catharanthus</i> leaf extract	5	52.65 (46.52)d	64.42 (53.38)b	100.00 (89.38)a
T <sub>7</sub> – Control	-	100.00 (87.14)g	0.00 (2.87)e	0.00 (0.63)
SEd		2.37	1.54	2.08
CD (p=0.05)		5.07	3.31	4.47

\*Mean of three replications; Figures in parentheses are arcsine transformed values  
In a column, means followed by similar letters are not different statistically (p=0.05)

The present study revealed that the antifeedant effect was maximum in NSKE with 74.55 per cent stem protection from YSB larval feeding followed by *Millettia* leaf extract with 67.54 per cent stem protection, over control (Figure 1). Maximum anti-oviposition effect (100 %) was observed in the botanicals *Millettia*, NSKE, *Acorus*, *Vitex* and *Catharanthus* followed by *Ocimum* (96.67 %) (Table 3). Chakraborty (2011) reported least damage of stem borer with NSKE (5%) and maximum damage with *V. negundo* extract (5%). Rao *et al.*, (2002), reported antifeedant effect (80%) against third instar larvae of *Earias vitella* (Fab.) on combined application of neem, sweet-flag and pungam extracts (1:1:1). The combination of aqueous extracts of NSKE (5%) and *A. calamus* (5%) exhibited strong ovicidal action and ovipositional deterrence against *Leucinodes orbonalis* (Guenee) (Yasodha and Natarajan, 2007). NSKE (5%) has been reported as the suitable alternative to synthetic insecticides for management of rice stem borers (Ogah *et al.*, 2011).

## Conclusion

Management of YSB dead heart and white ear damage is difficult as the larval stage is concealed inside the stem. Hence, management of this pest is easy and effective at the egg stage which prevents the further development of dead heart and white ear that leads to yield loss. Natural parasitism of YSB egg masses was observed at a maximum rate which leads to the effective control of YSB at the egg stage itself. Hence, keeping in view the natural occurrence of egg parasitoids in rice ecosystems, measures must be taken to avoid insecticide spray to conserve them. Thus, the application of efficacious botanical derivatives like NSKE (5 %), *Acorus* rhizome extract (5 %) and *Millettia* leaf extract (5 %) with ovicidal and repellent action on YSB can be developed as an alternative tool to insecticides for successful, eco-friendly and cost effective management strategy with safety to the egg parasitoids predominantly found in the rice ecosystem.

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## Variability of rice sheath blight pathogen, *Rhizoctonia solani* from Tamil Nadu

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### Abstract

A survey was conducted in five different major rice growing areas of Tamil Nadu viz., Mayiladuthurai, Aduthurai, Madurai, Vellore and Thiruvarur for the occurrence of Sheath Blight (ShB) disease, which revealed that Aduthurai in Thanjavur district is the hot spot area for ShB with highest disease incidence of 64%. Among the five isolates, *Rhizoctonia solani* (Rs<sub>2</sub>) collected from Aduthurai recorded the higher severity of ShB (53.80%) followed by Thiruvarur isolate (Rs<sub>5</sub>) (40.23%). *R. solani* isolates (Rs<sub>2</sub>) were successfully grown on different solid media viz., potato dextrose agar, beetroot dextrose agar, carrot dextrose agar, Czapek's dox agar and Richard's agar. Among the nitrogen sources tested for supporting the growth of *R. solani*, peptone recorded the maximum mycelial growth of 8.57 cm with sclerotial production of 86.67 per plate. Of the six carbon sources tested for supporting the growth of *R. solani*, glucose recorded the maximum mycelial growth of 7.92. All the five isolates of *R. solani* were molecularly identified using ITS 1F and ITS 4 R primer pairs. The results indicated an amplicon of 600 bp in all the isolates of *R. solani*.

**Key words:** *R. solani*- nitrogen sources-carbon source- pH-Internal transcribed spacer

### Introduction

Rice (*Oryza sativa* L.) is one of the widely cultivated important food crops in the world and over half of the world population relies on it. It is a chief food for more than 65% of the world's population and India's pre-eminent crop. Rice provides 20 per cent of the world's dietary supply and good source of thiamine, riboflavin, niacin and dietary fiber. In Asia, where 95 per cent of the world's rice is produced and consumed, it contributes 40 – 80 per cent of the calories of Asian diet (Kanchana *et al.*, 2012). Globally, more than three million people have rice as staple food, and it accounts for 50 to 80 per cent of their daily calorie intake (Delseny *et al.*, 2001). Over the next 20 years it is expected that demand for rice will grow by 2.5 per cent per year (Hobbs, 2001). In India, rice is grown in 43.86 million ha with the production of

104.80 million tonnes and the productivity of about 2390 kg/ha (Statistics, 2005). In Tamil Nadu, rice crop was cultivated in about 20 lakh hectares with an annual production of about 75.17 lakh tonnes and productivity of 3758 kg/hectare during the year 2015-16 (INDIASTAT).

Diseases are serious constraints to rice cultivation in India. More than 70 diseases caused by fungi, bacteria and viruses have been recorded on rice (Ou, 1985). Among them, sheath blight caused by *Rhizoctonia solani* (Teleomorph: *Thanatephorus cucumeris* (Frank) Donk) anastomosis group 1 IA (AG-1 IA) is a serious disease worldwide (Rush and Lee, 1983). The sheath blight disease of rice is prevalent in almost all the rice growing countries of the world. Sheath blight is an important soil borne fungal disease causing annually up to 40 per cent of yield losses. The



symptoms of the disease include greenish grey water-soaked spots development on the outer most sheaths of rice. As the disease progresses, spots enlarge and coalesce forming larger lesions with grayish-white center surrounded by tan to brown irregular borders or outlines. Sclerotia surviving in the soil are the major source of infection (Damicone and Jackson, 1996). When sclerotia come into contact with a rice plant, they germinate and the hyphae produce infection cushions on the exposed leaf sheath. Haustoria grow from the infection cushions, penetrate the host tissue, and develop typical green gray, water-soaked lesions (Dodman and Flentje, 1970).

Among the cultivated rice varieties, the level of resistance to rice sheath blight is low. The conventional method for the control of fungal diseases has been mainly the use of synthetic fungicides. However, chemical control is less acceptable due to the increase in the development of resistance, lack of specificity towards the target pathogen and adverse effect on environment and beneficial microbes (Mcneil *et al.*, 2010). Fungicide application may not be economically feasible and the time of application appears to be important.

Keeping this background, the following objectives were formulated to manage the sheath blight disease of rice, viz., 1) Isolation of the causal organism of rice sheath blight (*Rhizoctonia solani*) and proving the pathogenicity 2) Physiological and nutritional requirement by the pathogen for its growth and 3) Morphology and molecular characterization of *R. solani* by using ITS primers.

## Materials and Methods

### Plant materials and experimental site

Paddy variety ADT 49 was used throughout the experiment. Seeds were obtained from Tamil Nadu Rice Research Institute (TRRI), Aduthurai. The experiments on proving pathogenicity of rice sheath blight (ShB) pathogen *R. solani*, isolation of bacterial antagonists from rice rhizosphere, *in vitro* studies and induced systemic resistance were conducted at the TRRI, Aduturai, Thanjavur district.

### ShB disease assessment - *Symptomatology*

ShB disease of rice caused by *R. solani* inflicts damage to the outer most sheath covering the stem portion at maximum tillering or panicle initiation stage. Symptoms initially occur on leaf sheaths near the water line as water-soaked lesions which are greenish gray in colour. As the disease progresses, they enlarge and tend to coalesce. Secondary infections are caused by hyphae growing upward towards uninfected plant parts, producing additional lesions and sclerotia to complete the 1<sup>st</sup> cycle. Such sclerotia fall down and spread to adjacent plants through irrigation water. These sclerotia can survive in soil for longer period and the inoculum can survive in the infected plant debris. Large lesions formed on infected sheaths of lower rice leaves may lead to softness of the stem and lodging thereby causing considerable yield loss. Visible disease symptoms include formation of lesions, plant lodging and presence of ill-filled grains.

### Survey

A survey was conducted during the year 2017 to assess the severity of sheath blight disease in different rice growing areas of Tamil Nadu viz., Mayiladuthurai, Aduthurai, Madurai, Vellore and Thiruvavur districts during August, 2017. Per cent disease index (PDI) and severity was calculated by the following formula using the Relative Lesion Height (Singh *et al.*, 2015) and ShB grade chart (IRRI SES, 2014).

$$PDI = \frac{\text{Sum of individual ratings}}{\text{Total number of plants observed}} \times \frac{100}{\text{Maximum grade}}$$

### Rice sheath blight grade chart

Disease grade	Lesion height
0	No infection observed
1	Lesion limited to lower 20 per cent of the height of the plant
3	Lesion limited to 21-30 per cent of the height of the plant
5	Lesion limited to 31-45 per cent of the height of the plant
7	Lesion limited to 46-65 per cent of the height of the plant
9	Lesion more than 65 per cent of the height of the plant

$$\text{Relative Lesion Height (RLH)} = \frac{\text{Lesion height}}{\text{Plant height}} \times 100$$

The severity of the disease was calculated by using the following formula (Knaus *et al.*, 1985).

$$\text{Severity} = \frac{0(N_0) + 5(N_1) + 10(N_3) + 30(N_5) + 50(N_7) + 100(N_9)}{\text{Total number of tillers observed}} \times 100$$

Where, N<sub>0</sub>-N<sub>9</sub> = Number of tillers classified as grade 0-9

### Isolation and purification of rice ShB pathogen

**Preparation of Potato Dextrose Agar (PDA)** - PDA was prepared as described by Islam *et al.*, (2009). Two hundred grams of peeled and sliced potato was boiled in 500 ml water in a bowl for about half an hour. The extract of potato was filtered through a filter. The other ingredients *viz.*, 20g each of dextrose and agar were added in the extract and the volume was made up to 1 liter. The prepared PDA was poured in 1000ml conical flask and sterilized in autoclave 121°C with 1.1 kg/cm<sup>2</sup> pressure for 15 minutes.

**Isolation of *R. solani* from infected samples** - The infected rice sheaths showing the typical symptoms of rice sheath blight collected from different rice growing areas were used for the isolation of the pathogen. The diseased sheath tissues were washed in running tap water, cut into small bits of approximately 5mm long by means of a surface sterilized scalpel. The bits were further surface sterilized in 0.1 per cent mercuric chloride for 30 seconds and subsequently, washed three times in repeated changes of sterile distilled water. They were then placed on sterilized Petri dish containing PDA medium amended with streptomycin sulphate (16mg/l) by means of forceps. All these steps were carried out under aseptic conditions in the culture room. The plates were incubated at room temperature (28 ±2°C) for 4 days and observed for growth of the pathogen. The pure cultures of the pathogen were obtained by single hyphal tip method (Rangaswami and Mahadevan, 1998). The purified cultures of five isolates from five districts were maintained on PDA slants for further studies. They were designated as Rs<sub>1</sub>, Rs<sub>2</sub>, Rs<sub>3</sub>, Rs<sub>4</sub> and Rs<sub>5</sub>. Based on the morphological and

microscopic observations, the isolates were confirmed as *R. solani*.

### Pathogenicity and Virulence study

**Preparation of inoculums** - Fresh stem bits of rice plants were collected from the field. Stem bits were washed with running tap water to remove soil particles. They were cut into small pieces of 2cm length by using sterilized scissor, washed with sterile water and spreaded on blotter paper. Then the bits were filled in conical flask and plugged with nonabsorbent cotton and sterilized in autoclave at 1.4 kg cm<sup>2</sup> pressure for 30 minutes. The flasks containing stem bits were transferred to laminar air flow chamber. Three numbers of 8 mm mycelial discs taken from 5 days old *R. solani* culture were transferred to each conical flask. They were incubated at room temperature for 15 days. Similar inoculation was done for all the isolates of *R. solani*. The mycelial growth covered all the bits in flask and produced brown sclerotia after fifteen days of inoculation. These stem bits without sclerotia were used as source of *R. solani* inoculum for artificial inoculation.

**Inoculation for proving pathogenicity** - The pathogenicity of *R. solani* isolates was proved by Koch's postulates on the paddy variety ADT 49 under pot culture conditions. The plants were raised in earthen pots of 30cm diameter containing 3kg of pot mixture soil. Three seedlings per pot were maintained. At panicle initiation stage, the rice stem bit inoculum of *R. solani* was placed between the leaf sheath and culm just above the water level. Three sheaths of inner tillers per hill and three hills per pot were inoculated and replicated thrice. Similar inoculation was done for all the isolates separately. Inoculated plants were observed for the ShB symptoms regularly. After six days, the lesions produced by different isolates were measured by following the Standard Evaluation System (SES) developed by IRRI (1980). The plants inoculated with uninoculated autoclaved stem bits served as control. The pathogen was re-isolated from the typical ShB lesions and the characters were compared with those of original. The ShB severity



and Relative Lesion Height (RLH) was measured for each isolate. The isolate which showed maximum ShB severity and RLH was considered as most virulent one and taken for further studies.

### Growth and cultural characters of *R. solani*

**Morphological characters** - Nine mm mycelial disc was cut from 5 days-old culture using sterilized cork

borer. Then it was placed at the center of petri plates containing 20 ml of solidified PDA, replicated thrice and incubated at room temperature ( $28 \pm 2^\circ\text{C}$ ). The growth and morphological characters *viz.*, colour of mycelium, colony diameter and morphology, number of sclerotia per Petri plate, colour of sclerotia and arrangement pattern on PDA were recorded. The colony diameter was measured using a meter scale.



Stem bit culture



Inoculation of stem bits at Centre of the sheath portion



Expression of symptoms on outer leaf sheath

Figure 1: Artificial inoculation of *Rhizoctonia solani* in rice plants by stem bit culture method

### Cultural characters

**Growth on different media** - Seven different growth media *viz.*, PDA, Czapek's Dox agar, carrot dextrose agar, Richards's agar and beetroot dextrose agar were prepared separately and autoclaved at  $1.4 \text{ kg/cm}^2$  pressure for twenty minutes. Nine mm mycelial disc of *R. solani* was cut from 5 days-old culture using cork borer and placed at the center of each Petri plate containing 20 ml of different growth media. The plates

were incubated for five days at room temperature ( $28 \pm 2^\circ\text{C}$ ). Three replications were maintained for each treatment. The mycelial growth on different growth media was observed regularly.

**Growth in different carbon, nitrogen sources, pH of solid media** - The Czapek's Dox medium with carbon sources *viz.*, sucrose or glucose, or dextrose or starch or maltose and of nitrogen sources *viz.*, ammonium nitrate or peptone or sodium nitrate or potassium



nitrate or ammonium sulphate along with different pH viz., 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 using 0.1 N HCl or NaOH was prepared and autoclaved at 1.4 kg/cm<sup>2</sup> pressure for 20 minutes. Czapek's Dox medium without any carbon and nitrogen sources was kept as control. Three replications were maintained for each treatment. Nine mm mycelial disc of *R. solani* was cut from 5 days old culture using cork borer and placed at the center of each Petri plate containing 20 ml of medium. The plates were incubated for five days at room temperature (28±2°C) and growth was observed regularly.

### **Molecular characterization of *R. solani* isolates**

**Genomic DNA extraction from *R. solani*** - Genomic DNA was extracted from the pure cultures of *R. solani* isolates using CTAB (Zolan and Pukkila, 1986). Fresh mycelium of approximately 100 - 200g was ground by using liquid nitrogen until it gets powdered. The powdered mycelium was transferred into Eppendorf tube and added with 700 µl of CTAB buffer (700 mM of NaCl, 50 mM of Tris HCl with pH 8.0, 10 mM of EDTA, 2% CTAB, and 1% mercapto-ethanol). The samples were then incubated at 65°C for 15 minutes and added with 750µl of Phenol: Chloroform: Isoamyl alcohol (25:24: 1, v/v). The tube was inverted to form an emulsion and centrifuged at 10000 rpm for 10 min at 4°C. The upper phase was transferred into new tube, added with equal volume of Phenol: Chloroform: Isoamyl alcohol, (25:24: 1, v/v) and centrifuged at 4°C for 10 minutes. The upper phase was transferred into new Eppendorf tube and added with 0.5 vol (150 µl) of 3M sodium acetate or 5M NaCl and 600 µl of ice cold isopropanol. The content was mixed well and incubated at -20°C for overnight. Finally, the supernatant was precipitated by centrifugation at 10,000 rpm and the pellet was washed twice with 70 per cent cold ethanol, dried and dissolved in 50 µl TE buffer. DNA was stored at -20°C for further studies.

### **PCR amplification of ITS region of *R. solani* isolates**

- The Internal Transcribed Spacer (ITS) gene region

of *R. solani* isolates was amplified using following primer pairs as per the protocol (White *et al.*, 1990).

Forward primer: ITS1 (5'-TCCTGTAGGTGAACCTGCGG-3')

Reverse primer: ITS4 (5'-TCCTCCGCTTATTGATATGC - 3')

The PCR mixture consisted of 5-10 ng of genomic DNA, 1µM each of ITS1 and ITS4 primers, 3 mM MgCl<sub>2</sub>, 200µM each of dNTP and 2.5 U of Taq DNA polymerase in a total volume of 50 µl. The PCR was carried out with a Master cycler gradient (Eppendorf, Germany) programmed for preheating at 95°C for 5 minutes followed by 40 cycles consisting of denaturation at 94°C for 45 seconds, annealing at 58°C for 1 min and extension at 72°C for 2 min with a final extension at 72°C for 8 min and final hold at 4°C. The PCR products were separated by electrophoresis in a 1.2 per cent agarose gel stained with Ethidium bromide and visualized under UV light and documented using Alpha imager (Alpha Innotech, California, USA). The size of PCR products was measured using 100 bp DNA ladder (Bangalore Genei, Pvt. Ltd., Bangalore).

### **Results and Discussion**

**Survey on rice ShB** - The rice ShB disease severity ranged from 16 to 64 per cent in different rice growing areas of Tamil Nadu. The maximum severity index of 64 per cent was recorded at TRRI, Aduthurai in Thanjavur district of Tamil Nadu followed by Tiruvayur district with 56% PDI.. This might to be due to the high population and virulence of the pathogens, sclerotia present in the soil, continuous cropping of rice, intensive cultivation and the susceptibility of host to pathogen. Neha *et al.*, (2016) reported higher incidence of sheath blight in Naduthittu followed by Vadakkumangudi in Cuddalore district of Tamil Nadu. Savary *et al.*, (2000) estimated a yield loss up to 69% by rice sheath blight in Tamil Nadu. Losses due to rice sheath blight generally varies from 30 to 40 per cent (Srinivas *et al.*, 2013).

**Table 1: Survey on assessment of sheath blight disease in rice in Tamil Nadu**

S. No	Isolate	Places	District	Stage of the crop	Percent Disease Index (PDI)	Sclerotial colour	Sclerotial pattern	No of sclerotia / plate	Disease severity (Artificial condition)
1.	Rs <sub>1</sub>	Mayiladuthurai	Nagapattinam	Grain filling	30	Dark brown	Scattered	53	25.83
2.	Rs <sub>2</sub>	Aduthurai	Thanjavur	Tillering	64	Light brown	Periphery	86	53.80
3.	Rs <sub>3</sub>	Madurai	Madurai	Tillering	24	Light brown	Scattered	38	23.45
4.	Rs <sub>4</sub>	Vellore	Vellore	Grain filling	16	Light brown	Periphery	17	16.18
5.	Rs <sub>5</sub>	Thiruvarur	Thiruvarur	Tillering	56	Dark brown	Centre	82	40.23

**Symptomatology** - Under artificial inoculation, the symptoms of rice ShB appeared on the outer most sheath at maximum tillering and flowering stage of the plants near the water level. Initially the pathogen produces pale green soaked lesions seven days after inoculation

**Pathogenicity**- In the present study, rice ShB pathogen was inoculated by rice stem bit method. All the isolates were pathogenic and produced water soaked lesions on the sheath portion of plants under pot culture conditions. Among the five isolates of rice sheath blight, Rs<sub>2</sub> was identified as the most virulent one which recorded 53.80 per cent severity followed by Rs<sub>5</sub> with disease severity of 40.23 per cent (Table 1). The most virulent isolate Rs<sub>2</sub> was collected from TRRI, Aduthurai in Thanjavur district while the isolate Rs<sub>4</sub> collected from Vellore district, was less virulent. The results indicated variations in virulence depending on the continuous availability of host. Prasad *et al.*, (2010) inoculated *R. solani* in rice plants by straw bit method and the pathogen produced typical water soaked lesions seven days after inoculation.

**Morphological characters** - Morphological characters are important tool for identification and classification of fungus. The colour of the mycelium initially white later turned to light brown in all the five isolates and the angle of branching of mycelium

was right angle. Among five isolates, the mycelium of Rs<sub>1</sub>, Rs<sub>2</sub>, Rs<sub>3</sub> and Rs<sub>4</sub> isolates were flat in nature. Rs<sub>5</sub> isolate showed fluffy mycelial growth (Table 2). The mycelia of all the isolates were septate and showed right angle branching. The above characters were in agreement with those of the original descriptions given by Guleria *et al.*, (2007) and Lal *et al.*, (2014).

#### **Cultural characters of *R. solani***

**Growth of *R. solani* on different solid media** - Rs<sub>2</sub> isolate exhibited variation in their growth in different solid media. The maximum mycelial growth of 8.85 cm and sclerotial production of 89 per plate was observed in PDA medium followed by Beetroot Agar (8.62 cm) with sclerotial production of 85 per plate, while Richards's agar medium recorded the minimum mycelial growth of 7 cm with sclerotial production of about 73 per plate (Table 2). Similar findings were reported by several workers (Anilkumar and Sastry, 1982 ; Wasser *et al.*, 1988).

**Growth of *Rhizoctonia solani* on different Nitrogen sources** - The virulent isolate of *R. solani* (Rs<sub>2</sub>) obtained from Aduthurai was tested in different nitrogen sources. Among the different nitrogen sources, peptone recorded the maximum mycelial growth of 8.57 cm with number of sclerotia of 86.67 per plate followed by Ammonium sulphate with 7.5 cm growth and 73.67 sclerotia per plate. Potassium

**Table 2: Morphological characters and nutrient requirement of *Rhizoctonia solani* isolated from different places**

S. No	Isolate	Colony type	Colony colour	Growth rate of mycelium at 4 DAI	Media	Diameter of mycelial growth at 4 DAI (cm)*	Number of sclerotia / plate**	Nitrogen sources	Mycelial growth (cm)*at 4 DAI	Number of sclerotia per plate at 7 DAI*	Carbon sources	Mycelial growth (cm)*at 4 DAI	Number of Sclerotia per plate *
1.	Rs <sub>1</sub>	Flat	Dark brown	7.53	Potato dextrose agar	8.85	86	Ammonium nitrate	7.25	73.00	Maltose	6.00	4.00
2.	Rs <sub>2</sub>	Flat	Light brown	8.85	Richard's agar	7.20	70	Ammonium sulphate	7.50	73.67	Glucose	7.92	78.67
3.	Rs <sub>3</sub>	Flat	Light brown	7.20	Czapek's dox agar	7.60	73	Peptone	8.57	86.67	Sucrose	7.40	72.67
4.	Rs <sub>4</sub>	Flat	Light brown	6.76	Beetroot dextrose agar	8.62	83	Potassium nitrate	7.13	70.00	Dextrose	7.59	76.67
5.	Rs <sub>5</sub>	Light fluffy	Dark brown	8.27	Carrot dextrose agar	8.30	79	Sodium nitrate	7.25	72.00	Starch	6.96	70.00
					<b>SED</b>	<b>0.14</b>		Control	7.00	4.00	Control	5.40	2
					<b>CD</b>	<b>0.26</b>			<b>0.15</b>	<b>1.76</b>		<b>0.17</b>	<b>1.50</b>
									<b>0.33</b>	<b>3.84</b>		<b>0.36</b>	<b>3.95</b>

\*\* 7 Days after Inoculation; \*Mean of three replications; DAI – Days After Inoculation



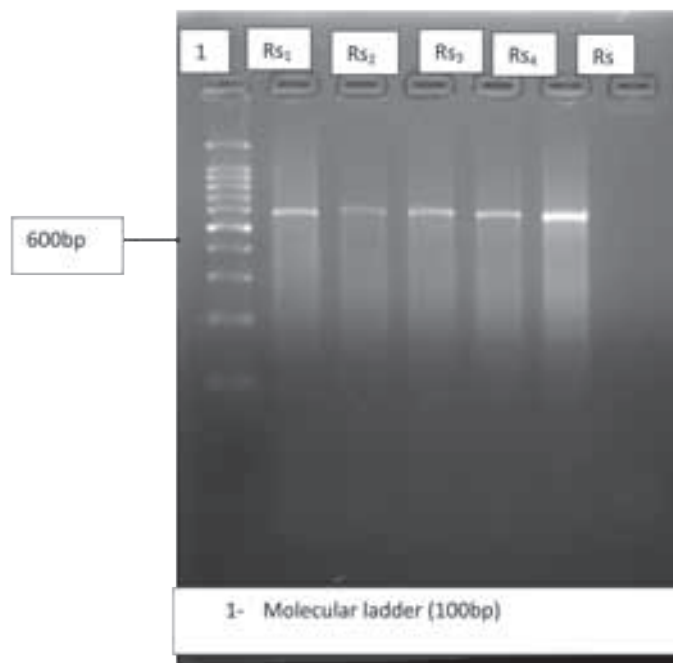
nitrate showed the minimum mycelial growth (7.13 cm) and sclerotia (70 per plate). Pal and Kaushik (2012) and Saha *et al.*, (2008) also reported the maximum growth of *R. solani* in peptone nitrogen source. (Table 2).

**Growth of *R. solani* on different carbon sources -** With respect to carbon sources, the results revealed that glucose was the best carbon source recording the maximum mycelial growth of 7.92 cm with sclerotial production of 78.67 per plate, followed by dextrose which showed a mycelial growth and sclerotial production of 7.59 cm and 76.67 per plate, respectively (Table 2). Maltose recorded the minimum mycelial growth of 6.8 cm and sclerotia of 64 per plate. Pal and Kaushik (2012) observed the maximum growth of *R. solani* in glucose source.

**Growth of *R. solani* on different pH levels -** In the present study, maximum mycelial growth was observed at pH 7.0 (8.7 cm) with 84.5 sclerotia per plate followed by pH 6 with a mycelial growth and sclerotia of 8.27 cm and 82 per plate, respectively (Table 3). The least mycelial growth (6.31 cm) and sclerotial production (66 per plate) was observed in pH 8.0. Goswami *et al.*, (2011) also reported that pH 7 supports the maximum mycelial growth followed by pH 6.

**Table 3: Effect of pH on the mycelial growth of *Rhizoctonia solani* (Rs<sub>2</sub>) under *in vitro* conditions**

S. No	pH	Mycelial growth (cm)*at 4 DAI	Number of sclerotia per plate*
1.	5	6.48	72.00
2.	5.5	6.33	68.3
3.	6	8.27	82.00
4.	6.5	8.13	79.67
5.	7	8.7	84.50
6	7.5	6.45	71.60
7	8	6.31	66.00
<b>SEd</b>		<b>0.09</b>	<b>1.52</b>
<b>CD</b>		<b>0.22</b>	<b>3.27</b>



**Figure 2: Molecular identification of *Rhizoctonia solani***

\*Mean of three replications ; DAI – Days After Inoculation

**Molecular identification of *R. solani* isolates -** All the isolates were molecularly characterized using ITS 1 and ITS 2 primer pairs. The result stated an amplicon of 600 bp and confirmed that all the five isolates belong to the genus *Rhizoctonia* (Figure 2). Molecular techniques such as using specific primers for species identification has quicker result compared to conventional methods (Moni *et al.*, 2016). Molecular characterization also gives a real time result and can distinguish isolates from each host and even from different geographic areas.

Salazar *et al.*, (2000) designed specific ITS 1F and ITS 4R based on specific sequences of the ITS regions in the *R. solani* species complex. They also showed that PCR detection has time saving advantages over traditional tool for identification. ITS region of the *R. solani* can be amplified using universal primers ITS1 and ITS 4 and it can yield an amplicon of 700 bp size in all the isolates of *R. solani*. Rani *et al.*, (2013) amplified ITS region of *R. solani* from cotton using ITS1 and ITS 4 primers. Nadarajah *et al.*, (2014) characterized the Malaysian isolates of *R. solani* at molecular level.

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## Evaluation of a liquid formulation of *Pseudomonas fluorescens* for management of sheath blight in paddy

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### Abstract

Sheath blight is a soil-borne disease caused by the fungus *Rhizoctonia solani* and one of the most economically significant rice diseases worldwide. Two year pooled results of field experiment revealed minimum disease intensity (11.8%) and maximum reduction (58.0%), maximum yield of paddy (3750.7 kg/ha) and cost benefit ratio (1.75) from the treatment *Pseudomonas fluorescens* @ 2.0 l/ha as foliar spray which was at par with *P. fluorescens* @ 3ml/kg as seed treatment followed by 1.5 l/ha as foliar spray (PDI 10.3 and PDC 64.5%) with grain yield (3911 kg/ha) as compared to control. However, standard check with fungicide Hexaconazole 5% EC @ 2ml/kg as seed treatment followed by 1.0 l/ha as foliar spray gave minimum disease intensity (9.3%), maximum disease control (67.9%) and highest yield of paddy (3944 kg/ha) with highest cost benefit ratio (1.95).

**Keywords:** Paddy, sheath blight, *Rhizoctonia solani*, *Pseudomonas fluorescens* and bio-agent

### Introduction

Rice (*Oryza sativa* L.) is the second most important cereal and the staple food for more than half of the world's population. It provides 20 per cent of the world's dietary energy supply followed by Maize and Wheat. Sheath blight incited by *R. solani* is one of the potentially devastating disease of rice in almost all over the rice growing countries of the world. In India, intensive and extensive cultivation of rice mostly under rice-wheat cropping system have resulted heavy losses to the crop ranging from 5.2 to 70 per cent in yield. Losses due to sheath blight disease generally vary from 30 to 40 per cent and may be even 100 per cent in endemic areas (Li *et al.*, 2009). The reduction in yield due to the disease has been estimated to vary from 5.2 to 50.0 per cent (Ou, 1985, Hori 1969). Per cent relative lesion height (RLH) was to the tune of 68.68 percent (Naik *et al.*, 2017). The disease development was favoured due to the application of high doses of nitrogenous

fertilizers, close plant spacing, widespread cultivation of high yielding varieties and weather conditions such as low light, cloudy days and high relative humidity. Infected plants are usually found in a circular pattern, locally referred to as 'bird's nest' (Hollier *et al.*, 2009). The disease appears at tillering stage on leaf sheath as elliptical or oval to irregular, 1-3 cm long, greenish gray spots with brown margin at or above the water line. Presence of many such spots on the leaf sheath gives the appearance of snake skin. Under favourable conditions, the infection spreads rapidly to the upper plant parts and also to the neighbouring plants by means of normal emergence and expansion of the ears and results in poor filling of the grains. The pathogen is also known to cause panicle infection resulting in production of unfilled or partially filled discoloured seed bearing brownish black spots or black to ashy gray patches (Acharya *et al.*, 2004). The use of pesticides caused an incredible harm to the environment and hazard in natural ecosystems, which resulted into replacement of chemicals with biological



approaches, which are considered more environment friendly in the long term. One of the emerging research area for the management of different phytopathogenic factors is the application of antagonistic plant growth promoting rhizobacteria (PGPR), which are capable of suppressing the phytopathogen damage. Therefore, the present study was undertaken to assess the bio-efficacy of *Pseudomonas fluorescens* for the management of Sheath blight in paddy.

## Materials and Methods

The experiment was conducted at Agricultural Research Station, Agriculture University, Kota during *Kharif* 2018 and 2019 to evaluate the bio-efficacy of liquid formulation of *P. fluorescens* in managing sheath blight in paddy. The experiment was laid out in a Randomized Block Design (RBD) with three replications and nine treatments comprised of testing of *P. fluorescens* as a positive check *i.e.* Hexaconazole 5% EC @ 1.0 l/ha and one untreated control plot for comparison. Paddy (*cv.*P-1509) was raised by using all recommended agronomical package of practices in both the seasons. Treatments were sprayed twice using a hand operated knapsack sprayer fitted with hollow cone nozzle and water volume of 500 lit/ha was maintained. Seedling dip treatment and first spraying was given just after the appearance of the disease and second was given 14 days after the first spray. Observations on diseases were recorded 15 days after each spray. The PDI was calculated on five plants / sampling unit by counting the number of infected tillers. Each selected plant was approximately divided into three portions as bottom, middle and top. The disease severity was assessed based on the Standard Evaluation System (0-9) of IRRI during the experimentation.

The percent disease Index (PDI) was finally calculated using the following formula:

$$PDI = \frac{\text{Sum of numerical rating}}{\text{Total number of hills observed} \times \text{maximum of score}} \times 100$$

Scoring of the diseases was done as per the scales given below.

### Scale for sheath blight disease

Score	Description
0	No infection
1	Vertical spread of the disease up to 20% of plant height
3	Vertical spread of the disease up to 21-30% of plant height
5	Vertical spread of the disease up to 31-45% of plant height
7	Vertical spread of the disease up to 46 – 65% of plant height
9	Vertical spread of the disease more than 66% of plant height

**Yield:** Plot wise yield recorded after harvest and then calculated in to Kg/ ha.

## Results and Conclusion

The results of field experiment revealed that there were significant differences among the treatments in reducing sheath blight severity (Table 1). Results revealed that Hexaconazole 5% EC @ 1lit/ha significantly reduced disease severity (68.00%) and disease intensity (9.3%) compared to control (29.0%). Significant differences of *P. fluorescens* treatment effects were also recorded on percent disease incidence as well as on tillers and yield. Among the different doses of *P. fluorescens*, the disease intensity was found significantly lowest (10.3%) and maximum reduction (64.5%) in the treatment (T7) *P. fluorescens* @ 3ml/kg as seed treatment followed by 1.5 lit/ha as foliar spray which was at par with (T6) *P. fluorescens* @ 2.0 lit/ha as foliar spray (PDI 11.8 and PDC 59.3%). The highest yield of paddy (3944.2 kg/ha) was recorded in Hexaconazole 5% EC @ 2ml/kg as seed treatment followed by 1.0 lit/ha as foliar spray (T8) which was at par with *P. fluorescens* @ 3ml/kg as seed treatment followed by 1.5 lit/ha as foliar spray (3911.7 kg/ha) and *P. fluorescens* @ 2.0 lit/ha as foliar spray (3750.7



kg/ha) as compared to control (2766.3 kg/ha). The highest cost benefit ratio of 1.95 was obtained with Hexaconazole 5% EC @ 2ml/kg as seed treatment followed by 1.0 lit/ha as foliar spray. Whereas, among

the biological treatments, maximum cost benefit ratio (1.75) was recorded by *P. fluorescens* @ 2.0 lit/ha as foliar spray against sheath blight of paddy.

**Table 1: Bio-efficacy of *P. fluorescens* on sheath blight in rice (pooled)**

Treatments	Infected tillers (%)	Yield /ha (kg)	% Disease control	B:C Ratio
T1: <i>P. fluorescens</i> @ 2ml/kg as seed treatment	27.2 (5.2)	2836.3	6.6	1:1.12
T2: <i>P. fluorescens</i> @ 3ml/kg as seed treatment	22.5 (4.7)	2933.3	22.4	1:1.17
T3: <i>P. fluorescens</i> @ 4ml/kg as seed treatment	20.7 (4.5)	3200.8	28.6	1:1.35
T4: <i>P. fluorescens</i> @ 1.0 lit/ha as foliar spray	18.0 (4.2)	3264.2	37.9	1:1.44
T5: <i>P. fluorescens</i> @ 1.5 lit/ha as foliar spray	14.3 (3.8)	3427.5	50.7	1:1.54
T6: <i>P. fluorescens</i> @ 2.0 lit/ha as foliar spray	11.8 (3.6)	3750.7	59.3	1:1.75
T7: <i>P. fluorescens</i> @ 3ml/kg as seed treatment followed by 1.5 lit/ha as foliar spray	10.3 (3.2)	3911.7	64.5	1:1.73
T8: Hexaconazole 5% EC @ 2ml/kg as seed treatment followed by 1.0 lit/ha as foliar spray	9.3 (3.1)	3944.2	67.9	1:1.95
T9: Untreated check	29.0 (5.4)	2766.3	-	1:1.11
LSD @ 5%	2.61	212.52	-	-

Values in parentheses are square root transformed values of three replications

Results are inconformity with Johnson *et al.*, (2013) who found hexaconazole as an effective fungicide against sheath blight of rice. Naik *et al.*, (2017) found that the field efficacy of hexaconazole 5EC (0.1%) and propiconazole 25EC (0.1%) could be used effectively for the management of sheath blight disease of rice and thus helped for getting higher grain yield and B: C ratio. *P. fluorescens* is ideal antagonist as they have ability to produce various inhibitory antimicrobial compounds (Schroth and Hancock, 1982). Maximum increase of all the growth parameters was recorded with combination of *P. fluorescens* and *T. harzianum*. Singh and Sinha (2009) observed growth promotion due to seed treatment with *T. harzianum* and *P. fluorescens* which enhanced nitrogen uptake and alleviated drought stress in rice crop. Tiwary and Trimurthy (2009) also reported lowest disease severity under field conditions through seed treatment along with two foliar sprays of *P. fluorescens* over foliar

application of carbendazim 50% WP as untreated control. Neha *et al.*, (2016) also recorded that seed treatment with *P. fluorescens* @ 10 ml kg<sup>-1</sup> along with seedling dipping of roots @ 3.0 l ha<sup>-1</sup> significantly enhanced the germination (75.82%), plant height (25.52), number of tillers (16.99) and grain yield (48.23 g pot<sup>-1</sup>). *P. fluorescens* has emerged as a potential bio control agent due to different modes of action such as competition for nutrients and space, antibiosis, mycoparasitism, induced systemic resistance, production of siderophore, phytohormones, production of lytic enzymes and various secondary metabolites such as DAPG, Phenazine, pyoluteorin, pyrrolnitrin, which are helpful in reducing pathogen population within soil (Gupta *et al.*, 2002). Thus, the finding of present investigations is quite in conformity with the reports of earlier workers. However, hexaconazole (triazole fungicide) is a potent ergosterol biosynthesis inhibitor and sterol in most fungi and hence resulted



comparatively higher effectiveness than bio-agents, because it persists for a longer period inside the plant system and protects crop against sheath blight constantly for a longer period.

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## Incidence of false smut on rice genotypes and the influence of weather factors on the disease

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### Abstract

False smut disease of rice is evolving as severe grain disease in most of the rice growing regions of the world. During *Kharif*, 2020 heavy incidence of false smut was observed. Hence, assessment of the incidence of the disease on different rice genotypes under natural field conditions was done at Rajendranagar farm, IIRR. The results revealed that average disease incidence ranged between 26.5 - 87.8% and the average number of smut balls per panicle varied from 1-26 with the mean value of 57.77% and 10.15, respectively. The weather data indicated that maximum rainfall recorded during the months of September and October provided the most favourable environmental conditions for the pathogen *Ustilaginoidea virens*. This also coincided with the 50 % flowering date of the most of the genotypes leading to the increase in the disease.

**Keywords:** False smut disease, *Ustilaginoidea virens* and Disease incidence

### Introduction

Rice (*Oryza sativa* L.) is one of the most significant cereal crops in India. It is nutritionally and agronomically most essential crop of the world and feeds nearly 3.2 billion people. Rice production in India has increased from 82.5 million tonnes during the period of 1997-98 to 114 million tonnes in 2018-19 (Anonymous, 2019). But projected figures for future indicate that the present levels of production are not sufficient to feed the population of the country. The low productivity of rice in India is ascribed to many abiotic and biotic stress factors. With respect to biotic factors, diseases *viz.*, blast, bacterial leaf blight, sheath blight, sheath rot, false smut, and brown spot are wide-spread and economically important. In recent decades, a number of minor diseases have attained the status of major importance in rice. One such disease is the rice false smut (RFS) disease which is a threat to yield and grain quality. The disease has been reported in more than 40 countries, especially in rice growing Asian nations such as China, India and Burma (Wang *et al.*, 2019).

RFS caused by *Ustilaginoidea virens* (teleomorph: *Villosiclava virens*) has become the most devastating grain disease of rice. The pathogen infects during booting to flowering stage and symptom can be observed on grains where individual grain is transformed into chlamydospores mass which results in reduced yield as well as grain quality. The disease can cause 2.8–81% yield losses in different rice-producing areas depending on the rice variety and disease intensity. Earlier the disease was considered as minor due to its sporadic occurrence but in recent decades there has been an increase in incidence because of extensive cultivation of rice hybrids and wider application of nitrogenous fertilizers (Ladhalakshmi *et al.*, 2019). RFS occurrence can be seen when incidence of rainfall coincides with the booting and heading stages of the rice crop. However, epidemics vary between varieties and season. In this study, we have undertaken a survey to know the natural incidence of false smut in various genotypes grown in Rajendranagar farm, ICAR-IIRR.



## Materials and Methods

Assessment of incidence of rice false smut disease was carried out at grain maturity stage during *Kharif* 2020 at Rajendranagar farm, IRR, Hyderabad. Data were recorded in 50 genotypes by randomly selecting 5 hills for observation. Disease incidence was assessed based on total number of tillers, number of infected tillers per hill and number of smut balls per infected panicle recorded on randomly selected hills to know the disease incidence under natural disease infestation. Percentage of infected tillers in each genotype was worked out. Weather parameters were recorded to know their effect on disease incidence.

## Results and Discussion

Incidence of false smut was observed in most of the genotypes at Rajendranagar farm, IRR. The number of yellow and greenish black coloured smut balls per panicle ranged from 1-50 with maximum disease incidence of 87.8 % (Table 1). The range, mean and standard error of mean was calculated for average disease incidence and number of smut balls. Disease incidence varied between 26.5 - 87.8% with the mean value of 57.77%. The range for smut balls per panicle varied from 1 to 26 with the mean value of 10.15 (Figure 1).

**Table 1: False smut disease incidence in different genotypes at IRR, Rajendranagar in *Kharif* 2020.**

Sl. No	Genotype Numbers	Cross combinations	Disease incidence (%)	Average No. of smut balls	Range on the no. of Smut balls/ Panicle
1.	1799	ISM X Yield genes	78.92	26.0	10 - 49
2.	1755	HWR-23 X BPT	35.99	16.4	9-24
3.	1713	MTU1010 X Yield genes	61.51	22.0	15-28
4.	1708	HWR-23 X BPT	59.33	9.0	7-10
5.	2120	ISM X Yield genes	40.99	18.6	7-50
6.	1902	HWR-23 X BPT	49.95	8.8	4-20
7.	1963	HWR-23 X BPT	83.11	16.6	8-30
8.	2067	ISM X Yield genes	74.77	12.2	8-15
9.	1932	HWR-23 X BPT	46.56	3.4	2-5
10.	1583	ISM POPULATION	26.50	6.4	5-10
11.	1824	AKD Population	80.69	9.2	7-15
12.	3008	HWR-23 X BPT	41.37	4.8	1-10
13.	1564	HWR-23 X BPT	61.94	10.2	7-14
14.	2041	MTU1010 X Yield genes	73.65	11.4	3-20
15.	1966	ISM POPULATION	36.52	10.2	5-16
16.	816	HWR-23 X BPT	45.05	8.6	3-13
17.	1955	HWR-23 X BPT	79.01	18.6	14-22
18.	2724	ISM POPULATION	53.68	4.8	3-6
19.	3396	HWR-23 X BPT	49.79	12.4	8-20
20.	3061	MTU1010 X Yield genes	40.33	3.6	3-5
21.	2804	Swarna x Yield population	52.94	6.8	4-11
22.	2726	ISM POPULATION	45.05	5.6	2-11
23.	2410	ISM X Yield genes	57.61	4.0	1-9
24.	2387	ISM Parent	52.59	2.0	1 -3
25.	2728	AKD Population	47.46	11.6	7-19
26.	2972	HWR-23 X BPT	72.43	8.0	1-14

Sl. No	Genotype Numbers	Cross combinations	Disease incidence (%)	Average No. of smut balls	Range on the no. of Smut balls/ Panicle
27.	3133	HWR-23 X BPT	57.53	1.0	0-2
28.	2722	HWR-23 X BPT	33.40	3.6	1 -5
29.	3230	MTU1010 X Yield genes	51.14	8.4	5-12
30.	2798	AKD Population	50.32	3.2	1 -5
31.	2279	HWR-23 X BPT	55.37	5.2	4-7
32.	2400	ISM POPULATION	45.38	3.2	0-6
33.	2716	ISM Parent	65.21	15.6	5-29
34.	3450	ISM POPULATION	44.04	4.75	1-20
35.	2295	ISM X Yield genes	86.42	20.4	9-27
36.	2286	HWR-23 X BPT	61.00	6.4	4-9
37.	2096	HWR-23 X BPT	69.11	16.4	2-27
38.	2137	ISM POPULATION	68.90	7.8	2 - 17
39.	3080	ISM POPULATION	55.14	3.8	2-6
40.	2161	HWR-23 X BPT	55.72	11.4	5-23
41.	2039	ISM POPULATION	44.36	7.6	4-11
42.	1950	HWR-23 X BPT	87.83	24.0	10-45
43.	756	Swarna x Yield population	61.18	12.4	8-15
44.	2072	Swarna parent	75.29	3.2	2-4
45.	1830	HWR-23 X BPT	69.52	14.8	5-42
46.	65	ISM POPULATION	51.07	7.6	6-10
47.	903	HWR-23 X BPT	70.23	19.8	12-32
48.	750	ISM X Yield genes	55.51	12.4	5-23
49.	1637	MTU1010 X Yield genes	64.12	11.0	3-19
<b>Range</b>			<b>26.5 - 87.8</b>	<b>1-26</b>	
<b>Mean</b>			<b>57.7</b>	<b>10.15</b>	
<b>SEM</b>			<b>2.1</b>	<b>0.86</b>	

Weather data revealed that there was heavy rainfall (208.2 mm) in the third week of September 2020 followed by 4<sup>th</sup> week (85.8 mm) along with low maximum (29.4°C) and minimum (21.3°C) temperatures and sun shine hours (2.3 hrs). High rainfall continued in second and third weeks of October with total rainfall of 196.8 and 147.8 mm, respectively. Similarly, the maximum and minimum temperature was low in 2<sup>nd</sup> and 3<sup>rd</sup> weeks compared to 1<sup>st</sup> and 4<sup>th</sup> weeks of October, 2020 (Table 2).

The data also revealed that for different genotypes, days to 50% flowering ranged from 3<sup>rd</sup> week of September to last week of October, 2020, coinciding with the favourable weather conditions *viz.*, low maximum temperature (28°C - 29.5°C); high humidity (96 to 98%) and high amount of rainfall (147 - 208 mm), which together played a major role in increasing the disease incidence in the field.

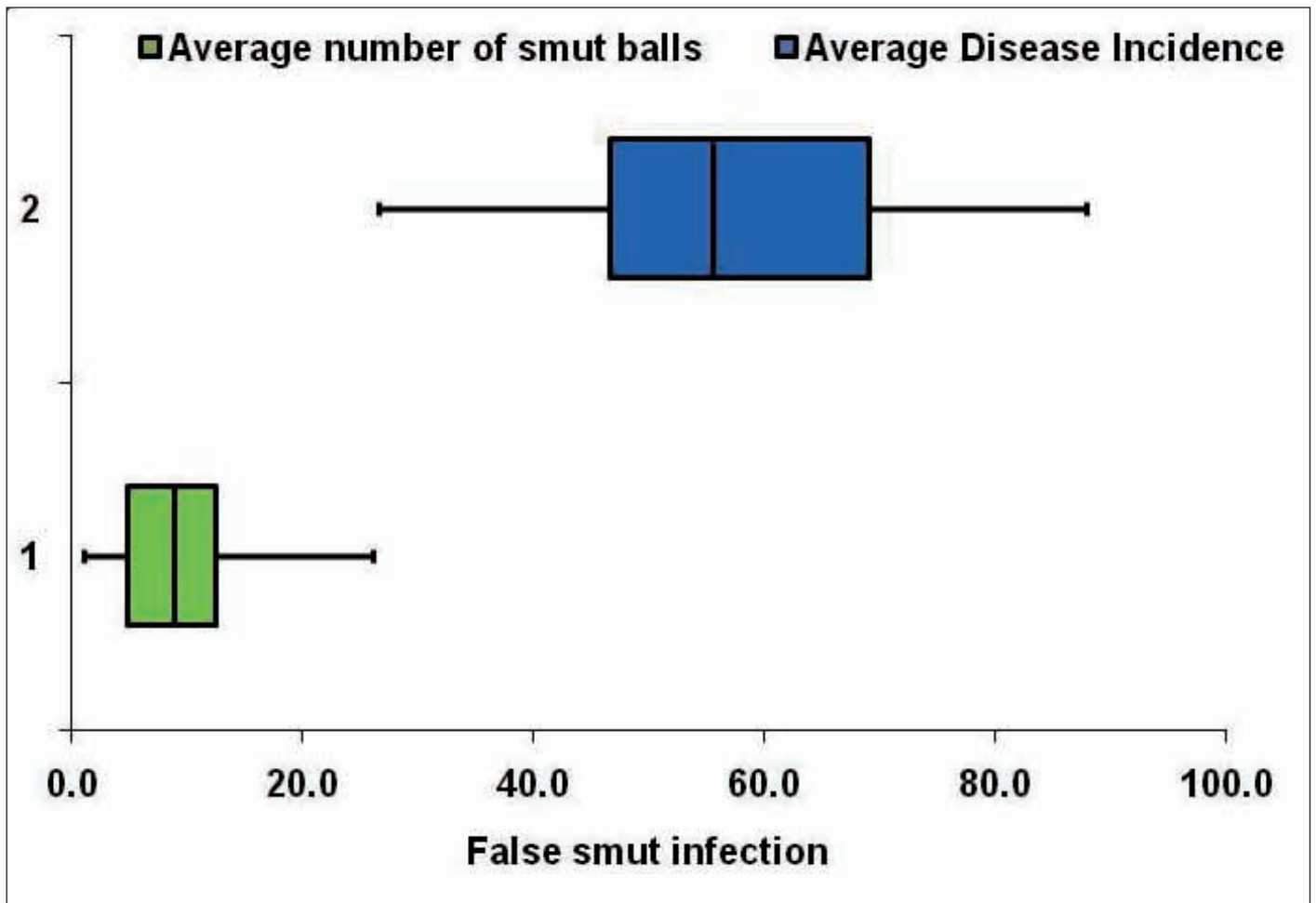


Figure 1: Box plot representation on average no. of smut balls and average disease incidence

Table 2: Meteorological data recorded at Rajendranagar for the month of September and October 2020

Weeks	Temperature (° C)		R.H. (%)		Total rainfall (mm)	Total Rainy days	SUNSHINE (hrs.)
	MAX.	MIN.	I	II			
<b>September, 2020</b>							
1 <sup>st</sup> week	32.6	22.8	91	60	5.4	1	8.6
2 <sup>nd</sup> week	31.6	21.4	96	72	69.6	5	4.8
3 <sup>rd</sup> week	29.4	21.3	97	30	208.2	6	2.3
4 <sup>th</sup> week	28.7	21.8	96	91	85.8	2	3.9
<b>October, 2020</b>							
1 <sup>st</sup> week	31.42	22.0	89.8	61.1	0	0	6.0
2 <sup>nd</sup> week	28.0	21.6	97	82	196.8	3	1.7
3 <sup>rd</sup> week	29.5	20.6	98	77	147.8	4	4.9
4 <sup>th</sup> week	30.9	17.9	94.8	60.2	0	0	7.1

Based on average disease incidence, the genotypes were categorized into three different groups *viz.*, group I with disease incidence of 26.5% to 50.32%, group II with 51.07% to 65.21% and group III with 68.9% to 87.83%. Table 3 revealed that with few exceptions, MTU 1010 and yield genes cross combinations recorded highest percentage of false smut disease

infection (68.9% to 87.83%) wherein the days to 50% flowering dates of the genotypes coincided with weather condition of October, 2020. Despite categorization, the overall false smut infection was moderate to high, indicating that conducive weather conditions are also the key pre-disposing factors for false smut incidence.

**Table 3: Grouping of genotypes based on the false smut average incidence along with the details of days to 50 % flowering**

False smut Disease incidence range (%) / Genotypes					
26.5 – 50.32%	Day to 50 % flowering	51.07 – 65.21%	Day to 50 % flowering	68.9 – 87.83%	Day to 50 % flowering
HWR-23 X BPT	25/09/20	ISM Parent	10/10/20	Swarna x Yield population	28/10/20
HWR-23 X BPT	12/10/20	ISM Population	18/10/20	MTU1010 X Yield genes	18/10/20
HWR-23 X BPT	23/09/20	HWR-23 X BPT	12/10/20	HWR-23 X BPT	23/09/20
ISM X Yield genes	22/10/20	ISM Population	12/10/20	HWR-23 X BPT	22/09/20
ISM Population	22/10/20	HWR-23 X BPT	10/10/20	ISM Population	21/10/20
Swarna parent	24/10/20	ISM Population	22/10/20	MTU1010 X Yield genes	15/10/20
ISM Population	22/10/20	AKD Population	16/10/20	MTU1010 X Yield genes	14/10/20
ISM Population	16/10/20	ISM Population	27/09/20/	MTU1010 X Yield genes	18/10/20
MTU1010 X Yield genes	10/10/20	Swarna x Yield population	24/10/20	HWR-23 X BPT	26/09/20
HWR-23 X BPT	21/09/20	ISM Population	16/10/20	ISM X Yield genes	22/10/20
HWR-23 X BPT	12/10/20	HWR-23 X BPT	15/10/20	HWR-23 X BPT	26/09/20
HWR-23 X BPT	13/10/20	HWR-23 X BPT	28/09/20	ISM X Yield genes	22/10/20
ISM X Yield genes	21/10/20	AKD Population	16/10/20	AKD Population	12/10/20
HWR-23 X BPT	11/10/20	HWR-23 X BPT	24/09/20	ISM X Yield genes	22/10/20
ISM Parent	26/10/20	HWR-23 X BPT	28/09/20		
ISM X Yield genes	21/10/20	HWR-23 X BPT	25/09/20		
ISM Population	13/10/20	HWR-23 X BPT	26/09/20		
		HWR-23 X BPT	11/10/20		



In the present study, the mean of average number of smut ball per tillers (10.15) and average disease incidence (57.77%) was higher compared to earlier reports of Baite *et al.*, (2017), Quintana *et al.*, (2016) and Singh and Pophaly (2010). However, Singh *et al.*, (2014) reported higher disease incidence between 5-80% in surveyed districts of Uttar Pradesh. Similarly, Ladhakshmi *et al.*, (2012) also reported disease incidence of 2-75% in North western states and 5-85% in southern state of Tamil Nadu.

The study conducted at IRR showed that rice false smut pathogen infected most of the genotypes under the natural field conditions when the weather factors were more favourable. Earlier reports have revealed higher disease incidence mostly in hot spot areas, however, the it is evident from the present study that when the weather conditions are more conducive and coincides with booting to flowering stage of the crop, the disease may occur in severe form even if the location is not a hot spot. A comprehensive study covering large areas reporting the false smut disease backed up by relevant weather data is needed to understand and prevent further spread of this disease to more areas in future.

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## An estimate on the adoption of ICAR-IIRR released varieties and their impact on rice production in India during 2015-20

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### Abstract

Rice is the most important food crop of India. ICAR-Indian Institute of Rice Research (ICAR-IIRR) was established in 1965 as All India Coordinated Rice Improvement Project (AICRIP) and has been playing a key role in the improvement of rice technologies. Extensive testing under the All India Coordinated Rice Improvement Project, which is one of the largest networks for evaluation of any single crop in India, has contributed to the release of 1375 rice varieties including 117 hybrids for all the major rice growing ecosystems of the country during the last five and half decades (1965-2020). Of the 1375 varieties released, about 267 varieties are still in the breeder seed production (BSP) chain. In the present study, an estimate was made on the area covered by the varieties released by ICAR-IIRR and their contribution to the rice production in India during 2015-16 to 2019-20. It was estimated that, annually, the ICAR-IIRR varieties contribute to about 8 % of the total production of rice in the country. The revenue impact of ICAR-IIRR varieties is estimated at 3161 Million USD annually, which is in the form of gross returns from cultivation.

**Keywords:** Rice, ICAR-IIRR varieties, area, production, gross returns

### Introduction

Globally rice is planted in an area of about 162 million ha and 755 million tonnes of paddy is harvested annually (FAO, 2020). Of this, Asia accounts for 90% of the production and consumption, but only about 46 million tonnes of rice is traded through international markets. Leading rice exporting countries are India, Thailand, USA, Vietnam, and Pakistan. India is the leading exporter of rice with the export of about 9.5 million tonnes (APEDA, 2021). India has the world's largest area under rice with 44 million ha and is the second largest producer (117.94 million tonnes in 2019-20) next only to China (3<sup>rd</sup> Advance Estimates, GoI, 2020). It contributes to 24 percent of global rice production. Within the country, rice occupies one-quarter of the total cropped area, contributes about 40 per cent of total food grain production and continues to play a key role in the national food and livelihood

security system. Rice export contributes to nearly 20% of revenue from total agricultural exports of the country.

Rice is the most important food crop of India. The area under rice crop was 31 million ha in 1950-51 which has increased by 42 percent to 44 million hectares during 2019-20,. However, the rice production has registered an appreciable increase of nearly 5.7 times from 20.58 million tonnes in 1950-51 to 118 million tonnes during 2019-20 (GoI, 2021). The milled rice yield of 668 kg/ha in 1950-51 has increased to 2682 kg/ha during 2019-20.

The Central Rice Research Institute, now named as National Rice Research Institute (NRRI) was established in the year 1946 to work on the crop and subsequently, ICAR-Indian Institute of Rice Research was established in 1965 as All India Coordinated Rice Improvement Project (AICRIP) with 12 main centres.



Later, it was elevated to Directorate status in 1983 and to a full-fledged Institute in December, 2014. AICRP on Rice is the largest research network on a single crop comprising of 45 funded and over 100 voluntary centres covering all the rice growing states in the country. Realizing the limitations imposed by the ecosystem complexities, destabilizing insect pests and disease problems as well as grain quality requirements of different varieties/ cultivars of rice consumed, AICRIP evolved need based programmes / trials over the years to identify suitable genotypes of high yield potential along with appropriate crop management practices. During the initial phase i.e. in the first decade, emphasis was given to achieve higher yields through improved plant type largely for irrigated areas. This led to the development of short statured high yielding varieties (HYVs), which heralded the process of “Green Revolution” in India. During 80s, yield stability and quality improvement received major attention. This led to the development of varieties possessing major biotic stress tolerance and desirable quality to develop prototype of semi-dwarf basmati rice varieties. Nineties witnessed efforts in developing suitable hybrid rice technology and multi-location testing to validate their superiority over varietal checks at least by 10% as well as non-basmati quality trials targeting the export markets. During 2000s emphasis was on soil stress trials for problem soil areas, aerobic trials laying emphasis on developing genotypes for water limited environments and hill trials for incorporating cold tolerance. In recent times, emphasis has been on the near isogenic line development for quick evaluation of marker assisted selection (MAS) derived products introgressed with genes conferring resistance/tolerance against biotic and abiotic stresses. Additionally, nutritional security is stressed by enriching the grain with micronutrients such as zinc, iron along with protein (Babu *et al.*, 2016).

First semi-dwarf high yielding variety “Jaya” released by AICRIP in 1968 ushered the green revolution in India. This transformed the country to a state of self-sufficiency by mid eighties and stalled rice imports beginning an era of exporting rice, earning high

foreign exchange by early nineties. Till date more than 28,300 elite lines developed by different cooperating centres were tested in multi-location trials across the country under the umbrella of All India Coordinated Rice Improvement Programme (AICRIP) at funded, voluntary centres and in partnership with private sector for hybrid rice. The dynamic time tested multi-location three tier testing programme involving one year of Initial Varietal Trial (IVT) and two years of Advance Varietal Trial (AVT – 1 and AVT -2) as well as screening of elite breeding lines at hotspot locations for generating information on their pest/disease resistance/tolerance, grain quality attributes and agronomic performance has led to release of varieties including hybrids suitable for all the ecosystems. Extensive testing under AICRIP has contributed to the release of 1375 varieties including 117 hybrids for all the major rice ecosystems of the country during the last five and half decades (1965-2020). Of the 1375 varieties released in the country, about 267 varieties are still in the breeder seed production (BSP) chain.

Among the several varieties/hybrids developed and released by ICAR-IIRR, Improved Samba Mahsuri is a product of Marker-Assisted Selection breeding that has successfully introgressed three bacterial blight resistance genes viz., *xa5*, *xa13* and *Xa21* in the background of one of the most popular rice varieties, Samba Mahsuri (BPT5204) without sacrificing either the yield or the cooking quality (Sundaram *et al.*, 2008). Recently, Improved Samba Mahsuri has been recorded to have low glycaemic index of 50.99, which is desirable for rice consumers suffering from Type II diabetes (Sundaram *et al.*, 2018). The development of Improved Samba Mahsuri has paved the way for more precise molecular breeding programs in rice.

The research efforts in agriculture initially aimed at achieving self-sufficiency of food grains. Presently, bio-fortification of major food crops is considered as a viable strategy to address malnutrition and to ensure nutritional security. The development of high zinc rice varieties, DRR Dhan 45, 48 and 49 by Indian Council of Agricultural Research-Indian Institute of Rice Research (ICAR-IIRR) is an effort to address

malnutrition, through bio-fortification in rice (Nirmala *et al.*, 2016).

ICAR-IIRR contributes significantly to the production of breeder seed and currently there are about 11 ICAR-IIRR varieties indented for breeder seed production through Department of Agriculture and Co-operation (DAC) in India. Therefore, the objectives of the present study were to estimate the area covered by the ICAR-IIRR varieties and to assess the contribution of ICAR-IIRR varieties to the overall rice production during 2015-20 and the income gains of the country.

## Methodology

In the present study, breeder seed indents of GOI and production, seed produced in mega seed project, truthfully labelled seed sale at IIRR godown, seed distributed through blight-out program and mega seed project was considered mainly for the estimation of area, production and gross-returns.

### Estimation of area under ICAR-IIRR varieties

It was considered that one kg of breeder seed (BS) was required to produce 60 kg of foundation seed (FS), which in turn produces another 60 kg of certified seed (CS) (Pathak *et al.*, 2018). It was also considered that 50 kg of certified seed or truthfully labelled seed (TLS) is required to plant one ha of rice area.

The area covered by IIRR varieties was estimated using the following equation:

$$\text{Area (Mha)} = ((\text{BSP of IIRR varieties (kg)} * 60 * 60) + \text{TLS (kg)} / 50 (\text{kgha}^{-1}) / 10^6)$$

Where:

BSP is the Breeder Seed Produced

TLS is Truthfully Labelled Seed

10<sup>6</sup> is conversion factor to million

### Estimation of rice production with ICAR-IIRR varieties

Production of rice (unmilled) with ICAR-IIRR varieties was calculated by using the following equation:

$$\text{Production of IIRR rice varieties (Mt)} = [\text{Area of IIRR varieties (Mha)} / \text{Total area (Mha)}] * \text{Total paddy production (Mt)}$$

### Estimation of gross returns with ICAR-IIRR varieties

Gross return with ICAR-IIRR varieties was calculated using the following equation:

$$\text{Value of IIRR rice (Million USD)} = \text{Production of IIRR rice (t)} * \text{MSP (Rs. t}^{-1}) / \text{USD rate} / 10^6$$

Where, MSP is minimum support price; USD rate is USD exchange rate in rupees; 10<sup>6</sup> is conversion factor to million.

## Results and Discussion

Availability of pure seed is one of the crucial constraints for bridging the gap between technology development and its adoption. Breeder seed production (BSP) is being organized by ICAR-IIRR, Hyderabad under the National Seed Project (NSP) – Crops as per the DAC indents at 32-35 centres every year. BSP activity in rice began with 66 varieties in 1995 and the number of varieties has steadily increased to 234 by 2012 and 336 by 2020. The breeder seed production has increased from 541 tonnes (t) in 2015-16 to 1196 tonnes in 2019-20 (Figure 1).

The ICAR-IIRR plays a significant role in the supply of quality breeder seeds to the country. The major ICAR-IIRR varieties indented through breeder seed indent include Improved Samba Mahsuri, IR 64, Jaya, Varadhan, Mahsuri, Dhanrasi, Rasi, Sampada and Akshayadhan. Annually, in the past five years, at least eleven ICAR-IIRR varieties were indented for breeder seed in India. During the year 2015-16, DRR Dhan 44, Improved Samba Mahsuri and Sampada together constituted more than 70 % of the breeder seed production of the total ICAR-IIRR varieties (Table 1). In the year 2016-17, DRR Dhan 44, Sampada, Improved Samba Mahsuri, DRR Dhan 45 and 46 together constituted about 69 % of the breeder seed production of the total ICAR-IIRR varieties. In the subsequent years, Improved Samba Mahsuri, Sampada, IR 64 and DRR Dhan 44 were the major varieties indented through breeder seed indents.

Improved Samba Mahsuri (ISM) is a fine and medium-slender grain variety having excellent grain and cooking quality with yield potential of 5.5-6 t/ha. It is

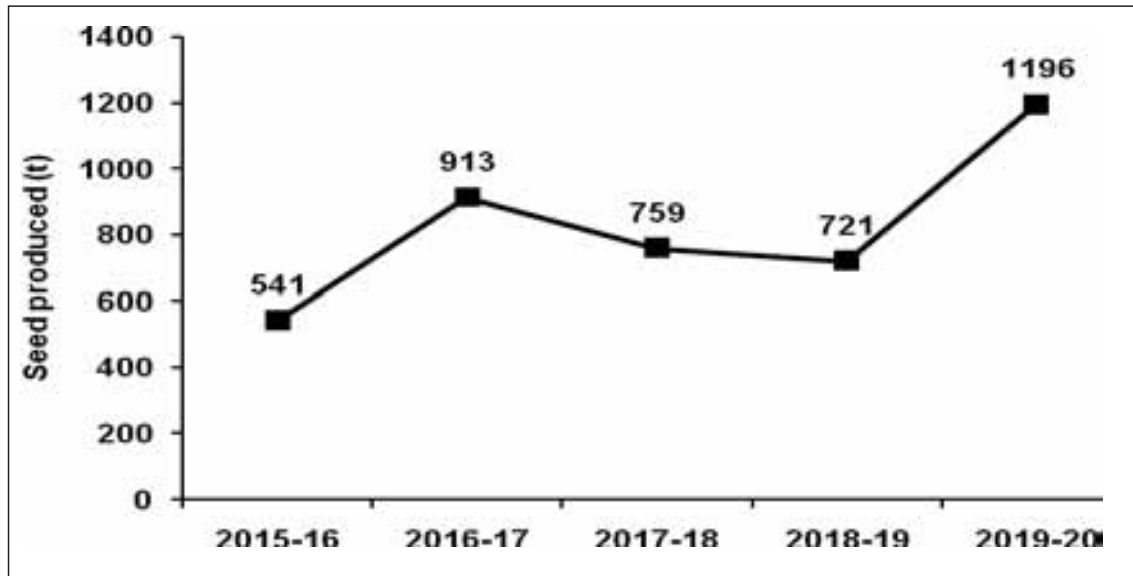


Figure 1: Breeder seed production (t) during 2015 to 2020

Table 1. Breeder Seed Production (tonnes) of ICAR-IIRR rice varieties

Sl. No.	Name of variety	2015-16	2016-17	2017-18	2018-19	2019-20
1.	DRR Dhan-50	-	-	-	-	0.85
2.	DRR Dhan-45	-	4.2	0.18	-	0.25
3.	DRR Dhan-39 Jagjeevan	-	0.84	0.4	0.7	0.6
4.	DRR Dhan-43	-	2	0.4	0.28	0.24
5.	DRR Dhan-44	8	6.3	3	-	1
6.	DRR Dhan-46	-	2.4	0.05	0.7	0.85
7.	DRR Dhan-51	-	-	-	-	0.24
8.	Improved Samba Mahsuri	6.5	3.7	3.55	3	2.3
9.	IR-64	5	5.5	4	6.45	2.1
10.	Jaya	1	0.84	1.4	0.42	0.9
11.	Krishna Hamsa	0.1		0.12	0.03	0.05
12.	Mahsuri	0.3	0.21	0.18	0.28	0.12
13.	Rasi	0.3	0.3	0.1	0.05	0.05
14.	Sampada	3.3	6.38	1.6	4.78	1.14
15.	DRR Dhan 47	-	-	-	0.35	-
16.	Dhanrasi	0.1	0.05	0.05	-	-
17.	Jarava	0.1	-	0.1	-	-
18.	Swarna Dhan	-	0.35	0.15	-	0.12
19.	Akshaya Dhan	0.1	0.3	-	-	-
20.	Vara Dhan	0.3	0.3	-	-	-
21.	IET-23832 (RP 5886 HP3 IR 80463-B39)	0.15	-	-	-	-
22.	Basmati Kasturi	0.1	-	-	-	-
	<b>Total</b>	25.35	33.67	15.28	17.04	10.81

Source: DRR Progress Reports, Varietal Improvement Vol 1, 2015 to 2019, IIRR, Hyderabad.

highly resistant to bacterial blight disease. It is almost similar to Samba Mahsuri in yield, grain and cooking quality and agro-morphological traits besides having resistance to bacterial blight. Under bacterial blight infestation, ISM gives more yield than the susceptible varieties. It is one of the first biotechnology derived products in the country. Improved Samba Mahsuri has been recorded to have low glycaemic index of 50.99, which is desirable for rice consumers suffering from Type II diabetes. Sampada, another high yielding variety has medium slender grains with intermediate amylose (23%) and recorded 24% more yield than BPT 5204. It is tolerant to blast, tungro disease and white backed plant hopper (Somasekhar *et al.*,

2009). DRR Dhan 44 is characterized by very high yield under limited water conditions. It performs exceedingly well under dry direct seeded conditions (sprinkler irrigation) with minimal inputs. Due to their unique traits, more number of indents was received for breeder seed production of these varieties.

The ICAR-IIRR plays a pivotal role in the supply of quality Breeder Seed (BS) and Truthfully Labelled Seed to the farmers of the country (Figure 2). The total IIRR seed supplied to the farmers has increased from 134 tonnes in 2015-16 to 143 tonnes in 2019-20 indicating an increase of about 7 percent of the supply of the seed of IIRR varieties.

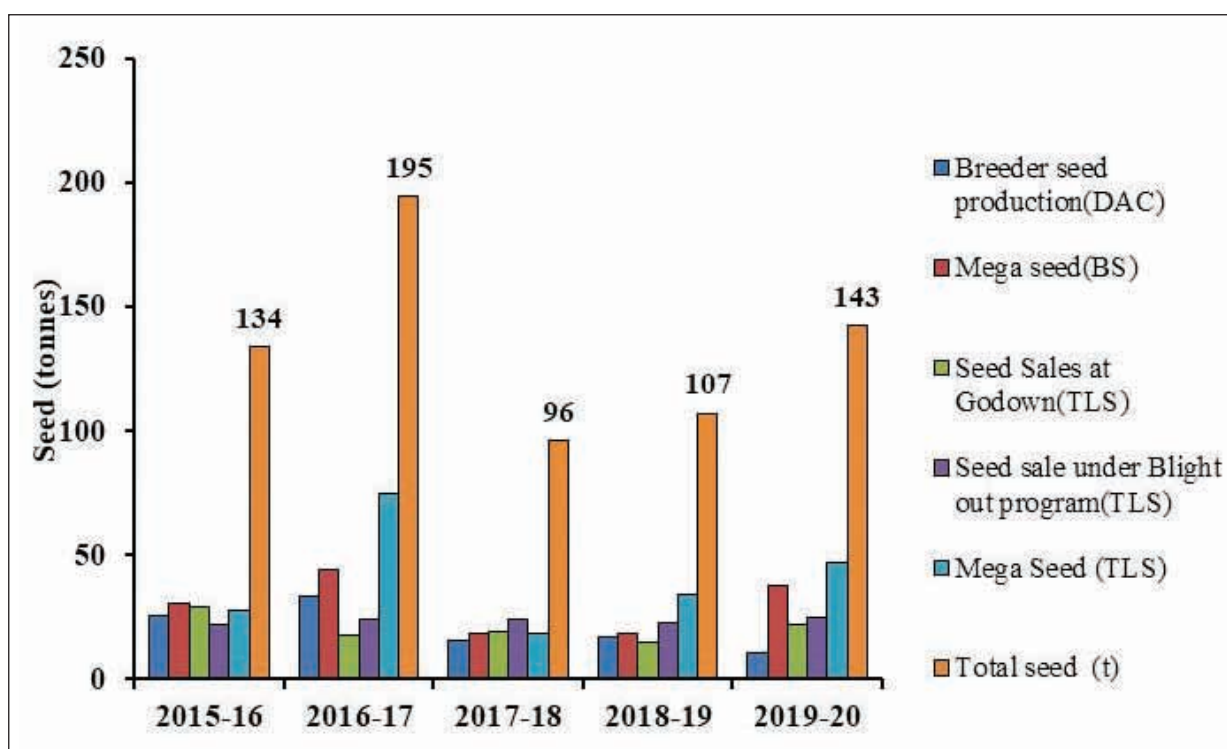


Figure 2: ICAR-IIRR varieties seed (tonnes) distribution details during 2015 to 2020

Source: Computed from i) breeder seed production data from DRR Progress Reports, Varietal Improvement Vol 1, 2015 to 2019 and ii) seed sales data from IIRR iii) seed produced and distributed under Mega seed and Blight Out programs

Rice was cultivated on an area of 44 million hectares in India with a record production of 118 million tonnes in 2019-20. The estimated area under IIRR varieties ranged from 2.5 Mha to 5.6 Mha in the past five years. On an average, annually, IIRR varieties occupied an area of about 3.4 Mha in India, for the past five years (Table 2). The cultivation of IIRR varieties resulted in the production of about 14 million tonnes (MT) of

paddy (9 MT of rice) in India. Thus, approximately 8% of rice production in India comes from the cultivation of varieties released from ICAR- IIRR. Annual gross returns from the cultivation of ICAR-IIRR released rice varieties in India were estimated to be around 3161 Million USD based on the average estimates for the past five years.



**Table 2: Contribution of ICAR-IIRR varieties to area, production and gross returns (2015-20)**

Sl. No.	Year	Area under IIRR Varieties (Mha)	Production of rice of IIRR Varieties (Mt)	Gross returns from IIRR Varieties (Mill.USD)
1	2015-16	2.5	9.40	2071
2	2016-17	4.0	15.33	3364
3	2017-18	5.6	21.50	5127
4	2018-19	2.5	10.34	2661
5	2019-20	2.5	10.23	2580
	Average	3.4	13.40	3161

Source: Computed from i) breeder seed production data from DRR Progress Reports, Varietal Improvement Vol 1, 2015 to 2019 and ii) seed sales data from IIRR iii) seed produced and distributed under Mega seed and Blight Out programs

## Conclusion

The study provides insights on the impact of ICAR-IIRR varieties and quantified the contribution of the IIRR varieties to the total area and production of rice at the national level. It was estimated that, annually, the IIRR varieties contribute to about 8 % of the total production of rice in the country. The significant impact of IIRR varieties is the generation of gross returns to the tune of 3,161 Million USD annually and cumulative returns of 15,803 Million USD for the period from 2015-16 to 2019-20.

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## Performance of zinc rich rice cultivar - DRR Dhan 48 in farmers' fields

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### Abstract

Biofortified variety 'DRR Dhan 48' was recently notified and released [CVRC S.O. 1379 (E) dt. 27.03.2018]. It has a high zinc content of 27 and 22 ppm in brown and polished rice, respectively and introgressed with three bacterial blight resistant genes (*xa5*, *xa13* and *Xa21*). It also has desirable grain quality parameters and agro-morphological characteristics akin to the popularly grown Samba Mahsuri. DRR Dhan 48 is distinguishable from other rice varieties of comparable grain type and duration using morphological, biochemical and molecular markers. Seed production was taken up in participatory mode in the farmer's field in Aroor village, Valigonda mandal of Yadadri-Bhuvanagiri district in Telangana State during *khariif* 2020. Roughing at regular intervals was done by breeders to maintain seed purity. DRR Dhan 48 is well accepted by the farmers and gradually gaining popularity. This cultivar is spreading from farmer to farmer and seed produced in farmer's field during *khariif* 2020 was distributed among farmers across 3 states viz., Telangana, Andhra Pradesh and Karnataka for cultivation during *Rabi* 2020-21.

**Keywords:** DRR Dhan 48, biofortification, zinc, bacterial leaf blight, maintenance breeding, MS cultivar

### Introduction

DRR Dhan 48 [RP 5898-182-22-4-3-2-1 (IET 24555)], a high zinc, bacterial leaf blight resistant medium slender grain rice variety derived from the cross of RPBio226\*1/CSR27, developed at ICAR-IIRR was notified and released in 2018 [S.O. 1379 (E) dt. 27.03.2018] for cultivation in the five southern states of India (Andhra Pradesh, Telangana, Tamil Nadu, Karnataka and Kerala). DRR Dhan 48 resembles popularly grown BPT5204 (Samba Mahsuri) in general agro-morphological and grain quality characteristics, in addition also possesses high zinc content (27 ppm in brown rice and 22 ppm in polished rice) and shows good level of resistance to bacterial leaf blight (BLB) and leaf blast. It is marker positive for three BB resistance genes (*xa5*, *xa13* and *Xa21*) and also resistant to lodging as it possesses strong culm. It possesses desirable grain quality parameters viz., medium slender (MS) grains, high milling recovery (69.4%), head rice recovery (HRR-

60.9%) and good cooking quality with intermediate amylose content (AC-24%), soft gel consistency (GC-28mm) and alkali spreading value (ASV-7.0).

### Maintenance of Pure Seed of DRR Dhan 48

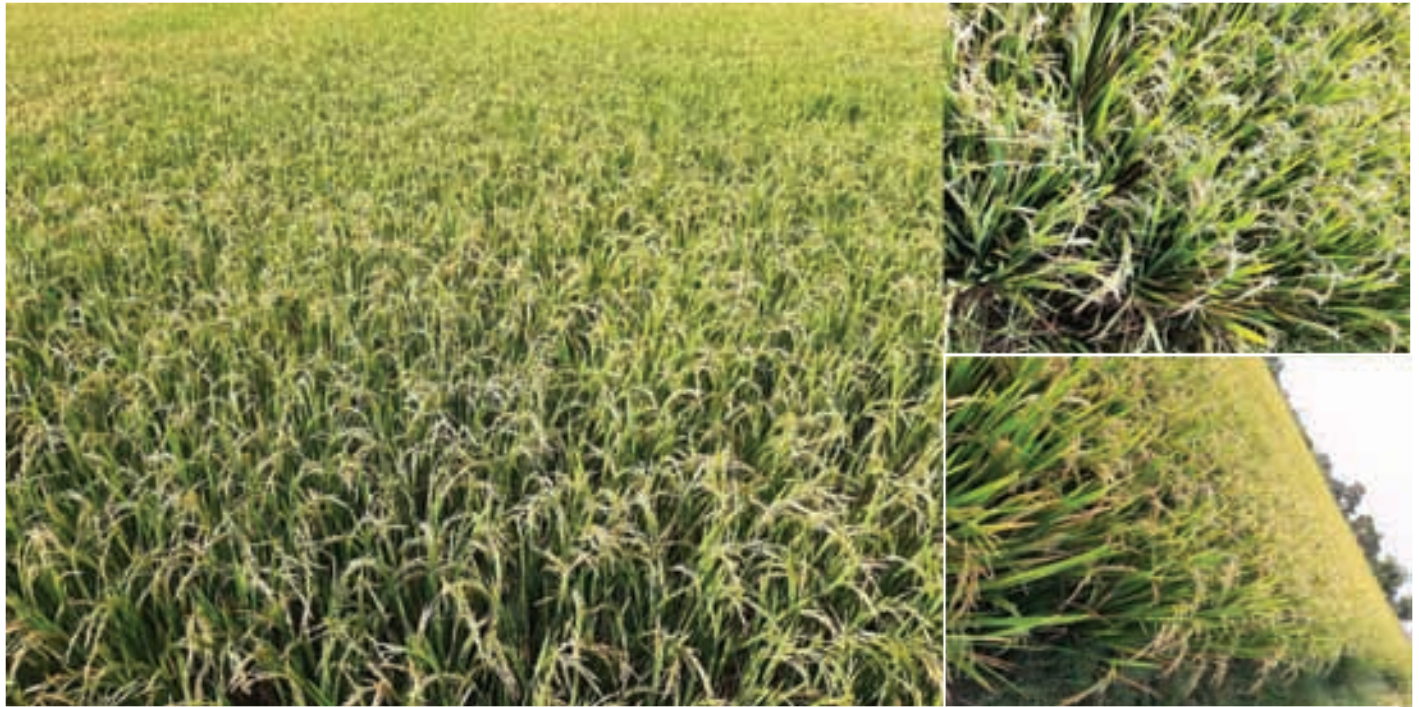
DRR Dhan 48 is distinguishable from rice cultivars of similar grain type and duration using morphological, biochemical and molecular markers

**Morphological markers-** plant type, grain type, eating and cooking quality characters akin to BPT 5204, resistance to bacterial blight and leaf blast

**Biochemical markers-** High zinc content (>20%) in brown and polished rice

**Molecular markers-** Presence of three BB resistance genes- *xa5*, *xa13* and *Xa21*

Nucleus seed production of released cultivars is a regular breeding activity. Hundred panicles were selected randomly during 2019 dry season (*Rabi*) at Ramachandra Puram farm of ICAR-IIRR located



Field view of DRR Dhan 48

in ICRISAT campus and were grown during *Kharif* 2019 following ear to row/panicle to row (PRT) method. Seeds from the individual panicle were grown in two rows leaving one row between each PRT. Observations were recorded on yield and yield related traits along with disease reaction to bacterial leaf blight. In field, selection was carried out based on DRR Dhan 48 designated traits (90-95cm height, semi-dwarf, compact, heavy and exerted panicle type,

medium slender grain type, days to 50% flowering being 105-110 days and seed to seed duration being 135-140 days) and lines showing the same were chosen. Also, the PRTs were inoculated with bacterial blight pathogen at maximum tillering stage by leaf clipping method under field conditions.

The freshly harvested grain from fourteen PRTs possessing morphological similarity with BPT 5204 and resistance to BLB (Figure 1a) were subjected

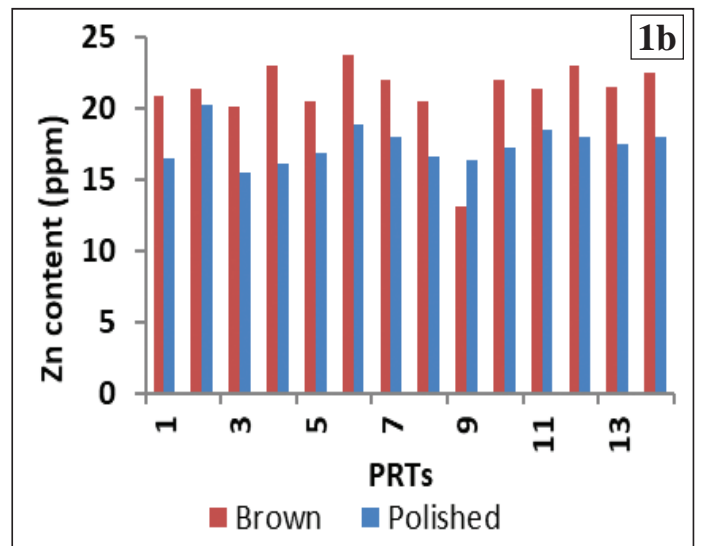
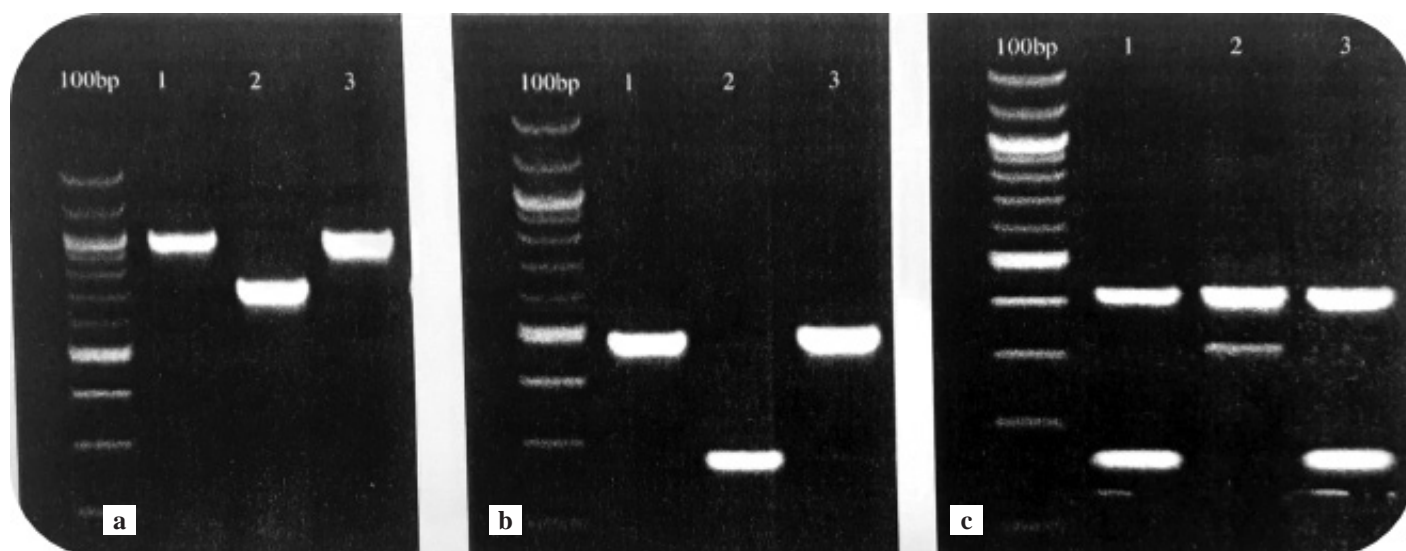


Figure 1a: Resistance to bacterial leaf blight (score 1) in DRR Dhan 48 similar to Improved Samba Mahsuri.  
1b. Zinc (ppm) content in PRTs grown during kharif 2019





100bp=ladder. 1. Positive parent, 2. Negative parent and 3. DRR Dhan 48

Figure 2: DRR Dhan 48 with BLB genes a. *Xa 21* b. *xa 13* and c. *xa 5*

to both biochemical and molecular analysis. In biochemical analysis, Zinc was estimated in both brown and polished grains using XRF at ICAR-IIRR (Figure 1b). In molecular analysis, DNA isolated from leaf samples from 14 PRTs was tested for the presence of all three BLB resistant genes viz., *xa5*, *xa13* and *Xa21* (Figure 2). Seed harvested during *Kharif* 2019 from 'PRT2' with morphological similarity to BPT 5204, resistance to bacterial leaf blight, marker positive to *xa5*, *xa13* and *Xa21* and zinc content of 24.4 ppm and 21.2 ppm for brown rice and polished rice respectively was chosen for seed multiplication during *Rabi* 2020.

#### Performance of DRR Dhan 48 in Farmer's field:

Pure seed of DRR Dhan 48 multiplied during *Rabi* 2020 from 'PRT2' was grown in farmer's field of Shri. Sangi Nagaiah/Tummala Muralidhar of Aroor village, Valigonda mandal, Yadadri Bhuvanagiri District of Telangana, during *Kharif* 2020 in four acres. Breeders carried out regular field visits and undertook rouging to maintain the seed purity and explained the importance of carrying out rouging in seed production plots to the farmers (Figure 3). Farmers checked for the presence of roughs along with their regular farming activities. Farmers appreciated the traits of DRR Dhan 48 in



Figure 3: Field visit and rouging in DRR Dhan 48 in farmer's field during *kharif* 2020



terms of its desirable plant height (slightly shorter than BPT 5204), resistance to bacterial blight despite not taking up plant protection measures and grain type akin to BPT 5204 and appreciated high (15-18) productive tillers per plant. An average yield of 5104 kg/ha was obtained in the farmer's field under late sown conditions indicating the suitability of DRR Dhan 48 cultivation under late sown conditions. Seed samples collected from farmer's field as well as from IIRR Rajendranagar farm were used for zinc estimation at International Rice Research Institute South Asia Hub (IRRISAH), ICRISAT, Hyderabad. The average zinc content in seed samples collected in farmer's field ranged from 25.2 to 27.4 and 22.2 to 23.3 ppm in brown and polished rice, respectively,

while in seed samples of IIRR farm ranged from 24.9 to 27.1 and 21.0 to 21.3 ppm in brown and polished rice, respectively. Varietal release to field performance of DRR Dhan 48 is schematically represented in Figure 4.

Owing to the desirable morpho-agronomic plant type and grain type similarity to BPT 5204, resistance to bacterial leaf blight and high zinc content, DRR Dhan 48 is well accepted by the farmers and is gradually gaining popularity. This cultivar is spreading from farmer to farmer and seed produced in farmer's field of Nalgonda district during *Kharif* 2020 was distributed among farmers across 3 states *viz.*, Telangana, Andhra Pradesh and Karnataka for cultivation during *Rabi* 2020-21.

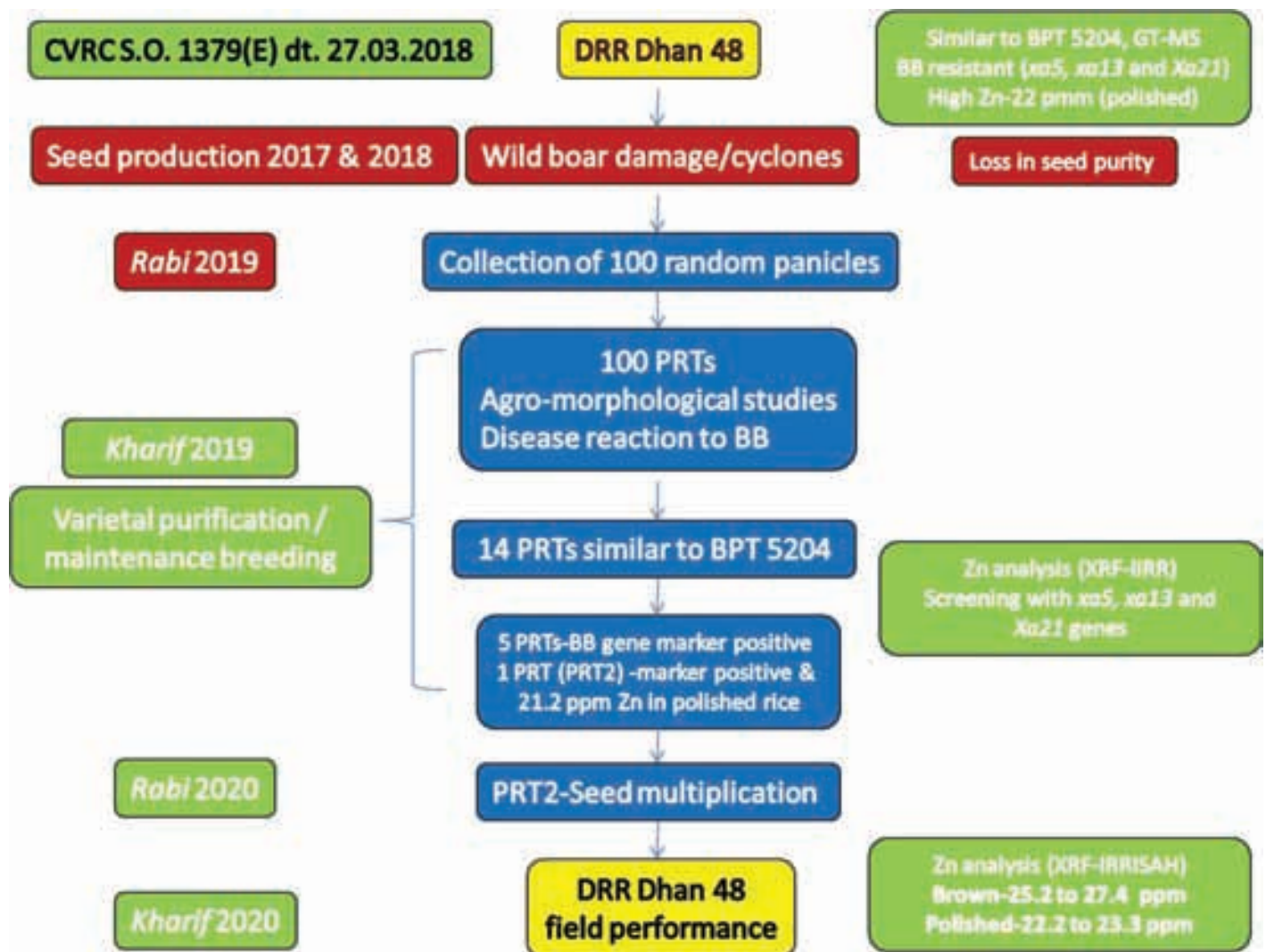


Figure 4: Schematic representation of varietal release to field performance of DRR Dhan 48

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

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

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