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# Society For Advancement of Rice Research

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- To facilitate research and industry collaboration and public private partnership at national level.
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Volume 16 : Issue No. 2 December 2023

| Contents | Page No. |
|---|---|

**Review Article**

**Unlocking the Energy-Water-Carbon Nexus in Rice Cultivation: A Comprehensive Review** 1
Vijayakumar S and Sayam Padma

**Research Article**

**Genetic Variability and Correlation Studies in High-yielding Varieties of Rice (*Oryza sativa* L.)** 22
Dayala Sai Teja, Divya Balakrishnan, Sonali Kar, Salam JL, Singh DP, Arun Prem Kumar, Swamy AVSR and Subba Rao LV

**Studies on Promising Restorer Lines for Yield and Yield Components in Rice (*Oryza sativa* L.)** 31
Dungu Vasudeva Reddy, Suneetha Y, Ravi Kumar BNVSR, Ramesh D, Manoj Kumar D and Srinivas T

**Evaluation of Pre-Released Rice Cultures at Different Nitrogen Levels** 43
Anny Mrudhula K, Krishna Veni B, Suneetha Y and Sunil Kumar M

**Assessment of Grain Zinc, Iron and Protein Content in Selected Red Rice (*Oryza Sativa* L.) Mutant Lines** 51
Lekha PS, Shridevi Amoghappa Jakkeral, Dhananjaya BC, Dushyantha Kumar BM, Jayashree S, Halingali BI, Gaurav Kumar and Shashikala Kolkar

**Exploration of Genetic Variability and Trait Association for Root Architecture Related Traits under Aerobic Conditions in Rice (*Oryza sativa* L.)** 58
Vishal Reddy, Kalyani MB, Nakul DM, Padmashree R, Waghmode BD, Manjunatha B, Divya B, Gireesh C, Anantha MS, Dushyantha Kumar BM, Satish KM, Usha TN, Kalleshwara Swamy CM, Prem Sagar SP, Madhavilatha K, Anil A Hake and Lokesha R

**Study on Genetic Variability and Correlation Coefficient for Grain Quality Parameters in South Indian Rice (*Oryza sativa* L.) Varieties** 66
Krishnamrutha T. Suresh, Sai Prasad SV, Sameer Kumar CV and Ramesh T

**Evaluation for Multiple Abiotic Stress Tolerance in Rice (*Oryza Sativa*) Genotypes** 73
Veronica N, Srinivas T, Venkata Ramana Rao P and Vani Praveena M

**Growth and Yield of Rice (*Oryza sativa* L.) are Influenced by Different Levels of Nitrogen, Phosphorus and Biofertilizers** 81
Kacha DJ, Patel AS, Kacha RP, Thorat SS and Parmar MB

**Insights of Colorimetric Estimation of Phytic Acid in Rice Grain** 87
Jaldhani V, Sanjeeva Rao D, Suman K, Srikanth B, Neeraja CN, Raghuveer Rao P and Sundaram RM

**Characterization of Engineered Zinc Oxide (ZnO) Nanoparticles Using Different Techniques** 95
Srivalli C, Gobinath R, Tedia, K, Surekha K, Manasa V, Brajendra Latha PC, Bandeppa and Vijayakumar S

**Efficacy of Fungicides on Rice Sheath Blight and Grain Discolouration Diseases in Rice** 101
Surendran M, Silpa Sasikumar, Nimmy Jose, Jyothi Sara Jacob and Aiswarya Jagadees

**Contents** **Page No.**

**Studies on Compatibility of Insecticides and Fungicides against Brown Plant Hopper and Blast in Rice** 108

Anand Kumar ADVSLP, Nanda Kishore M, Bhuvaneswari V, Srinivasa Rao N, Anusha B and Jogi Naidu G

**Impact of Farmers' Participatory Rice (*Oryza sativa* L.) Demonstration Programme on Crop Yield and Economics Under Temperate Hill Ecology** 114

Tasneem Mubarak, Ab. Shakoor Khanday and Haseeb-ur-Rehman

**Short Communication**

**DRR Dhan 65 (IET 27641) - A High Yielding Low Soil Phosphorous Tolerant and Climate Resilient Rice Variety Developed from Wild Introgression Lines** 120

Divya Balakrishnan, Sarla Neelamraju, Vijai Pal Bhadana, Jyothi Badri, Subba Rao LV, Sundaram RM, Subrahmanyam D, Voleti SR, Padmavathi G, Swamy AVSR, Senguttuvel P, Magrauithia SK, Anantha MS, Aravind Kumar J, Prasad MS, Laha GS, Ladhalakshmi D, Jhansi Lakshmi V, Padmavathi Ch, Padmakumari AP, Jhansi Rani B, Mahendrakumar R, Sreedevi B, Mangaldeep Tuti, Surekha K, Brajendra, Suneeta K, Revathi P, Gireesh C, Fiyaz RA, Kalyani MB, Suvarna Rani Ch, Sruthi K, Nirmala B, Nagarjuna E, Vijay Kumar M, Thaseen M, Pradeep Reddy M, Prasad Babu Adari, Malathi Surapaneni, Vishnu Prasanth V, Siddique EA

**Genetic Stocks**

Rice Germplasm Registered during July-December 2023 at ICAR-National Bureau of Plant Genetic Resources, New Delhi 123

**Tributes**

**Dr. M. S. Swaminathan -** The father of India's Green Revolution

**REVIEW ARTICLE** https://doi.org/10.58297/PSJA9488

# Unlocking the Energy-Water-Carbon Nexus in Rice Cultivation: A Comprehensive Review

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## Abstract

Rice cultivation, as a cornerstone of global food security, holds significant environmental implications due to its carbon, water, and energy footprints. Energy, carbon and water footprint assessments can be powerful tools to guide sustainable food production systems. Due to higher water losses in conventional rice culture, the irrigation water footprint associated with rice cultivation increases, thereby elevating the energy and carbon footprint. Improper use of resources like fertilizers, pesticides, labour and fuel may lead to higher energy consumption. Several alternative rice production systems like Direct Seeded Rice (DSR), Alternate Wetting and Drying (AWD), System of Rice Intensification (SRI) as well as better nutrient management practices have been developed and refined to reduce energy, carbon and water footprint associated with rice cultivation. This review presents a comprehensive analysis of the intricate interplay between these footprints, highlighting potential trade-offs and synergies that warrant attention within the context of rice cultivation. Moreover, this review discusses in detail the significance of selecting appropriate rice cultivation techniques, such as direct seeded rice, SRI and alternate wetting and drying suitable for different ecologies in comparison to transplanted method of rice cultivation.

**Keywords:** Carbon, DSR, Energy, Footprint, SRI, Water.

## Introduction

Rice plays a pivotal role in the food and livelihood security of the Asian people. As the continent wise data shows, more than 90% of rice production and consumption takes place in Asia and more than two billion people are getting 60-70% of their energy requirement from rice and its derived products (FAO, 2021). In India, it is the staple food for more than two-thirds of the Indian population contributing to 40% of the total food grain production (Nayak *et al.,* 2020). Globally, rice is grown in 164.8 million hectares with an annual production of about 507.2 million metric tons of paddy (USDA, 2020-21). Globally, India holds first position in terms of rice area and second position in terms of production of rice after China. In India, the rice crop is grown on about 43 million hectares area, with a production of 122 million tones and productivity of 3878 kg ha$^{-1}$ (Nayak *et al.,* 2020). The states including West Bengal, Uttar Pradesh, Andhra Pradesh, Telangana and Punjab alone contribute to more than 50% of the total rice production of the country. The demand for rice is projected to increase in the next 30 years by nearly 70% to maintain the present per capita availability which is 69 kg per annum (Muthayya *et al.,* 2014). However, it is difficult to meet the increasing demand for rice with conventional methods of rice cultivation.

Producing more rice with less water is a formidable challenge. A lot of irrigation water is used to produce rice through conventional method, as a result water scarcity is increasing, especially in most of the rice growing regions (Vijayakumar *et al.,* 2023a). The amount of water applied to produce 1 kg of rice ranges from 800 to 5000 L (Surendran *et al.,* 2021). Rice growing farmers, often apply more irrigation water although rice crop needs a much lower amount for normal growth and yield. The inefficient irrigation in rice causes a rapid decline in ground water table, groundwater pollution and greenhouse gas (GHG) emissions (Vijayakumar *et al.,* 2018). Therefore, it is difficult to meet the increasing demand for rice with conventional methods of cultivation.

Along with water, energy is another major component of rice production. In rice cultivation, energy is used as well as produced, most notably in the form of bioenergy (Alam *et al.,* 2005; Vijayakumar *et al.,* 2019). The energy requirement of rice cultivation is directly related to the management techniques followed and inputs used during the growing season (Mariano *et al.,* 2012; Yadav *et al.,* 2017). Greater energy efficiency in food production systems is required since the projected energy production growth is inadequate and conventional energy sources are limited (Vijayakumar *et al.,* 2023b). Understanding the energy budget in rice cultivation helps in making informed decisions regarding resource allocation to enhance energy use efficiency. Energy footprint (EF) of rice is the equivalent energy associated with various farm operations *viz.,* land preparation, sowing, transplanting, weeding, harvesting and post-harvest management. A production system is considered efficient when it produces higher energy output and consumes comparatively lesser energy (Kumar *et al.,* 2021). By quantification of energy footprints, farmers can choose the most efficient energy sources to maximize the yield by spending less input energy to various farm operations. Energy analysis is also an important tool for judging the rice-based production system efficiency and achieving the Sustainable Development Goals (SDGs).

Growing rice in flooded fields create the ideal anaerobic conditions for bacteria to thrive on decomposing organic matter (mainly rice straw residue) and release methane (Mahato, 2014; Kumar *et al.,* 2016). Poor absorption of nitrogen by rice crop, often overused by farmers, leads to $N_2O$ emissions (Vijayakumar *et al.,* 2022). Burning of rice residues and waste in the value chain add to GHG emissions (Bhaduri *et al.,* 2023). Burning is a convenient way for farmers to quickly dispose large volumes of leftover rice straw (Vijayakumar *et al.,* 2021). Thus, all these practices in rice cultivation increase the GHG emission and ultimately Global warming potential (GWP). According to the Kyoto protocol, carbon footprint (CF) is the total amount of GHGs in terms of carbon dioxide ($CO_2$) equivalent coming from the product's life cycle, including its storage, use, and disposal (Kijewska and Bluszcz, 2016). Not all GHGs affect climate change in the same way. To easily compare the CF of various products, they are converted to the amount of $CO_2$ using appropriate factors. The GWP of $N_2O$ and $CH_4$ are 298 and 25 times that of $CO_2$, respectively. The CF in rice include the total amount of $CO_2$, nitrous oxide ($N_2O$) and methane ($CH_4$) that are generated during the rice cultivation and the rice value chain (Danish and Wang, 2019; Bhaduri *et al.,* 2023, Grant *et al.,* 2004). Higher production and release of these gases will increase the CF of rice cultivation. In general, the CF of 1 ton of rice varied from 1.11 to 1.57 ton $CO_2$-eq in the 100-year horizon (Alam *et al.,* 2016). It has recently been estimated that the global food system is responsible for about a third of GHG, second only to the energy sector; it is the number one source of methane and biodiversity loss. The effects of changing climate, rising temperatures, more frequent droughts, floods, and intense typhoons are devastating rice farms and farmer livelihoods (Vijayakumar *et al.,* 2023d). Thus, cultivation of rice in conventional transplanted system may accelerate the global climate change.

Due to higher CF in the recent years, there has been a shift in the regular climate. Climate change induced global warming had a significant impact on rainfall pattern (Vijayakumar *et al.,* 2023e). There was either late onset of monsoon or early cessation of rains. The late onset of monsoon resulted in delay of sowing due to which there were unfavourable conditions at critical growth stages there by reducing the yields. The early cessation of rains caused water deficit during peak period of water requirement which in turn had a great impact on the yields (Vijayakumar *et al.,* 2023d). Irrigation to rice crop is limited in many rice growing areas due to unavailability of power (electricity) and water scarcity. Climate change increased food insecurity from 135 million in 2019 to 345 million in 82 countries by June 2022, as the war in Ukraine, supply chain disruptions, and the continued economic fallout of the COVID-19 pandemic pushed food prices to all-time high. The farming families in Sub-Saharan Africa, South Asia, and Southeast Asia are disproportionately poor and vulnerable. About 80% of the population in this region is at risk from crop failures and hunger due to climate change. A severe drought caused by an El Nino weather pattern or climate change could push millions more people into poverty. The above reasons indicate how the assessment of energy, carbon and water footprint is important.

The carbon, energy, and water footprints are interlinked with each other. Water footprint influences both the energy and carbon footprint by consuming more electricity or fuel and by emitting $CH_4$. Along with water, fertilizers and other inputs in energy footprint are also reasons for higher carbon footprint by producing $CO_2$ and $N_2O$ (Surekha *et al.,* 2023). Hence, energy, carbon and water footprints are interrelated with each other **(Figure 1)**. In comparison to other field crops, rice has a higher carbon, energy and water footprint in India and abroad (Sah *et al.,* 2018). Different rice production systems have varying impacts on GHG emissions, and the choice of system should consider both short-term and long-term goals. Some systems may be better suited for immediate GHG emission reduction, while others may offer better long-term sustainability. Therefore, the identification of energy, carbon and water efficient rice cultivation system is important to food security, and sustainable intensification. In this review, we meticulously reviewed the nexus between energy, water and carbon footprint of rice under different rice establishment methods.

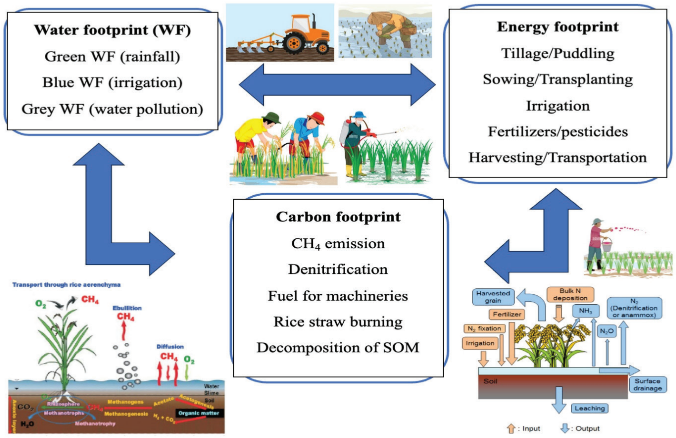

**Figure 1: Water, carbon and energy nexus in rice cultivation**

## Energy footprint of different rice production systems

The identification of energy-efficient rice production systems is gaining importance due to factors such as the increasing demand for rice resulting from population growth, changing societal energy consumption patterns, the recent oil crisis, and pollution caused by the fuel used in agricultural operations` (Bhardwaj *et al.,* 2016). Agriculture is experiencing a faster rate of energy consumption growth than other economic sectors due to the use of mechanized cultivation techniques and soil nutrient materials, particularly fertilizers (Kamoshita *et al.,* 2010). Numerous energy-intensive processes are necessary to produce rice, including tillage, transplanting, irrigation, fertilizer application, pesticide spray, harvesting, transportation, etc. (Mohanty *et al.,* 2014; Vijayakumar *et al.,* 2023c). The use of fertilizers, fossil fuels for machinery, and pesticides has resulted in GHG emissions and environmental pollution (Mansoori *et al.,* 2012). Conventional transplanted method of rice cultivation requires larger energy inputs particularly for water, chemical fertilizers, pesticides, and seeds. This not only contributes to the degradation of soil, water, and air resources, but also reduces economic benefits for farmers and the nation (Pooja *et al.,* 2021). Thus, improving energy use efficiency is one of the criteria for achieving agricultural sustainability, as it lowers production costs and environmental pollution (Mohammadi *et al.,* 2010).

Singh *et al.,* (2019) investigated the energy expenditure in rice cultivation and identified irrigation water use as the largest energy-consuming component, followed by chemical fertilizers. The distribution of energy inputs in rice cultivation was as follows: seed (0.3%), human labour (0.5%), agri-machinery (0.8%), biocides (7.2%), diesel fuel (8.8%), electricity (17.7%), chemical fertilizers (24.7%) and irrigation water (40.0%). Another study by Paramesha *et al.,* (2022) reported that fertilizers (42.7%) had the highest share of non-renewable energy sources, followed by diesel (12.4%) and machines (8.6%). A similar finding was reported by Bockari-Gevao *et al.,* (2005). They found that fertilizers account for the 7700 MJ of energy per hectare with a total energy input of 12400 MJ. A study in North Eastern Region of India showed that the land preparation, application of chemical fertilizers, farm yard manure and seeding and/or transplanting operations consumed more than 80% of energy input in different rice cultivation systems (Mandal *et al.,* 2015). These results emphasize the significant role of land preparation, irrigation and chemical fertilizers in energy consumption during rice cultivation. The total input energy of rice cultivation could be significantly reduced by supplementing chemical fertilizers with FYM, as the use of chemical fertilizers makes farming exceptionally energy-intensive (Billore *et al.,* 1994).

In the modern production methods, direct sowing of seeds on to puddled soil (wet seeding by drum seeder or broadcasting of either sprouted or direct seeds) holds unique relevance because it reduces time, labour, and energy consumption while boosting profitability (Subbaiah and Balsubramanian, 2000) therefore, considered more economical as compared to transplanting. Similarly, the adoption of system of rice intensification (SRI), alternate wetting and drying (AWD), direct seeded rice (DSR) and mechanical transplanting practices in rice cultivation makes the crop cultivation economically viable and environmentally sustainable (Vijayakumar *et al.,* 2023a). Farmers are facing the problem of labour shortage during peak season that delay the timely transplanting and sowing of succeeding crop in the rice-based system. The shortage of labour during the peak period and escalating fuel prices, in turn increase the production cost.

### SRI vs TPR

There are differences in energy consumption and energy efficiency among different rice establishment methods. SRI requires less water, fertilizer, seed, and labour inputs, thereby minimizes overall energy requirements and contributing to energy savings in rice cultivation (Srinivas *et al.,* 2022). The SRI method saves approximately 2 MJ of energy per kilogram of rice produced compared to the conventional method. The energy consumption for producing 1 kg of rice is reported as 4.41-4.51 MJ in SRI, while it is 6.36-6.47 MJ in the conventional method. The SRI method achieved a significant reduction in input consumption while increasing output, resulting in improved energy efficiency. SRI method uses 75% lower seed rate than conventional method. SRI method uses only 19 kg of seeds for a hectare of paddy field, as opposed to the conventional method's 76 kg. Because of this, around 1000 MJ of energy is saved (Truong *et al.,* 2017). SRI contributes to a decreased use of chemical fertilizers and pesticides since weeds are controlled through mechanical weeding or manual labor and 50% of the external plant nutrient requirement is supplied through organic sources.

Additionally, SRI contributes to energy savings by utilizing a lesser amount of water for irrigation. SRI significantly reduces irrigation water use by keeping the soil moist rather than in a flooded condition. Das *et al.,* (2014) compared the energy productivity of SRI with conventional rice culture. They found that SRI exhibited the highest energy productivity (0.68 kg $MJ^{-1}$), while conventional rice culture had lower energy productivity (0.59 kg $MJ^{-1}$). The mean specific energy of DSR was 3.31 MJ/kg, which was significantly higher than those of SRI (2.76 MJ/kg), modified SRI (2.73 MJ/kg), and transplanted rice (2.89 MJ/kg) (Htwe *et al.,* 2021). Additionally, the highest energy use efficiency (EUE) was observed in the modified SRI (9.60), followed by SRI (9.46), transplanting (8.55) and DSR (8.35). Similarly, Nirmala *et al.,* (2021) reported that the SRI method exhibited higher energy use efficiency (6.6), energy productivity (0.21 kg MJ-1), and lower specific energy (4.69 MJ kg-1) compared to conventional practices. This indicates that SRI is more efficient in terms of energy utilization, resulting in higher productivity per unit of energy input.

### DSR vs TPR

The transplanting system consumes more energy in terms of diesel fuel, electricity, irrigation and human labour. In contrast, in DSR, herbicides accounted for the major input energy (Eskandari and Attar, 2015). The total energy output was higher in the transplanting system (114,720 MJ ha$^{-1}$), while the highest energy ratio was observed in DSR. The DSR method also had higher energy efficiency (2.8) compared to the transplanting method (2.3). DSR utilized more energy to produce one unit of rice grain. The direct seeding method required 85% more energy for weed control and inter-cultivation compared to the transplanted method (Chaudhary *et al.,* 2017). This higher energy requirement was attributed to the use of a greater quantity of herbicides in DSR. In contrast, flooding in the transplanted method reduced the weed burden, leading to lower herbicide use (Rao *et al.,* 2021).

Contrary to previous results, Lal *et al.,* (2020) reported an 18.4% lower energy input in DSR compared to transplanted rice (TPR). The major energy savings were observed in diesel (160%), machinery and labor (66%), making dry-DSR more energy-efficient with only a minor yield penalty. The net energy of TPR was 2.3 and 13.4% higher than wet-DSR and dry-DSR, respectively. Tillage operations for land preparation account for a considerable amount of energy input in rice production. One of the main advantages of No-Tillage (NT) systems over traditional tillage was the minimal energy needed for land preparation. Energy input in DSR and conventional TPR systems was 13% and 19% more than that for the NT-DSR and NT-TRP, respectively. The mechanized TRP system required a larger energy input for sowing and transplanting than any other approach because it used machines for transplanting and more labour to set up a mat-type nursery. On the other hand, direct seeding required 22% less energy than TPR because of the absence of nursery preparation (Mandal *et al.,* 2015).

### Energy budget of different rice seeding methods

The energy use efficiency and energy productivity were found to vary among the different rice establishment methods. The use of seed-cum-fertilizer drill is the energy efficient method for establishing rice under dry direct seeding, compared to manual line and broadcast seeding. The drill-seeding of rice increased energy use efficiency by 13% compared to line-seeding and broadcast seeding (Saha *et al.,* 2021). For each unit of energy consumed in the fields, drill seeding resulted in 0.47 yield units, manual line-seeding achieved 0.42 yield units and broadcast seeding obtained 0.38 yield units (Saha *et al.,* 2021). Utilizing drill-seeding method for rice crop establishment will maximize the energy use efficiency and energy productivity in rice cultivation. Rice fields are submerged for most of their growth period. Therefore, puddled transplanted rice (PTR) is considered an energy-intensive and more GHG-emissive crop compared to other cultivated crops. To achieve higher productivity, the PTR system primarily relies on indirect and non-renewable energy sources, such as fertilizers, machinery, chemicals, irrigation, seeds, fuel and electricity (Mansoori *et al.,* 2012).

The investigation on specific energy requirement of various rice cultivation practices revealed that drum-seeded rice requires significantly lower specific energy, with reductions of 19.0% and 16.8% compared to broadcasting of dry seeds and sprouted seeds, respectively (Bhardwaj *et al.,* 2016). It suggests that using the drum-seeding method can lead to energy savings in rice cultivation compared to broadcasting of seeds. The transplanting method of rice cultivation demands more water and this system suffers from more surface evaporation and percolation loss of water, which, in turn, increases the frequency and duration of irrigation. Additionally, this system requires puddling to make the soil soft and easy for transplanting. All of these processes lead to an increased input energy requirement in the transplanted rice system (Begum *et al.,* 2006). Using surface water resources rather than groundwater sources can help reduce the amount of electricity and diesel fuel needed to deliver water for rice cultivation.

The tillage method employed in rice cultivation has a significant role in fuel consumption, water input and operational efficiency. Compared to puddling, non-puddled strip and zero tillage reduced fuel consumption for mechanical transplanting by 11-18%. Additionally, strip tillage reduced tillage time and fuel consumption by 50-70% (Hossen *et al.,* 2018). Adoption of conservation tillage and efficient residue management enhanced energy productivity from 15.8% to 21.0% and energy use efficiency from 17.1% to 22.4% compared to conventional practice (Singh *et al.,* 2022). The experiment on No till (NT) and Conventional tillage (CT) with different mulching systems revealed that NT rice system required 48.3% less energy (8,479 MJ ha$^{-1}$) than CT system (16,465 MJ ha$^{-1}$) and the energy productivity was higher in NT (45437 MJ ha$^{-1}$) than CT (44834 MJ ha$^{-1}$). The NT system had higher net energy (36,958 MJ ha$^{-1}$), energy use efficiency (5.36), energy productivity (0.36 yield kg MJ$^{-1}$) and lower specific energy (2.76 MJ kg$^{-1}$) compared to CT (Yadav *et al.,* 2020). Absence of tillage operations like plowing, tilling and leveling under NT led to a reduction in energy input, whereas the CT with multiple tillage operations required nearly double the amount of fossil fuel as energy input in operating the field machinery **(Table 1)**.

**Table 1: Comparison of energy use between no-till and conventional tillage systems in rice**

| S. No. | Energy use | Input energy for NT (MJ ha$^{-1)}$ | Input energy for CT (MJ ha$^{-1}$) | Energy saving in NT over CT (%) |
|---|---|---|---|---|
| 1. | Machine operations | 157 | 4546 | 96.5 |
| 2. | Diesel consumption | 3942 | 4195 | 93.9 |
| 3. | Pesticides | 981 | 621 | 36.7 |
| 4. | Other operations | 3399 | 7103 | 52.1 |
| 5. | Total consumption | 8479 | 16465 | 48.3 |

*Source: Yadav et al., (2020)*

Hence, an appropriate tillage system selection is an important consideration for crops which helps in reducing energy consumption and plays a great role in energy budgeting. Farmers should be taught to decrease unnecessary energy use in order to optimize energy use in rice production systems. It is essential to use machinery, fertilizer, and other inputs under the supervision of agricultural specialists. One of the most significant strategies to reduce energy usage in the context of herbicides is to increase farmers' awareness about non-chemical weed control. Despite the fact that direct seeding systems have the advantage of consuming less energy, improving the productivity of rice under this system will motivate farmers to adopt it on a large scale **(Table 2)**.

**Table 2: Energy budget of different rice production systems**

| S. No. | Production system | Input energy MJ ha$^{-1}$ | Output energy MJ ha$^{-1}$ | EUE | References |
|---|---|---|---|---|---|
| 1. | SRI | 25378 | 221221 | 8.70 | Troung *et al*., (2017) |
| | Conventional transplanting | 32794 | 199372 | 6.07 | |
| 2. | SRI | 6895 | 149884 | 21.7 | Das *et al*., (2014) |
| | Integrated rice culture | 6925 | 151942 | 21.9 | |
| | Conventional rice culture | 7250 | 132232 | 18.2 | |
| 3. | Drum seeding | 11255 | 64240 | 5.71 | Bhardwaj *et al*., (2016) |
| | Broadcasting | 11208 | 53660 | 5.64 | |
| | Transplanting | 11520 | 64940 | 4.79 | |
| 4. | Wet DSR | 15809 | 162210 | 10.3 | Lal *et al*., (2020) |
| | Dry DSR | 14156 | 143123 | 10.1 | |
| | Transplanted rice | 16051 | 176286 | 11.0 | |
| 5. | DSR | 34623 | 98677 | 2.85 | Eskandari and Attar (2015) |
| | Transplanting | 49878 | 114720 | 2.30 | |
| 6. | No tillage | 8479 | 45437 | 5.36 | Yadav *et al*., (2020) |
| | Conventional tillage | 16465 | 44834 | 2.72 | |
| 7. | No tilled DSR | 9162 | 100782 | 11.00 | Mandal *et al*., (2015) |
| | Mechanized TRP | 15371 | 132191 | 8.60 | |

### Carbon footprint

Agricultural operations, such as tillage, irrigation, fertilizer application, inter-cultivation, and harvesting, contribute to greenhouse gas (GHG) emissions, with a substantial effect on global warming and climate change (Yadav *et al.,* 2018). A significant portion of GHG emissions (10-14%) that contribute to climate change is produced during agricultural production (Jantke *et al.,* 2020). In India, agriculture is one of the significant contributors to the national economy, accounting for 19% of the total GHG emissions (Sharma *et al.,* 2011). Within agriculture, wetland rice production contributes to 55% of agricultural GHG emissions globally. Rice production accounts for the emission of 97 million metric tonnes of carbon dioxide equivalent annually, ranking fourth in importance after enteric fermentation (40%), livestock manure management (23%) and fertilizer use (13%) (FAO, 2017). Therefore, any new technology with the potential to reduce GHG emissions from wetland rice could make a significant contribution to global warming mitigation.

In rice production, irrigation water contributes to methane emissions, NPK fertilizer applications contribute to nitrous oxide emissions (Hoben *et al.,* 2011; Venterea *et al.,* 2012) and the use of diesel machinery contributes to carbon dioxide emissions (Afiyanti *et al.,* 2018). These emissions collectively contribute to the CF of rice production. Continuous flooding, nitrogenous fertilizers, and machinery are responsible for higher GHG emissions from rice field (Pathak *et al.,* 2014). Constant flooding and the use of organic manures are the primary sources of methane emissions in conventional rice culture (Pathak *et al.,* 2014). When compared to other crops, transplanted rice produces the most GHGs, with emissions reaching 1112 kg $CO_2$ eq./ha (Soni *et al.,* 2013). Rice cultivation practices that optimize irrigation water usage may offer a means to reduce the CF, contributing to climate change mitigation. The CF of rice cultivation varies with the season and the method of rice crop establishment. For example, in Indonesia, the highest CF during the dry and rainy seasons was observed in the Belitung Islands and East Nusa Tenggara province, respectively. This is primarily due to paddy cultivation in these regions, which demands more water due to their topography and dry weather conditions, leading to significant water requirements in these areas.

Meanwhile, the lowest CF in both the dry and rainy seasons was recorded in Yogyakarta province, which employs several agricultural practices that are more water-efficient, including SRI and AWD practices.

During the dry season, complete AWD is an effective water management practice to replace conventional flooding, as it can help mitigate GHG emissions, conserve water and increase yield. Incomplete AWD reduces methane emissions by 10.62% but increases nitrous oxide emissions by 5.94%, while complete AWD reduces $CH_4$ emissions by 23.10% but increases $N_2O$ emissions by 14.79% (Sriphirom *et al.,* 2019). Although both AWD systems increase $N_2O$ emissions, their total GWP remain lower than those of conventional flooding, with a reduction of 5.32% under incomplete AWD and 10.83% under complete AWD. In terms of rice yield, enhancements are observed only under complete AWD, with a 2.42% increase attributed to a higher number of tillers and panicles. The CF is reduced by 13.95% under complete AWD but increases by 3.44% under incomplete AWD. In another study, the AWD method reduced seasonal $CH_4$ emissions by 47% per hectare and the $CH_4$ emission factor by 88% per hectare per day. Moreover, AWD decreased the overall GWP by 41% and improved water productivity by 32% compared to the conventional flooding method. AWD also increased paddy productivity by 3% while reducing irrigation water consumption by 27% and associated costs by 24% (Mohammad *et al.,* 2018).

Production inputs such as fertilizers, insecticides, organic manure, fossil fuels, machinery, and irrigation systems have a major impact on GHG emissions (Soni and Soe, 2016). Fertilizer, especially nitrogenous fertilizer, is a significant contributor to the CF and energy consumption. If nitrogen use efficiency is enhanced or properly managed through improved agronomic practices, it can reduce total emissions by 30 to 50% (Liu *et al.,* 2016). Similarly, Paramesha *et al.,* (2022) observed that the highest GHG emissions were from nitrogenous fertilizers (72.1 kg $CO_2$ eq./ha), followed by machinery (68.5) and diesel fuel (67.9), with the least GHG emissions from insecticides (5.9) due to their low usage. In contrast to previous study results, Kramer *et al.,* (1999) reported that the combustion of diesel fuel by farm machinery had a greater contribution to GHG emissions, followed by fertilizers in the Dutch region. Periodic soil testing, as well as the use of organic sources of nutrients such as green manure, Azolla, and farmyard manure (FYM), can help limit the indiscriminate use of fertilizers (Mohammadi *et al.,* 2013).

Growing fertilizer-responsive, high-yielding cultivars in nutrient-poor soil results in the increased use of chemical fertilizers and higher GHG emissions. The increased usage of diesel fuel, due to intensive tillage and increased mechanization, results in additional GHG emissions. It emphasizes the potential for conservation tillage to save energy and reduce GHG emissions by reducing the use of machinery and fossil fuel combustion. To conserve energy and reduce GHG emissions, farmers must implement conservation tillage and better crop management techniques. The GWP of dry direct-seeded rice (DDSR) and wet direct-seeded rice (WDSR) was lower by 76.9% and 58.5% in 2014, and 75.4% and 62.2% in 2015, compared to transplanted rice (Tao *et al.,* 2016). The use of DDSR can decrease the CF of rice by more than 30%, mainly by reducing input requirements for irrigation and energy, resulting in a lower GWP (Kumar *et al.,* 2018). Transplanted rice (TPR) recorded the highest CF of 2470 kg CO2-e./ha, which were 3.3% and 8.4% higher than those of wet and dry DSR, respectively (Lal *et al.,* 2020).

The CF of rice cultivation varies between regions and states, with differences in crop management practices and input utilization contributing to these variations. Excessive consumption of fertilizers, pesticides, and fuel can result in higher carbon footprints in specific areas. For example, in Karnataka state, Raichur district recorded the highest CF (1532 kg-CE $ha^{-1}$), closely followed by Ballari and Koppal districts, each with 1368 kg-CE $ha^{-1}$, compared to the state's average carbon input of 1081 kg-CE $ha^{-1}$ (Sridhara *et al.,* 2023). The CF of Raichur district and Ballari and Koppal districts was 42% and 27% higher than the state average due to excess consumption of fertilizers, pesticides, and fuel by 129%, 32% and 140%, respectively, over the state average (Sridhara *et al.,* 2023). Dash *et al.,* (2023) quantified the CF of major rice production systems, namely aerobic rice (AR), shallow lowland rice (SLR), SRI, deep water rice (DWR), and zero-tilled direct-seeded rice (ZTR) in India. They concluded that DWR had the highest seasonal cumulative $CH_4$ emission (115.1 kg $ha^{-1}$), while AR had the lowest cumulative $CH_4$ emission (34.5 kg $ha^{-1}$). The higher seasonal cumulative $N_2O$ emission was observed in the AR system (1.40 kg $ha^{-1}$), followed by SRI (1.10 kg $ha^{-1}$), and the least was in DWR (0.86 kg $ha^{-1}$). Among these systems, DWR had the highest estimated seasonal mean GWP (3.92 t $ha^{-1}$), while AR had the lowest (1.48 t $ha^{-1}$).

The CF per tonne of rice production among these systems varied from 0.57-0.87 t C-eq $t^{-1}$ rice, with the lowest value found under ZTR, while the SRI system recorded the highest CF. The zero-tilled direct-seeded rice system saved 28.3%, 34.0%, 48.6% and 53.3% of C-eq emissions per tonne of rice production compared to DWR, AR, SLR and SRI, respectively. However, total GHG emissions were lower in AR compared to ZTR due to a lower carbon stock. Therefore, if the focus is on short-term or immediate GHG emission reduction, AR appears to be a good option. However, for a long-term strategy, ZTR, with its lower CF and higher soil carbon stock potential, needs to be promoted with incentives. They also concluded that although CF in SRI was higher, this system is potentially higher yielding and sequesters more carbon in the soil.

Yadav *et al.,* (2020) conducted a field trial to determine the carbon-efficient rice production system among No till (NT) and Conventional tillage (CT) with different mulching systems (RSM-Rice straw mulch, GLM-Gliricidia mulch, BMM-Brown manuring mulch of cowpea, and NM-No mulch). They found that total $CO_2$-e emissions from NT were lower (1,080 kg $CO_2$-e $ha^{-1}$) compared to CT (1,292 kg $CO_2$-e $ha^{-1}$). The difference of 212 kg $CO_2$-e $ha^{-1}$ between CT and NT was attributed to the increased use of diesel-operated power tillers for field preparation under CT (247 kg $CO_2$-e $ha^{-1}$) compared to NT (15 kg $CO_2$-e $ha^{-1}$). An increase in diesel consumption under CT had a major contribution to high GWP and total $CO_2$-e emissions. Regarding the mulching treatments, GLM and BMM mulches had slightly higher CF (3-8%) compared to RSM and NM. However, yield improvement, energy use efficiency, and economic profitability were significantly higher in these mulches compared to RSM and NM. The reduction in tillage operations resulted in lower energy consumption and saved fossil fuel, leading to the lowest GWP under NT (Pratibha *et al.,* 2015). This implies that the adoption of conservation agriculture practices, such as no tillage or reduced tillage along with in-situ mulching, will reduce the energy footprint by saving diesel, water, and other intensive inputs. This approach represents a better option for maximizing yield and profit in direct-seeded upland rice cultivation **(Table 3)**.

**Table 3: Carbon footprint of different rice production systems**

| S. No. | Rice production system | $CO_2$ e kg $ha^{-1}$ | Reference |
|---|---|---|---|
| 1. | Transplanted rice | 2099 | Lal *et al*., (2020) |
| 2. | Wet DSR | 2035 | Lal *et al*., (2020) |
| 3. | Dry DSR | 1939 | Lal *et al*., (2020) |
| 4. | Manual broadcasting | 4984 | Nguyen *et al*., (2022) |
| 5. | Blower seeding | 4991 | Nguyen *et al*., (2022) |
| 6. | Drum seeding | 4995 | Nguyen *et al*., (2022) |
| 7. | Mechanical transplanting | 4679 | Nguyen *et al*., (2022) |
| 8. | No tillage rice | 1080 | Yadav *et al*., (2020) |
| 9. | Conventional tilled rice | 1292 | Yadav *et al*., (2020) |

The combination of ZT and rice residue retention could potentially be an option to build up soil carbon, lower GHG emissions, with a relatively less negative impact on crop yield compared to rice residue retention/incorporation and green manuring alone in lowland transplanted rice in the tropics (Dash *et al.,* 2017). The comparison of drip irrigation with a plastic-film-mulch system (DP) with conventional flooding (CF) reveals that the GWP was 36 and 4 g $m^{-2}$ $season^{-1}$ for CF and DP, respectively (Fawibe *et al.,* 2019). The GWP was reduced by 89% under DP compared to CF. The potential loss of soil organic carbon (SOC) caused by higher soil aeration under the non-flooded system has the capability of increasing GWP. Nevertheless, the use of plastic-film-mulch could possibly mitigate the loss of SOC. This indicates that drip irrigation with ground cover rice production using mulching will reduce both the water and carbon footprint (Samoy *et al.,* 2022).

The carbon footprint of rice production is a complex issue influenced by various factors, including water management, fertilizer use, regional variations, and specific practices. Efforts to reduce GHG emissions in rice production should consider a combination of practices and techniques that promote sustainability, increased yields, and economic profitability while minimizing the impact on climate change.

**Water footprint**

Water is the most essential ingredient for all living things. Three major sectors i.e., agriculture, domestic consumption and industry are competing for water, thus it is going to be a scarce commodity worldwide. The irrigation water utilized for land preparation processes does not find utilization in plant transpiration, thus leading to loss from paddy fields (Mallareddy *et al.,* 2023). This phenomenon distinguishes rice cultivation from other forms of irrigated crops. The water requirement of rice depends on many factors encompassing environmental conditions, the growing season, length of the growing period (LGP), weather parameters, soil type, and other hydrological parameters (Nayak *et al.,* 2022). Numerous studies have reported a range of 1000–2000 mm ± 350 mm as water demand in rice cultivation **(Table 4)**. The compilation of data from various studies reflects a broader range of seasonal water usage, spanning from 660 to 5280 mm. The wide variation in seasonal water requirement for rice farming was mainly attributed to deep percolation losses which notably varies across different soil types (clay loam: 1566 mm; sandy loam: 2262 mm). Other factors include climate, varied management practice and hydrological circumstances.

**Table 4: Typical seasonal water outflows and input in lowland rice**

| S. No. | Item | Water outflow and input (mm) |
|---|---|---|
| 1. | Land preparation | 160-1560 |
| | Crop growth period requirement | |
| 2 | *Evapotranspiration* | |
| | i) Wet season | 400-500 |
| | ii) Dry season | 600-700 |
| 3. | *Seepage and percolation* | |
| | i) Heavy clays | 100-500 |
| | ii) Loamy/sandy soils | 1500-3000 |
| | **Total seasonal water input** | **660-5280** |

*Source (Tuong and Bouman, 2003)*

DSR not only reduces the reliance on fresh and groundwater resources, but also demonstrates an enhanced ability to harness rainwater effectively. DSR has the capacity to reduce the total water footprint associated with rice production (Chakrabarti *et al.,* 2014) and boasts a substantially lower water footprint (953.8 $m^3$ per ton), in stark contrast to the transplanted rice (1071.1 $m^3$ per ton). The effects of different crop establishment methods *viz.,* Dry Seeding (DS), Wet Seeding (WS) and Transplanted method (TP) on irrigation input and water productivity in the Muda Irrigation Scheme, Malaysia from 1988 to 1994 revealed that crop establishment methods such as DS and WS significantly reduced irrigation and total water input during the pre-crop establishment period, due to reduced land preparation compared to TP. However, during the crop growth period in the main field, TP had a significantly shorter crop growth duration (110 days) and lower total water input compared to DS and WS. DS rice required significantly less irrigation water for unit production and exhibited higher water productivity (1.48 kg m$^{-3}$) compared to WS (0.62 kg m$^{-3}$) and TP (1.00 kg m$^{-3}$) (Cabangon *et al.,* 2002). The advantage of WS rice over TP rice depends on the balance between the reduction in depletion and outflow before crop establishment and the increase in the same during the crop growth period. Dry seeding can advance the establishment of the wet-season crop, does not require pre-saturation irrigation, and shortens the land preparation period considerably compared with WS and TP rice. These factors lead to a reduction in seepage and percolation, evaporation and evapotranspiration, and irrigation water amount.

Crop evapotranspiration accounted for 26.8% and 27.9% of the total water input in TPR and DSR, respectively. Runoff accounted 20.4% and 7.9%, while deep percolation beyond 100 cm depth accounted for 55.9% and 67.5% in TPR and DSR, respectively. This indicated that DSR had 14.6% more deep percolation, which has the potential to contribute to groundwater recharge. Additionally, 23.6% of irrigation water was saved under DSR fields compared to TPR during the crop period (Gulati *et al.,* 2022). DDSR yielded 6040 kg/ha (only 5.5% less than PTR) and saved 32.6% of irrigation water, and 48.9% of labor compared to PTR (Ramesh *et al.,* 2023). Thus, DDSR is a promising solution for areas with water scarcity and labor shortage.

The SRI practices combined with the AWD method of irrigation resulted in a remarkable water saving (22.2%) compared to continuously flooded rice cultivation (Thakur *et al.,* 2011). Similarly, in sandy loam soils of the ICRISAT farm, SRI demonstrated water savings of 22% and 38% during the dry and wet seasons, respectively compared to conventional methods (Viraktamath and Kumar, 2007). The water savings in the AWD system were primarily attributed to the reduction in seepage and percolation losses. Furthermore, the SRI method required 1,463 liters of water to produce 1 kilogram of rice, whereas continuously flooded rice cultivation required 2,778 liters of water for the same rice production (Thakur *et al.,* 2011). This highlights the significant water-saving potential of the SRI method. The water productivity with AWD-SRI management practices was nearly double (0.68 grams per liter) compared to the water productivity of continuously flooded rice cultivation (0.36 grams per liter).

AWD practices reduced water input, amounting to 26-29% during the *kharif* season and 22-27% in the *rabi* seasons. The AWD practice also improved the water use efficiency by 27-33% during the *kharif* season and 20-29% in the *rabi* season. Furthermore, the consumptive water footprint was reduced by 2-3% and 2-5%, and blue water footprints were reduced by 7% and 4-5% in *kharif* and *rabi* seasons, respectively (Biswas *et al.,* 2021). The reduction in evapotranspiration by approximately 6% in both *kharif* and *rabi* seasons contributed to water saving. Pan *et al.,* (2017) reported a 24 to 71% reduction in water input under AWD based on a two-year study. AWD method resulted in a 3% increase in paddy productivity, accompanied by a significant decrease in irrigation water consumption by 27% and associated costs by 24%. As a result, it improved water productivity by 32% compared to the CF method (Mohammad *et al.,* 2018).

Dry Direct Seeding (DDS) method required approximately 983 mm of water, while providing a water productivity of 6.27 kg ha$^{-1}$ mm$^{-1}$. In contrast, the transplanting method required 1238 mm of water with a water productivity of 5.03 kg ha$^{-1}$ mm$^{-1}$. The DDS method, by avoiding puddling and facilitating rainfed cultivation up to 45 days post sowing, reduced the duration of water requirement and resulted in reduced overall water consumption compared to transplanting (Suresh *et al.,* 2004). Moreover, the study by Tao *et al.,* (2016) contributes further insights by establishing a reduction of 24.7% in irrigation water consumption for DDSR compared to wet DSR and a reduction of 13.3% in comparison to transplanted rice. Additionally, the water productivity of DDSR was 11.6% higher than transplanted rice, while wet DSR had a water productivity 13.4% higher than transplanted rice. It is worth noting that wet DSR recorded higher water productivity compared to transplanted rice and DSR due to increased grain yield **(Table 5)**.

**Table 5: Water productivity of different rice production systems**

| S. No. | Production system | Total water productivity (kg hamm$^{-1}$) | Irrigation water Productivity (kg hamm$^{-1}$) | Reference |
|---|---|---|---|---|
| 1. | Conventional tilled puddled transplanted rice | 1.93 | - | Guru *et al.* (2017) |
| | Reduced tilled DSR in vatter conditions | 2.43 | - | |
| 2. | SRI | 5.8 | 15.8 | Raj *et al.,* (2017) |
| | PTR (Puddled transplanted rice) | 3.5 | 8.0 | |
| | DSR (Direct seeded rice) | 3.4 | 8.2 | |
| 3. | ICM (Integrated cultural management) | 2.86 | - | Das *et al.,* (2014) |
| | SRI | 2.98 | - | |
| | CRC (Conventional rice culture) | 2.63 | - | |
| 4. | Aerobic rice irrigation at 75% CPE | - | 8.61 | Duary *et al.,* (2022) |
| 5. | Aerobic rice drip irrigation 1.5 Epan in raised bed system | 4.10 | - | Bhavana (2022) |
| | Aerobic rice with surface irrigation | 3.26 | - | |
| 6. | Surface irrigation (Aerobic rice) | 4.6 | 4.5 | Pascual *et al.,* (2022) |
| | 40 cm drip line spacing | 5.5 | 5.5 | |
| | 60 cm drip line spacing | 7.7 | 7.4 | |
| | 80 cm drip line spacing | 6.1 | 6.0 | |
| 7. | Continues ponding | 3.5 | 3.6 | Poddar *et al.,* (2022) |
| | AWD | 3.9 | 4.0 | |
| | Saturation | 4.6 | 4.8 | |

The irrigation input for wet seeded rice on puddled soil was significantly higher (2817 mm) compared to other establishment methods (puddled transplanted rice (PTR), non-puddled transplanted rice (NPTR), surface seeded rice on non-puddled soil (NWSR) and dry seeded rice (DSR) in both wet and dry seasons (Evangelista *et al.,* 2014). This higher irrigation requirement can be attributed to several factors, including the need for water during the puddling process, the cracking of puddled soil during crop establishment which necessitates additional irrigation and the longer duration of the wet seeded crop in the main field compared to transplanted crops. These factors collectively contribute to the higher irrigation input associated with direct seeded rice cultivation. In general, DSR recorded higher water productivity compared to transplanting method. For example, the comparison of water productivity of DSR and transplanting methods reveal that the water productivity in DSR ranged from 0.40 to 0.46 kg grain m$^{-3}$ of irrigation water, while under transplanting, it varied from 0.29 to 0.39 kg grain m$^{-3}$ of irrigation water (Gill *et al.,* 2006). Adopting improved DSR resulted in labor savings (40-45%), water savings (30-40%), fuel/energy savings (60-70%) and reduced greenhouse gas emissions (Yaduraju *et al.,* 2021).

The total water input of transplanted flooded rice with the average flooding depth of 3.9 cm and the average flooding period of 114.0 days was 1255.0 mm. In contrast, for direct seeded flooded rice, the total water input (1022.8 mm) was significantly lower than transplanted rice as the average flooding depth and the average flooding period were 2.6 cm and 93.5 days, respectively. Furthermore, for direct seeded rice with AWD, the average flooding depth was even lower (0.8 cm), the average flooding period was significantly reduced to 13.5 days and the total water input was 607.5 mm. This indicates that compared to transplanted flooded rice, both direct seeded flooded rice and direct seeded rice with AWD significantly reduced flooding depth, flooding period and total water input. Specifically, when compared to transplanted flooded rice, direct seeded flooded rice demonstrated a 34.1% reduction in flooding depth, a 17.8% reduction in flooding period and a 22.1% reduction in total water input. Similarly, direct seeded rice with AWD exhibited even greater reductions, with a 79.5% decrease in flooding depth, an 88.2% reduction in flooding period and a 53.7% decrease in total water input.

Conservation agriculture plays a major role in reducing water footprint. For example, strip and zero tillage methods reduced irrigation water input for transplanting by 22% and 28%, respectively (Hossen *et al.,* 2018). Strip and zero tillage also improved soil physio chemical properties. The delay of first flood irrigation until 55 days after sowing (DAS) of DDSR (Dry DSR) decreased the number of irrigations required from eight to four in 2014 and from twelve to seven in 2015. Furthermore, the amount of irrigation water applied was significantly reduced from 376 mm to 185 mm in 2014 and from 477 mm to 284 mm in 2015. The slight drought stress in the early vegetative growth stage did not negatively affect the plant growth or yield (Jiang *et al.,* 2016). This emphasizes that number and frequency of irrigation influence the crop water requirement.

The highest grain yield of 4.56 t ha$^{-1}$ was obtained with continuous ponding, which outperformed AWD with a yield of 4.30 t ha$^{-1}$ and saturation with a yield of 3.97 t ha$^{-1}$. However, when considering crop water productivity (CWP), saturation achieved a CWP of 0.428 kg m$^{-3}$, which was 13.5% higher than AWD (0.377 kg m$^{-3}$) and 24.9% higher than continuous ponding (0.343 kg m$^{-3}$). Despite having the highest grain yield, continuous ponding had a lower CWP compared to saturation and AWD (Poddar *et al.,* 2022). Enriquez *et al.,* (2021) conducted a study comparing the water use between traditional continuous flooding and AWD methods. Their findings revealed that AWD reduced water use by 16-28% in both pump and canal-based irrigation systems compared to traditional continuous flooding. This indicates that AWD is an effective approach for reducing water consumption in rice production. Aerobic rice saved water by 11.2% and 28.4% in 2018 and by 5.72% and 32.98% in 2020, compared to AWD and conventional flooding (CF), respectively. This suggests that aerobic rice has the potential to significantly reduce water usage compared to AWD and CF methods (Hussain *et al.,* 2021).

### Perennial rice

Perennial rice holds the promise of substantially reducing the carbon, water, and energy footprints associated with traditional annual rice cultivation. This innovative approach to rice farming entails cultivating rice varieties that have longer life cycles and can persist for multiple years, as opposed to the conventional practice of replanting each season. Perennial rice systems can contribute to a decreased carbon footprint by minimizing the need for frequent soil disturbance through tillage and replanting. Reduced soil disturbance prevents the rapid decomposition of organic manures, thereby reducing the release of carbon dioxide and other GHGs stored in the soil. The establishment of a perennial root system also enhances carbon sequestration in the soil, further mitigating atmospheric carbon levels. Similarly, perennial rice systems generally exhibit deeper and more extensive root systems, enabling them to access water resources more effectively. This increased water use efficiency is especially valuable in water-scarce regions, where traditional rice cultivation may require intensive irrigation. By tapping into deeper water sources and reducing surface evaporation, perennial rice can conserve water resources and contribute to improved water management. Perennial rice systems promote healthier soil structures due to reduced disturbance and continuous root growth. This, in turn, enhances soil water retention and nutrient cycling. The longer life cycle of perennial rice reduces the frequency of land preparation, planting, and other labor-intensive tasks. This results in lower energy requirements for machinery operation and reduced use of fossil fuels. Furthermore, the decreased need for annual replanting and associated inputs like fertilizers and pesticides can contribute to energy savings. The adoption of perennial rice has the potential to revolutionize rice cultivation by offering a more sustainable and environmentally friendly alternative to traditional annual systems. By addressing the carbon, water, and energy footprints of rice production, perennial rice contributes to a more resilient and sustainable agricultural future.

## Conclusion

Based on this review it is concluded that the method of rice crop establishment, irrigation method and crop management practices followed, climatic conditions and resource availability during crop growing season are the major determinants of energy, carbon and water footprint of rice crop. The conventional transplanted rice cultivation leads to more water and energy carbon footprint while alternate rice production systems such as SRI, AWD, DSR (drum-seeding and broadcasting) and better nutrient management practices like SSNM, use of nutrient decision support tools like a nutrient expert, leaf colour chart, chlorophyll meter, nano-fertilizers, slow releasing fertilizers and legumes in the off-season will help to reduce the carbon, water and energy footprint in rice cultivation. Although micro-irrigation and fertigation were found to be more efficient in terms of energy, water and nutrients than conventional transplanted rice, still its adoption is less. Appropriate interventions are required for all farming communities through proper subsidy policies to ensure large-scale adoption. To reduce the utilization of fossil fuels in rice cultivation, the development of renewable energy (solar energy, biofuels) driven machinery and its adoption is vital. Suitable governmental policies/promoting schemes and subsidies to machinery and irrigation accessories will ensure its large-scale adoption. Hence, method of establishment, irrigation method followed, better crop management practices, climatic conditions and resource availability during crop growing season will decide the energy, carbon and water footprint of rice crop.

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**RESEARCH ARTICLE** https://doi.org/10.58297/GXWQ9357

# Genetic Variability and Correlation Studies in High-yielding Varieties of Rice (*Oryza sativa* L.)

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## Abstract

Rice is the major staple food crop of India and there is an anticipated high demand due to increasing population. Information on yield contributing traits and the level of heritable variation in these traits is essential for establishing the genotype's potential for future breeding programmes. The study was conducted to evaluate the variability in yield contributing traits and their correlation in a set of released rice varieties from ICAR - Indian Institute of Rice Research, Hyderabad. The objective of the study was to estimate the genotypic and phenotypic coefficient of variation, heritability and genetic advance of yield and related traits using thirty-two rice cultivars. For all evaluated traits, the phenotypic coefficient of variation was found to be significantly higher than the genotypic coefficient of variance, implying that the expression of these traits is influenced by the environment. High heritability coupled with high genetic advance was observed for panicle weight whereas low heritability coupled with low genetic advance over per cent mean was recorded for productive tiller number. The traits *viz.,* tiller number, productive tiller number, flag leaf width, panicle weight, number of grains per panicle, number of filled grains per panicle and test weight exhibited a positive association, whereas the number of unfilled grains per panicle and plant height exhibited negative association with grain yield.

**Keywords:** Genetic variability, coefficient of variation, heritability, genetic advance and correlation.

## Introduction

Rice (*Oryza sativa* L.) is the principal staple food crop for human race and one of the world's foremost crops, with over a hundred nations cultivating it. Rice is grown in a variety of climatic conditions around the world, including tropical and subtropical climates. During the main crop season (*Kharif*), the principal rice ecology is found across temperatures ranging from 20-40 °C in flooded environments and with a solar radiation requirement of 25-95 per cent. Rice is grown in more than 150 million hectares around the world, with India accounting for one-third of the total (*i.e.*, 44 million hectares). However, India's rice production (122 million tonnes) is only a fifth of global rice production (503 million tonnes). India is first in the world in terms of area and second in terms of rice production. Rice is a calorie source for more than 70% of India's population and a key source of income for millions of farmers.

The global population is steadily rising, with an anticipated 9.1 billion people by 2050, but agricultural productivity is not keeping pace. By 2050, global agricultural production is expected to increase by 100-110 per cent to feed the whole population (FAO 2009). Rice production is being threatened by dwindling natural resources, socio-economic constraints, biotic and abiotic stresses and climatic uncertainty as the demand grows.

Variation is the prerequisite for every breeding programme. The range of genetic variability in selected genotypes is determined by the degree of genetic variation among genotypes, which provides more selection chances. In establishing the genotype's potential for future breeding programmes, the level of heritable variation in the attributes is particularly useful. To measure the degree of diversity in a germplasm sample, genetic parameters such as the genotypic coefficient of variation (GCV) and the phenotypic coefficient of variation (PCV) can be considered. Estimating heritability is a useful method for proving the consistency of phenotypic value. As a result, increased heritability benefits in the character selection procedure. Heritability is a term used to describe how much genetic variation across individuals in a community causes variation in phenotypic traits. In evaluating the gain under selection, heritability estimates combined with genetic advancement are usually more informative than heritability estimates alone.

Grain yield is a multifaceted trait that is influenced by a number of various aspects, and it responds poorly to direct selection when decisions are made solely on the basis of yield. Knowledge of the relationship between grain yield and its component features will be useful in improving grain yield. As a result, correlation analysis is required to determine the relationship between yield and yield components (Akhtar *et al*., 2011). Correlation measures the degree and direction of association between two or more variables and provides information about yield contributing characters. This information is useful to a plant breeder in the selection of elite genotypes from diverse genetic populations. On the other hand, the genotypic correlation, which represents the genetic component of the phenotypic correlation, is one of inheritable nature and is thus utilized to drive breeding programmes. In plant breeding, estimates of the correlation coefficient are useful in determining yield components, which can be used for genetic improvement of yield.

The focus of this study is to estimate the correlation among the various quantitative traits of rice in order to understand the relationship between grain yield and its constituent features and aid the effective selection for a successful breeding program. The high-yielding varieties released from ICAR-Indian Institute of Rice Research, Hyderabad were used in this study to understand the range of available yield potential. The finding of this research will assist in the identification of suitable genetic material and for further development of high-yielding varieties in the subsequent breeding programmes to support varietal improvement.

## Materials and Methods

The experiment was conducted during *kharif*, 2021 on IIRR farm located at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad, Telangana, India. The experiment material comprises of 32 rice genotypes (DRR Cultivars) **(Table 1)** and the experiment was planned in a randomized block design. Each experimental unit consists of a plot size of 2m$^2$. All genotypes were sown separately in an experimental unit with three replications. 30 days old seedlings were transplanted with a spacing of 20 cm × 20 cm. The crop was grown with the recommended package of practices. Fertilizers were applied to the crop on a regular basis, as per conventional recommendations. Intercultural operations were carried out as per the requirement. Observations for 12 traits *viz*., plant height, tiller number, productive tiller number, flag leaf length, flag leaf width, panicle length, panicle weight, number of grains per panicle, number of filled grains per panicle, number of unfilled grains per panicle, single plant yield and test weight were recorded from three randomly selected plants in each replication. The genotypic and phenotypic coefficient of variation was calculated as per the formula suggested by Burton and Devane (1953). Heritability in broad sense was calculated as suggested by Allard (1960). From the heritability estimates, the genetic advance (GA) was calculated as suggested by Johnson *et al.,* (1955). The correlation between characters was calculated by using the method suggested by Dewey and Lu (1959).

**Table 1: List of rice genotypes used in the experiment**

| S. No. | Cultivars | S. No. | Cultivars | S. No. | Cultivars |
|---|---|---|---|---|---|
| 1 | DRR Dhan 39 | 12 | DRR Dhan 50 | 23 | Binadhan 17 |
| 2 | DRR Dhan 40 | 13 | DRR Dhan 51 | 24 | Salivahana |
| 3 | DRR Dhan 41 | 14 | DRR Dhan 54 | 25 | RP Bio 226 |
| 4 | DRR Dhan 42 | 15 | DRR Dhan 55 | 26 | Varadhan |
| 5 | DRR Dhan 43 | 16 | DRR Dhan 56 | 27 | Swarnadhan |
| 6 | DRR Dhan 44 | 17 | DRR Dhan 62 | 28 | Akshaydhan |
| 7 | DRR Dhan 45 | 18 | Sugandamati | 29 | Jaya |
| 8 | DRR Dhan 46 | 19 | IR 64 | 30 | Sampada |
| 9 | DRR Dhan 47 | 20 | Vasumati | 31 | Dhan rasi |
| 10 | DRR Dhan 48 | 21 | Kasturi | 32 | Jarava |
| 11 | DRR Dhan 49 | 22 | Binadhan 11 | | |

## Results and Discussion

The degree of diversity for any trait is critical for crop improvement through breeding. The estimates of genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) heritability and genetic advance for the traits under study were presented in **(Table 2)**. The extent of the differences between GCV and PCV explains the amount of the influence of the growing environment on those characteristics. The higher divergence between PCV and GCV indicates that the expression of the specific trait is heavily influenced by the environment. For all evaluated traits, the phenotypic coefficient of variation was found to be significantly higher than the genotypic coefficient of variance, implying that the expression of these traits is influenced by the environment.

The estimate of the phenotypic coefficient of variation was found to be high in the number of unfilled grains per panicle (107.46). A higher magnitude of the phenotypic coefficient of variation was recorded in panicle weight (37.57), number of grains per panicle (26.44), number of filled grains per panicle (26.25), tiller number (23.53) and productive tiller number (22.38). Jahan *et al*., (2020) observed a similar effect in panicle weight while Chavan *et al*., (2022)'s findings were similar to findings for number of filled grains per panicle. The traits such as flag leaf length (19.98), test weight (19.27), single plant yield (17.75) and flag leaf width (16.07) exhibited a moderate level of phenotypic coefficient of variation. Low estimates of PCV were recorded in plant height (11.31) and panicle length (10.89). Similarly, Gupta *et al*., (2022) observed a similar effect in the case of test weight and panicle length.

**Table 2: Components of genetic parameters for yield and its attributing characters in rice**

| Traits | Range |  | Mean | Heritability in broad sense ($h^2$) | GCV | PCV | GA | GA % mean |
|---|---|---|---|---|---|---|---|---|
|  | Min | Max |  |  |  |  |  |  |
| Plant height | 94.00 | 133.00 | 112.22 | 81.24 | 10.20 | 11.32 | 21.25 | 18.94 |
| Tiller number | 9.30 | 20.30 | 15.10 | 23.14 | 11.32 | 23.53 | 1.69 | 11.22 |
| Productive tiller number | 9.30 | 18.00 | 14.84 | 14.27 | 8.46 | 22.39 | 0.98 | 6.58 |
| Flag leaf length | 15.30 | 39.30 | 32.60 | 74.51 | 17.25 | 19.99 | 10.00 | 30.67 |
| Flag leaf width | 1.20 | 2.30 | 1.73 | 62.43 | 12.70 | 16.08 | 0.36 | 20.68 |
| Panicle length | 21.10 | 34.10 | 25.87 | 75.34 | 9.45 | 10.89 | 4.37 | 16.90 |
| Panicle weight | 2.10 | 8.10 | 4.17 | 68.89 | 31.19 | 37.58 | 2.23 | 53.32 |
| Total number of grains per panicle | 127.30 | 316.00 | 235.41 | 47.52 | 18.23 | 26.44 | 60.93 | 25.88 |
| Number of filled grains per panicle | 114.70 | 282.00 | 199.50 | 29.91 | 14.36 | 26.26 | 32.27 | 16.18 |
| Number of unfilled grains per panicle | 5.00 | 137.30 | 35.91 | 62.31 | 84.84 | 107.47 | 49.52 | 137.95 |
| Single plant yield | 20.00 | 35.30 | 30.08 | 27.61 | 9.33 | 17.75 | 3.04 | 10.10 |
| Test weight | 13.50 | 30.50 | 22.43 | 93.87 | 18.68 | 19.28 | 8.36 | 37.28 |

The high estimates of GCV were observed for traits *viz*., number of unfilled grains per panicle (84.84) and panicle weight (31.19), whereas a low value for GCV was found in panicle length (9.45), single plant yield (9.32) and productive tiller number (8.45). A similar effect for the number of unfilled grains per panicle was observed by Gebrie *et al.,* (2022). Similarly, low estimates for productive tiller number were observed in the findings of Singh *et al*., (2022). Test weight (18.68), number of grains per panicle (18.23), flag leaf length (17.25), number of filled grains per panicle (14.36), flag leaf width (12.7), tiller number (11.32) and plant height (10.2) showed a moderate estimate for GCV.

For panicle length and plant height, lower PCV and GCV values were observed which was previously reported by Gupta *et al*., (2022). It indicates a limited range of total variability and a strong influence of the environment on the expression of these traits. As a result, there was not much scope for direct selection for this character.

### Heritability in broad sense $h^2b$

Heritability in a broad sense was found to be higher in test weight (93.87) followed by plant height (81.24), panicle length (75.33), flag leaf length (74.5), panicle weight (68.88), flag leaf width (62.43) and number of unfilled grains per panicle (62.31). Low heritability estimates were found in filled grains per panicle (29.9), single plant yield (27.61), tiller number (23.14) and productive tiller number (14.26) while moderate estimates were recorded in number of grains per panicle (47.52).

### Genetic advance

While the genetic advance is a valuable indicator for achieving desired results on the trait of interest after selection, as estimated heritability is not much dependable due to the inclusion of the effects of both additive and non-additive genes. In comparison to genetic advance, genetic advance as a percentage of mean provides a more precise conclusion and comparable across the traits. Genetic advance over mean exhibited higher values among the number of unfilled grains per panicle (137.95), panicle weight (53.32), test weight (37.27), flag leaf length (30.67), number of grains per panicle (25.88) and flag leaf width (20.67), while the character productive tiler number (6.57) exhibited a low value and remaining characters recorded moderate values.

Heritability estimates combined with genetic advancement are more effective than heritability estimates alone in predicting gain under selection (Johnson *et al*., 1955). High heritability coupled with high genetic advance was observed for panicle weight and similar effect was observed by Keerthiraj *et al.,* (2020), whereas low heritability coupled with low genetic advance over per cent mean was recorded in productive tiller number which was previously reported by Singh *et al.,* (2022). It suggests that non-additive genes have a role in the inheritance of these traits, implying that heterosis breeding rather than selection could be used to improve the trait.

### Correlation studies

Correlation analysis of the yield and its contributing characters indicated that the genotypic correlation coefficients were higher than the phenotypic correlation coefficients in most cases, suggesting that the association was primarily due to genetic factors (Bhattacharyya *et al*., 2007). The correlation studies for yield and its contributing characters were presented in the **(Table 3)**.

**Table 3: Coefficients of phenotypic ($r_p$) and genotypic ($r_g$) among different yield components**

| Characters | Correlation | PH | TN | PTN | LL | LW | PL | PW | TG | FG | UFG | SPY | TW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **PH** | $v_p$ | 1 | -0.327 | -0.328 | 0.121 | 0.21 | 0.576** | 0.555** | 0.115 | 0.291 | -0.161 | -0.177 | 0.179 |
| **PH** | $v_g$ | 1 | -0.514** | -0.604** | 0.104 | 0.249* | 0.607** | 0.635** | 0.14 | 0.42** | -0.204* | -0.257* | 0.1777 |
| **TN** | $v_p$ |  | 1 | 0.941** | -0.493** | -0.177 | -0.2967 | -0.446** | 0.0077 | -0.2215 | 0.2653 | 0.291 | -0.0596 |
| **TN** | $v_g$ |  | 1 | 0.971** | -0.714** | -0.285** | -0.419** | -0.656** | 0.0456 | -0.393** | 0.422** | -0.1319 | -0.0673 |
| **PTN** | $v_p$ |  |  | 1 | -0.22 | -0.279 | -0.289 | -0.556** | -0.006 | -0.287 | 0.318 | 0.213 | -0.18 |
| **PTN** | $v_g$ |  |  | 1 | -0.364** | -0.544** | -0.504** | -0.936** | 0.039 | -0.586** | 0.597** | -0.483** | -0.286** |
| **LL** | $v_p$ |  |  |  | 1 | -0.045 | 0.118 | 0.014 | 0.07 | 0.081 | 0.012 | -0.242 | -0.275 |
| **LL** | $v_g$ |  |  |  | 1 | -0.059 | 0.131 | -0.012 | 0.064 | 0.077 | 0.012 | -0.325** | -0.29** |
| **LW** | $v_p$ |  |  |  |  | 1 | -0.159 | 0.208 | 0.357* | 0.368* | 0.115 | 0.3 | 0.01 |
| **LW** | $v_g$ |  |  |  |  | 1 | -0.181 | 0.226* | 0.438** | 0.488** | 0.149 | 0.395** | 0.015 |
| **PL** | $v_p$ |  |  |  |  |  | 1 | 0.697** | 0.038 | 0.222 | -0.197 | -0.069 | 0.481** |
| **PL** | $v_g$ |  |  |  |  |  | 1 | 0.769** | -0.018 | 0.232* | -0.248* | -0.051 | 0.513** |
| **PW** | $v_p$ |  |  |  |  |  |  | 1 | 0.188 | 0.579** | -0.380* | 0.083 | 0.503** |
| **PW** | $v_g$ |  |  |  |  |  |  | 1 | 0.143 | 0.686** | -0.445** | 0.123 | 0.555** |
| **TG** | $v_p$ |  |  |  |  |  |  |  | 1 | 0.746** | 0.648** | 0.047 | -0.395* |
| **TG** | $v_g$ |  |  |  |  |  |  |  | 1 | 0.708** | 0.745** | 0.091 | -0.463** |
| **FG** | $v_p$ |  |  |  |  |  |  |  |  | 1 | -0.022 | 0.212 | -0.202 |
| **FG** | $v_g$ |  |  |  |  |  |  |  |  | 1 | 0.044 | 0.381** | -0.266** |
| **UFG** | $v_p$ |  |  |  |  |  |  |  |  |  | 1 | -0.172 | -0.362* |
| **UFG** | $v_g$ |  |  |  |  |  |  |  |  |  | 1 | -0.252* | -0.399** |
| **SPY** | $v_p$ |  |  |  |  |  |  |  |  |  |  | 1 | 0.0029 |
| **SPY** | $v_g$ |  |  |  |  |  |  |  |  |  |  | 1 | 0.0238 |
| **TW** | $v_p$ |  |  |  |  |  |  |  |  |  |  |  | 1 |
| **TW** | $v_g$ |  |  |  |  |  |  |  |  |  |  |  | 1 |

**Note:** PH= plant height, TN= tiller number, PTN= Productive tiller number, LL (cm)= Flag leaf length, LW (cm)= Flag leaf width, PL (cm) = Panicle length, PW(g)= Panicle weight, TG= total number of grains per panicle, FG= number of filled grains per panicle, UFG= number of unfilled grains per panicle, SPY(g)= single plant yield, TW(g) = Test weight

* indicates significant at 5% level of probability

** indicates significant at 1% level of probability

At the phenotypic level, a significant positive correlation was observed for plant height with panicle length (r =0.576**) and panicle weight (r = 0.555**), whereas tiller number, productive tiller number, single plant yield and number of unfilled grains had a negative relationship with plant height; and rest of the characters exhibited a positive relationship with plant height. Similar findings were observed for plant height with panicle length and panicle weight by Parimala *et al*., (2020) and Rukminidevi *et al.,* (2022). At genotypic level, plant height possessed positive and highly significant correlation with panicle weight (r = 0.635**), panicle length (r = 0.607**), number of filled grains per panicle (r = 0.42**) and flag leaf width (r = 0.249*) while a negative and significant correlation was associated with number of unfilled grains per panicle (r = -0.204*), single plant yield (r = -0.258*), tiller number (r = -0.515**) and productive tiller number (r = -0.604**).

Tiller number exhibited a highly positive and significant association with productive tiller number (r = 0.941**), which was previously reported by Saha *et al*., (2019) whereas, a negative and significant association with panicle weight (r = -0.446**) and flag leaf length (r = -0.494**), while number of grains per panicle, number of unfilled grains and single plant yield showed a positive relationship; and rest of the characters showed a negative association at the phenotypic level. At genotypic level, tiller number with productive tiller number (r = 0.971**) and number of unfilled grains per panicle (r = 0.422**) exhibited a high positive and significant association, while a negative and significant association with flag leaf width (r = -0.285**), number of filled grains per panicle (r = -0.394**), plant height (r = -0.515**), panicle weight (r = -0.657**) and flag leaf length (r = -0.714**). Number of filled grains per panicle and single plant yield exhibited positive and negative relationships respectively.

Productive tiller number had a negatively significant association with panicle weight (r = -0.556**) whereas, a positive relation was observed with number of unfilled grains per panicle and single plant yield at the phenotypic level. Productive tiller number exhibited positive and significant association with number of unfilled grains per panicle (r = 0.597**); while, a negative and significant association was observed with test weight (r = -0.287**), flag leaf length (r = -0.365**), single plant yield (r = -0.483**), panicle length (r = -0.504**). Similar effect was observed by Lakshmi *et al*., (2014) for flag leaf width (r = -0.544**), plant height (r = -0.604**) and panicle weight (r = -0.936**) at genotypic level.

A positive relationship was observed for flag leaf length with number of unfilled grains per panicle, panicle weight, total grains per panicle, number of filled grains per panicle and panicle length, while the rest of the characters exhibited a negative relationship at a phenotypic level while at the genotypic level, there was a negative and significant association for flag leaf length with test weight (r = -0.290**) and single plant yield (r = -0.325**). Flag leaf width and panicle weight were negatively associated, whereas the rest of the characters have a positive relationship with flag leaf length. Significant positive relationship for flag leaf width was observed with number of filled grains per panicle (r = 0.368**) and total grains per panicle (r = 0.357**) at phenotypic level. At genotypic level, flag leaf width exhibited significantly positive association with panicle weight (r = 0.226*), single plant yield (r = 0.395**), number of grains per panicle (r = 0.438**) and number of filled grains per panicle (r = 0.488**).

A significant highly positive association for panicle length was observed with panicle weight (r = 0.697**), and test weight (r = 0.481**) at the phenotypic level whereas a significantly positive association was exhibited with characters *viz*., panicle weight (r = 0.769**), test weight (r = 0.513**) and the number of filled grains per panicle (r = 0.232*) and significantly negative association with the number of unfilled grains per panicle (r = -0.248*) at the genotypic level. Panicle weight exhibited a positive and significant association with number of filled grains per panicle (r =0.579**) and test weight (r = 0.503**) while a negative and significant association was observed with number of unfilled grains per panicle (r = -0.380*) at phenotypic level whereas panicle weight exhibited a positively significant association with number of filled grains per panicle (r = 0.686**) and a significantly negative association with the number of unfilled grains per panicle (r = -0.445**) at genotypic level.

At both the phenotypic level and genotypic level high positive significant values were observed for number of grains per panicle with number of filled grains per panicle ($r_p$ = 0.746**; $r_g$ = 0.708**) and number of unfilled grains per panicle ($r_p$ = 0.648**; $r_g$ = 0.745**). Similar results were obtained by the study conducted by Singh *et al*., (2022) while a negative and significant association was observed with test weight ($r_p$ = -0.395**; $r_g$ = -0.463**).

At the genotypic level number of filled grains per panicle was observed to be negative and significantly correlated with test weight (r = -0.266**) similar results were observed by Saha *et al*., (2019).

A negative and significant association was observed for number of unfilled grains per panicle with test weight ($r_p$ = -0.362*; $r_g$ = -0.399**) at both phenotypic and genotypic level. A negative and significant relationship was exhibited by single plant yield (r = -0.252*) at the genotypic level.

Single plant yield exhibited a positive relationship with flag leaf width, productive tiller number, number of filled grains per panicle, panicle weight, the total number of grains per panicle and test weight whereas the rest of the characters exhibited a negative relationship at the phenotypic level **(Figure 1)**. A positive and significant association was observed with number of filled grains per panicle (r = 0.381**) and flag leaf width (r = 0.395**) while a negative and significant association was observed with number of unfilled grains per panicle (r = -0.252*), plant height (r = -0.257*), flag leaf length (r = -0.325**) and productive tiller number (r = -0.483**) at genotypic level.

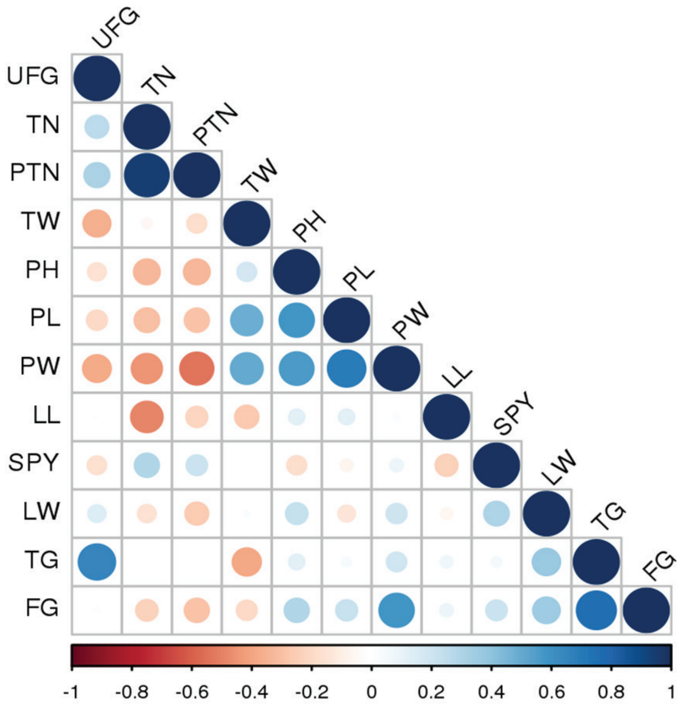

**Figure 1: Correlograms showing Coefficients of phenotypic correlation among different yield components**

## Conclusion

The varieties under study exhibited a high variability among the traits studied. A higher estimate of the phenotypic coefficient of variation was recorded for panicle weight (37.57), the total number of grains per panicle (26.44). Low estimates of PCV were recorded in plant height (11.31) and panicle length (10.89). High GCV values were observed for traits *viz*., the number of unfilled grains per panicle (84.84) and panicle weight (31.19) whereas a low value for GCV was found in panicle length (9.45), single plant yield (9.32). Single plant yield ranged from 20g to 35.3g while the highest single plant yield was recorded in DRR Dhan 50 (35.3 g) followed by Dhanrasi (35.1 g), DRR Dhan 40 (35 g), Swarnadhan (34.7 g) and DRR Dhan 56 (34.4 g). Based on the correlation studies single plant yield exhibited a positive association with flag leaf width, productive tiller number, number of filled grains per panicle, panicle weight, the total number of grains per panicle and test weight. The variety DRR Dhan 50 was identified to have a high productive tiller number; Dhanrasi and Swarnadhan showed a high number of filled grains per panicle; DRR Dhan 40 exhibited higher values for the number of filled grains per panicle coupled with productive tiller number which represents conclusive evidence for their high single plant yield and are potential donors for yield improvement in further breeding programmes.

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# Studies on Promising Restorer Lines for Yield and Yield Components in Rice (*Oryza sativa* L.)

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## Abstract

The present investigation was carried out at Regional Agricultural Research Station, Maruteru with a set of 30 experimental hybrids developed by crossing three male sterile lines with 10 testers in Line × Tester mating design during *kharif*, 2022. The resultant 30 hybrids were evaluated in Randomized Block Design with two replications along with the parents and hybrid check, HRI-174 during *rabi*, 2022-23. Analysis of variance for yield and yield components revealed significant differences among the genotypes, parents, hybrids and parents vs. crosses for most traits, indicating significant differences among parents and hybrids, in addition to significant levels of heterosis for the traits studied. In general, the hybrids had recorded higher grain yield per plant, compared to the lines and were observed to be early and relatively tall with a more number of productive tillers per plant and increased panicle length compared to the parents. The hybrids, APMS 15A×RGL 5613, APMS 15A×MTU 1213, APMS 17A×RGL 5613 and APMS 17A ×MTU 2055had recorded significant and positive relative heterosis, heterobeltiosis and standard heterosis of more than 25 per cent for grain yield per plant and were identified as promising heterotic combinations for further evaluation and commercial exploitation.

**Keywords:** Rice, Hybrids, Heterosis, Grain yield, and Yield components.

## Introduction

Rice (*Oryza sativa* L.) is a staple food crop that plays a crucial role in global food security, feeding nearly half of the world's population (Manoj *et al*., 2023). India is one of the top rice producers globally, with predominant area, production, and productivity (www.indiastat.com, 2018-19). Globally, rice is being cultivated in 162.06 million hectares with a production of about 500 million metric tonnes and average productivity of 5.0t ha$^{-1}$ (FAO, 2021). India has been the largest producer after Chinawith a cultivated area of paddy is 46.3 million hectares and production and productivity of 129.5 million tonnes and 2798 kg ha$^{-1}$, respectively during 2021-22 (Ministry of Agriculture and Farmer Welfare, GOI, 2022). In Andhra Pradesh, it is grown in an area of 2.6 million ha with a production and productivity of 13.1 million tonnes and 5130 kg ha$^{-1}$, respectively (Ministry of Agriculture and Farmers Welfare, Directorate of Economics and Statistics, 2021-2022).

With the increasing global population, the demand for rice is expected to rise, and production needs to increase by almost two million tons per year to meet this demand (Buelah *et al*., 2021). To achieve this, adopting hybrid rice technology is a viable alternative (Buelah *et al*., 2021), which has the potential to yield significantly higher than traditional high-yielding varieties (HYVs) by exploiting the genetic expression of heterosis or hybrid vigor (Virmani, 1994). In India, first rice hybrids (APHR 1 and APHR 2) were released in 1993 (Vishnuvardhan *et al*., 2022). Gradually, hybrid rice technology has gained importance in India (Singh *et al.,* 2016). Hybrid rice varieties account for about 50 per cent of rice genotypes in China, producing 148.27 million tonnes of paddy annually (Kushal *et al.,* 2023). Thus, on an average, hybrid rice in China yields about 27 per cent (1.5 mt $ha^{-1}$) more than inbred high-yielding varieties. The potential of hybrid rice technology to enhance yield and contribute to global food security has been demonstrated by its success in China (Yuan, 2003). However, the poor adoption of hybrid rice outside China, particularly, in India is due to the absence of significant levels of heterosis (Peng *et al*., 2008). Twenty per cent of dietary protein, 3 per cent of dietary fat, and other essential nutrients are provided by rice (Bhargavi *et al*., 2022). Efforts to develop and promote high yielding, heterotic hybrids are therefore, essential to address the growing food demands of the world's population (Vennela *et al.,* 2022). In this context, the present investigation was undertaken to identify heterotic experimental rice hybrids for grain yield and yield component traits.

## Materials and Methods

The study was undertaken at Regional Agricultural Research Station (RARS), Maruteru, West Godavari District of Andhra Pradesh. The experimental site is situated at 26°38'N latitude, 81° 44'E longitude and 5 m above mean sea level with semi-humid climate with black alluvial clayey soils. The experimental material for the present study comprised of three male sterile lines *viz.,* APMS 15A, APMS 17A and APMS 18A and 10 restorer lines *viz*., RGL 5613, MTU 2716, MTU 1224, RM 409-26-1-1-1, MTU 2055, MTU 2347-158-3-1-1, RM 67-60-1-1-1, UTR 76, MTU 2846-34-1-1and MTU 1213. The salient features of parents and the check are summarized in **(Table 1)**. Thirty rice hybrids were developed by crossing the three male sterile lines with the ten testers in Line × Tester mating design during *kharif*, 2022. Healthy male sterile plants of the female male sterile lines with just emerged panicles were uprooted and potted in the evening hours of the day in plastic buckets filled with mud and were transferred to the net house **(Plate 1)**. Productive tillers of these plants with healthy panicles were selected and leaf sheaths were removed carefully. Further, florets that had completed anthesis (at the top) and young florets at the bottom of the panicle were also removed. Florets due to flower on the next day alone were used for crossing. Top 1/3rd of each floret was clipped with scissors and the clipped florets were covered with butter paper bags and labelled properly. On the next day morning, panicles ready for anthesis were selected from healthy male parents and were brought to the crossing chamber, in which temperature relative humidity and light conducive for anthesis were maintained. When the male parent was ready for dehiscence, the female parent was brought inside the crossing chamber and butter paper bags covering clipped panicles of the female parents were removed. Further, the panicles of the female parent were gently shaken so that the sterile extruded anthers fell off. Later, panicles of male parents were gently shaken over the female parents (CMS lines) until adequate pollen was deposited on stigmas of the clipped spikelets. The pollinated spikelets were then covered with fresh butter paper bags, duly labelled and fixed against the support of bamboo stakes. The process of pollination was continued upto 11 AM in the morning. Crossed seeds were collected after four weeks from the plant maintained in the buckets in the net house. The hybrid seeds were then sun dried, dehusked, counted and placed in small envelops and stored under ideal conditions for use in the next season for evaluation along with parents.

**Table 1: Salient features of experimental material**

| S. No | Genotype | Source | Salient features |
|---|---|---|---|
| **Lines** | | | |
| 1 | APMS 15A | RARS, Maruteru | Medium duration, medium slender, straw glume, moderately tolerant to BPH |
| 2 | APMS 17A | RARS, Maruteru | Medium duration, medium slender, straw glume, fine |
| 3 | APMA 18A | RARS, Maruteru | Medium duration, medium slender, straw glume, tolerant to leaf blast |
| **Testers** | | | |
| 1 | RGL 5613 | ARS, Ragolu | Medium duration, long slender, straw glume, moderately tolerant to leaf blast |
| 2 | MTU 2716 | RARS, Maruteru | Medium duration, medium slender, straw glume |
| 3 | MTU 1224 | RARS, Maruteru | Medium duration, medium slender, straw glume |
| 4 | RM 409-26-1-1-1 | RARS, Maruteru | Medium duration, medium bold, straw glume |
| 5 | MTU 2055 | RARS, Maruteru | Medium duration, long slender, straw glume |
| 6 | MTU 2347-158-3-1-1 | RARS, Maruteru | Medium duration, medium bold, straw glume, moderately tolerant to leaf blast |
| 7 | RM 67-60-1-1-1 | RARS, Maruteru | Medium duration, medium slender, bold grain |
| 8 | UTR 76 | ARS, Utukuru | Medium duration, long slender, straw glume, moderately tolerant to leaf blast |
| 9 | MTU 2846-34-1-1 | RARS, Maruteru | Medium duration, medium slender, straw glume |
| 10 | MTU 1213 | RARS, Maruteru | Early duration, medium slender, straw glume |
| **Check** | | | |
| 1 | HRI-174 | Bayer Bioscience, Hyderabad | Early duration, long bold, straw glume |

Evaluation of the 30 hybrids along with their parents, *i.e.,* three CMS lines and 10 restorers and the hybrid check, HRI-174 was carried out in a randomized block design with two replications during *Rabi*, 2022-2023 at Regional Agricultural Research Station, Maruteru. The seedlings were transplanted into the main field 21 days after sowing in the nursery. Normal, healthy and vigorous seedlings of each genotype were selected and transplanted in two rows plot of 4.5 m length with a spacing of 20 x 15 cm. All the recommended package of practices were adopted throughout the crop growth period to raise a healthy crop. Observations were recorded for grain yield per plant and yield attributing characters, namely, plant height, productive tillers per plant, panicle length spikelet fertility and grain density, measured as grain number divided by panicle length, by randomly choosing five plants from each entry in each replication and their means were used for the statistical analysis. The plants were selected from the middle rows to minimize the error due to border effect. However, days to 50 per cent flowering was recorded on plot basis. In contrast, observations for test weight were obtained from a random grain sample drawn from each plot in each genotype and replication using standard procedures. Further, the observations for yield and yield component traits were recorded on maintainer (B) lines of the respective male sterile lines, while panicle exertion and duration of floret opening were recorded on the male sterile lines. The data collected were subjected to standard statistical procedures (Panse and Sukhatme, 1967).

APMS 15A APMS 17A APMS 18A

**Male sterile lines studied**

Panicle of male parents in crossing chamber

Pollen dusting

Bagging after pollination

**Plate 1: Hybridization techniques**

## Results and Discussion

Commercial exploitation of heterosis in crop plants is regarded a major breakthrough in the realm of plant breeding. Heterosis in rice was first reported by Jones (1926). Later on, several workers had reported considerable heterosis for yield and other important economic characters. The aim of heterosis analysis in the present study was to identify the best combination of parents giving high degree of useful heterosis. In this direction, hybrid vigour over mid parent, better parent and the standard hybrid check, HRI-174 was studied for the 30 hybrids obtained by crossing of three male sterile lines with ten restorers in a Line ×Tester fashion for grain yield and yield component characters. The results obtained are presented in **Tables 2-5**.

### Days to 50 per cent flowering and maturity

Negative heterosis is desirable for the trait as earliness is preferred over delayed and late flowering. Relative heterosis for the trait ranged from -9.31 (APMS 18A × MTU 1224) to 4.06 per cent (APMS 17A x UTR 76), while heterobeltiosis was noticed to range from -10.29 (APMS 17A × MTU 1224) to 4.06 per cent (APMS17A ×UTR 76). However, standard heterosis for the trait ranged from -2.67(APMS 15A ×MTU 2055, APMS 17A ×MTU 2055) to 9.63 per cent (APMS 17A ×UTR 76). Further, significant and desirable heterosis over mid parent was observed for 15 hybrids,16 hybrids over better parent and none of the hybrids over the standard check, HRI-174 **(Figure 1 and 2)**.

Heterosis over mid-parent for days to maturity ranged from -6.43 (APMS 17A × MTU 1224) to 3.61 per cent (APMS 17A × MTU 2347-158-3-1-1), while heterosis over better parent ranged from -8.40 (APMS 17A × MTU 1224) to 2.79 per cent (APMS 17A × MTU 2347-158-3-1-1). Further, standard heterosis for the trait ranged from-3.23 (APMS 17A × MTU 1224) to 4.03 per cent (APMS 17A × MTU 1224, APMS 17A × UTR 76). Significant and desirable heterosis was recorded for six hybrids over mid-parent; nine hybrids over better parent and none of the hybrids over the standard check, HRI-174. Among these, six hybrids had recorded significant and desirable heterosis over mid-parent and better parent.

Devi *et al.,* (2014) and Dar *et al.,* (2015) had reported lack of hybrids with significant and negative heterosis over standard hybrid check, similar to the findings of the present investigation.

**Table 2: Analysisof variance (mean squares) for yield and yield components in rice**

| Source of variation | Degrees of freedom | Days to 50% flowering | Days to maturity | Plant height (cm) | Productive tillers per plant | Panicle length (cm) | Filled grains per panicle | Un-filled grains per panicle | Spikelet fertility (%) | 1000 grain weight (g) | Grain density | Grain yield per plant (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Replications | 1 | 1.14 | 8.28 | 0.0290 | 0.25 | 0.57 | 44.25 | 6.88 | 10.64 | 0.1020 | 0.04 | 0.76 |
| Genotypes | 43 | 18.92** | 15.91* | 153.57** | 3.66** | 5.23** | 9995.21** | 3326.67** | 482.57** | 10.14** | 8.87** | 147.59** |
| Parents | 12 | 6.51 | 10.32 | 181.91** | 2.06 ** | 8.77 ** | 6543.70** | 721.06 ** | 26.15 * | 13.17 ** | 12.87 ** | 109.78 ** |
| Hybrids | 29 | 18.15** | 14.88 | 102.79 ** | 4.18 ** | 3.54 ** | 10603.41** | 3597.75** | 571.15 ** | 5.06 ** | 7.09 ** | 170.58 ** |
| Parent vs. Crosses | 1 | 193.41 ** | 126.40** | 1303.56** | 7.99 ** | 10.76 ** | 7832.81 ** | 27980.15** | 3472.69** | 5.01 ** | 2.95 ** | 44.16* |
| Error | 43 | 6.02 | 8.98 | 24.46 | 0.15 | 1.38 | 130.93 | 8.14 | 11.70 | 0.56 | 0.18 | 14.35 |

## Table 3: *Per se* performance of parents and hybrids for grain yield and yield component characters in rice

| Character | Mean | | | Range | | | Best genotype | | |
|---|---|---|---|---|---|---|---|---|---|
| | Lines | Testers | Hybrids | Lines | Testers | Hybrids | Lines | Testers | Hybrids |
| Grain yield Per plant | 24.90 | 29.16 | 26.61 | 21.70 to 27.00 | 19.00 to 38.50 | 17.50 to 46.50 | APMS 18A | MTU 2846-34-1-1 | APMS 17A × MTU 2055 |
| Days to 50% flowering | 99.83 | 98.25 | 95.35 | 98.50 to102.00 | 96.00 to 102.00 | 91.00 to 102.50 | APMS 17A | MTU 1213 | APMS 15A × MTU 2055,<br>APMS 17A × MTU 2055 |
| Days to maturity | 127.83 | 126.65 | 124.28 | 125.50 to 130.00 | 123.00 to 131.00 | 120.00 to 129.00 | APMS 17A | MTU 1213 | APMS 15A × MTU 2055 |
| Plant Height | 113.81 | 107.74 | 117.62 | 111.05 to 115.75 | 88.50 to 119.70 | 101.00 to 133.80 | APMS 15A | MTU 1224 | APMS 15A × MTU 1224 |
| Productive tillers per plant | 8.41 | 9.32 | 9.78 | 7.75 to 8.90 | 7.90 to 10.50 | 6.65 to 12.85 | APMS 18A | MTU 1224 | APMS 17A × RGL 5613 |
| Panicle length | 25.80 | 24.90 | 25.88 | 25.30 to 26.15 | 21.15 to 28.90 | 23.45 to 30.10 | APMS 18A | MTU 2716 | APMS 18A × MTU 2055 |
| Filled grains per panicle | 315.05 | 221.25 | 212.12 | 289.00 to 365.25 | 164.60 to 289.05 | 49.50 to 419.15 | APMS 17A | UTR 76 | APMS 18A × MTU 2055 |
| Un-filled grains per panicle | 67.80 | 27.45 | 76.04 | 61.00 to 75.85 | 17.20 to 39.00 | 26.50 to 192.00 | APMS 18A | RM 409-26-1-1-1 | APMS 17A × MTU 2055 |
| Spikelet fertility | 82.18 | 88.94 | 73.54 | 79.35 to 84.65 | 85.05 to 92.45 | 20.50 to 91.50 | APMS 17A | RM 409-26-1-1-1 | APMS 17A × RGL 5613 |
| 1000-grain weight | 14.31 | 17.02 | 16.92 | 12.90 to 17.05 | 13.35 to 21.00 | 13.45 to 19.30 | APMS 15A | MTU 2347-158-3-1-1 | APMS 15A × MTU 1213 |
| Grain density (grain numbercm$^{-1}$) | 14.88 | 9.95 | 11.49 | 13.50 to 17.10 | 7.60 to 12.15 | 7.55 to 15.85 | APMS 17A | UTR 76 | APMS 17A × MTU 2055 |

## Table 4: Relative heterosis, heterobeltiosis and standard heterosis for grain yield and yield component character

| Character | Relative heterosis | | | Heterobeltiosis | | | Standard heterosis | | |
|---|---|---|---|---|---|---|---|---|---|
| | Range | No. of desirable & significant heterotic hybrids | Best hybrid combination | Range | No. of desirable & significant heterotic hybrids | Best hybrid combination | Range | No. of desirable & significant heterotic hybrids | Best hybrid combination |
| Grain yield Per plant | -45.74 to 118.45 | 7 | APMS 17A × RGL 5613 | -54.55 to 107.37 | 6 | APMS 17A × RGL 5613 | -44.44 to 47.62 | 5 | APMS 17A ×MTU 2055 |
| Days to 50% flowering | -9.31 to 4.06 | 15 | APMS 18A × MTU 1224 | -10.29 to 4.06 | 15 | APMS 17A × MTU 1224 | -2.67 to 9.63 | - | - |
| Days to maturity | -6.43 to 3.61 | 6 | APMS 17A × MTU 1224 | -8.40 to 2.79 | 9 | APMS 17A × MTU 1224 | -3.23 to 4.03 | - | - |
| Plant Height | -7.64 to 16.17 | - | - | -9.05 to 12.15 | - | - | -18.15 to 8.43 | 7 | APMS 15A ×MTU 1224 |
| Productive tillers per plant | -20.83 to 52.98 | 15 | APMS 17A × RGL 5613 | -25.28 to 49.42 | 10 | APMS 17A × RGL 5613 | -19.39 to 55.76 | 21 | APMS 17A × RGL 5613 |
| Panicle length | -6.81 to 17.35 | 2 | APMS 18A × MTU 2055 | -11.25 to 15.11 | 2 | APMS 18A × MTU 2055 | -14.57 to 9.65 | 1 | APMS 18A ×MTU 2055 |
| Filled grains per panicle | -82.79 to 67.19 | 2 | APMS 18A × MTU 2055 | -82.79 to | 1 | APMS 18A × MTU 2055 | -86.40 to 15.15 | 1 | APMS 18A ×MTU 2055 |
| Un-filled grains per panicle | -48.44 to 270.40 | 7 | APMS 17A × RGL 5613 | -69.05 to 163.26 | 13 | APMS 17A × MTU 2055 | -34.71 to 508.56 | 1 | APMS 17A ×MTU 2055 |
| Spikelet fertility | -75.93 to 8.34 | 2 | APMS 17A × MTU 2055 | -77.47 to 7.58 | - | - | -71.70 to 1.63 | - | - |
| 1000-grain weight | -13.86 to 45.73 | 16 | APMS 17A × MTU 2055 | -30.48 to 43.82 | 5 | APMS 17A × MTU 2055 | -45.77 to 22.18 | - | - |
| Grain density (grain number. cm$^{-1}$) | -34.49 to 19.17 | 6 | APMS 17A × MTU 2055 | -44.74 to 3.33 | - | - | -47.75 to 9.69 | 1 | APMS 17A × MTU 2055 |

## Table 5: Details of Promising hybrids identified

| Hybrids | Characterization of parents with respect to *per se* performance | Grain yield per plant (g) | Relative heterosis (%) | Heterobeltiosis (%) | Standard heterosis (%) | Significant and positive standard heterosis recorded for other characters | Grain type |
|---|---|---|---|---|---|---|---|
| APMS 17A × MTU 2055 | Low × High | 46.50 | 58.43** | 25.68** | 47.62** | Un-filled grains per panicle, grain density, grain yield per plant | Medium slender, Straw glume |
| APMS 17A × RGL 5613 | Low × Low | 45.00 | 118.45** | 107.37** | 42.86** | Productive tillers per plant, grain yield perplant | Medium slender, Straw glume |
| APMS 15A × MTU 1213 | High × Low | 42.50 | 70.00** | 63.46** | 34.92** | Grain yield per plant | Medium bold, Straw glume |
| APMS 15A × RGL 5613 | High × Low | 42.00 | 84.62** | 61.54** | 33.33** | Productive tillers per plant, grain yield perplant | Medium slender, Straw glume |
| APMS 15A × MTU 2055 | High × High | 41.50 | 31.75** | 12.16** | 31.75** | Productivetillers per plant, grain yield perplant | Medium bold, Straw glume |

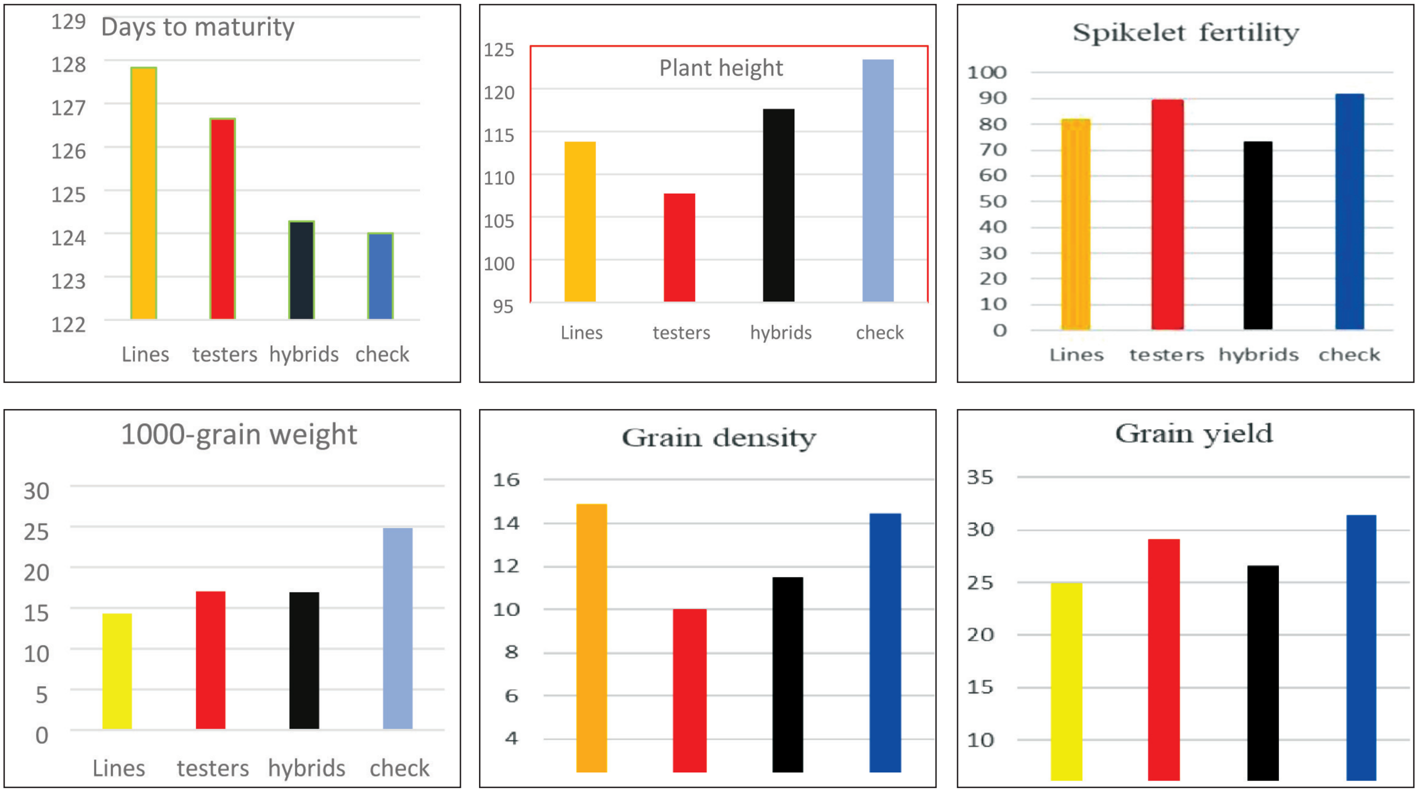

**Figure 1: Mean performance of grain yield per plant and important yield attributes**

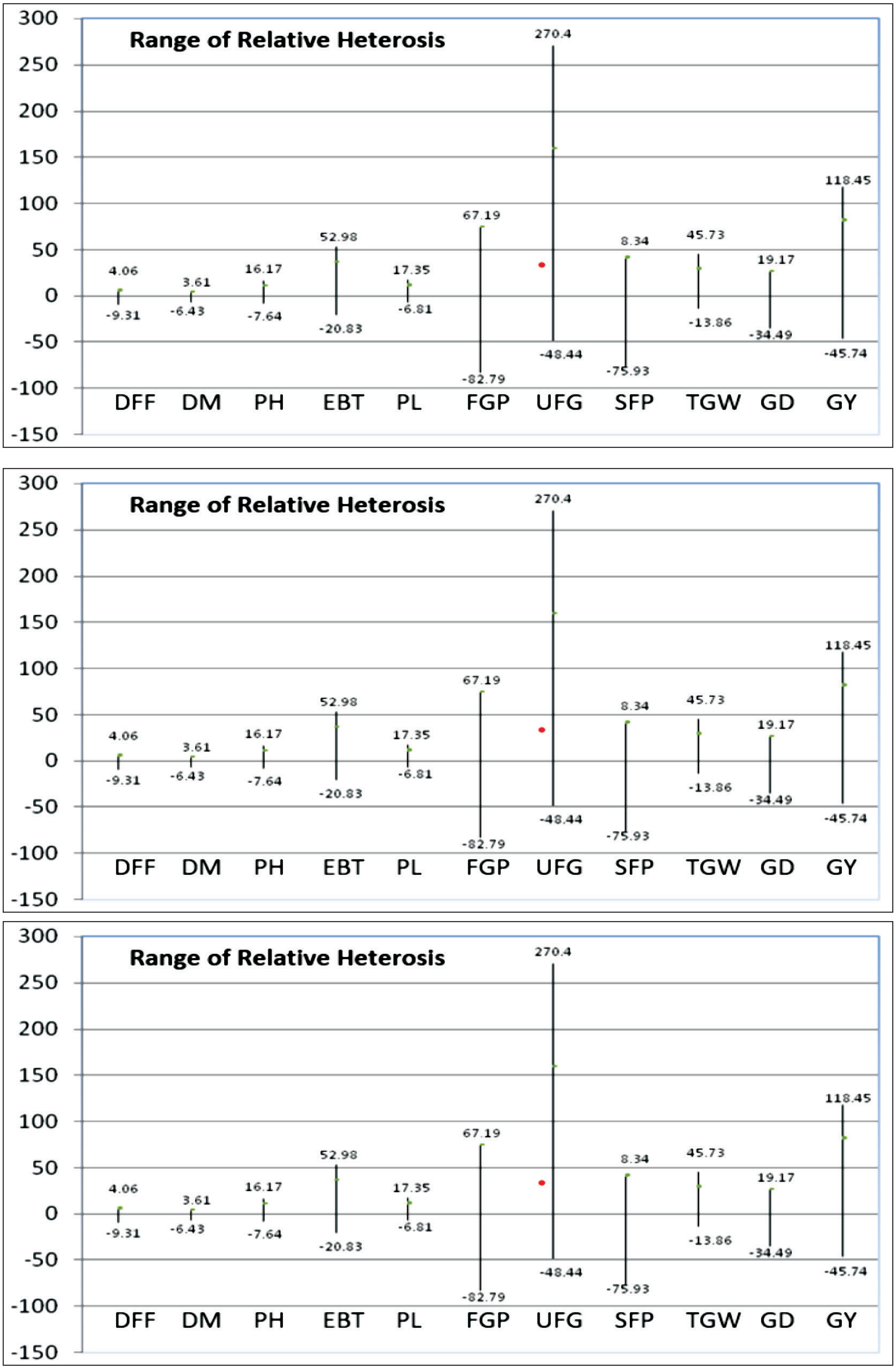

**Figure 2: Range of relative heterosis, heterobeltiosis and standard heterosis for yield and yield component traits**

### Plant height

Negative heterosis is also desirable for the trait as dwarfness confers resistance to lodging. Relative heterosis was noticed to range from -7.64 (APMS 18A ×MTU 2347-158-3-1-1) to16.17 per cent (APMS 15A ×MTU 2055). However, heterobeltiosis ranged from -9.05 (APMS 15A ×MTU 1224) to 12.15 per cent (APMS 15A ×MTU 2055), while heterosis over the standard check ranged from -18.15 (APMS 15A ×MTU 1224) to 8.43 per cent (APMS 15A ×MTU 2055). Further, six crosses had recorded significant and desirable heterosis for the trait over the standard check, HRI-174. Similar results were reported by Bhati *et al.,* (2015) and Sri Lakshmi *et al.,* (2019). Of these, APMS15A× MTU 1224 had recorded more than 15 per cent significant and desirable heterosis over the standard check.

### Productive tillers per plant

More number of productive tillers per plant resulting in higher number of panicles and ultimately more number of grains and grain yield per plant. Hence, positive heterosis is considered as desirable for the character. Heterosis over mid-parent range for this trait ranged from -20.83 (APMS 18A ×MTU 1213) to 52.98 per cent (APMS 17A ×RGL5613), while, heterobeltiosis was noticed to range from-25.28 (APMS 18A ×MTU 1213) to 49.42 per cent (APMS 17A ×RGL 5613). Standard heterosis ranged from -19.39 (APMS 18A ×MTU 1213) to 55.76 per cent (APMS 17A ×RGL 5613). Further, 15 crosses exhibited positive and significant heterosis over mid-parent, 10 hybrids over better parent and 21 over the standard hybrid check, HRI-174. Among these, 11 hybrids had recorded significant and desirable heterosis over mid-parent, better parent, and standard hybrid check, HRI-174. Of these, APMS17A×RGL 5613hadrecorded more than 45 per cent significant and desirable heterosis over the mid-parent, better parent and standard hybrid check. Similar levels of heterosis were reported by Rahman *et a*l., (2022).

### Panicle length

Long panicles are a desirable trait and positive value has been considered as desirable for yield improvement. Relative heterosis for the trait ranged from -6.81 (APMS 18A × RGL 56133) to 17.35 per cent (APMS 18A × MTU 2055), while, heterobeltiosis ranged from -11.25 (APMS 18A × MTU 2716) to 15.11 per cent (APMS 18A × MTU 2055). However, standard heterosis was observed to range from -14.57 (APMS 15A × MTU 1224) to 9.65 per cent (APMS 18A × MTU 2055). Further, significant, and positive heterosis over mid parent and better parent was observed for two hybrids and one hybrid over the standard check, HRI-174. Among these, APMS 18A × MTU 2055 had recorded more than 10 per cent significant and desirable heterosis over mid-parent, better parent and the standard check. The findings agree with the reports of Ramakrishna *et al.,* (2023).

### Filled grains per panicle

For this character, positive value has been considered desirable as it is directly associated with spikelet fertility percentage in rice. Relative heterosis estimates ranged from -82.79 (APMS 18A ×RGL 56133) to 67.19 per cent (APMS 18A ×MTU 2055). Two crosses exhibited positive and significant heterosis in the desired direction. On the other hand, heterobeltiosis ranged from-83.03 (APMS 17A × MTU 2716) to 44.09 per cent (APMS 18A × MTU 2055). Further, only one hybrid exhibited significant and positive heterosis over better parent. Heterosis over the standard check ranged from -6.40 (APMS 18A × MTU 2716) to15.15 per cent (APMS 18A × MTU 2055). One hybrid showed significant and positive standard heterosis. Among these, one hybrid APMS 18A × MTU 2055 had recorded more than 15 per cent significant and desirable heterosis over mid-parent, better parent and the standard check. These findings are in conformity with the results reported by Prasad *et al.,* (2019) and Vennela *et al*., (2022).

**Un-filled grains per panicle**

Heterosis in negative direction is desirable for this character. Relative heterosis for the trait ranged from -48.44 (APMS 17A × RGL 5613) to 270.40 per cent (APMS 17A × MTU 2716), respectively. Seven crosses showed significant and negative relative heterosis for the trait. Heterotic effects over better heterobeltiosis ranged from -11.25 (APMS 18A × MTU 2716) to 15.11 per cent (APMS 18A × MTU 2055). However, standard heterosis was observed to range from -14.57 (APMS 15A × MTU 1224) to 9.65 per cent (APMS 18A × MTU 2055). Further, significant, and positive heterosis over mid parent and better parent was observed for two hybrids and one hybrid over the standard check, HRI-174. Among these, APMS 18A × MTU 2055 had recorded more than 10 per cent significant and desirable heterosis over better parent and the standard check, indicating increased grain filling in the hybrid. These findings are in conformity with the results reported by Sri Lakshmi *et al.,* (2019).

**Spikelet fertility**

The estimates of heterosis over mid parent for spikelet fertility per centranged from -75.93 (APMS 18A × MTU 2716) to 8.34 per cent (APMS 17A × MTU 2055). Two crosses had exhibited significant and positive relative heterosis for this character. The estimates of heterobeltiosis ranged between -77.47 (APMS18A × MTU 2716) to 7.58 per cent (APMS 17A × RGL 5613). None of the crosses recorded significant and positive heterobeltiosis for the trait. On the other hand, economic heterosis ranged from -71.70 (APMS 17A × MTU 2716) to 1.63 per cent (APMS 17A ×MTU 2055). None of the crosses exhibited positive and significant standard heterosis for the character. Similar results were reported by Srivastava and Jaiswal (2016).

**1000-Grain weight**

Heterosis over mid parent ranged from -13.86 (APMS18A × MTU 2347-158-3-1-1) to 45.73 per cent (APMS 17A × MTU 2055). Sixteen crosses recorded positive and significant heterosis for the trait. Heterosis over better parent varied from -30.48 (APMS 18A × MTU 2347-158-3-1-1) to 43.82 per cent (APMS 17A × MTU 2055); and five crosses had recorded significant and positive heterobeltiosis for the trait. None of the crosses recorded significant and positive standard heterosis for the trait. Results of similar trend were also reported by Vanisree *et al*., (2011) and Prem kumar *et al.,* (2017).

**Grain density**

Heterosis over mid parent for grain density exhibited minimum and maximum values of -34.49 (APMS 15A × MTU 1224) to 19.17 per cent (APMS17A × MTU 2055) respectively. Six crosses exhibited significant and positive heterosis over mid parent. Estimates of heterosis over better parent varied between -44.74 (APMS17A× MTU 2716) to 3.32 per cent (APMS15A × UTR 76). None of the hybrids recorded significant and positive over better parent. On the other hand, standard heterosis ranged from -47.75 (APMS 15A ×MTU 1224) to 9.69 per cent (APMS 17A × MTU 2055). Of these, only one hybrid registered significant and positive heterosis over the check, HRI-174.

**Grain yield per plant**

Heterosis over mid parent for grain yield per plant ranged from -45.74 (APMS 15A × MTU 2846-34-1-1) to 118.45 per cent (APMS17A × RGL 5613). Similarly, high levels of relative heterosis for grain yield per plant were reported earlier (Buelah *et al.,* 2021). Seven crosses exhibited significant and positive heterosis over mid parent. Estimates of heterosis over better parent varied between -54.55 (APMS 15A × MTU 2846-34-1-1) to107.37 per cent (APMS 17A × RGL 5613). Similarly, high levels of heterobeltiosis for grain yield per plant were reported earlier (Vennela *et al*., 2022). Six crosses recorded positive and significant estimates of heterobeltiosis. On the other hand, standard heterosis ranged from -44.44 (APMS 15A × MTU 2846-34-1-1) to 47.62 per cent (APMS 17A

×MTU 2055). Similar levels of standard heterosis for grain yield per plant were reported earlier (Sudeepthi *et al.,* 2017). Further, Swaminathan *et al*., (1972) and Virmani (1996) had reported that about 20 to 30 per cent standard heterosis is sufficient to offset the extra cost of hybrid seed. In the present investigation, five crosses recorded significant and positive heterosis more than 30 per cent over the standard check, indicating their potential for commercial exploitation. Among these, APMS 17A × RGL 5613, APMS 15A × MTU 1213, APMS 15A × RGL 5613 and APMS 17A × MTU 2055 recorded significant and positive heterosis over mid and better parent also.

A perusal of the results on heterosis revealed several heterotic hybrids with significant and desirable heterosis for grain yield per plant and other yield attributes. Several workers have also reported similar significant and desirable heterosis for yield and yield components (Dar *et al*., 2015 and Srivastava and Jaiswal, 2016). Further, the hybrids, APMS 15A × RGL 5613, APMS 15A × MTU 1213, APMS 17A × RGL 5613 and APMS 17A × MTU 2055 were identified as promising and high yielding heterotic hybrids in the present study with significant and positive relative heterosis, heterobeltiosis and standard heterosis of more than 25 per cent for grain yield per plant. These hybrids had also recorded desirable levels of relative heterosis and heterobeltiosis for days to 50 per cent flowering.

## Conclusion

The crosses, APMS 17A × MTU 2055, APMS 17A × RGL 5613, APMS 15 A × MTU 1213, APMS 15 A × RGL 5613 and APMS 15 A × MTU 2055are identified as promising heterotic combinations with potential for commercial exploitation and need to be tested over locations and years for their stability in performance, before utilization.

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# Evaluation of Pre-Released Rice Cultures at Different Nitrogen Levels

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## Abstract

A field experiment was conducted to evaluate the pre-released rice cultures at different nitrogen levels during *kharif,* 2020-21 at Agricultural Research Station, Bapatla. The experiment was laid-out in split plot design with three replications. The four rice varieties *viz*, BPT 2776, BPT 2766, BPT 2824 and BPT 2846 were considered as main treatments and four nitrogen levels *viz,* 80 kg ha$^{-1}$, 120 kg ha$^{-1}$, 160 kg ha$^{-1}$ and 200 kg ha$^{-1}$were considered as sub treatments. The results revealed that BPT 2846 recorded the highest plant height, productive tillers, panicle length, grain yield (5417 kg ha$^{-1}$) and straw yield (7073 kg ha$^{-1}$). The highest nitrogen uptake both in grain and straw also recorded highest values with BPT 2846 pre-released culture. Among the nitrogen levels 200 kg N ha$^{-1}$ recorded the highest growth, yield attributes, yield and nutrient uptake of pre-released cultures. The data pertaining to economics 160 kg N ha$^{-1}$ recorded the highest gross returns, net returns and B:C ratio.

**Keywords:** Pre-released culture, yield, nutrient uptake.

## Introduction

Rice (*Oryza sativa* L.) is a primary source of nutrition for over 50% of the world's population, catering to their daily dietary needs. Asia accounts for approximately 92% of the overall global rice production and 90% of the worldwide rice consumption. India is recognised as the second largest global contributor to rice production, constituting approximately 20% of the total global output. Rice cultivation in India encompasses a vast expanse of approximately 46.3 million ha$^{-1}$, yielding an annual production of approximately 129.5 million tonnes, with a productivity rate of approximately 2,798 kg ha$^{-1}$. Rice cultivation in Andhra Pradesh spans an area of 22.9 lakh ha$^{-1}$, resulting in an annual production of 77.6 lakh tonnes and a productivity of 3,392 kg ha$^{-1}$ (Ministry of Agriculture and Farmer Welfare, GOI, 2022).

In comparison to other essential nutrients, the rice crop necessitates a substantial quantity of nitrogen. Biradar (2005) revealed that in rice production Nitrogen (N) is the most limiting nutrient. Xing and Zhu (2000) reported that the N losses from puddled, flooded soils are often large, through ammonia volatilization which accounts for 40% to 60%. Moreover, the denitrification losses from puddled soils depend on the rate of N fertilizer application. The application of nitrogen fertilisation is a significant agronomic practise that has a notable impact on both the yield and quality of rice crops (Venkatanna *et al*., 2022).

Annually, farmers worldwide utilise approximately 112.36 million tonnes of nitrogen for agricultural purposes (IFA, 2021). Approximately 35% (equivalent to 39.32 million tonnes) of nitrogen is utilised by the crop, while the remaining 82 million tonnes are discharged into rivers, lakes and natural ecosystems. The over utilization of nitrogen fertiliser has resulted in diverse forms of nitrogen losses, which can have detrimental consequences on the environment. The emission of nitrous oxide ($N_2O$) gas into the atmosphere from agricultural fields has a significant impact on the warming of the Earth's atmosphere (Vijayakumar *et al*., 2022). In fact, the warming potential of 1 pound of $N_2O$ is approximately 265 times greater than that of 1 pound of carbon dioxide. The process of nitrate leaching into rivers and lakes can give rise to eutrophication, a phenomenon characterised by the proliferation of aquatic weeds and algae. The decline in fish population and the subsequent reduction in the recreational value of water were observed. Furthermore, this phenomenon has resulted in the contamination of groundwater with nitrate-N ($NO_3$-N), a substance that can pose a health hazard to both humans and livestock when its concentration exceeds 10 mg $L^{-1}$ in drinking water (Cameron *et al*., 2013 and Sainju *et al*., 2020).

Mahajan and Timsina (2011) reported that the yield of rice increased with N rate up to 120 kg N $ha^{-1}$ in Punjab, India. In a recent study from Bangladesh, Ahmed *et al*., (2016) reported that grain yield of rice increased significantly up to 160 kg N $ha^{-1}$ for Aman rice and 180 kg N $ha^{-1}$ for Boro rice. Application of optimum quantity of N at the right time is one of the most important factors to realize high yield and N use efficiency in rice. Furthermore, optimum rate and time of fertilizer N application for rice may depend on soil type, climate and genetic potential of rice cultivar. The probability of increasing rice production depends on the ability to incorporate better crop management for the different varieties into existing cultivation systems (Mikkelsen *et al*., 1995). Nitrogen is the most essential element in determining the yield potential of rice and the nitrogenous fertilizer is one of the major inputs for rice production (Mae, 1997). The rice varieties differ from one variety to another in response to different levels of nitrogen. Fixing optimum dose of N fertilizer for each pre- released cultures is important to avoid excess application of fertilizers. Keeping the above points in view, the present investigation was conducted at Agricultural Research station, Bapatla during 2020-21 to optimize the nitrogen level for the pre-release rice cultures and observe the influence of nitrogen fertilizer on grain yield.

## Materials and Methods

A field experiment was conducted during *kharif,* 2020-21 at Agricultural Research Station, Bapatla. The soil is clay loam in texture. The soil is neutral (pH 7.5) in reaction with low electrical conductivity (0.32 dS $m^{-1}$). The soil is medium in organic carbon content, low in available nitrogen, medium in available phosphorus and potash. The experiment was laid out in split plot design with 4 main plots treatments and 4 sub-plot treatments replicated thrice. Main treatments were pre-released cultures *viz,* $V_1$-BPT 2776, $V_2$-BPT 2766, $V_3$-BPT 2824 and $V_4$-BPT 2846; sub-treatments were four nitrogen levels *viz,* $N_1$- 80 kg $ha^{-1}$, $N_2$-120 kg $ha^{-1}$, $N_3$-160 kg $ha^{-1}$ and $N_4$-200 kg $ha^{-1}$. Rice pre-released cultures were sown separately in nursery and 25 days old seedlings were transplanted at 20 cm x 15 cm spacing @ two seedlings per hill in three years. Nitrogen (Urea) was applied as per treatments in three equal splits (1/3 as basal, 1/3 at maximum tillering and 1/3 at panicle initiation stage). Phosphorus and potassium were applied through single super phosphate and muriate of potash. Irrigation and weed management were done time to time. The plant height was measured from ground level to the apex of last fully opened leaf during vegetative period and up to the tip of the panicle after flowering. Panicle length of ten randomly selected panicles from each plot was measured from neck node to the tip of panicle and then averaged and expressed in cm. Number of grains of 10 randomly selected panicles from each plot were counted and then averaged as grains $panicle^{-1}$. Samples of grain collected separately at the time of threshing from each plot were dried properly. 1000-grains from each of these samples were taken and their weights were recorded and expressed in grams. The border rows were harvested first and then, the net plot area was harvested and the produce was threshed by beating on a threshing bench, cleaned and sun dried to 14 per cent moisture level. Grain from net plot area was thoroughly sun dried, threshed, cleaned and weight of grains was recorded and expressed in yield per hectare. The data were analyzed statistically following the method given by Panse and Sukhatme (1978) and wherever the results were calculated at 5 per cent level of significance.

## Results and Discussion

Plant height was significantly affected by nitrogen levels in all rice pre-released cultures. The highest plant height of 129.7 cm was observed with BPT 2776 pre-released culture which was significantly superior to BPT 2846 whereas, the lowest plant height of 118.4 cm was observed with BPT 2846 variety. Among the nitrogen levels, application of 200 kg N ha$^{-1}$ recorded significantly the highest plant height (125.4 cm) followed by 160 N kg ha$^{-1}$ treatment. There is significant interaction affect among nitrogen levels and different rice varieties in the case of plant height. Increase in the level of nitrogen application might have increased nitrogen availability to the crop which might have enhanced cell division, photosynthesis, metabolism, assimilate production and cell elongation resulting in taller plants. Such a favourable effect of nitrogen on increase in plant height of rice has been reported by many researchers (Prasad Rao *et al.,* 2011 and Contreras *et al.,* 2017).

There is significant difference among the nitrogen levels and varieties in tiller number. Significantly more number of tillers was observed in BPT 2846 variety (13.8) and lowest number of tillers (13) was recorded with BPT 2766 variety. In different nitrogen levels applied to rice varieties 200 kg N ha$^{-1}$ treatment recorded maximum number of tillers (13.9) followed by 160 kg N/ha applied treatment. Nitrogen

**Table 1: Effect of nitrogen levels on growth and yield attributes of different pre-released rice cultures**

| | Plant height (cm) | No. of productive tillers/plant | Panicle length (cm) | No of filled grains/ panicle | Test weight (g) |
|---|---|---|---|---|---|
| **Varieties** | | | | | |
| $V_1$-BPT 2776 | 129.7 | 13.1 | 26.3 | 232.3 | 14.7 |
| $V_2$-BPT 2766 | 124.1 | 13.0 | 27.0 | 252.7 | 15.6 |
| $V_3$-BPT 2824 | 121.0 | 13.7 | 25.8 | 245.6 | 15.2 |
| $V_4$-BPT 2846 | 118.4 | 13.8 | 27.2 | 250.8 | 16.1 |
| SEm± | 2.2 | 0.2 | 0.4 | 5.3 | 0.3 |
| CD (P=0.05) | 6.6 | 0.6 | 1.2 | 15.9 | 1.0 |
| CV (%) | 9.2 | 8.5 | 10.0 | 9.3 | 6.2 |
| **Nitrogen doses** | | | | | |
| $N_1$-80 kg ha$^{-1}$ | 110.3 | 12.1 | 24.6 | 217.3 | 13.6 |
| $N_2$-120 kg ha$^{-1}$ | 117.0 | 12.9 | 25.7 | 235.0 | 14.7 |
| $N_3$-160 kg ha$^{-1}$ | 125.1 | 13.7 | 26.8 | 253.6 | 15.8 |
| $N_4$-200 kg ha$^{-1}$ | 125.4 | 13.9 | 26.9 | 263.8 | 16.0 |
| SEm± | 1.8 | 0.2 | 0.3 | 4.5 | 0.3 |
| CD (P=0.05) | 5.3 | 0.6 | 0.9 | 13.2 | 0.9 |
| CV (%) | 8.1 | 10.1 | 9.3 | 12.9 | 6.6 |
| **Interaction** | | | | | |
| SEm± | 3.2 | 0.4 | 0.5 | 7.8 | 0.5 |
| CD (P=0.05) | 9.2 | NS | 1.5 | NS | NS |
| SEm± | 3.3 | 0.4 | 0.6 | 8.2 | 0.5 |
| CD (P=0.05) | 9.5 | NS | 2.5 | NS | NS |

fertilization plays a vital role in cell division and might have supported the increase in number of tillers m$^{-2}$. Similar results were also reported by Mamata Meena *et al.,* (2013). The data indicated that the nitrogen levels and rice varieties significantly influenced the panicle length. The highest panicle length was recorded with BPT 2846 (27.2 cm) and the lowest was recorded with BPT 2824 (25.8 cm) variety (**Table 1**). There was significant difference in case of panicle length at different nitrogen levels. Application of 200 kg N ha$^{-1}$ treatment recorded significantly longer panicle (26.9cm) and the shortest panicle was observed at 80 kg N ha$^{-1}$ applied treatment (24.7cm). The increase in panicle length with N fertilization was also reported by Tabar (2013) and Gewaily *et al.,* (2018).

Yield attributes were significantly affected by nitrogen levels in different pre-released rice cultures. More number of filled grains per panicle was recorded with BPT 2766 variety (252.7) which was significantly superior to BPT 2776 variety (232.3). Significantly highest number of filled grains panicle$^{-1}$ was recorded with 200 kg N ha$^{-1}$ treatment (263.8) followed by 160 kg N ha$^{-1}$ treatments. Lowest number of filled grains panicle$^{-1}$ was recorded with 80 kg N ha$^{-1}$ treatment (217.3). There is no significant interaction among rice varieties and nitrogen levels in number of grains/ panicle. The increase in the number of filled grains with increase in N rates indicates that N fertilization is important for both source and sinks development (Yesuf and Balcha, 2014).

Significant difference in test weight was observed in rice varieties at different nitrogen levels. Among the rice varieties BPT 2846 recorded highest test weight (16.1 g) followed by BPT 2766 and the lowest test weight was observed in BPT 2776 variety (14.7 g). Among nitrogen levels 200 kg N ha$^{-1}$ recorded significantly highest test weight (16.0 g) followed by 160 kg N ha$^{-1}$ which compared to 120 & 80 kg N ha$^{-1}$. Such an increase in 1000 grain weight with the application of nitrogen was also noticed earlier (Zaidi *et al.,* 2007 and Pandey *et al.,* 2008).

The data pertaining to the grain yield revealed significant influence of the nitrogen levels on rice varieties. Among the four rice varieties, BPT 2846 produced significantly the highest grain yield (5417 kg ha$^{-1}$) and the lowest grain yield (5039 kg ha$^{-1}$) was recorded with BPT 2776 variety. There was no significant difference in interaction in case of grain yield. Data revealed that the maximum grain yield (5515 kg ha$^{-1}$) was recorded with 200 kg N ha$^{-1}$followed by 160 kg N ha$^{-1}$. The linear response was observed in grain yield is also supported by the similar trend noticed with all growth and yield attributing characters studied. The increase in grain yield might be due to nitrogen application enhancing the dry matter production and improved growth rate. These results are in confirmation with the findings of Singh *et al.,* (2012), Gaind and Nain (2012) and Mrudhula *et al*., (2021).

The data pertaining to straw yield was significantly affected by different nitrogen levels and rice varieties. Significantly highest straw yield (7570 kg ha$^{-1}$) was recorded with BPT 2776 variety followed by BPT 2766 variety whereas, the lowest straw yield (6880 kg ha$^{-1}$) was recorded with BPT 2824 variety. Among the nitrogen levels applied to different rice varieties 200kg N ha$^{-1}$ recorded significantly the highest straw yield (7657 kg ha$^{-1}$) followed by 160 kg N ha$^{-1}$and lowest straw yield (6186 kg ha$^{-1}$) was recorded in 80 kg N ha$^{-1}$ applied treatment. Overall, the increase in straw yield with these treatments might be due to better growth reflected in these treatments in terms of plant height, dry matter accumulation and tillering. These results are in conformity with Singh *et al.,* (2006) and Zayed *et al.,* (2011). There was no significant difference in rice varieties, nitrogen levels and interaction in case of harvest index (**Table 2**).

**Table 2: Effect of nitrogen levels on grain yield, straw yield and harvest index of different pre-released rice cultures**

| | Grain yield (kg $ha^{-1}$) | Straw yield (kg $ha^{-1}$) | Harvest index (%) |
|---|---|---|---|
| **Varieties** | | | |
| $V_1$-BPT 2776 | 5039 | 7570 | 43.9 |
| $V_2$-BPT 2766 | 5146 | 7291 | 43.9 |
| $V_3$-BPT 2824 | 5246 | 6880 | 43.8 |
| $V_4$-BPT 2846 | 5417 | 7073 | 44.7 |
| SEm± | 87.8 | 188.2 | 0.5 |
| CD (P=0.05) | 303.9 | 651.4 | NS |
| CV (%) | 9.8 | 11.1 | 4.1 |
| **Nitrogen doses** | | | |
| $N_1$-80 kg ha $ha^{-1}$ | 4536 | 6186 | 42.3 |
| $N_2$-120 kg $ha^{-1}$ | 4921 | 6896 | 43.3 |
| $N_3$-160 kg $ha^{-1}$ | 5406 | 7595 | 44.1 |
| $N_4$-200 kg $ha^{-1}$ | 5515 | 7657 | 44.3 |
| SEm± | 97.8 | 240 | 0.6 |
| CD (P=0.05) | 285.3 | 665 | NS |
| CV (%) | 12.5 | 15.7 | 4.4 |
| **Interaction** | | | |
| SEm± | 169.3 | 203.2 | 1.1 |
| CD (P=0.05) | 494.2 | 593.2 | NS |
| SEm± | 165.2 | 239.9 | 0.9 |
| CD (P=0.05) | 457.8 | 665 | NS |

The data pertaining to nitrogen content in rice grain, there was significant difference was observed among the pre-released cultures of rice varieties. Among the rice varieties BPT 2846 recorded significantly highest grain nitrogen content (1.78%) followed by BPT 2776 and BPT 2824. The lowest grain nitrogen content (1.52%) was observed in BPT 2766 variety. Significant difference was observed at different nitrogen levels. The highest nitrogen content (1.7%) was observed with 200 kg N $ha^{-1}$ followed by 160 kg $Nha^{-1}$ (1.66%). No significant difference was observed in interaction among rice varieties and nitrogen levels. Maximum nitrogen uptake in rice grain was recorded with BPT 2846 variety (95.9 kg $ha^{-1}$) and the lowest nitrogen uptake was recorded with BPT 2776 variety (88.1 kg $ha^{-1}$). Significantly high nitrogen uptake was recorded with 200 kg N $ha^{-1}$ treatment (93.7 kg $ha^{-1}$) followed by 160 kg N $ha^{-1}$ treatment (89.7 kg N $ha^{-1}$) **(Table 3)**.

**Table 3: Effect of nitrogen levels on grain and straw nitrogen content and uptakes of different pre-released rice cultures**

| | Nitrogen content in grain (%) | Nitrogen uptake in grain (kg ha$^{-1}$) | Nitrogen content in straw (%) | Nitrogen uptake in straw(kg ha$^{-1}$) |
|---|---|---|---|---|
| **Varieties** | | | | |
| $V_1$-BPT 2776 | 1.76 | 88.1 | 1.17 | 82.4 |
| $V_2$-BPT 2766 | 1.52 | 78.3 | 1.21 | 88.6 |
| $V_3$-BPT 2824 | 1.65 | 86.6 | 1.28 | 86.9 |
| $V_4$-BPT 2846 | 1.78 | 95.9 | 1.37 | 96.8 |
| SEm± | 0.05 | 2.9 | 0.03 | 2.6 |
| CD (P=0.05) | 0.16 | 10.3 | 0.12 | 9.0 |
| CV (%) | 9.9 | 11.9 | 9.36 | 10.0 |
| **Nitrogen doses** | | | | |
| $N_1$-80 kg ha$^{-1}$ | 1.54 | 69.8 | 1.02 | 63.1 |
| $N_2$-120 kg ha$^{-1}$ | 1.60 | 78.7 | 1.14 | 78.6 |
| $N_3$-160 kg ha$^{-1}$ | 1.66 | 89.7 | 1.26 | 95.7 |
| $N_4$-200 kg ha$^{-1}$ | 1.70 | 93.7 | 1.27 | 97.2 |
| SEm± | 0.06 | 2.6 | 0.04 | 1.4 |
| CD (P=0.05) | NS | 7.5 | 0.11 | 4.1 |
| CV (%) | 12.5 | 10.4 | 10.6 | 13.3 |
| **Interaction** | | | | |
| SEm± | 0.1 | 4.5 | 0.07 | 2.4 |
| CD (P=0.05) | NS | 13.0 | NS | 7.0 |
| SEm± | 0.1 | 4.6 | 0.06 | 3.1 |
| CD (P=0.05) | NS | NS | 0.19 | 8.8 |

Significant difference was noticed among the pre-released cultures and nitrogen levels in nitrogen content of rice straw. Data revealed significantly high nitrogen content (1.37%) and uptake (96.8 kg ha$^{-1}$) in BPT 2846 followed by BPT 2766 (1.21% and 88.6 kg ha$^{-1}$) in case of paddy straw. Among the nitrogen doses 200 kg/ha nitrogen application treatment recorded significantly highest nitrogen content (1.27%) and uptake (97.2 kg ha$^{-1}$) and the lowest nitrogen content (1.02%) and uptake (63.1 kg ha$^{-1}$) were recorded with 80 kg N ha$^{-1}$ treatment in rice straw.

Gross returns, net returns and benefit cost ratio was affected by nitrogen levels and all pre-released rice cultures. The highest gross returns, net returns and benefit cost ratio of Rs. 85,615, Rs.16,534/- and 1.24 was observed with BPT 2846 pre-released culture which was superior to BPT 2776 whereas, the lowest gross and net returns was Rs. 80,641/- and Rs. 11,057/- with BPT 2776 pre-released rice culture (**Table 4**). Application of 200 kg N ha$^{-1}$ recorded the maximum gross returns (Rs.84225/-), net returns (Rs.14759/-) and benefit cost ratio (1.21). These results are in agreement with the findings of Singh *et al*., (1998) and Mishra *et al.,* (2015).

**Table 4: Effect of nitrogen levels on economics of different pre-released rice cultures**

| | Gross Returns (Rs.ha$^{-1}$) | Net Returns (Rs.ha$^{-1}$) | B:C Ratio |
|---|---|---|---|
| **Varieties** | | | |
| $V_1$-BPT 2776 | 80641 | 11057 | 1.17 |
| $V_2$-BPT 2766 | 81906 | 12822 | 1.19 |
| $V_3$-BPT 2824 | 82845 | 13761 | 1.20 |
| $V_4$-BPT 2846 | 85615 | 16534 | 1.24 |
| **Nitrogen doses** | | | |
| $N_1$-80 kg ha$^{-1}$ | 81084 | 12855 | 0.19 |
| $N_2$-120 kg ha$^{-1}$ | 82266 | 13568 | 0.20 |
| $N_3$-160 kg ha$^{-1}$ | 84225 | 14759 | 1.21 |
| $N_4$-200 kg ha$^{-1}$ | 83432 | 13494 | 1.19 |

## Conclusion

The experimental results revealed that grain yield was significantly affected by the application of nitrogen at different levels in rice cultivars. BPT 2846 pre-released rice culture recorded significantly the highest grain yield, yield attributes and nitrogen uptake. Incremental doses of nitrogen influenced the grain yield significantly. Application of 200 kg N ha$^{-1}$ recorded significantly the highest grain yield of BPT 2846 pre-released rice culture when compared to all other pre-released rice cultures and nitrogen levels.

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**RESEARCH ARTICLE** **https://doi.org/10.58297/YMGM1210**

# Assessment of Grain Zinc, Iron and Protein Content in Selected Red Rice (*Oryza Sativa* L.) Mutant Lines

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## Abstract

Traditional red rice cultivars grown in coastal regions of Karnataka are popular as food and medicine and specifically in promoting lactation. Effect of zinc, iron and protein deficiency is more in children and lactating women. Analysis of variance evidenced that treatment with gamma rays created significant variability and all the mutant lines exhibited wide range of values for zinc (20-41.5ppm), iron (10-35 ppm), protein content (8-11.9g/100 g) and yield traits. Grain zinc exhibited negative significant association with grain yield per plant, while protein and iron content showed non-significant and negative association with grain yield per plant and very low residual effect of 0.2721 indicated that the traits included in this study explained high percentage of variation in the grain yield and grain zinc, iron and protein content. BMRM15 and BMRM13 mutants showed reduced plant height, early maturity, recorded one percentage increase of protein (9.5% and 10.0%), higher zinc (28 and 25 ppm) and iron content (17 and 13 ppm). These mutant lines can supplement the micronutrient requirement of consumers and also help prevent the micronutrient deficiency.

**Key words**: Gamma irradiation, Induced variability, Short stature, Early maturity, Fe and Zn Deficiency.

## Introduction

In India, red rice occupies a special position since time immemorial (Ahuja *et al*., 2007). Because of their medicinal value and exclusive taste, a number of red grained varieties are cultivated in Kerala, Tamil Nadu, Karnataka and the north eastern states in areas with unfavourable conditions such as deep water, drought, sandy soils, salinity and cold conditions (Chandhni, 2015). A great diversity in cultivars exists within red rice and one such traditionally grown long red kernelled rice in Coastal Karnataka is *Kajejaya,* which is very popular in this region and is consumed as *Kuchalakki* (special processed parboiled rice). Malnutrition is one of the significant problems in the developing countries and imbalanced supply of zinc, iron and protein leads to deficiency affecting the human metabolism (Khan *et al*., 2022 and Galani *et al*., 2022). Rice enriched with iron, zinc, protein along with higher yield and superior agronomic characters can support the health and livelihood of the millions of people who depend on the rice (De Oliveira *et al*., 2020).

Worldwide, there is an increasing emphasis on the relationship between food, nutrition and health and also food production that meets the demands of population and the market (Fukagawa and Ziska 2019). Therefore, strengthening the research and development of rice varieties plays an important role in improving the nutritive values of the grain and in promoting public health. The traditional red rice-

*Kajejaya* is the most sought after variety in spite of its lower yield and susceptibility to lodging in Coastal region. Hence, it is essential to improve the yield of traditional red rice varieties like *Kajejaya* to meet the food demand with nutritional security.

In this context, an attempt was made to develop an improved variety through mutation breeding. The prime strategy in mutation breeding is to upgrade the well adapted local varieties by altering one or two major traits that limit their productivity or enhance their quality value. Gamma rays are known to influence plant growth and development by including cytological, morphogenetic, biochemical and physiological changes in cells and tissues. The present experiment was conducted to assess the genetic variability induced by gamma rays in selected ($M_6$) mutant lines of *Kajejaya* for grain zinc, iron, protein content and yield traits.

## Materials and Methods

The experiment was conducted during *kharif* 2022 at Zonal Agricultural and Horticultural Research Station, Brahmavar. Initially 200 seeds of the long bold traditional red rice variety *Kajejaya* were exposed to 15kR, 25kR, 35kR and 45kR gamma ray treatment from Cobalt 60 at BARC, Mumbai. Plant to progeny method was followed to forward the individual plants from $M_1$ to $M_2$ and semi-dwarf, early and medium bold red grain type were primarily selected and forwarded to $M_3$ and $M_5$ generation (106 mutant lines). The material ($M_6$) for the present study consisted of 5 each superior mutant lines which were selected based on higher grain yield per plant from 15kR, 25kR, 35kR and 45kR gamma treatments, respectively. A total of 20 mutant lines ($M_6$) with one untreated parent check (*Kajejaya*) laid out in a Randomized Complete Block Design (RCBD) with two replications. The seedlings were raised in wet nursery and twenty-one days old healthy seedlings from each treatment along with the check were transplanted in the well-prepared puddled field. The spacing was maintained with plant to plant spacing of 15 cm within a row and 20cm row to row spacing of 5 meters. The observations were recorded by randomly selecting ten plants in each mutant line. The experimental data was collected on 10 yield attributing traits yield and grain zinc, iron and protein (**Table 1**) were subjected to standard statistical procedure prescribed by Cochran and Cox (1957). The phenotypic coefficients of correlations were made as suggested by Al-Jibouri (1958). The

**Table 1: Analysis of Variance for yield components, zinc, iron and protein content in the mutant lines of $M_5$ generation of red rice variety Kajejaya**

| Source of Variation | Degrees of freedom | Days to 50% flowering | Days to Maturity | Plant height (cm) | Number of productive tillers per plant | Number of grain per panicle | Test weight (g) | Protein (g/100g) | Iron (ppm) | Zinc (ppm) | Grain yield per plant (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Replication | 1 | 0.095 | 0.0952 | 6.881 | 10.500 | 10.50 | 0.0688 | 0.00595 | 0.857 | 13.149 | 1.2758 |
| Mutant lines | 19 | 35.631** | 25.4286** | 102.181** | 18.107** | 167.31** | 6.8689** | 1.18657** | 42.907** | 47.541** | 14.5312** |
| Error | 20 | 1.445 | 2.2952 | 18.581 | 5.050 | 30.50 | 2.3028 | 0.19895 | 1.607 | 1.261 | 2.5760 |
| **CD (5%)** | | 2.5077 | 3.1602 | 8.9917 | 4.6876 | 11.5201 | 3.1654 | 0.9304 | 2.6444 | 2.3427 | 3.3480 |
| **CD (1%)** | | 3.4206 | 4.3107 | 12.2650 | 6.3941 | 15.7139 | 4.3178 | 1.2691 | 3.6071 | 3.1955 | 4.5668 |
| ** Significance at 1% level, *significance at 5% level | | | | | | | | | | | |
| **Mean** | | 76.10 | 122.29 | 65.74 | 18.36 | 139.93 | 26.49 | 10.04 | 24.64 | 31.96 | 29.41 |
| **Range** | **Min** | 72.0 | 113.0 | 51.0 | 12.0 | 120.0 | 21.1 | 8.0 | 10.0 | 20.0 | 23.7 |
| | **Max** | 88.0 | 129.0 | 96.0 | 25.0 | 159.0 | 32.2 | 11.9 | 35.0 | 41.5 | 34.6 |
| **PCV (%)** | | 5.65 | 3.04 | 11.82 | 18.53 | 7.10 | 8.08 | 8.29 | 19.14 | 15.45 | 9.94 |
| **GCV (%)** | | 5.43 | 2.78 | 9.83 | 13.91 | 5.91 | 5.70 | 7.00 | 18.44 | 15.04 | 8.31 |
| **$H^2$ broad see (%)** | | 92.2 | 83.44 | 69.23 | 56.39 | 69.16 | 49.78 | 71.28 | 92.78 | 94.83 | 69.88 |
| **GAM (%)** | | 10.75 | 5.23 | 16.86 | 21.53 | 10.13 | 8.29 | 12.18 | 36.59 | 30.19 | 14.32 |

phenotypic coefficients of correlation and path coefficients were analyzed by Windstar Version 9.2 from Indostat services.

**Quality parameters**

Top five superior mutant lines (a total of 20) from each treatment with higher grain yield per plant were analyzed for zinc, iron and protein content including untreated parent check. The protein content was determined by Micro Kjeldhal method, in three steps, namely digestion, distillation and titration. Zinc and iron content of grain samples were assessed through Atomic Absorption Spectrophotometer by feeding the prepared mineral solution to the AAS having appropriate hallow cathode lamps after getting values for standard solutions. The per cent elements concentrations of zinc and iron were calculated in ppm.

## Results and Discussion

The mean sum of squares due to mutant lines were highly significant for the ten traits studied, indicating that gamma irradiation generated the variability in the experimental material (**Table 1**). The mutant lines exhibited wide range of values for days to maturity (113-129), plant height (51-96cm), number of productive tillers per plant (12-25), number of grains per panicle (120-159), test weight (21-32.2g), zinc (20-41.5 ppm), iron (10-35 ppm), protein content (8-11.9g/100g) and grain yield per plant (23.7-34.6 g). The observations are evidence that a desirable variation has been generated in the grain quality parameters and also yield component traits in the mutant lines of all treatments.

Moderate phenotypic and genotypic coefficient of variation was exhibited by plant height (11.82 and 9.83), number of productive tillers per plant (18.53 and 13.91), iron (19.14 and 18.44), and zinc content (15.45 and 15.04), (**Table 1**). Similar results were reported by Ullah *et al*., (2023). This result specified the presence of variations and the possibility of improvement of these traits by direct selection when the effect of external environment is considerably low.

High heritability coupled with high GAM was observed for iron (92.78 and 36.59) and zinc content in the grain (94.83 and 30.19). The results were similar with those of Singh *et al*., (2020) and high heritability with moderate GAM was exhibited in days to fifty per cent flowering (92.2 and 10.75), plant height (69.23 and 16.86), number of grains per panicle (69.16 and 10.13), protein content (71.28 and 12.18) and grain yield per plant (69.88 and 14.32). Similar findings were delineated by Demeke *et al.,* (2023). These traits were less affected by the environment and the traits appear to be governed by additive gene action

**Table 2: Phenotypic correlation for yield components, zinc, iron and protein in the mutant lines of $M_5$ generation of red rice variety Kajejaya**

| | DF | DM | PH | PT | NG | TW | Pr | Fe | Zn | GY |
|---|---|---|---|---|---|---|---|---|---|---|
| **DF** | **1**** | | | | | | | | | |
| **DM** | -0.160 | **1**** | | | | | | | | |
| **PH** | 0.154 | 0.345* | **1**** | | | | | | | |
| **PT** | -0.036 | 0.183 | 0.243 | **1**** | | | | | | |
| **NG** | -0.106 | 0.091 | 0.008 | 0.459** | **1**** | | | | | |
| **TW** | 0.040 | -0.085 | -0.169 | 0.499** | 0.279 | **1**** | | | | |
| **Pr** | 0.017 | -0.004 | -0.365* | -0.168 | -0.265 | 0.216 | **1**** | | | |
| **Fe** | -0.111 | 0.068 | -0.537** | -0.301 | -0.077 | 0.194 | 0.414** | **1**** | | |
| **Zn** | 0.218 | -0.476** | -0.377* | -0.352* | -0.401** | -0.059 | 0.290 | 0.188 | **1**** | |
| **GY** | -0.167 | 0.054 | 0.285 | 0.685** | 0.672** | 0.348* | -0.304 | -0.204 | -0.364* | **1**** |

**DF**: Days to 50 per cent flowering; **DM**: Days to maturity; **PH** : Plant height (cm); **NG** : Number of grains per panicle; **PT** : Number of productive tillers; **TW** : Test weight (g); **Fe** : Iron (ppm); **Pr** : Protein (g/100g); **Zn** : Zinc (ppm); GY :Grain yield per plant suggesting ample scope for genetic improvement through selection.

The results of correlation coefficient are presented in **Table 2**. Grain yield per plant (g) exhibited positive and significant association with productive tillers per plant, number of grains per panicle and test weight. These results are in collaboration with Srihari *et al*., (2023) and Suman *et al*., (2006) for number of grains per panicle; Monalisa *et al*., (2006) for number of productive tillers per plant; Gholipoor *et al*., (1998) and Habib *et al.,* (2007) for test-weight. Productive tillers per plant, grains per panicle and test-weight are useful in increasing the grain yield. Therefore, the selection of these traits will be beneficial in the process of yield improvement.

There was a positive correlation between grain iron, protein and zinc content results are in accordance with Jeom Ho *et al*., (2008). Grain zinc exhibited negative significant association with grain yield per plant while protein and iron content showed non-significant and negative association with grain yield per plant. These results are accordance with Nagesh *et al*., (2012) and Kanatti *et al*., (2009). This negative association may be the result of pleiotropy or linkage. Therefore, a cautious selection of these component nutritive traits is imperative for the concurrent development.

The path coefficient analysis was computed to estimate the contribution of individual traits to grain yield. In this study, the phenotypic direct and indirect effect of different traits on grain yield is presented in **(Table 3)**. The results of path coefficient analysis revealed that among the traits number of grains per panicle showed highest the direct and positive effect on grain yield, followed by productive tillers per plant, plant height, test weight, iron and zinc content the results were on par with Bagudam *et al*., (2018), indicating the effectiveness of direct selection for these traits in improvement of grain yield per plant. Days to maturity had highest negative direct effect followed by days to fifty per cent flowering and protein content. Similar results were found for number of productive tillers per plant by Kole *et al.,* (2008); for number of grains per panicle by Yogameenakshi *et al.,* (2004) and Panwar *et al.,* (2007) for test weight by Habib *et al.,* (2007) towards grain yield.

**Table 3: Phenotypic path coefficient analysis for yield components, zinc, iron and protein in the mutant lines of $M_5$ generation of red rice variety Kajejaya**

| | DF | DM | PH | PT | NG | TW | Pr | Fe | Zn | r Values |
|---|---|---|---|---|---|---|---|---|---|---|
| **DF** | **-0.18277** | 0.03505 | 0.05642 | -0.01699 | -0.05002 | 0.00066 | -0.00095 | -0.01931 | 0.01002 | **-0.16789** |
| **DM** | 0.02937 | **-0.21809** | 0.12654 | 0.08519 | 0.04271 | -0.0014 | 0.00023 | 0.01187 | -0.02182 | **0.0546** |
| **PH** | -0.02817 | -0.07537 | **0.36614** | 0.11331 | 0.00375 | -0.00278 | 0.01972 | -0.0934 | -0.0173 | **0.2859** |
| **PT** | 0.00667 | -0.03991 | 0.08912 | **0.4655** | 0.21561 | 0.00818 | 0.00908 | -0.05242 | -0.01613 | **0.6857** |
| **NG** | 0.0195 | -0.01987 | 0.00293 | 0.2141 | **0.46882** | 0.00457 | 0.0143 | -0.01338 | -0.01838 | **0.67259** |
| **TW** | -0.00735 | 0.0186 | -0.06224 | 0.23273 | 0.13094 | **0.01637** | -0.01168 | 0.03374 | -0.00271 | **0.3484** |
| **Pr** | -0.00322 | 0.00092 | -0.13393 | -0.0784 | -0.12438 | 0.00355 | **-0.05392** | 0.07209 | 0.01329 | **-0.304** |
| **Fe** | 0.02031 | -0.0149 | -0.19676 | -0.14041 | -0.0361 | 0.00318 | -0.02236 | **0.17381** | 0.00864 | **-0.20459** |
| **Zn** | -0.03997 | 0.1039 | -0.13829 | -0.16392 | -0.18809 | -0.00097 | -0.01564 | 0.03278 | **0.0458** | **-0.3644** |

**Residual effect= 0.2721**

DF: Days to 50 per cent flowering; DM: Days to maturity; PH : Plant height (cm); NG: Number of grains per panicle; PT: Number of productive tillers; TW: Test weight (g); Fe: Iron (ppm); Pr: Protein (g/100g); Zn: Zinc (ppm); GY: Grain yield per plant.

In the present study, very low residual effect of 0.2721 indicated that the traits included in this study explained high percentage of variation in the grain yield. The highest indirect positive effect on grain yield was exhibited by productive tillers per plant through test weight, followed by number of grains per panicle, plant height and days to maturity, thus selection based on number of productive tillers per plant, number of grains per panicle, plant height and test weight would be most effective, since test weight, number of productive tillers per plant and number of grains per panicle were had maximum direct effect as well as indirect effect on other characters *via* these traits.

Out of 20 mutant lines, two productive mutant lines ranked top, based on early maturity and reduced plant height as compared to check (**Table 4**). BMRM15and BMRM13 mutant lines mature early (114 and 117 days), which showed reduced plant height (84 and 80 cm), exhibited more number of productive tillers per plant (19 and 18), number of grains per panicle (141.5 and 140.5), test-weight (29.5 and 28.6 g) and grain yield per plant (30.5 and 30.6 g) as compared to the check. Mutations could create novel and unique variations as natural variability could not have provided the alleles for desired traits and application of radiation for induction of mutation must have resulted in direct development of 89% of mutant varieties in rice (Velmurugan *et al.,* 2010).

**Table 4: Mean performance of high zinc, iron and protein content productive mutant lines of each treatment based on higher grain yield in $M_6$ generation of red rice variety Kajejaya**

| Gamma rays treatment | Mutant lines | Days to maturity | Plant height (cm) | Number of productive tillers per plant | Number of grains per panicle | Test weight (g) | Protein (%) | Zinc (ppm) | Iron (ppm) | Grain yield per plant (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| 35kR | BMRM15 | 114.0 | 84.0 | 19 | 121.5 | 29.5 | 9.5 | 28.0 | 17.0 | 30.5 |
| 45kR | BMRM13 | 117.0 | 80.0 | 18 | 120.5 | 28.6 | 10.0 | 25.0 | 13.0 | 30.6 |
| Check- Kajejaya | | 120-125 | 90-95 | 12 | 115 | 24.25 | 8 | 20 | 10 | 24 |

Rice is the main staple food playing an important role in meeting calorie needs of the people hence, there is an ever-increasing consumer demand for rice for its functional nutritive quality. In present investigation, protein content in BMRM15 was 9.5% and BMRM13 recorded about 10.0%. In India, 80% of children under five years of age are under nourished, for whom the recommended intake of protein is 13-19 g/day/ child. As recommended calorie intake is 1000-1400 cal/day/ child (200-300g of rice) out of which 150-450 calories are to be supplied by protein, even 1% increase in grain protein content would add significant amount of protein to the diet. The OsASN1 OX rice plants produced grains with increased N and protein contents without yield reduction compared to wild-type (WT) rice (Lee 2020).

These two mutant lines also showed higher zinc (28 and 25ppm) and iron (Fe) content (17 and 13ppm). Zhang *et al*., (2012) and Bashir *et al.,* (2013) reported that, the OsVIT2 is a vacuolar localized transporter, which plays an important role in the vacuolar sequestration of Fe to regulate Fe homeostasis. It has been demonstrated that the disruption of *OsVIT2* results in an increase in Fe and Zn concentration in rice grains. Kandwal *et al.,* (2022) and Cheng *et al.,* (2007) noted that *naat*1 mutation exhibited strong stimulation of the Fe(II) acquisition system and leads to a significantly higher concentration of iron in both brown and polished (*naat*1) rice grown under water logged field conditions. BMRM15 and BMRM13 mutant Lines should be tested in different environmental condition for further confirmation of protein, iron and zinc content and can be efficiently used as donor in varietal improvement programmes to develop healthier rice varieties with high yield potential.

## Conclusion

Biofortification is the only feasible way of reaching the malnourished population with vital nutrients and to produce nutrient packed rice grains in a sustainable way. Present investigation results documented that gamma ray treatment induced notable variability in mutant lines not only for yield attributing traits but also on grain zinc, iron and protein content. BMRM15and BMRM13 mutant lines can be used as parents in a breeding program targeting semi-dwarf and short duration characters. These two mutant lines should be evaluated in multi-location trial to assess their performance in a wide range of environments for nutritional quality and productivity.

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RESEARCH ARTICLE https://doi.org/10.58297/GMSL7598

# Exploration of Genetic Variability and Trait Association for Root Architecture Related Traits under Aerobic Conditions in Rice (*Oryza sativa* L.)

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## Abstract

In this study, we performed exploration of genetic variability for root architecture related traits in a mapping population derived from TI-128×BPT 5204, such as length, volume, fresh and dry weight, in influencing nutrient and moisture uptake during 60 days after sowing under aerobic conditions at three diverse environments for the selection of desirable lines. The variance analysis, revealed significant variations ($p \leq 0.005$) for all studied traits, indicating a broader sense of genetic variability for selection purposes. Significant positive correlation was recorded between all the traits under study. The rice lines *viz.,* TB 1, TB4, TB 8, TB 24, TB 51, TB 67 and TB 107 exhibited comparatively higher RL, RV and RDW, than all the checks used in the present study. The identified lines exhibiting better root architectural traits can benefit in developing a suitable DSR cultivar with better adaptability and performance under dry direct seeded rice conditions.

**Keywords:** Aerobic rice, root, DDSR, genetic variability, correlation.

## Introduction

Rice (*Oryza sativa* L.) stands as the primary food source for billions in developing nations, flourishing across diverse environments in terms of latitude, altitude, and topography, all with varying water availability. Despite its global significance irrigated rice cultivation is known for its inefficient water usage, requiring 3,000 to 5,000 liters of water to yield just one kilogram of grain. Startlingly, rice commands a substantial 85% share of the 70-80% of freshwater used in agriculture globally (Pathak *et al.,* 2011, Kommana *et al.,* 2023). Predictions sound an alarm, with around 39 million hectares of irrigated rice in Asia alone potentially facing yield reductions due to water scarcity (Tuong and Bouman, 2003, Reddy *et al.,* 2022). The forthcoming decades are poised for a critical shift toward more sustainable agricultural practices that integrate environmental well-being and socio-economic considerations. Therefore, transitioning from the conventional transplanted puddled rice (TPR) to alternative systems that enhance water productivity without compromising yields is a prudent choice. This kind of shift in rice cultivation is needed to cope with water scarcity as well as to maintain the groundwater table (Phule *et al.,* 2019). One such system is direct-seeded rice (DSR), which not only reduces water consumption and labour needs but also bolsters resource efficiency, productivity, and mitigates greenhouse gas emissions. In recent years, DSR has gained prominence as the primary rice cultivation method in developed countries and has been adopted in more than 25% of global rice cultivation areas (Kumar and Ladha, 2011). Overall, DSR offers numerous benefits, including substantial savings in water and labor expenses, improved crop rotation possibilities, and reduced greenhouse gas emissions (Corton *et al.,* 2000). DSR, in particular, makes efficient use of early-season monsoons in regions with limited moisture. Compared to TPR, the DSR system uses 60.3% less non-renewable energy and exhibits an average energy-use efficiency of 7.3 compared to 4.4 for TPR (Panda *et al.,* 2021).

Roots serve as the primary sensory and adaptive organs in response to various environmental stresses, including drought, flooding, salinity, and mineral deficiencies. An extensive root system helps support above ground plant growth by enabling better water and nutrient absorption from the soil, ultimately leading to higher yields (Padmashree *et al.,* 2022). Surprisingly, most breeding efforts have traditionally focused on improving above ground plant characteristics, largely neglecting root traits that contribute to nutrient acquisition efficiency (NAE), often due to limited awareness and inadequate screening methods. Unfortunately, these valuable root traits have been inadvertently disregarded or even selected against in the context of intensive agriculture. Tailoring root architecture to suit the requirements of DSR presents a promising opportunity to overcome adaptability and yield limitations. This approach could yield genotypes that thrive in DSR conditions, outperforming those suited for TPR (Sandhu *et al.,* 2021). The widespread acceptance and adoption of DSR, as well as the development of suitable rice varieties, hinge on overcoming the challenges associated with breeding varieties that thrive in low-water conditions and offer sufficient nutrient availability. In this study, we conducted a phenotypic evaluation of 150 Recombinant Inbred Lines (RILs) over 60 Days After Sowing (DAS) at three diverse locations under aerobic conditions, with a focus on traits related to root architecture.

## Materials and Methods

The experimental material comprised of mapping population (150 lines, TB1 to TB150) developed through the crossing of selected mutant line (TI-128) as the female parent with best root related traits (seedling vigour index, root length, root volume) and the wild type (BPT 5204) as the male parent. The Mapping population ($F_7$) comprised of 150 RILs were evaluated at three locations (ICAR-IIRR, Hyderabad ($E_1$), AHRS, Kathalagere, KSNUAHS Shivamogga ($E_2$), RARS, Karjat ($E_3$)) for root architecture related traits under aerobic conditions during *rabi* 2022. To evaluate the lines for root architecture related traits under aerobic conditions, the experiment was laid out in augmented block design with five blocks, wherein, each block consisted of 30 lines along with the parents (TI-128 and BPT-5204) and six checks (Sahabhagidhan, MAS 946-1, Sabita, TI-112, TI-3 and TI-17) were randomized in each block. Each RIL was sown in two-meter length line at a spacing of 20cm × 15cm. Agronomic practices were followed as recommended for aerobic rice cultivation, for the first four weeks after sowing (WAS), lines were irrigated once in three days for two to three hours. From the fifth week, irrigation was provided 2-3 times a week for two hours to reach field capacity. The seeds were directly sown and 15 days after sowing extra seedlings were thinned to maintain a single plant per hill. Timely weeding was performed, and the field was maintained as per the agronomic practices with need-based irrigation.

Randomly three plants in each line were carefully uprooted by carefully pulling them out from the soil without damaging the roots (destructive sampling). The roots were washed manually using a high-pressure water pump and observations were recorded for root architecture related traits *viz.,* root length (RL), shoot length (SL), total plant length (TPL), root fresh weight (RFW), shoot fresh weight (SFW), total fresh weight (TFW), root dry weight (RDW), shoot dry weight (SDW), total dry weight (TDW), and root volume (RV). The analysis of variance, genetic variability parameters and correlation were carried out in R studio (version 3.5.2) using R-scripts for statistical analysis (Aravind *et al.,* 2019).

## Results and Discussion

### Analysis of variance (ANOVA) for root architecture related traits

The Mean Sum of Squares (MSS) for the root architecture related traits under aerobic conditions in the mapping population during *rabi* 2022 is presented in **(Table 1)**. The ANOVA revealed that the MSS of test genotypes versus checks was highly significant at $p<0.05$ for all the traits under investigation. Highly variable traits are preferred in breeding programmes for maximizing the genetic base (Barde *et al.,* 2021). The MSS due to test genotypes exhibited highly significant differences at $p<0.05$ for all the traits under investigation across three diverse locations, revealing that sufficient variability was present in the mapping population.

**Table 1: Analysis of variance for root architectural related traits in RILs under aerobic condition during *rabi* 2022**

| Source | Location | *d. f.* | RL | SL | TPL | RFW | SFW | TFW | RDW | SDW | TDW | RV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | EI | 157 | 1.64** | 14.28** | 19.82** | 123.42** | 591.24** | 1024.12** | 13.24** | 57.28** | 109.24** | 228.26** |
|  | EII |  | 1.58** | 15.24** | 20.18** | 130.28** | 586.28** | 1041.28** | 14.28** | 54.12** | 107.28** | 231.42** |
|  | EIII |  | 1.69** | 16.22** | 20.93** | 126.47** | 604.72** | 1055.72** | 15.46** | 61.33** | 112.93** | 236.28** |
| Check | EI | 7 | 1.68** | 87.42** | 78.12** | 1010.32** | 2042.36** | 4230.28** | 124.24** | 218.24** | 476.24** | 556.82** |
|  | EII |  | 1.72** | 86.28** | 76.54** | 1024.28** | 2100.28** | 4310.24** | 130.54** | 216.22** | 472.24** | 550.28** |
|  | EIII |  | 1.98** | 95.89** | 81.41** | 1045.20** | 2179.20** | 4440.53** | 127.78** | 221.02** | 484.23** | 548.26** |
| Test genotypes | EI | 149 | 1.28** | 10.26** | 14.24** | 76.28** | 412.26** | 712.26** | 8.62** | 48.24** | 72.42** | 124.28** |
|  | EII |  | 1.32** | 10.46** | 13.26** | 71.14** | 421.58** | 734.28** | 8.84** | 42.24** | 74.26** | 126.24** |
|  | EIII |  | 1.48** | 11.98** | 16.68** | 75.22** | 438.93** | 740.45** | 9.20** | 44.52** | 78.91** | 132.22** |
| Test vs. Check | EI | 1 | 1.36** | 87.14** | 222.28** | 1228.24** | 13284.28** | 21364.28** | 154.28** | 1242.24** | 2326.28** | 1284.22** |
|  | EII |  | 1.28** | 88.24** | 216.24** | 1324.24** | 13862.24** | 22328.24** | 158.36** | 1342.28** | 2428.24** | 1324.24** |
|  | EIII |  | 1.41** | 91.42** | 230.01** | 1330.79** | 14286.17** | 24337.49** | 162.70** | 1448.96** | 2582.72** | 1266.28** |
| Block | EI | 4 | 7.41 | 56.28 | 74.26 | 163.28 | 1242.28 | 1324.26 | 44.22 | 112.24 | 124.24 | 110.28 |
|  | EII |  | 5.12 | 52.48 | 68.28 | 142.24 | 1366.48 | 1412.28 | 43.24 | 121.28 | 126.28 | 108.26 |
|  | EIII |  | 6.12 | 58.64 | 79.54 | 154.32 | 1382.38 | 1426.38 | 38.26 | 116.26 | 132.24 | 104.22 |
| Residuals | EI | 28 | 0.48 | 15.32 | 19.22 | 86.24 | 62.24 | 42.28 | 12.26 | 25.24 | 42.22 | 31.26 |
|  | EII |  | 0.56 | 16.84 | 18.24 | 76.32 | 74.28 | 54.26 | 11.24 | 28.34 | 44.26 | 32.48 |
|  | EIII |  | 0.32 | 14.26 | 17.28 | 79.30 | 54.32 | 52.26 | 12.38 | 22.26 | 38.28 | 30.22 |

EI- ICAR-IIRR, Hyderabad, EII- AHRS, Kathalagere, KSNUAHS, Shivamogga, EIII-RARS, Karjat

RL- Root length (cm), SL- Shoot length (cm), TPL- Total Plant length (cm), RFW, Root fresh weight (g), SFW- Shoot fresh weight (g), TFW- Total fresh weight (g), RDW- Root dry weight (g), SDW- Shoot dry weight (g), TDW- Total dry weight (g), RV- Root volume (cm3)

### Genetic variability parameters

The results of genetic variability parameters *viz.,* range, mean, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), broad-sense heritability and genetic advance as per cent mean were analyzed to estimate the extent and magnitude of genetic variation for root architecture related traits in mapping population. The mean performance for the traits showed a wide range of variation for most of the characters studied and the findings of the present experiment are given in **(Table 2)**. The heritability of broad-sense ($h^2$ bs) was divided into three categories: high (above 60%), medium (30%–60%), and low (less than 10%) (Robinson *et al.,* 1949). GAM is also divided into three

**Table 2: Estimation of genetic variability parameters for root architectural related traits in RILs under aerobic condition during *Rabi* 2022**

| Traits | Location | Range | | Mean | CV (%) | Genetic variability | | $h^2$(bs) (%) | GAM |
|---|---|---|---|---|---|---|---|---|---|
| | | Min | Max | | | GCV (%) | PCV (%) | | |
| **RL** | EI | 4.33 | 28.33 | 16.49 | 4.05 | 12.68 | 12.71 | 96.24 | 51.20 |
| | EII | 5.83 | 26.23 | 15.23 | 4.03 | 13.20 | 13.21 | 97.12 | 51.82 |
| | EIII | 5.89 | 24.24 | 14.81 | 4.97 | 13.90 | 14.10 | 97.26 | 52.40 |
| **SL** | EI | 19.90 | 53.23 | 31.77 | 5.20 | 10.82 | 10.86 | 94.12 | 45.28 |
| | EII | 22.97 | 44.13 | 31.81 | 5.33 | 10.16 | 10.24 | 93.26 | 46.24 |
| | EIII | 21.28 | 41.28 | 32.20 | 5.46 | 10.74 | 10.78 | 93.19 | 47.52 |
| **TPL** | EI | 24.40 | 62.83 | 40.25 | 7.59 | 9.18 | 9.72 | 97.12 | 39.28 |
| | EII | 29.00 | 53.87 | 41.04 | 6.76 | 9.26 | 9.64 | 94.28 | 41.26 |
| | EIII | 27.26 | 50.52 | 41.01 | 7.10 | 9.97 | 9.98 | 96.72 | 40.54 |
| **RFW** | EI | 5.70 | 60.30 | 23.11 | 8.04 | 13.22 | 13.24 | 95.24 | 57.26 |
| | EII | 10.24 | 63.20 | 24.32 | 8.81 | 13.82 | 13.86 | 94.24 | 57.40 |
| | EIII | 6.67 | 60.86 | 23.43 | 9.49 | 14.05 | 14.12 | 95.98 | 58.20 |
| **SFW** | EI | 16.20 | 125.10 | 58.73 | 12.12 | 35.42 | 36.42 | 94.12 | 57.26 |
| | EII | 15.12 | 127.12 | 58.48 | 13.77 | 36.28 | 37.12 | 93.88 | 59.28 |
| | EIII | 13.01 | 125.60 | 58.36 | 11.86 | 37.53 | 37.58 | 94.92 | 62.38 |
| **TFW** | EI | 23.8 | 175.5 | 81.84 | 9.88 | 38.24 | 38.68 | 85.28 | 51.28 |
| | EII | 25.96 | 171.40 | 82.80 | 11.25 | 37.26 | 37.88 | 84.26 | 52.24 |
| | EIII | 23.79 | 176.56 | 81.78 | 10.86 | 36.50 | 36.50 | 84.62 | 53.44 |
| **RDW** | EI | 1.91 | 20.97 | 7.73 | 4.05 | 36.28 | 36.33 | 93.12 | 32.68 |
| | EII | 3.40 | 21.06 | 8.08 | 4.03 | 35.29 | 35.31 | 94.04 | 32.88 |
| | EIII | 2.33 | 21.28 | 8.19 | 4.97 | 35.87 | 35.92 | 94.18 | 32.98 |
| **SDW** | EI | 5.06 | 39.09 | 18.35 | 5.20 | 36.28 | 36.42 | 76.28 | 66.24 |
| | EII | 3.26 | 37.61 | 16.56 | 8.33 | 34.26 | 34.28 | 74.26 | 67.28 |
| | EIII | 4.14 | 40.00 | 18.58 | 7.46 | 37.53 | 38.52 | 75.24 | 68.24 |
| **TDW** | EI | 7.56 | 56.10 | 26.08 | 7.59 | 32.28 | 33.52 | 82.24 | 26.54 |
| | EII | 6.86 | 52.32 | 24.64 | 6.76 | 31.26 | 32.28 | 84.26 | 25.24 |
| | EIII | 7.91 | 57.82 | 26.78 | 9.10 | 33.70 | 33.82 | 87.24 | 27.28 |
| **RV** | EI | 8.24 | 31.92 | 18.26 | 8.04 | 34.24 | 35.28 | 97.42 | 35.24 |
| | EII | 7.26 | 30.28 | 17.28 | 9.81 | 35.12 | 25.42 | 97.26 | 36.28 |
| | EIII | 7.28 | 32.24 | 17.36 | 9.49 | 35.87 | 36.82 | 98.12 | 36.94 |

EI- ICAR-IIRR, Hyderabad, EII- AHRS, Kathalagere, KSNUAHS, Shivamogga, EIII-RARS, Karjat

RL- Root length (cm), SL- Shoot length (cm), TPL- Total Plant length (cm), RFW, Root fresh weight (g), SFW- Shoot fresh weight (g), TFW- Total fresh weight (g), RDW- Root dry weight (g), SDW- Shoot dry weight (g), TDW- Total dry weight (g), RV- Root volume ($cm^3$).

categories: high (>20%), medium (10–20%), and low) (below 10%) (Johnson *et al.,* 1955). High GCV and PCV coupled with high heritability and GAM were observed for the traits *viz.,* shoot length, root length, shoot fresh weight, root fresh weight, total fresh weight, shoot dry weight, root dry weight, total dry weight and root volume. Moderate to high GCV and PCV coupled with high heritability and GAM were observed for total plant length and low GCV and PCV coupled with high heritability and high GAM. The narrow magnitude of difference between phenotypic and genotypic coefficients of variations was recorded for characters such as shoot length, root length, shoot fresh weight, root fresh weight, total fresh weight, shoot dry weight, total dry weight and root volume indicating the limited influence of environment in the expression of these characters. Selection based on the phenotypic performance of these characters would be effective in bringing about considerable genetic improvement. The narrow difference between GCV and PCV implies that most features are less influenced by the environment. The heritability estimates for a given trait determine the reliability of the phenotypic value. As a result, high heritability aids in the efficient selection of a specific trait, hence, quantitative trait genetic analysis is critical for breeding programmes. Our results are in accordance with the results of Koshle *et al.,* (2020) and Singh *et al.,* (2017) observed high heritability and GAM for the traits *viz.,* shoot length, root length, root to shoot length ratio, shoot fresh weight, root fresh weight, total fresh weight, root to shoot fresh weight ratio, shoot dry weight, root dry weight, total dry weight and root to shoot dry weight ratio and concluded that such characters would be considered for selection.

### Estimates of correlation coefficients

Correlation studies were carried out to know which parameters contributed to the maximum for root architecture traits and the details of the results are given in graphical representation of **(Figure 1)**. Root length exhibited the highest positive significant correlation with total plant length, total fresh weight, shoot fresh weight, shoot length, total dry weight, root volume, root dry weight and shoot dry weight. The shoot length exhibited the highest positive significant correlation with total plant length, root to shoot length ratio, root fresh weight, total dry weight, shoot fresh weight and shoot dry weight. Total plant length exhibited the highest positive significant correlation with shoot fresh weight, total fresh weight, shoot dry weight, total dry weight, root dry weight, root fresh weight and root volume. Root to shoot length ratio exhibited the highest positive significant correlation with root fresh weight, root to shoot fresh weight ratio, root dry weight, total fresh weight, total dry weight, root to shoot dry weight ratio and root length per volume, tiller number per plant with shoot fresh weight and total fresh weight with root dry weight and root average diameter. Shoot fresh weight showed a positive significant association with total fresh weight, root fresh weight, shoots dry weight, total dry weight and root dry weight. Root fresh weight exhibited the highest positive significant correlation with total fresh weight, shoot dry weight, total dry weight, root dry weight, root to shoot fresh weight ratio and root to shoot dry weight ratio. Total fresh weight showed a positive significant correlation with total dry weight, shoot dry weight, root dry weight and root length per volume. Results are in accordance with the results of Sandhu *et al.,* (2019) who observed a positive significant correlation between shoot fresh weight, total fresh weight, shoot dry weight, total dry weight, root dry weight, root fresh weight and root volume with root length. Subudhi *et al.,* (2015) also reported the similar results.

The positive and significant correlation among component traits indicated that these related traits could be used in combination for selection or initial phenotyping for seedling vigour. The root-related traits play a crucial role in nutrient acquisition, adaptation and need to be concentrated along with the yield-attributing traits (Padmashree *et al.,* 2023). Rice roots had an impact on plant growth and grain yield, which was highly connected to root dispersion as well as to root morphology (Yang, 2011). It has been reported that the uppermost layer (0–20 cm) of rice roots is crucial for moisture and nutrient absorption, which could improve the rate of grain filling, and, subsequently, the grain weight (Ishimaru *et al.,* 2017).

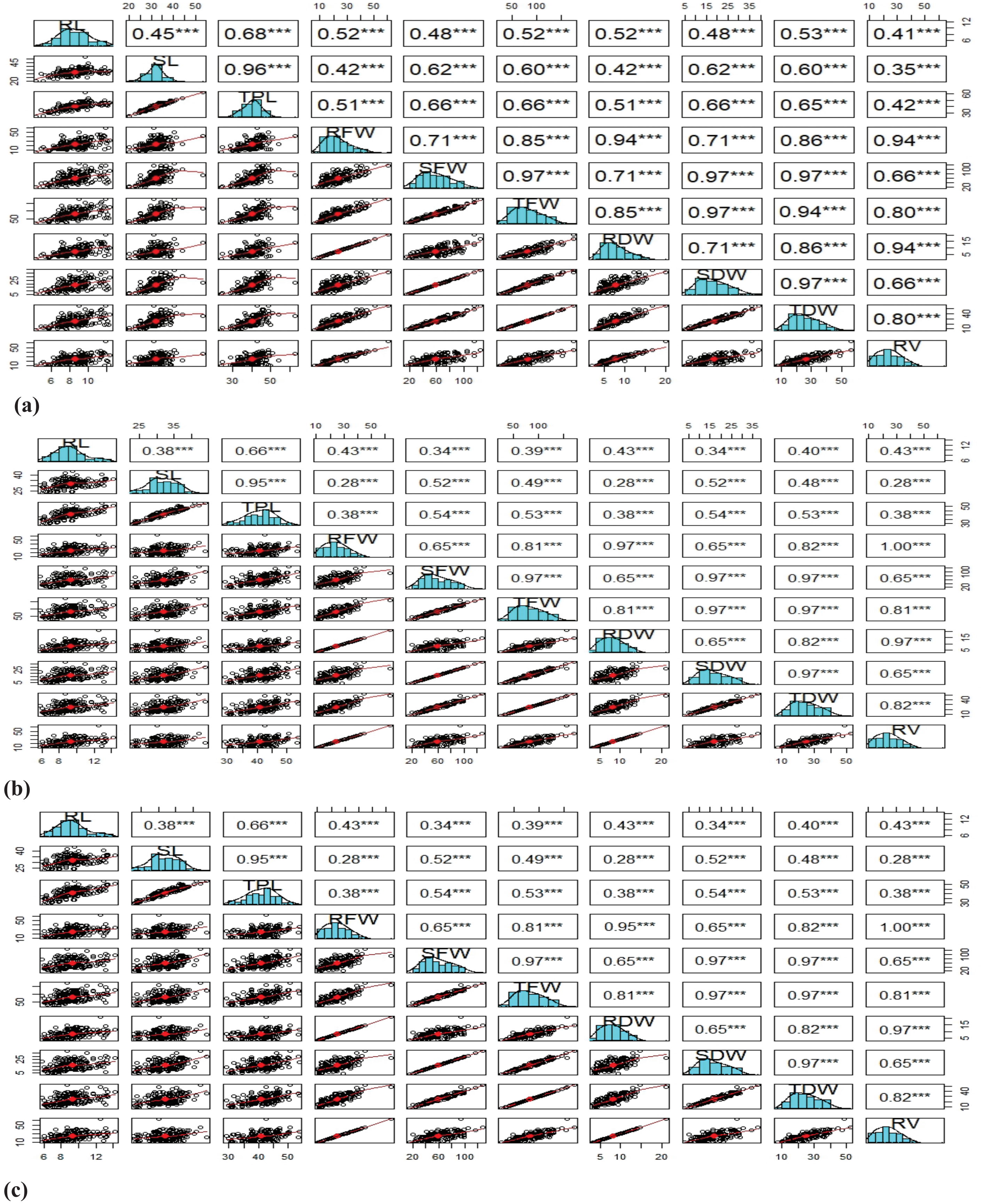

**Figure 1: Graphical representation of correlation coefficients for root architectural traits in RILs at 60 DAS stage under polyhouse condition during *Rabi* 2022 (a) ICAR-IIRR, Hyderabad, (b) AHRS, Kathalagere, (c) RARS, Karjat**

The rice lines TB 1, TB4, TB 8, TB 24, TB 51, TB 67 and TB 107 than the checks Sahabhagidhan, MAS 946-1, Sabita, TI-112, TI-3, TI-17. TB 1, TB 4 reported higher RL, TB 24 and TB 51 recorded higher RL and RV than the checks. These lines with the superior potential of RL and RV have a strong inherent genetic ability for performance under aerobic conditions. Root length and Root volume are important components of root architecture that are essential for survival in complex soil conditions, under deep sown conditions and primarily responsible for better adaptability and performance under DDSR, and are desirable for developing hybrid varieties for direct seeded genotypes with wide adaptability. Identifying the ideal root architecture and breeding new varieties with efficient root architecture has great potential to improve resource-use efficiency and grain yield, especially under DSR direct seeded genotypes with wide adaptability.

## Conclusion

The current study was aimed to explore the root architectural related traits in rice RILs and their manifestation under water-limited conditions. We hypothesized that the variability in root architecture related traits in the RILs could be a breeding resource for improving genotypic performance under limited water conditions. We anticipate that the information will allow rice breeders to determine and select genotypes at the seedling stage. The identified lines with better root traits can be used to breed for water limiting or DDSR conditions.

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## Authors' contribution

The study was planned by KMB, phenotyping the panel and analysis was done by KMB, VR, NDM, WBD, MB, PSP, MK, AAH; supervision and timely inputs were given by DB, GC, AMS, DBM, SKM, UTN, KCM; critically edited the manuscript KMB, LR.

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RESEARCH ARTICLE https://doi.org/10.58297/ITVW7951

# Study on Genetic Variability and Correlation Coefficient for Grain Quality Parameters in South Indian Rice (*Oryza sativa* L.) Varieties

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## Abstract

The demand for high quality rice among consumers has surged in recent decades due to improvements in living standards. Hence, improving grain quality without reducing grain yield is a major concern in rice breeding programmes to benefit the rice growers and consumers. Therefore, it is very crucial to study the genetic variability among the genotypes for selecting potential parents to exploit maximum heterosis and superior recombinants in terms of quality components. The present investigation was carried out at Indian Institute of Rice Research, Hyderabad during *kharif* 2022 with 96 rice lines to assess the genetic variability for grain quality parameters. The analysis of variance revealed a significant difference among the genotypes for all the traits studied, except volume expansion ratio, which indicates the presence of considerable amount of variation among these genotypes. Overall study on genetic variability revealed that the estimates of PCV were only slightly higher than the corresponding GCV for traits such as kernel length, kernel width, kernel length after cooking, elongation ratio, alkali spreading value, amylose content and gel consistency suggesting a lesser influence of the environment. Traits like kernel length, kernel length after cooking, alkali spreading value and gel consistency had high heritability coupled with high genetic advance which indicates the role of additive genes in the inheritance of these traits. Correlation studies revealed that, grain yield plot$^{-1}$ had a significant and positive association with quality traits such as hulling, milling and head rice recovery. Therefore, simultaneous improvement for these characters is possible through selection. Binadhan 17, DR 714-1-2R, DRR Dhan 44, IR 64 and JGL 18047 were found to be the best five genotypes for grain quality parameters.

**Keywords:** Rice, Genetic variability, Heritability, Genetic advance, Quality parameters

## Introduction

Rice (*Oryza sativa* L.) is one of the most significant cereal crops in the world and provides the daily dietary requirements for majority of the global population. Most crop improvement programmes on rice are generally focused on breeding for higher yield. But in recent decades, as the living standards of the population have steadily improved, human demand for high-quality rice has continuously increased, making the incorporation of improved grain quality parameters the most important objective, next to yield enhancement.

Unlike other cereals, rice is consumed mainly as a whole grain contributing to 20% of daily calories as staple food for a large population throughout the world (Bhattacharjee *et al.,* 2002). As a result, physical features such as size, shape, homogeneity and overall appearance is critical. The economic value of rice is mainly determined by the physico-chemical properties, cooking and processing quality of grain (Pushpa *et al*., 2018). Hence, improving grain quality without reducing yield needs to be a major concern in rice breeding programmes in order to benefit all rice growers and consumers.

Consumer preferences for good quality rice varies from one geographical region to another (Rathi *et al*., 2010). In India, long grain type, especially, Basmati varieties and fine or short slender grain varieties with intermediate amylose, alkali spreading value and gel consistency along with high volume expansion ratio is preferred over other grain types (Hossain *et al*., 2009). The rice grain quality traits, include milling quality, appearance quality, nutritional quality, cooking and eating quality are most important for the consumers. Hence, selection for improved milling, cooking, eating and processing qualities is crucial to meet consumer's preference and industry standards.

An extensive amount of genetic variability has been documented for quality traits in the past, but there is still untapped genetic variability in germplasm, which is of extreme importance in selecting potential parents to exploit maximum heterosis and superior recombinants in terms of quality components.

The success of crop breeding programmes mainly depends upon amount of genetic variability available for exploitation and the extent to which the desirable characters are heritable (Tiwari *et al*., 2011). Genetic parameters such as genotypic coefficient of variation and phenotypic coefficient of variation are useful in determining the amount of variability present in the germplasm. Heritability coupled with high genetic advance helps in determining the influence of environment on the expression of genotype and reliability of characters. The degree of heritable variation in the variables evaluated is extremely valuable in assessing the potential of genotype for future breeding programme.

The correlation between different traits is frequently obtained through gene linkage (Mather and Jinks, 1971). The degree and direction of genetic correlations aid in the selection of one or more traits. The knowledge of correlation between yield and quality traits are significant in the selection of elite genotypes for breeding high yielding varieties with premium quality grain (Dhavaleshvar *et al*., 2019).

The desired quality characteristics in rice include hulling, milling, head rice recovery, kernel length, kernel width, LB ratio, volume expansion ratio, water uptake, kernel length after cooking, elongation ratio, alkali spreading value, amylose content and gel consistency. In the present investigation, an attempt has been made to elucidate information on genetic variability of south Indian rice varieties for these 13 important quality parameters.

## Materials and Methods

The experimental material for the present investigation comprised of 96 South Indian rice genotypes collected from IIRR Hyderabad, RARS Maruteru, RARS Jagtial and RARS Mandya. Field trials were laid out in simple lattice design with two replications and five blocks. For each replication, individual genotypes were assigned a plot size of 2 sq.m with four rows and a spacing of 20 cm between rows and 15 cm spacing within the rows. All necessary measures were taken to maintain a uniform population in both replications. Observations on physico-chemical characters such as hulling, milling, head rice recovery, kernel length, kernel width, L/B ratio, volume expansion ratio, water uptake, kernel length after cooking, elongation ratio, amylose content and gel consistency were recorded. The data were subjected to analysis of variance by using SAS statistical software and the genetic parameters such as phenotypic coefficient of variation and genotypic coefficient of variation (PCV and GCV), heritability in broad sense ($h^2$) and genetic advance as per cent of mean were calculated. The genotypic and phenotypic correlations were calculated as per the method recommended by Singh and Chaudhary (1977).

## Results and Discussion

The analysis of variance **(Table 1)** revealed a significant difference among the genotypes for all the traits studied, except volume expansion ratio, which indicates a considerable amount of variation present among these genotypes. The variation present in these parameters can be exploited by the rice breeders in their hybridization programmes.

**Table 1: Analysis of variance for quality parameters**

| Sl. No. | Source | Type III SS (Mean square) | | |
|---|---|---|---|---|
| | | Replication | Block | Varieties |
| | **Degrees of freedom** | **1** | **4** | **95** |
| 1 | Hulling (%) | 4.66 | 14.50** | 8.74** |
| 2 | Milling (%) | 0.41 | 2.63 | 12.10** |
| 3 | Head rice recovery (%) | 62.37 | 101.00* | 72.80** |
| 4 | Kernel length (mm) | 0.00 | 0.01 | 1.24** |
| 5 | Kernel width (mm) | 0.01 | 0.003 | 0.08** |
| 6 | L/B ratio | 68.70** | 7.21** | 0.53** |
| 7 | Volume expansion ratio | 0.32 | 0.54 | 0.21 |
| 8 | Water uptake (ml) | 1360.00 | 9394.00** | 2795.00** |
| 9 | Kernel length after cooking (mm) | 2.50** | 1.24** | 3.29** |
| 10 | Elongation ratio | 0.05* | 0.017 | 0.04** |
| 11 | Amylose content (%) | 2.18 | 7.52** | 9.17** |
| 12 | Gel consistency (mm) | 19.40 | 19.90* | 347.00** |

The estimates of mean, range, coefficient of variation, heritability in broad sense and genetic advance as per cent of mean for different characters have been presented in **Table 2.** A perusal of the results revealed maximum range for water uptake (100-300 ml). The genotype, SW-RMS-1 (100 ml) recorded lowest water uptake, while Phalguna (300ml) recorded highest water uptake. Hulling percentage ranged between 70.3% to 83.4% with a general mean of 77.7%. Milling percentage exhibited a wide variation ranging from 61.3% to 75.7% with a general mean of 69.4%. The genotype, Ranjeet recorded lowest and Krishna Hamsa recorded highest in hulling and milling percentage. Head rice recovery ranged from 45.0% (FBM 145-6-6) to 69.3% (MTU 1155) with a mean value of 60.7%. Kernel length (mm) varied from 3.30 mm to 7.46 mm with a general mean of 5.90mm. The genotype, Chittimutyalu (3.30 mm) recorded the shortest grain, while NDR8002 (7.46 mm) recorded the longest grain. Kernel width ranged between 1.77 mm (Karjat 6) to 2.75 mm (Vikra marya) with a general mean of 2.10 mm. The genotypes with high L/Bratio were slender and were more preferred by consumers. The genotype, MTU 1262 (1.46) recorded lowest L/B ratio, whereas the genotype CR DHAN 300 (3.43) recorded highest L/B ratio with a general mean value of 2.21. Volume expansion ratio varied between 4.1 (Salivahana) to 5.6 (KMP149) with a general mean of 4.8. Kernel length after cooking (mm) ranged from 6.7 mm (MTU 1262) to 12.4 mm (PR 121) with an average value of 9.8 mm. Elongation ratio ranged between 1.39 (MTU1262) to 2.33 (Chittimutyalu) with a general mean of 1.67. The mean values for Alkali spreading value ranged between 3 to 7 with a general mean of 4.69. The Amylose content estimated for 96 genotypes ranged between13.74% (SW-RMS-1) to 27.25% (Akshaydhan) with a general mean of 23.09%. Genotypes were classified as low (9-20%), intermediate (20-25%) and high (25-33%) with respect to Amylose content. The genotypes, Swarnadhan (16.91), SW-RMS-1 (13.74), Kasturi (14.87), DRR Dhan 54 (19.68) were having low Amylose content which was not preferred by consumers as they tend to cook soft and sticky. Whereas, 16 genotypes were having high amylose content and these genotypes tends to cook firm and dry. The 76 genotypes have intermediate amylose content and these were most preferred by the consumers. Gel consistency ranged between 22 mm to 71 mm with a general mean of 29.4 mm. A total of 66 genotypes exhibited low gel consistency, whereas the genotype, Kasturi (71 mm) had the highest gel consistency. Out of 96 genotypes, only 21 genotypes were having medium gel consistency which were preferred over soft and hard types.

Coefficient of variance is the percentage ratio of standard deviation of a sample to its mean. Analysis of variance provides estimates of phenotypic, genotypic and environmental variance, which are used for the estimation of respective coefficient of variance (Burton, 1952). Overall study on genetic variability **(Table 2)** revealed that the estimates of PCV were slightly higher than the corresponding GCV for traits such as kernel length, kernel width, kernel length after cooking, elongation ratio, alkali spreading value, amylose content and gel consistency, which suggests that expression of these traits is mainly due to the genetic makeup of the genotypes. Greater the difference between the values of PCV and GCV, the greater will be the influence of the environment on the performance of the genotype. Low PCV and GCV were observed for traits such as hulling, milling and kernel width, indicating that selection directly based on these traits would not be much rewarding. Similar results were reported by Sala *et al.,* (2015) for kernel width. Traits such as kernel length and kernel length after cooking exhibited moderate level of PCV and GCV. These results are in conformity with Gangashetty *et al.,* (2013) and Devi *et al*., (2017) for kernel length and Subbaiah *et al*., (2011) for kernel length after cooking. Alkali spreading value and gel consistency had high PCV and GCV indicating the presences of high variability available in these traits. Similar results were reported by Patel *et al.,* (2014) and Allam *et al.,* (2015) for ASV and Gampala *et al.,* (2015) for gel consistency.

Heritability and genetic advance are important parameters that directly affect the response to selection (Johnson *et al.,* 1955). Heritability is the measure of extent of phenotypic variance caused by the actions of genes. Therefore, high heritability helps in effective selection for a particular character. The characters studied in the present investigation expressed low to high heritability estimates ranging from 8.90 to 95.30. Characters such as kernel length (92.40), kernel width (90.80), kernel length after cooking (80.80), elongation ratio (63), alkali spreading value (82.20), amylose content (69.40) and gel consistency (95.30) had high heritability in broad sense. Similar results were reported by Sanjukta *et al*., (2013) and Gampala *et al.,* (2015) for kernel length and width, Patil *et al.,* (2003) for elongation ratio, Rathi *et al.,* (2010) for ASV and amylose content. High heritability values indicate that the characters under study are less influenced by environment in their expression. The plant breeder, therefore, may make his selection

**Table 2: Estimation of genetic parameters**

| S. No. | Charac-ters | Mean | Range | | Coefficient of variation | | Heritability (Broad sense) (%) | GA as % of the mean |
|---|---|---|---|---|---|---|---|---|
| | | | Minimum | Maximum | PCV (%) | GCV (%) | | |
| 1 | Hulling | 77.7 | 70.3 | 83.4 | 3.31 | 1.87 | 31.8 | 2.17 |
| 2 | Milling | 69.4 | 61.3 | 75.7 | 4.34 | 2.50 | 33.1 | 2.96 |
| 3 | HRR | 60.7 | 45.0 | 69.3 | 12.37 | 6.68 | 29.21 | 7.44 |
| 4 | KL | 5.90 | 3.30 | 7.46 | 13.6 | 13.1 | 92.4 | 25.9 |
| 5 | KW | 2.10 | 1.77 | 2.75 | 10.0 | 9.5 | 90.8 | 18.7 |
| 6 | L/B | 2.21 | 1.46 | 3.43 | 31.5 | 9.4 | 8.9 | 5.78 |
| 7 | VER | 4.80 | 4.1 | 5.6 | 10.2 | 3.98 | 15.3 | 3.21 |
| 8 | WU | 190 | 100 | 300 | 22.4 | 16.5 | 54.3 | 25.1 |
| 9 | KLAC | 9.8 | 6.6 | 12.4 | 13.7 | 12.3 | 80.8 | 22.8 |
| 10 | ER | 1.67 | 1.39 | 2.33 | 10.4 | 8.29 | 63.0 | 13.6 |
| 11 | ASV | 4.69 | 3.00 | 7.00 | 26.8 | 24.4 | 82.2 | 45.5 |
| 12 | AC | 23.09 | 13.7 | 27.2 | 10.1 | 8.39 | 69.4 | 14.4 |
| 13 | GC | 29.4 | 22 | 71 | 45.4 | 44.3 | 95.3 | 89.1 |

safely based on the phenotypic expression of these characters in individual plant by adopting simple selection methods.

The genetic advance is a useful indicator of the progress that can be expected as result of exercising selection on the pertinent population. Heritability in conjunction with genetic advance would give a more reliable index of selection value (Johnson *et al.*, 1955). Characters such as kernel length (25.90), water uptake (25.10), kernel length after cooking (22.80), alkali spreading value (45.50) and gel consistency (89.10) had high Genetic advance as per cent of the mean. High genetic advance coupled with high heritability estimates offers the most suitable condition for selection. Traits like kernel length, kernel length after cooking, alkali spreading value and gel consistency had high heritability along with high genetic advance. It indicates the presence of additive genes in the trait and further suggest reliable crop improvement through selection of such traits. Amylose content and elongation ratio had high heritability along with moderate genetic advance as per cent of the mean, suggesting a combined or conditional function of additive and non-additive gene activity in controlling these traits. In these case, high heritability may come from the positive effect of environmental variables. As a result, selection for these characteristics may be counter-productive. L/B ratio and volume expansion ratio exhibited low heritability and low genetic advance as per cent of mean indicating greater influence of environmental factors on this character.

Correlation between yield and quality traits were carried out for ninety-six genotypes and the results were presented in **Table 3**. Correlation studies revealed that, grain yield plot$^{-1}$ had a significant and positive association with quality traits such as hulling (0.28), milling (0.26) and head rice recovery (0.32). Therefore, selection based on these characters helps in simultaneous improvement of both yield and quality. But it had a negative and significant association with volume expansion ratio and water uptake.

Based on the mean performance of 96 genotypes for traits such as amylose content, gel consistency, alkali spreading value, L/B ratio and volume expansion ratio, the genotypes, Binadhan17, DR 714-1-2R, DRR Dhan 44, IR 64 and JGL 18047 were found to be the best five genotypes for grain quality parameters **(Table 4)**.

**Table 3: Phenotypic correlation coefficient for quality traits**

| | Hulling | Milling | HRR | KL | KB | L/B | VER | WU | KLAC | ER | ASV | AC | GC | Y/P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hulling | 1.00 | 0.82** | 0.55** | 0.35** | 0.21* | 0.13 | 0.03 | 0.02 | 0.27** | -0.12 | 0.05 | 0.02 | 0.05 | 0.28** |
| Milling | | 1.00 | 0.66** | 0.28** | 0.32** | -0.01 | 0.04 | 0.01 | 0.22* | -0.13 | 0.10 | -0.01 | -0.10 | 0.26** |
| HRR | | | 1.00 | -0.07 | -0.04 | 0.07 | 0.09 | -0.05 | -0.16 | -0.14 | -0.002 | -0.15 | -0.09 | 0.32 ** |
| KL | | | | 1.00 | 0.32** | 0.43* | -0.03 | 0.10 | 0.80** | -0.32 ** | -0.03 | -0.06 | 0.35** | 0.07 |
| KB | | | | | 1.00 | -0.14 | 0.01 | 0.13 | 0.38** | 0.05 | 0.23* | 0.11 | 0.00 | 0.19 |
| L/B | | | | | | 1.00 | -0.02 | -0.08 | 0.40** | -0.04 | -0.16 | -0.08 | 0.17 | 0.07 |
| VER | | | | | | | 1.00 | 0.06 | 0.01 | 0.06 | 0.05 | 0.00 | -0.04 | -0.20* |
| WU | | | | | | | | 1.00 | 0.20* | 0.16 | 0.7** | 0.27** | -0.13 | -0.27** |
| KLAC | | | | | | | | | 1.00 | 0.28** | 0.05 | -0.05 | 0.25* | -0.002 |
| ER | | | | | | | | | | 1.00 | 0.08 | -0.03 | -0.13 | -0.16 |
| ASV | | | | | | | | | | | 1.00 | 0.35** | -0.22* | -0.16 |
| AC | | | | | | | | | | | | 1.00 | -0.38** | 0.05 |
| GC | | | | | | | | | | | | | 1.00 | 0.11 |
| Y/P | | | | | | | | | | | | | | 1.00 |

Significant at 5 per cent level; **Significant at1 per cent level

**HRR:** Head rice recovery, **KL:** Kernal length, **KW:** Kernal width, **VER:** Volume expansion ratio, **WU:** Water uptake, **KLAC:** Kernallength after cooking, **ER:** Elongation ratio, **ASV:** Alkali spreading value, **AC:** amylose content, **GC:** gel consistency, **Y/P:** Grainyield/plot

**Table 4: Mean performance of top five genotypes for grain quality parameters**

| Genotype | L/B ratio | Volume expansion ratio | Alkali Spreading value | Amylose content | Gel consistency |
|---|---|---|---|---|---|
| Binadhan 17 | 3.04 | 4.2 | 4 | 20.85 | 51 |
| DR714-1-2R | 3.13 | 5.5 | 4 | 20.09 | 57.5 |
| DRRDhan 44 | 3.15 | 4.6 | 4 | 21.98 | 59.5 |
| IR64 | 3.29 | 4.9 | 4 | 20.61 | 43.5 |
| JGL18047 | 2.06 | 5.2 | 4 | 22.73 | 63.5 |

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# Evaluation for Multiple Abiotic Stress Tolerance in Rice (*Oryza Sativa*) Genotypes

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## Abstract

Abiotic stresses are the major constraints that affect the morphological, physiological and biochemical attributes of plants resulting in reduction of yield. Recently, combined occurrence of stresses has become a greater cause of concern in farmers' fields. Hence, there is a need to identify genotypes that are tolerant to more than one stress. A study on physiological characterization for water and salinity stress was conducted in twenty rice genotypes during *kharif* 2022 at Regional Agricultural Research Station, Maruteru, Andhra Pradesh, India. Results showed that among all the treatments, root length, shoot length, root and shoot dry weight and chlorophyll content were greater under control followed by 1% mannitol, 2% mannitol and salinity stress. Visual scoring for water stress at seedling stage revealed that under mild water stress (1% M) in genotypes *viz.,* CR4423-8, FL 478 and Pantara recorded 75-90% of survival and under severe water stress (2% M) 40-50% survival. Under salinity stress, the score 3 was recorded in FL 478 and 5 in Naveen, Pantara and CR 4423-75. For combined stresses, FL 478 and Pantara were identified as tolerant and can be suggested as donors.

**Key words:** Chlorophyll content, Rice, Salinity, SES, Water stress

## Introduction

In Asian countries, rice is the primary staple food crop on which more than half of the population is dependent. Rice cultivation provides livelihood, serves as a source of calories and when exported provides support to the nation's economy. There is an incessant need for increasing the rice production keeping in view the increasing demand. However, rice production faces several constraints especially in the form of abiotic stresses, such as water stress and salinity, both being the major threats. Under the changing climatic scenario, a plant in its life cycle may face a single stress, combination of stresses at same physiological stage or two different stresses at different stages of the crop (Rosa *et al*., 2022). Hence, a plant tolerant to more than one stress is desirable.

Morphological, physiological and biological traits of plants are severely affected by abiotic stresses. In particular, water stress and salinity affect various aspects of plant growth and physiology. Exposure of crops to abiotic stresses during seedling stage leads to poor crop establishment due to early death of the seedlings. Drought stress decreases turgor pressure, an important physiological mechanism that affects cell growth. It has been reported that onset of water stress at early seedling stage inhibits rice germination and growth of the seedling (Pirdashti *et al*., 2003) and also decrease the leaf chlorophyll content and hence, affecting the metabolism (Chutia, and Borah, 2012). Salinity at early stages causes reduction in water absorption leading to stomatal closure and thereby reduces plant growth in terms of both height and biomass (Yamamoto *et al*., 2011). The growth of the roots is affected as a result of increasing osmotic pressure outside the roots (Yadav, 2020). Salt stress has detrimental effects on plant physiological processes such as photosynthesis and also affects the pigment composition such as chlorophylls, anthocyanins and carotenoids (Panda *et al*., 2013).

To assess the tolerance level of rice genotypes to abiotic stress in the early seedling phase, static hydroponic culture is the best viable approach that is being used frequently to screen the germplasm (Ali *et al*., 2014). With this background, a hydroponic study was designed and setup with a major objective to identify tolerant rice genotypes for both water stress (mild and severe) as well as salinity. The identified genotypes could be used as a donor in further crop improvement programmes.

## Materials and Methods

The experiment comprised of twenty rice genotypes as listed in **(Table 1)** that were tested for their multiple stress tolerance during *kharif* 2022 at Regional Agricultural Research Station, Maruteru. The seeds of genotypes were surface sterilized with 70% ethanol solution for 5 min followed by thorough washing for two-three times with sterilized distilled water. Pre-germinated seeds of each variety were taken and placed in hydroponic setup in 4 sets with 3 replications. The hydroponic setup was laid down in CRD. The plants were initially grown for 2-3 days in fresh water. Later it was shifted to Hoagland's solution containing the essential macro and micro nutrients. At 3-4 leaf stage, stress was imposed by addition of Mannitol for 1% and 2% for mild and severe water stress treatments, respectively. The amount of mannitol was calculated based on the quantity of the nutrient solution taken in the tray (~5 lit). For salinity stress, sodium chloride was added. An EC of 6 was maintained for first 3 days till the plants were acclimatised. The EC was increased to 12 and maintained till the end of the experiment. The nutrient solutions were changed and replaced by fresh solution every 2-3 days by draining the old solution completely followed by rinsing three–four times with fresh solutions so as to avoid increased stress level due to Mannitol and sodium chloride. The pH of the solution was adjusted daily to 5.5. Observations such as shoot length, root length, shoot dry weight, root dry weight and chlorophyll content were recorded at the end of the experiment.

**Table 1: List of genotypes used for phenotyping for water (mild and severe) and salinity stress conditions**

| S. No. | Genotypes |
|---|---|
| 1 | CR4423-8 |
| 2 | IC-256564 |
| 3 | CR4423-10 |
| 4 | FL 478 |
| 5 | CR4111-B-1-4-S-1-Sub-B |
| 6 | Vandana |
| 7 | CR4423-17 |
| 8 | Naveen |
| 9 | CR3483-29-M-4-B-Sub-79-1 |
| 10 | Pantara |
| 11 | CR4423-20 |
| 12 | IR20 |
| 13 | CR4423-75 |
| 14 | CR 4111-B-1-10-S-1-Sub-B |
| 15 | AC 1125A |
| 16 | CR4423-101 |
| 17 | CR4423-111 |
| 18 | AC847A |
| 19 | IC-256508 |
| 20 | IC-256605 |

Shoot and roots were separated at the shoot-root junction and the length of the of both shoot and root was measured. Both were expressed in cm. The samples were oven dried for 48 h at 60 $^{o}$C and the weight was recorded as shoot and root dry weight and it was expressed in g.

Chlorophyll content in the samples were recorded at the end of the experiment. 25 mg of leaf sample was taken and placed in 80% acetone as per the methodology described by Porra *et al*., (1989). Using a UV-VIS spectrophotometer, absorbance of chlorophyll a and chlorophyll b were measured at 663.2 nm and 646.8 nm respectively and the chlorophyll content was expressed in mg g$^{-1}$ fresh weight (mg g-1 FW). Chlorophyll a content, chlorophyll b content and the total chlorophyll content were calculated according to Lichtenthaler and Wellburn, (1983).

### Statistical analysis

Two-way analysis of variance (ANOVA) was performed using Statistix 8.1 package. Statistical significance of the parameter means was determined by performing Fisher's LSD test to test the statistical significance.

## Results and Discussion

### Shoot and root length

Imposition of stress resulted in the reduction of shoot and root lengths under all the three stress treatments. Under control conditions, the shoot length varied from 11.6 cm in CR 4423-20 to 24.5 cm in AC 847 A. Under mild and severe water stress (1% M and 2% M), shoot length was maximum in Pantara (19.6 cm and 19.2 cm) followed by AC 847 A (17.6 cm and 16.4 cm) and Vandana (17.5 cm and 14.7 cm), respectively. The shoot length was minimum under 1% M in CR4111-B-1-4-S-1-Sub-B (10.1 cm) followed by CR 4423-20 (10.4 cm). Under 2% M, minimum was in CR 4423-8 (9.4 cm) and CR4111-B-1-4-S-1-Sub-B (9.7 cm). Under salinity stress maximum shoot length was in Pantara (20.0 cm), AC 847 A (15.0 cm) and Vandana (12.1 cm). Minimum shoot length was observed in CR4111-B-1-4-S-1-Sub-B (5.3 cm) and CR4423-10 (7.5 cm) **(Table 2)**.

**Table 2: Impact of water stress (1% M and 2% M) and salinity stress on shoot length (cm) and root length (cm) of rice genotypes.**

| Entry | Shoot length (cm) | | | | Root length (cm) | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 1% Mannitol | 2% Mannitol | NaCl | Control | 1% Mannitol | 2% Mannitol | NaCl |
| CR4423-8 | 18.5 | 16.3 | 9.4 | 9.2 | 7.4 | 7.1 | 4.8 | 3.3 |
| IC-256564 | 19.7 | 14.4 | 12.0 | 10.1 | 4.5 | 4.4 | 4.3 | 2.8 |
| CR4423-10 | 16.7 | 12.7 | 10.9 | 7.5 | 9.6 | 6.2 | 5.2 | 3.6 |
| FL 478 | 14.2 | 14.1 | 12.3 | 10.6 | 7.7 | 7.4 | 7.3 | 6.9 |
| CR4111-B-1-4-S-1-Sub-B | 15.2 | 10.1 | 9.7 | 5.3 | 5.7 | 5.1 | 5.2 | 2.6 |
| Vandana | 23.6 | 17.5 | 14.7 | 12.1 | 6.9 | 5.8 | 5.2 | 5.0 |
| CR4423-17 | 14.4 | 11.0 | 10.0 | 9.2 | 5.5 | 5.3 | 5.0 | 4.9 |
| Naveen | 14.0 | 11.1 | 10.3 | 9.4 | 6.7 | 5.8 | 5.7 | 5.3 |
| CR3483-29-M-4-B-Sub-79-1 | 14.4 | 11.5 | 10.1 | 9.2 | 6.8 | 5.1 | 4.6 | 4.5 |
| Pantara | 21.3 | 19.6 | 19.2 | 20.0 | 8.9 | 8.7 | 7.8 | 8.4 |
| CR4423-20 | 11.6 | 10.4 | 10.1 | 8.8 | 4.9 | 4.7 | 4.4 | 4.3 |
| IR20 | 13.3 | 12.4 | 11.1 | 8.2 | 7.2 | 6.9 | 6.1 | 6.0 |
| CR4423-75 | 13.8 | 13.1 | 12.7 | 11.4 | 7.8 | 7.5 | 6.2 | 5.3 |
| CR 4111-B-1-10-S-1-Sub-B | 13.6 | 12.4 | 12.2 | 11.5 | 8.2 | 7.8 | 7.5 | 4.9 |
| AC 1125A | 19.6 | 16.0 | 12.8 | 9.7 | 6.2 | 5.6 | 5.4 | 4.7 |
| CR4423-101 | 17.6 | 12.0 | 11.6 | 7.9 | 5.1 | 4.8 | 4.7 | 3.8 |
| CR4423-111 | 20.8 | 11.8 | 10.5 | 9.1 | 6.5 | 6.1 | 6.0 | 5.4 |
| AC847A | 24.5 | 17.6 | 16.4 | 15.0 | 7.5 | 7.2 | 6.8 | 6.5 |
| IC-256508 | 21.0 | 15.8 | 13.6 | 8.4 | 6.5 | 6.2 | 5.7 | 4.4 |
| IC-256605 | 23.9 | 12.4 | 12.2 | 11.4 | 5.1 | 4.6 | 4.8 | 4.2 |
| Mean | 17.6 | 13.6 | 12.1 | 10.2 | 6.7 | 6.1 | 5.6 | 4.8 |
| LSD (T) | | 0.38 | | | | 0.19 | | |
| LSD (V) | | 0.86 | | | | 0.42 | | |
| LSD (TxV) | | 1.72 | | | | 0.85 | | |
| CV (%) | | 7.9 | | | | 9.1 | | |

Root length under control varied from 9.6 cm in CR4423-10 to 4.5 cm in IC-256564. Under 1% M, root length was highest in Pantara (8.7 cm) followed by CR 4111-B-1-10-S-1-Sub-B (7.8 cm) and CR 4423-75 (7.5 cm). Lowest was in IC-256564 (4.4 cm) followed by IC-256605 (4.6 cm) and CR 4423-20 (4.7 cm). Under 2% M, highest root length was in Pantara (7.8 cm) followed by CR 4111-B-1-10-S-1-Sub-B (7.5 cm) and FL 478 (7.3 cm) and lowest was recorded in IC-256564 (4.3 cm) followed by CR4423-20 (4.4 cm). Under salinity stress, Pantara had maximum root length of 8.4 cm followed by FL 478 (6.9 cm) and AC 847A (6.5 cm) while minimum was in CR 4111-B-1-4-S-1-Sub-B (2.6 cm) followed by IC-256564 (2.8 cm) (**Table 2**).

It has been reported that water stress at early seedling stage inhibits the growth of the rice seedling as reflected by reduction in the length and dry matter (Madabula *et al*., 2016). Similar observation was recorded in our study as well. Both mild and severe water stress resulted in reduction in 22.7 and 8.9% of mean shoot and root length respectively. Similarly, Negrao *et al*., (2016) reported that imposition of salinity at early stages reduced plant growth in terms of both height and biomass. Inhibition in plant growth is mainly due to ionic toxicity and osmotic stress due to high

**Table 3: Impact of water stress (1% M and 2% M) and salinity stress on shoot dry weight (g) and root dry weight (g) of rice genotypes**

| Entry | Shoot weight (g) | | | | Root weight (g) | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 1% Mannitol | 2% Mannitol | NaCl | Control | 1% Mannitol | 2% Mannitol | NaCl |
| CR4423-8 | 1.72 | 1.49 | 1.26 | 1.21 | 0.34 | 0.28 | 0.33 | 0.32 |
| IC-256564 | 1.92 | 1.53 | 1.28 | 1.16 | 0.42 | 0.37 | 0.33 | 0.27 |
| CR4423-10 | 1.95 | 1.56 | 1.51 | 1.31 | 0.46 | 0.42 | 0.39 | 0.32 |
| FL 478 | 1.65 | 1.54 | 1.48 | 1.43 | 0.51 | 0.42 | 0.39 | 0.30 |
| CR4111-B-1-4-S-1-Sub-B | 1.58 | 1.29 | 1.25 | 1.22 | 0.53 | 0.42 | 0.33 | 0.30 |
| Vandana | 1.59 | 1.59 | 1.62 | 1.04 | 0.82 | 0.43 | 0.34 | 0.27 |
| CR4423-17 | 1.62 | 1.35 | 1.31 | 0.92 | 0.54 | 0.44 | 0.35 | 0.15 |
| Naveen | 1.59 | 1.29 | 1.18 | 1.30 | 0.51 | 0.49 | 0.44 | 0.40 |
| CR3483-29-M-4-B-Sub-79-1 | 1.33 | 1.00 | 0.95 | 0.87 | 0.40 | 0.35 | 0.27 | 0.26 |
| Pantara | 1.54 | 1.40 | 1.25 | 1.31 | 0.49 | 0.40 | 0.39 | 0.43 |
| CR4423-20 | 1.56 | 1.25 | 1.11 | 0.91 | 0.49 | 0.37 | 0.34 | 0.22 |
| IR20 | 1.53 | 1.39 | 1.35 | 1.10 | 0.62 | 0.44 | 0.31 | 0.20 |
| CR4423-75 | 1.42 | 1.19 | 1.17 | 1.03 | 0.66 | 0.53 | 0.41 | 0.37 |
| CR 4111-B-1-10-S-1-Sub-B | 1.61 | 1.25 | 0.95 | 0.88 | 0.45 | 0.37 | 0.35 | 0.22 |
| AC 1125A | 1.78 | 1.36 | 1.29 | 1.07 | 0.46 | 0.35 | 0.24 | 0.25 |
| CR4423-101 | 1.06 | 0.77 | 0.82 | 0.85 | 0.41 | 0.34 | 0.32 | 0.26 |
| CR4423-111 | 1.54 | 1.19 | 1.06 | 1.17 | 0.59 | 0.46 | 0.35 | 0.16 |
| AC847A | 1.55 | 1.19 | 1.23 | 0.89 | 0.50 | 0.40 | 0.30 | 0.11 |
| IC-256508 | 1.56 | 1.15 | 1.01 | 0.84 | 0.52 | 0.40 | 0.35 | 0.26 |
| IC-256605 | 1.65 | 1.23 | 0.92 | 0.86 | 0.49 | 0.33 | 0.28 | 0.21 |
| Mean | 1.59 | 1.30 | 1.20 | 1.07 | 0.51 | 0.40 | 0.34 | 0.26 |
| LSD (T) | 0.032 | | | | 0.014 | | | |
| LSD (V) | 0.073 | | | | 0.033 | | | |
| LSD (TxV) | 0.146 | | | | 0.066 | | | |
| CV (%) | 7.05 | | | | 7.01 | | | |

salt concentration (Singhal *et al.,* 2022). A reduction in both root and shoot length in rice seedlings when exposed to salinity was also reported by Rasel *et al.,* (2020). These findings are in agreement with the results of this study where a reduction of both root and shoot length in all the tested genotypes was noted.

### Shoot and Root Dry weight

Similarly, there was reduction in both root and shoot weight under both water stresses as well as salinity stress. Shoot weight varied from 1.06 g in CR4423-101 to 1.95 g in CR 4423-10. Maximum shoot weight under 1% M and 2% M was in Vandana (1.59 g and 1.62 g) followed by CR 4423-10 (1.56 g and 1.51 g) and FL 478 (1.54 g and 1.48 g). Minimum was in CR 4423-101 (0.77 g) followed by CR3483-29-M-4-B-Sub-79-1 (1.0 g) and IC-256508 (1.15 g). Under salinity, highest weight was in FL 478 (1.43 g) followed by Pantara and CR 4423-10 (1.31 g each). Lowest was observed in IC-256508 (0.84 g) followed by CR4423-101 (0.85 g) and IC-256605 (0.86 g) (**Table 3**).

Root weight under control varied from 0.82 g in Vandana to 0.34 g in CR 4423-8. Under 1% M, CR4423-75 had maximum root weight of 0.53 g followed by Naveen (0.49 g) and CR 4423-111 (0.46 g). Minimum was in CR4423-8 (0.28 g) followed by IC-256605 (0.33 g) and CR4423-101 (0.34 g). Under 2% M, Naveen recorded highest shoot weight of 0.44 g followed by CR4423-75 (0.41 g) and Pantara (0.39 g). Lowest was in AC 1125 A (0.24 g) (**Table 3**).

Reduction in the growth in terms of biomass was reported by Madabula *et al*., (2016) under water stress and by Rahman *et al*., (2016a) and Negrao *et al*., (2016) under salinity stress in rice seedlings. These findings are in tune with the results of this study where under stress both root and shoot biomass were reduced in all the genotypes tested.

### Chlorophyll content (mg/g FW)

The mean chlorophyll content reduced by 31.4% under 1% M, 42.0% under 2% M and by 62.3% under salinity stress. Under 1% M, less than 20% reduction of total chlorophyll content was observed in Pantara (19.8%) followed by CR4423-8 (19.4%), IC-256564 (19.0%) and FL 478 (13.3%). More than 40% was noted in IR20 (43.0%) and CR4111-B-1-4-S-1-Sub-B (45.5%). Under 2% M stress, less reduction was in FL 478 (23.8%) followed by CR4423-8 (25.7%), Pantara (29.4%) and IC-256564 (30.5%). Whereas more reduction was seen in CR 4111-B-1-10-S-1-Sub-B (60.2%) and CR4423-101 (57.5%). Under salinity stress, reduction in chlorophyll content was less in Pantara (43.1%) followed by IC-256508 (48.6%), FL 478 (49.2%), Naveen (51.6%) and CR4423-75 (51.7%). Higher reduction was in CR4423-101 (87.9%) followed by AC847A (76.4%) and CR4111-B-1-4-S-1-Sub-B (76.3%) (**Figure 1**).

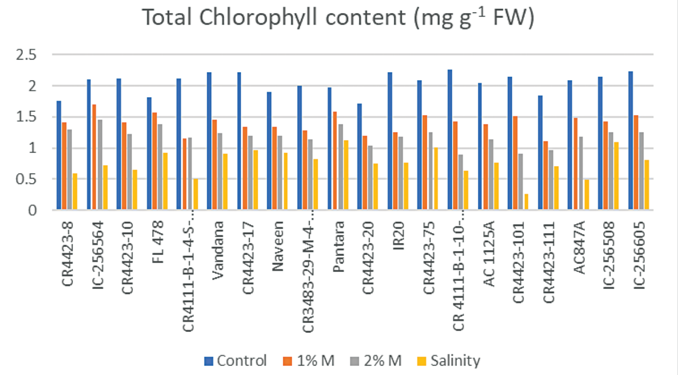

**Figure 1: Impact of water stress (1% M and 2% M) and salinity stress on total chlorophyll content (mg/g FW) of rice genotypes**

Chlorophyll is one of the major components of chloroplast as well as an essential pigment for sustenance of any plant as it is involved in the process of photosynthesis. Abiotic stress greatly affects the pigment composition. Imposition of water stress at early seedling stage is known to affect the chlorophyll content and in turn affect the plant metabolism (Swapna and Shylaraj, 2017). A reduction in chlorophyll content in PEG-induced drought stressed rice seedlings was reported by Dalal and Tripathy, (2012) and Hsu and Kao, (2003). In our study, chlorophyll content was sensitive to both mild and severe water stress compared to normal condition in all the tested genotypes. The main reason being that the imposition of stress leads to production of reactive oxygen species that cause lipid peroxidation as well as damage to the chlorophyll pigment (Hirt and Shinozaki, 2004). It is the increased activity of chlorophyllase enzyme that leads to the degradation of chlorophyll.

Similarly, salinity also causes a reduction in chlorophyll and caretonoid content (Sairam *et al*., 2002). The reduction in total chlorophyll content was more in susceptible genotypes when compared to tolerant (Panda *et al*., 2013). Rahman *et al*., (2016b) in his study on 12-d old rice seedling exposed to 150 mM of salinity stress reported a reduction in the chlorophyll content by 46 and 48% of chlorophyll a and b, respectively. In this study too, tolerant genotypes such as Pantara, FL 478, Naveen and CR4423-75 had less reduction in their chlorophyll content under salinity stress.

### Visual scoring under water and salinity stresses

Visual scoring for water stress at seedling stage revealed that under mild water stress (1% M) in genotypes *viz.,* CR4423-8, FL 478, Pantara, IR 20, CR4423-75, CR 4111-B-1-10-S-1-Sub-B almost 75-90% of plants survived. Under severe water stress (2% M) almost 40-50% of the seedlings survived in CR4423-8, FL 478, Pantara, CR4423-20 (**Tables 4 and 5**). Under salinity stress the score 3 was recorded in FL 478, and 5 in Naveen, Pantara and CR 4423-75 (**Table 6**).

**Table 4: Visual scoring for water stress (1% Mannitol) at seedling stage**

| Observation | Genotypes |
|---|---|
| Almost all plants dead | CR4423-111, IC-256508, IC-256605 |
| <25% survival | AC 1125A, CR4423-101, AC847A |
| <40% survival | CR4423-17, Naveen |
| 50-60% survival | IC-256564, CR4423-10, CR4111-B-1-4-S-1-Sub-B, Vandana, CR3483-29-M-4-B-Sub-79-1, CR4423-20 |
| 75-90% survival | CR4423-8, FL 478, Pantara, IR 20, CR4423-75, CR 4111-B-1-10-S-1-Sub-B |

**Table 5: Visual scoring for water stress (2% Mannitol) at seedling stage**

| Observation | Genotypes |
|---|---|
| Almost all plants dead | CR4423-17, Naveen, CR3483-29-M-4-B-Sub-79-1, IR20, CR4423-75, CR 4111-B-1-10-S-1-Sub-B, AC 1125A, CR4423-101, CR4423-111, AC847A, IC-256508, IC-256605 |
| <10% survival | CR4423-10 |
| 25%- 40% survival | IC-256564, CR4111-B-1-4-S-1-Sub-B, Vandana, |
| 40%-50% survival | CR4423-8, FL 478, Pantara, CR4423-20 |

**Table 6: Modified standard evaluation score (SES) for salinity stress at seedling stage**

| Score | Observation | Tolerance | Genotypes |
|---|---|---|---|
| 1 | Normal growth, no leaf symptoms | Highly tolerant | - |
| 3 | Nearly normal growth, but leaf tips or few leaves whitish and rolled | Tolerant | FL 478 |
| 5 | Growth severely retarded; most leaves rolled; only a few are elongating | Moderately tolerant | Naveen, Pantara, CR4423-75 |
| 7 | Complete cessation of growth; most leaves dry; some plants dying | Susceptible | CR4111-B-1-4-S-1-Sub-B, CR3483-29-M-4-B-Sub-79-1, CR4423-20, IR20, AC 1125A, CR4423-101 |
| 9 | Almost all plants dead or dying | Highly Susceptible | CR4423-8, IC-256564, CR4423-10, Vandana, CR4423-17, CR 4111-B-1-10-S-1-Sub-B, CR4423-111, AC847A, IC-256508, IC-256605 |

## Conclusion

The data revealed that under mild and severe water stress the genotypes CR 4423-8, FL 478 and Pantara were found to be tolerant. Similarly, under salinity stress, FL 478, Naveen, Pantara and CR 4423-75 were tolerant. For the combined stress (water stress and salinity), FL 478 and Pantara could be identified as promising genotypes. This was also indicated by lesser reduction in the shoot and root lengths, shoot and root dry weight and total chlorophyll content. The above cultures identified for different abiotic stress situations may be used as physiological donors for respective stresses.

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# Growth and Yield of Rice (*Oryza sativa* L.) are Influenced by Different Levels of Nitrogen, Phosphorus and Biofertilizers

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## Abstract

A field experiment was conducted during the rainy (*Kharif*) seasons of 2013 to 2015 at Agriculture Research Station (Irrigation crops), Anand Agricultural University, Thasra.The farm is located in hot semi-arid eco-region with medium deep black soils and geographically situated at $22.40^0$ latitude, $73.12^0$ longitude and 32.4 m above the mean sea-level.To study the response of different levels of Nitrogen (N), Phosphorus (P) and Biofertilizers on Rice (*Oryza sativa* L.) under middle Gujarat Agro climatic zone III, 12 treatment combinations consisting three levels of Nitrogen, two levels of Phosphorus and two levels of Biofertilizer were tried in factorial randomized block design with four replications. The three-year experimental results revealed that different treatments for nitrogen levels were found significant for most of the growth and yield contributing characters, while for phosphorus and bio-fertilizer found non-significant. In pooled data the N level $N_3$ (120 kg N $ha^{-1}$) gave significantly higher grain yield of 5508 kg $ha^{-1}$and was found significantly superior over N level of 80 kg ($N_1$). The result revealed that the potential production and profit from the rice crop could be secured by applying120 kg Nitrogen $ha^{-1}$ only to get higher yield in middle Gujarat Agro climatic zone III.

**Key Words**: Rice, Growth, Yield, Nitrogen, Phosphorus and Biofertilizer

## Introduction

Rice is grown in over hundred countries and is the primary food for half of the people in the world. World population is expected to be 8.5 billion by 2025 and to maintain the self-sufficiency in rice an increase of 2% -3% per year in rice production had to be maintained within the limited available land (Vallino *et al*., 2009). Due to continuous use of chemical fertilizers in rice production, soil health related problems are emerging. While integration of organic sources to sustain the productivity is necessary, it is difficult to meet the crop-nutrient requirements with bulky organic manure alone and there is a need for integrated application of different sources of nutrients including biofertilizers for sustaining the desired crop productivity (Gogoi *et al*., 2010). The efficiency of fertilizer use for nitrogen is lower than 50%, for phosphorus lower than 10% and for potassium 40%. This low efficiency of fertilizer use is also associated with other losses by immobilization, volatilization, denitrification, leaching, and clay adsorption (Ruiz *et al*., 2012). Therefore, the use of biofertilizer along with chemical fertilizers to maintain soil health as well as soil fertility and productivity is a need of the time. Biofertilizer is needed to increase the yield and thereby provide eco-friendly solution by adding organic matter in soil. The availability and efficiency of these mineral elements depend on the organic matter content and the biological activity of soils. In this context, the biofertilizers, products based on microorganisms, are an alternative for plant nutrition as these microorganisms enhance nutrients availability by biological activity, which reduces the amount of chemical fertilizers applied (Arévalo, 2009).

## Materials and Methods

### Experimental site

A field experiment on the response of different levels of Nitrogen (N), Phosphorus (P) and Biofertilizers on Rice (*Oryza sativa* L.) under middle Gujarat conditions was conducted during the *kharif* 2013 to 2015 at Agriculture Research Station (Irrigation crops), Anand Agricultural University, Thasra. The farm is located in hot semi-arid eco-region with medium deep black soils and geographically situated at $22.40^0$ latitude, $73.12^0$ longitude and 32.4 m above the mean sea-level. A composite representative soil sample was collected from the site of experimentation and analysed for physico-chemical properties. The soils of experimental site were slightly clay loam and alkaline (*p*H value 7.6 with 1:2.5 soil and water ration). It consists of 0.33% organic carbon (Jacksons *et al.,* 1973), 0.049% total Nitrogen (Jacksons *et al.,* 1973), 34.50 kgha$^{-1}$ available $P_2O_5$ (Olsen *et al.,* 1954) and 189 kgha$^{-1}$available $K_2O$ (Jacksons *et al.,* 1973). The soil was low in organic matter and nitrogen content and medium in available phosphorus and available $K_2O$. The average rainfall (900 mm) and average minimum and maximum temperature ($20.30^0$ C and $33.66^0$ C) were recorded, respectively in the years 2013, 2014 and 2015. The experiment was laid out in factorial randomized block design (FRBD) with four replications on GAR 13 variety. The treatment consisted of three levels of Nitrogen N ($N_1$: 80 kg ha$^{-1}$, $N_2$:100 kg ha$^{-1}$ and $N_3$: 120 kg ha$^{-1}$), two levels of Phosphorus ($P_0$: 0 kg ha$^{-1}$, $P_1$: 25 kg ha$^{-1}$) and two levels of Bio-fertilizers B {$B_0$: Control and $B_1$: Azospyrillum + PSB (Root dipping 3-5 ml/liter Water)} and 12 treatment combinations in each replication.

The field was ploughed, irrigated, puddled and made ready for sowing. Twenty-five to thirty days old seedlings of rice variety GAR 13 established on 16th July, 2013, 20th July, 2014 and 17th July, 2015 on well puddled soil. The seedlings were collected from the seed bed and transplanting was done at 1-2 seedlings per hill. The crop was treated with three levels of nitrogen fertilizer *viz.*, 80, 100 and 120 kg N ha$^{-1}$, two levels of phosphorus fertilizer *viz.,* 0 and 25 kg $P_2O_5$ ha$^{-1}$and two levels of biofertilizer *viz.,* control and Azospirillum + PSB root dipping 10-15 minute in 3-5 ml/Liter water at the time of transplanting. Full dose of $ZnSO_4$ (25 kg/ha) was applied as basal dose. While nitrogen and phosphorus were applied treatment wise 50% N and 100% $P_2O_5$ as basal and remaining nitrogen was applied in two splits, 25% N at tillering (30 DAP) and 25% N at panicle initiation (60 DAP) as top dressing and the treatments were superimposed in the same plot every year to study the cumulative treatment effect. The unit plot size was gross and net 6.00 m x 4.40 m, 5.20 m x 3.60 m respectively. Planting configuration was 20 cm x 15 cm where plot and block were separated by 0.50 m and 1.00 m respectively. Irrigation, weeding and other agronomic practices were done as per recommendations.

### Data Collection

Data on different growth parameters, yield components and yield were recorded. For determination of yield attributes five hills were selected and plant height, panicles/m$^2$, panicle weight, panicle length and test weight were measured while grain yield and straw yield were measured after harvesting in each plot. The crop was harvested from an area of 5.2 m x 3.6 m (18.72 m$^2$) leaving four rows and five dibbles to avoid border effect. The harvested yield was converted into kg/ha at 12-14% moisture content.

## Results and Discussion

### Growth

Growth of rice variety was measured by plant height, panicle/m$^2$, panicle length and panicle weight as affected by various levels of nitrogen, phosphorus and biofertilizer. Data presented in **(Table 1)** showed that nitrogen levels exerted significant effect on plant height and panicles/m$^2$ while panicle length and panicle weight were not affected nsignificant. While the effect of phosphorous levels were found non-significant on yield attributes, biofertilizer levels registered significant effect on panicle length (cm) and panicle weight. Effect of nitrogen at $N_3$ level (120 kg N ha$^{-1}$) found to be significantly highest in plant height (114 cm) and panicle/m$^2$ (287) over the rest of treatments **(**Ebaid *et al.,* 2000). Lowest plant height, panicle/m$^2$, panicle length and panicle weight recorded in nitrogen at $N_1$ level while nitrogen level $N_2$ effect was at par with Nitrogen $N_3$ level in plant height and panicles/m$^2$. Growth promoting effect of N on plant can be explained on the basis of the fact that N supply increases the number and size of meristematic cells which leads to formation of new shoots (Lawlor, 2002). Furthermore, N application is known to increase the levels of cytokinin which affects cell wall extensibility (Arnold *et al*., 2006). It is therefore, logical to speculate that N was involved directly or indirectly in the enlargement and division of new cells and production of tissues which in turn were responsible for increase in growth characteristics particularly tiller numbers and plant height of the rice variety.

**Table 1: Growth attributes influenced by various treatments (Pooled 2013 to 2015)**

| Treatment | Plant height (cm) | Panicle/m$^2$ | Panicle length (cm) | Panicle wt.(g) |
|---|---|---|---|---|
| **Nitrogen (N) kg/ha** | | | | |
| $N_1$:80 | 109 | 272 | 23.79 | 3.78 |
| $N_2$:100 | 111 | 283 | 24.05 | 3.77 |
| $N_3$:120 | 114 | 287 | 24.28 | 3.85 |
| S.Em± | 0.99 | 3.22 | 0.21 | 0.04 |
| CD 0.5% | 2.86 | 9.27 | NS | NS |
| **Phosphorus (P) kg/ha** | | | | |
| $P_0$:00 | 111 | 279 | 23.90 | 3.79 |
| $P_1$:25 | 112 | 282 | 24.18 | 3.81 |
| S.Em± | 0.81 | 2.63 | 0.18 | 0.03 |
| CD 0.5% | NS | NS | NS | NS |
| **Biofertilizer (B)** | | | | |
| $B_0$:00 | 110 | 278 | 23.72 | 3.73 |
| $B_1$:* | 112 | 283 | 24.36 | 3.88 |
| S.Em± | 0.81 | 2.63 | 0.18 | 0.03 |
| CD 0.5% | NS | NS | 0.50 | 0.09 |
| C.V. % | 6.19 | 7.95 | 6.18 | 6.91 |
| Y1 | 116 | 276 | 25.03 | 3.57 |
| Y2 | 109 | 281 | 23.65 | 4.21 |
| Y3 | 108 | 286 | 23.43 | 3.62 |
| S.Em± | 0.99 | 2.27 | 0.22 | 0.04 |
| CD 0.5% | 2.81 | 6.41 | 0.63 | 0.11 |
| C.V. % | 6.20 | 5.60 | 6.48 | 7.02 |

* $B_1$: Azospyrillum + PSB (Root dipping)

## Yield

### Effect of Nitrogen

The data in **Table 2** indicated that 1000 grain weight, grain yield and straw yield were significantly affected due to various levels of nitrogen application. Nitrogen at $N_3$level (120 kg N ha$^{-1}$) recorded significantly higher 1000 grain weight (18.89 g), grain yield (5508 kg ha$^{-1}$) and straw yield (7349 kgha$^{-1}$) than the rest of nitrogen levels. It was at par with nitrogen level $N_2$ (100 kg N ha$^{-1}$) while lowest 1000 grain weight (18.09

g), grain yield (5095 kg ha$^{-1}$) and straw yield (6703 kg ha$^{-1}$) recorded under the nitrogen level $N_1$ 80 kg N ha$^{-1}$. Application of 120 kg N ha$^{-1}$ increases grain yield and straw yield (**Figure 1**). This result is in agreement with the findings of (Sivasabari *et al.,* 2021). The increment of grain yield in this study at higher nitrogen levels might be due efficient absorption of nitrogen and other elements which raise the production and translocation of dry matter from source to sink (Ebaid *et al*., 2000; Morteza *et al*., 2011).

### Effect of Phosphorus

Impact of Phosphorus on grain yield, straw yield and 1000 grain weight were not significantly affected by various levels of phosphorus (**Table 2**). The higher 1000 grain weight (18.66 g), grain yield (5368 kgha$^{-1}$) and straw yield (7138 kgha$^{-1}$ was found when the crop fertilized with 25 kg $P_2O_5$ and lowest value recorded from $P_0$ level (**Figure 1**). In case of 1000 grain weight, the variation is very low among the levels as it is known to be a genetically controlled character. Similar results were reported by other scientists (Ahmed *et al*., 2005; Maske *et al*., 1997). The results are in conformity with that of Natarajan *et al.,* (2017).

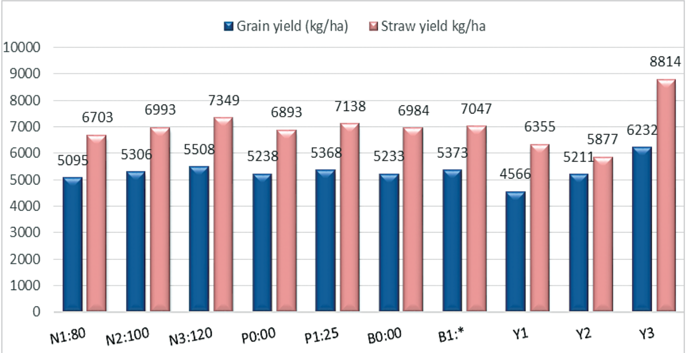

**Figure 1: Effect of different treatments on grain and straw**

**Table 2: Yield attributes influenced by various treatments (Pooled 2013 to 2015)**

| Treatment | 1000 grain weight (g) | Grain yield (kg/ha) | Straw yield (kg/ha) |
|---|---|---|---|
| **Nitrogen (N) kg/ha** | | | |
| $N_1$:80 | 18.09 | 5095 | 6703 |
| $N_2$:100 | 18.56 | 5306 | 6993 |
| $N_3$:120 | 18.89 | 5508 | 7349 |
| S. Em± | 0.16 | 63 | 95 |
| CD 0.5% | 0.45 | 183 | 274 |
| **Phosphorus (P) kg/ha** | | | |
| $P_0$:00 | 18.36 | 5238 | 6893 |
| $P_1$:25 | 18.66 | 5368 | 7138 |
| S.Em± | 0.13 | 52 | 78 |
| CD 0.5% | NS | NS | NS |
| **Biofertilizer (B)** | | | |
| $B_0$:00 | 18.28 | 5233 | 6984 |
| $B_1$:* | 18.74 | 5373 | 7047 |
| S.Em± | 0.13 | 52 | 78 |
| CD 0.5% | 0.37 | NS | NS |
| C.V. % | 5.86 | 8.28 | 9.93 |
| Y1 | 18.55 | 4566 | 6355 |
| Y2 | 18.69 | 5211 | 5877 |
| Y3 | 18.29 | 6232 | 8814 |
| S.Em± | 0.14 | 67 | 92 |
| CD 0.5% | NS | 188 | 261 |
| C.V. % | 5.23 | 8.70 | 9.13 |

* $B_1$: Azospyrillum + PSB (Root dipping)

**Table 3: Economics of various treatment**

| Treament | Grain yield kg/ha | Straw yield kg/ha | Gross income Rs/ha | Total expenditure Rs/ha | Net income Rs/ha | BCR |
|---|---|---|---|---|---|---|
| N1:80 | 5095 | 6703 | 83128 | 49880 | 33248 | 1.67 |
| N2:100 | 5306 | 6993 | 86583 | 50183 | 36400 | 1.73 |
| N3:120 | 5508 | 7349 | 89969 | 50487 | 39482 | 1.78 |
| P0:00 | 5238 | 6893 | 85463 | 48664 | 36799 | 1.76 |
| P1:25 | 5368 | 7138 | 87658 | 50021 | 37637 | 1.75 |
| B0:00 | 5233 | 6984 | 85479 | 48664 | 36815 | 1.76 |
| B1:* | 5372 | 7047 | 87628 | 48964 | 38663 | 1.79 |

*- Azospyrillum + PSB (Root dipping), BCR- Benefit cost ratio, Selling price: Paddy 15.00 Rs/ kg (Three year average), Paddy Straw 1.00 Rs / kg, Input cost: Urea Rs. 350/50 kg, DAP Rs. 1250/50 kg, $ZnSo_4$ 750 Rs / 25 kg, Biofertilizer, 150 Rs / Litre

### Effect of Biofertilizer

Effect of biofertilizer was found significant on 1000 grain weight (18.74 g) while it was non-significant on grain yield and straw yield. The highest grain yield (5373 kgha$^{-1}$) and straw yield (7047 kgha$^{-1}$) were obtained from biofertilizer $B_1$ level (**Figure 1**).

### Economics

The cost of cultivation and net return of rice was influenced to a great extent by different treatments. The economics of different treatments presented in **(Table 3)** indicated that the highest net income of Rs. 39482 was obtained with the treatment $N_3$ [120 kg N ha$_{-1}$] followed by treatment $B_1$ (Azospyrillum + PSB root dipping) with net income of Rs. 38663.

## Conclusion

Based on the study conducted during three years, it may be concluded that the potential production and profit from the rice crop (GAR 13) could be secured by fertilization of the crop with 120 kg Nitrogen ha$^{-1}$ only to get higher yield in middle Gujarat Agro-climatic zone III.

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# Insights of Colorimetric Estimation of Phytic Acid in Rice Grain

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## Abstract

Phytic acid present in rice grain is an anti-nutrient that hinders the absorption of iron (Fe) and zinc (Zn) ions in the human digestive system. Estimation of phytic acid is a part of the biofortification studies targeted to enhance micronutrient content in the rice grain. Among the various methods of phytic acid estimation, colorimetric method is highly amenable to most of the laboratories due to its simple requirements. Wade reagent has been widely used in phytic acid estimation in various crops. However, details like the range of phytic acid concentrations required for standard graph preparation and maximum limits of detection were not clearly mentioned and the volume of Wade reagent differed. Hence, the objective of this study is to identify the range of phytic acid concentrations that is suitable to develop phytic acid standard graph followed by the determination of the maximum limit of Wade reagent volume. The results of this study will be useful in the future studies to understand the inherent details like the volume of Wade reagent, volume of biological extract, optimum volume as well as concentration optimum for standard graph preparation and the factor for calculating phytate-P from phytic acid.

**Keywords:** Phytic acid, Colorimetric assay, Spectrophotometric assay, Wade reagent.

## Introduction

Rice is consumed as a staple diet by more than half of the world's population. Rice grain provides more energy than nutrients. Elements like iron, zinc, phosphorous, aluminium, cadmium, calcium, cobalt, copper, magnesium, manganese, molybdenum, etc. are also considered as micronutrients (Longvah, 2017). In general, elements are in minute quantities and are often lower than the prescribed recommended dietary allowance (RDA) which is 10-15 mg for iron and 12-15 mg for zinc (Sanjeeva Rao *et al*., 2014, 2020; Suman *et al*., 2021). Of these, iron and zinc are considered as priority elements due to the prevalence of anaemia and stunted growth in the global population.

Iron is required for the formation of heme (protoporphyrin) which is the prosthetic group of haemoglobin and redox components of various electron transfer chains of human beings, microbes and plants. Similarly, zinc is a prosthetic group of zinc fingers that control gene expression of some operons in eukaryotes and required for around 300 enzymatic reactions (McCall *et al*., 2000).

Phytic acid (PA), also referred to as the main phosphorus (P) storage compound found in seeds (Kumar *et al*., 2021), plays a crucial role in negatively binding positively charged minerals (**Figure 1**), rendering them inaccessible to the human digestive system (Raboy *et al*., 2001; Prasanna *et al*., 2021). Seeds accumulate an excess of phosphorus beyond what is necessary for normal cellular functions during germination. Rice grain contains around 7% phytic acid and binds to 70% of the phosphorus stored in the grain (Perera *et al*., 2018). This trait in rice germplasm was less studied or exploited with phytic acid content ranging from 22.64 to 41.31 µg/grain in brown rice, 2.67 to 6.42 µg/grain in embryo and 22.24 to 38.70 µg/grain in endosperm of wild and low phosphate mutant (Liu *et al*., 2004), 3.98 mg/g to 32.36 mg/g in the few rice germplasm sets and it noted negative association with grain zinc as well as iron contents (Gyani *et al*., 2021). It contains multiple negative charges due to the presence of phosphate groups that bind to divalent cations like iron and zinc and makes them unavailable for absorption in the human digestive tract (Graham *et al*., 2001).

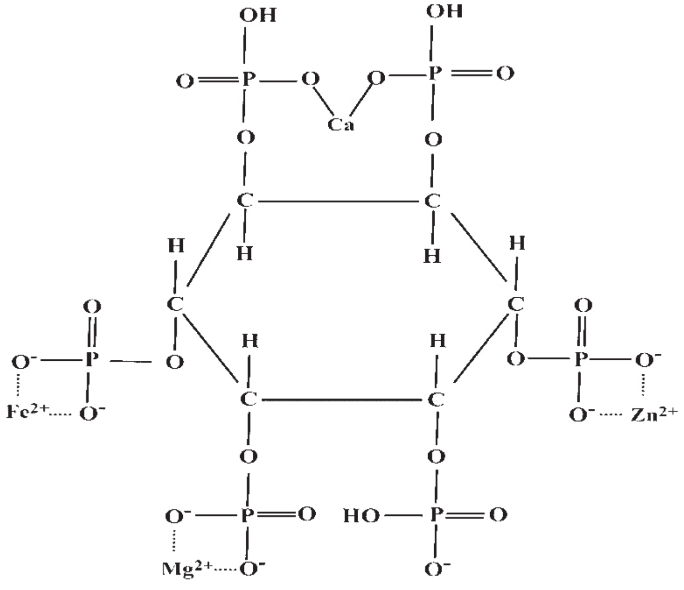

**Figure 1: Phytic acid multi-cation complex**

In addition to yield, enhancing nutrition in cereal crops is one of the targets of millennium development goals. As some of the other molecules in the grain can act as anti- or pronutrients and influence the absorption of the zinc or iron, assessing the bioavailability of the zinc or iron also became a pre-requisite. Since phytic acid is considered as an anti-nutrient, screening the germplasm or mapping population or advanced material for phytic acid is gaining focus in the past few years. Reducing PA content not only enhances the nutritional value of food but also addresses concerns related to malnutrition (Bohn *et al*., 2008; Ragi *et al*., 2022).

The two decades of consistent research on biofortification by various groups resulted in the release of few better zinc biofortified rice varieties through AICRPR biofortification (Sanjeeva Rao *et al*., 2014; Neeraja *et al*., 2018; Kapoor *et al*., 2019; Pradhan *et al*., 2020; Bollinedi *et al*., 2020; Sanjeeva Rao *et al*., 2020; Suman *et al*., 2021; Anusha *et al*., 2021; Uttam *et al*., 2022). Phytic acid is one of the phosphoric acid esters which was initially estimated by descending paper chromatography where phosphorus is released in the presence of acid and reacts with molybdate to form blue colour phosphor-molybdate complex (Hanes and Isherwood, 1949). However, in the presence of acid, paper is degraded that affects the further analysis. Hence, instead of acid molybdate solution, $FeCl_3$. $6H_2O$ in 80% alcohol followed by salicyl sulphonic acid in 80% alcohol were sprayed on the paper and these chemicals have been used as Wade reagent (Wade and Morgan, 1953).

A colorimetric method for the estimation of the phytic acid using Wade reagent (0.03% $FeCl_3$-$6H_20$ and 0.3% sulfosalicylic acid in distilled water) was developed (Latta and Eskin, 1980) without extracting phytate by precipitation as iron phytate or by ion-exchange chromatography (Vaintraub and Lapteva, 1988). This Wade reagent was modified (0.125% $FeCl_3$-$6H_20$ and 1.25% sulfosalicylic acid in distilled water) by adjusting the pH to 3.05 using NaOH (Lorenz *et al*., 2007).

Apart from these, phytic acid was also indirectly derived by estimating iron and multiplying it with a factor of 4.2 in Barley (Dai *et al*., 2007) or estimating inorganic phosphorus and dividing it with a factor of 3.55 in Soyabean (Raboy and Dickinson, 1984). Phytate-P was calculated by multiplying the estimated value of phytate with a conversion factor of 0.282 in Rice (Gyani *et al*., 2021). Eventually, colorimetric / spectrophotometric method is always simpler and can be carried out in many laboratories as spectrophotometer is a common equipment in comparison with sophisticated equipment like HPLC (Liu *et al*., 2007; Lee *et al*., 2014).

Although Wade reagent is popular, the quantity of the biological sample, volume of extract and volume of Wade reagent varied in the above reports. In the case of standard graph, regression values were mentioned without the range of working standard solution. Moreover, the intensity of the colour decreases with the increase in phytic acid concentration in the extract (Latta and Eskin, 1980) and the faint colour formed above the maximum limit of the reagent also fades in few minutes. Our efforts indicate that the identification of maximum limit of Wade reagent (1 mL to 2 mL) plays an important role in the phytic acid estimation. As the range of standard graph is not mentioned in the earlier reports, every researcher has to independently make efforts to identify the concentrations required for the standard graph.

Hence, the objective of this study is to identify the range of phytic acid concentrations to make a standard graph, identify the minimum and maximum detection limits of the Wade reagent to get a stable final colour intensity, to identify a plausible quantity of rice powder and the volume of extract. The results presented here will be useful to the researchers planning to work on phytic acid estimation in rice grain.

## Materials and Methods

### Preparation of reagents

Stock solution of phytic acid standard (5 mg/mL) was prepared using phytic acid dodecasodium salt from corn (Sigma P-8810) in 0.65 *M* HCl. The working standard solution was prepared by adding 25 mL of stock solution to 25 mL of 0.65 *M* HCl. Wade reagent was prepared by taking 0.125% $FeCl_3$. $6H_20$ and 1.25% 5- Sulfosalicylic acid hydrate 95% (Sigma-390275) in distilled water and pH was adjusted to 3.05 using NaOH. This is also called as modified Wade reagent which could be stored in a refrigerator for one month (Lorenz *et al*., 2007).

### Preparation of standard graph

Phytic acid ranging from 50 µg to 1000 µg was prepared by taking series of 10 µL to 200 µL of working standard solution into pre-labelled 2 mL Eppendorf tubes (**Table 1**). The final volume in each tube was made upto 300 µL with 0.65 *M* HCl. 1.5

**Table 1: Development of phytic acid standard graph with Wade reagent.**

| S. No. | Volume of WSS (µL) | Phytate (µg) | Volume of 0.65 M HCl (µL) | Volume of Wade reagent (mL) | Average OD at 490 nm |
|---|---|---|---|---|---|
| 1 | 10 | **50** | 290 | 1.5 | **1.48** |
| 2 | 20 | **100** | 280 | 1.5 | **1.34** |
| 3 | 30 | **150** | 270 | 1.5 | **1.23** |
| 4 | 40 | **200** | 260 | 1.5 | **1.13** |
| 5 | 50 | **250** | 250 | 1.5 | **1.01** |
| 6 | 60 | **300** | 240 | 1.5 | **0.92** |
| 7 | 70 | **350** | 230 | 1.5 | **0.80** |
| 8 | 80 | **400** | 220 | 1.5 | **0.76** |
| 9 | 90 | **450** | 210 | 1.5 | **0.68** |
| 10 | 100 | **500** | 200 | 1.5 | **0.64** |
| 11 | 110 | **550** | 190 | 1.5 | **0.53** |
| 12 | 120 | 600 | 180 | 1.5 | 0.50 |
| 13 | 130 | 650 | 170 | 1.5 | 0.46 |
| 14 | 140 | 700 | 160 | 1.5 | 0.42 |
| 15 | 150 | 750 | 150 | 1.5 | 0.38 |
| 16 | 160 | 800 | 140 | 1.5 | 0.38 |
| 17 | 170 | 850 | 130 | 1.5 | 0.35 |
| 18 | 180 | 900 | 120 | 1.5 | 0.33 |
| 19 | 190 | 950 | 110 | 1.5 | 0.29 |
| 20 | 200 | 1000 | 100 | 1.5 | 0.29 |

*****Bold values indicate the range of phytic acid that can be determined by 1.5 mL of Wade reagent.

mL of Wade reagent was added, contents were made uniform by mixing and all the tubes were incubated at room temperature (37 °C) for 20 minutes. The colour intensity was measured at 490 nm in a UV-Visible spectrophotometer (Biochrom Ultrospec™ 7000) using 0.65 *M* HCl as blank.

The phytic acid concentration was converted to phytate-P by dividing with 3.55 (Lorenz *et al*., 2007) or by multiplying with 0.282 (Gyani *et al*., 2021) following the range of phytic acid-P (1.2 to 1.8 mg/g) which is equal to 4.2 to 6.5 mg/g of phytic acid identified by applying low nutrient P for cultivation in soya bean (Raboy and Dickinson, 1984).

## Results and Discussion

In the past few years screening for phytic acid levels in rice grain gained attention due to its natural capacity (possess negative charge) to bind with the cations and making them unavailable for absorption in the human gut or digestive tract. Hence, identification of low phytic acid lines coupled with higher iron and or zinc contents, yield and the ability to complete all the other aspects of rice life-cycle (seed to seed) like high yielding rice varieties became a priority. As mentioned in the introduction, direct (Wade reagent) colorimetric or spectrophotometric method is preferred. However, the available reports varied with respect to the weight of the biological sample, volume of extract and volume of Wade reagent. Moreover, the range of standard solutions in the preparation of standard graph plays an important role in the final estimated values. Hence, the standard graph of phytic acid with Wade reagent is presented followed by maximum detection limits of the Wade reagent.

In the phytic acid standard graph, regression value of 0.99 was obtained with the known concentrations of phytic acid ranging from 50 µg to 350 µg **(Figure 2)**. The regression value decreased slightly from 0.99

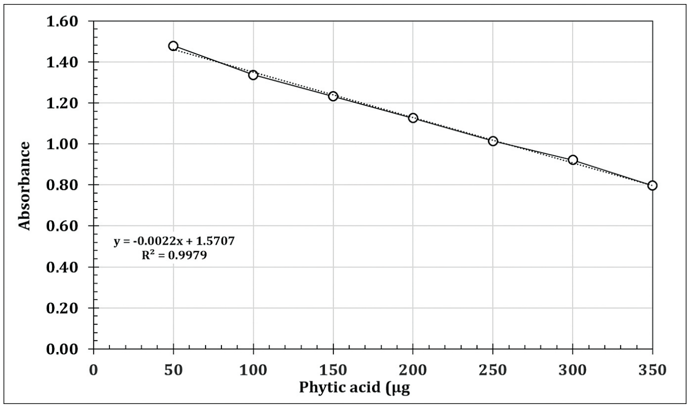

**Figure 2: Standard graph indicating phytate concentration from 50 µg to 350 µg**

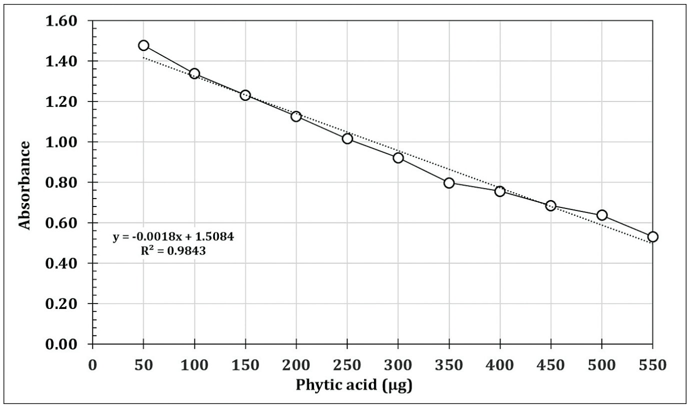

**Figure 3: Standard graph indicating phytate concentration from 50 µg to 550 µg**

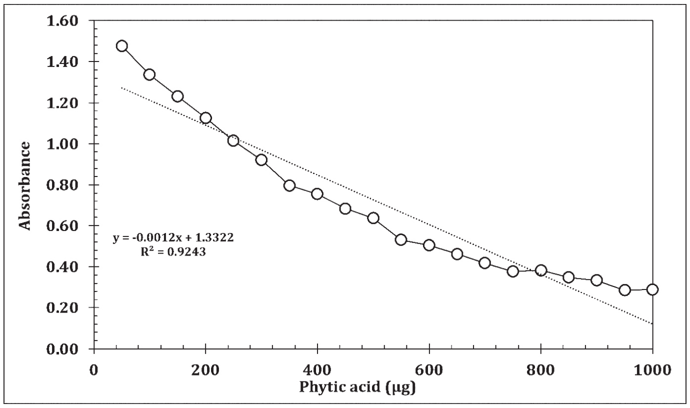

**Figure 4: Standard graph indicating phytate concentration from 50 µg to 1000 µg**

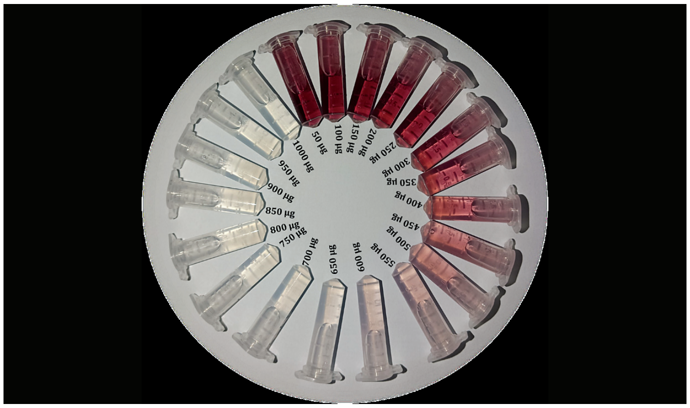

**Figure 5: Change in colour intensity with increase in phytate concentration from 50 µg to 1000 µg**

to 0.98 while including phytic acid concentration upto 550 µg in the standard graph (**Figure 3**). The regression value further decreased to 0.92 while phytic acid concentration upto 1000 µg in standard graph (**Figure 4**). Most importantly, the traces of pink colour also faded completely from 600 µg onwards. As the phytic acid reduces iron ion in Wade reagent the intensity of the pink colour decreased (**Figure 5**) with the increase in phytic acid concentration and eventually, the maximum detectable concentration of phytic acid was approximately 600 µg with 1.5 mL of Wade reagent. This maximum detectable phytic acid can change with the change in the usage of the volume of Wade reagent (Lorenz *et al*., 2007). In some studies, 0.2 mL of Wade reagent for 30 µL biological extract which is equivalent to 2.0 mL of Wade reagent and 300 µL of biological extract (Lorenz *et al.*, 2007), 1.25 mL (Gyani *et al*., 2021) of Wade reagent was used in both the preparation of standard graph as well as the determination of phytic acid in the biological samples. Moreover, it can also change with the change in the composition of ferric chloride concentration in the Wade reagent preparation.

In the case of biological samples, if faint pink colour appears, it indicates that the phytate concentration in the volume of the extract taken almost saturated or consumed or reduced most of the iron ions available in the Wade reagent. Hence, it is better to either decrease the volume of extract or dilute the extract to get dependable values or increase the volume of Wade reagent in both biological sample as well as the standard graph (the volume of the reagent, total volume of the reaction mixture must be same in all the gradations of the standard and biological samples). In our case, in some biological samples, even the faint pink colour that formed after adding the Wade reagent disappeared within 15 minutes of the incubation period.

The proportion of phytate-P in phytate was identified as 28.2% while cultivating soya bean at low nutrient P in pots (Raboy and Dickinson, 1984). This difference was converted into factor 3.55 to estimate phytic acid-P in maize (Lorenz *et al*., 2007; Nadeem *et al*., 2011; Abhijith *et al*., 2020), rice (Liu *et al*., 2004), Siberian oil seed (Russo and Reggiani, 2013; Colombini *et al*., 2014), in hemp seed (Russo and Reggiani, 2013), Marijuana (Galasso *et al*., 2016), Wheat (Safar-Noori *et al*., 2018) and Soya bean (Taliman *et al*., 2019). However, unlike maize where this factor 3.55 was determined, the factor is being applied in other crops too. Hence, although the global soils are deficient in phosphorus, it is also important to conduct studies on the relationship between phosphorous application and its uptake in other crops to know the applicability of this factor.

## Conclusion

The standard graph range for phytic acid from 50 to 550 µg can be used along with 1.5 mL of Wade reagent with a total reaction volume of 1.8 mL. If the colour of reaction mixture is unstable then the biological extract can be diluted or the initial quantity of the sample (weight) can be decreased to get a stable colour and dependable values. Further, the response of rice crop with graded levels of phosphate nutrition can be studied to decide on applicability of the factor 3.55 identified for soya bean.

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RESEARCH ARTICLE https://doi.org/10.58297/QPXE9763

# Characterization of Engineered Zinc Oxide (ZnO) Nanoparticles Using Different Techniques

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## Abstract

The characterization of chemically fabricated zinc oxide (ZnO) nanoparticles, involved the utilization of diverse techniques for characterization. The utilization of X-ray diffraction (XRD), *scanning electron microscope* (SEM), Transmission Electron Microscopy (TEM) and *Fourier-transform infrared spectroscopy (FTIR*) (FTIR) in the assessment of zinc nanoparticles unveiled their crystalline structure and high purity. Results exhibited that ZnO nanoparticles had a flattened spherical morphology with a textured surface, demonstrating an average size spanning from 80 to 130 nm and well-organized with minimal aggregation was observed using SEM imaging. The TEM analysis corroborated the particle size determined through XRD, indicating a dimension of around 65 nm. Notably, FTIR analysis unveiled discernible peaks, with a specific narrow band observed at 3450 $cm^{-1}$, confirming the presence of Zn-O bonds. These findings indicate that engineered ZnO particles were confined to the nano range, which has potential application in agricultural fields as nutrient carriers.

**Keywords:** ZnO nanoparticles, Electron Microscopy, X-Ray Diffraction

## Introduction

Nanotechnology, at the forefront of science and technology, arises from the convergence of various fields of study, leading to a domain of unprecedented advancement. In the agriculture and fertilizer sector, the use of nanotechnology-enabled nano nutrients has emerged as the main key player in different avenues of this industry *i.e.,* nano-fertilizers (enhancing nutrient use efficiency), new-generation pesticides and reclamation of salt-affected soils (DeRosa *et al*., 2010). Particles with one or more dimensions in the ≤ 100 nm or less range are referred to as nanoparticles (Huber, 2005). Nanoparticles possess unique properties such as small size and high specific surface area, which enhance their reactivity (Liscano *et al*., 2000; Milani *et al*., 2015) and due to this, these nanoparticles may act as ideal materials for efficient fertilizer use (Joseph and Morrison, 2006). Zinc oxide, in particular, is considered a promising and practical approach to environmental protection and the second most abundant metal oxide after iron, is cost-effective, safe, and easily prepared (Kalpana *et al*., 2018). The ZnO isomer is called a wurtzite-type and it is FDA-approved and can solubilize in acidic environments, making them promising nanocarriers for drug delivery (Gobinath *et al*., 2021; Lakshmipriya and Gopinath, 2021). ZnO nanoparticles were successfully synthesized using various methods, including the sol-gel approach, precipitation method, green leaf extract method, microwave method, wet chemical method, and hydrothermal method. Nano-sized zinc oxide was synthesized using various precursors and alkalis like acetate-based zinc salts with NaOH and

Oxalic acid as reactants, respectively (Rajendran *et al*., 2022). The prepared materials were studied by measuring the size, shape, and morphology under X-ray diffraction, Scanning electron microscopy, and Transmission electron microscopy. Crystalline oxide powders offer chances for acquiring better chemical, mechanical, optical, or electrical properties when mixed with other materials (Gobinath, R *et al*., 2015; Manjunatha *et al*., 2019). In the present investigation, we have characterized the ZnO nanoparticles for their size, shape, and composition using various techniques for their use in agricultural fields. We have employed different techniques namely, X-ray Diffraction, Scanning electron microscopy, Transmission electron microscopy and Fourier transmission infra-red (FTIR) for confirming the characteristics of engineered ZnO nanoparticles.

## Materials and Methods

### Characterization of ZnO nanoparticles

The Zinc Oxide nanoparticles (analytically pure) were purchased from Sigma-Aldrich (Germany) and characterized using techniques like XRD, SEM, TEM, and FTIR for their size, shape and crystallinity.

### X-ray diffraction (XRD)

X-ray diffraction, also known as XRD, is a scientific technique used to analyze the structure of materials. The crystallite and purity of ZnO nanoparticles are identified and determined by the XRD pattern by using the X-ray diffraction technique using Philips PW 1710 X-ray diffractometer. For this purpose, automated powder diffraction (APD) software with Cu kα radiation (λ=1.5418 Å) source was used. An operating system with a power of 15 kV and Kβ filter was used as a background filter. A powdered nanoparticle is placed in a sample holder and the sample was scanned at a diffraction angle of 2θ from 3 to 50°. The average particle diameter D of the nanoparticle is determined with the help of main peaks obtained from the XRD image; using Debye-Scherer's formula as follows:

$$\mathbf{D = k\lambda/(\beta \cos\theta)}$$

Here λ is the x-ray wavelength (Cu kα = 1.5418 Å)

*k* is the machine constant (0.916)

*β* is the full width at half maximum (FHWM) of the peak and θ is the peak position.

### Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) is used to reveal information about the sample including external morphology (texture), composition, and structure. Morphology and surface modification of synthesized nanoparticles were determined by EVO/MA10 scanning electron microscopy (SEM) of CARL Zeiss instrument equipment. ZnO nanopowder is trapped on double-sided sticky carbon tape covered copper stab and coated with a palladium layer in a vacuum of 10-3 torr for a minimum of 6 hours to fixation.

### Transmission electron microscopy (TEM)

Powdered nanoparticles were made into suspension placed on the grid, dried under a vacuum, and then placed on the grid plate to reduce the settlement of the nanoparticles. Suspension mounted grid was kept under an infra-red lamp for one hour for fixation and then analyzed under Joel JEM 1010, USA. Images were captured by Olympus digital camera and analyzed with the help of installed software.

### Fourier transmission infrared spectroscopy (FTIR)

Functional groups present in the compounds were analyzed by Fourier transmission infrared spectrometer. The analysis is carried out using the principle of vibrational a stretching motion of atoms or molecules. These compounds absorb electromagnetic energy in the infrared region of the spectrum. The position of a particular absorption band is specified by a particular wave number. The powdered sample is analyzed in the Brucker: ALPHA, FTIR, ATR system (Typically 24 scans, Resolution- 4cm$^{-1}$) with scanning between 4000-400 cm$^{-1}$.

## Results and Discussion

### X-ray diffraction (XRD)

The crystallite size and purity of the synthesized ZnO nanoparticle were determined by X-ray diffraction (XRD), where the X-ray diffraction patterns were as follows; diffraction angles (2θ) of 31, 34 and 35, and the peak list was given in **(Table 1)**. These peaks corresponded to the crystal planes of the ZnO structure, specifically the 100, 002 and 101 planes (**Figure 1**), with no impurity peaks. The Debye-Sherrer equation was used to calculate the particle size using with diffraction angle of the intense peak. Additionally, these peaks align with the standard wurtzite structure observed in the powder diffraction standard image of JCPDS card no. 36-1451 (Morkoc and Ozgur, 2008; Gobinath *et al.*, 2021). A similar result was recorded by Alwan *et al.*, (2015) in which ZnO nanoparticles produced using the sol-gel method exhibited platy structure orientation and an intense peak was observed at 58.3 nm from the width of dominant peaks (100) and (101) reflections according to the Debye - Scherrer equation. Furthermore, utilizing the Debye-Sherrer equation, the average size of the ZnO nanoparticles was determined to be 45-60 nm.

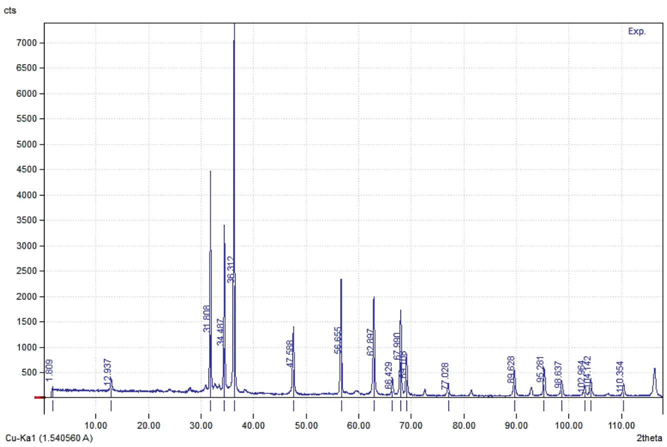


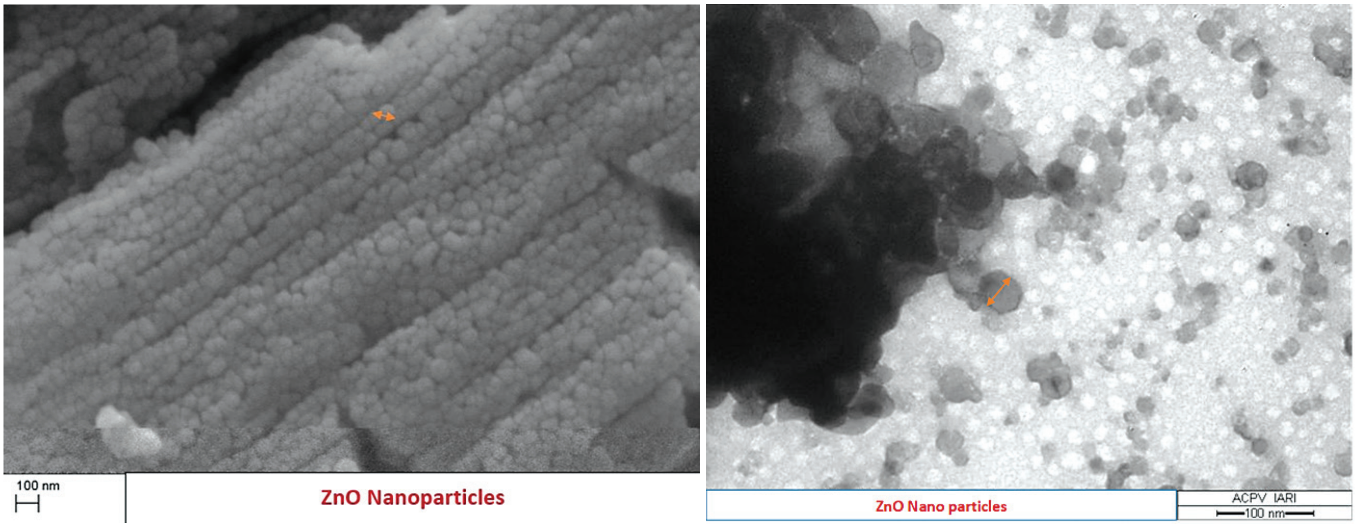
**Figure 1: X-Ray Diffraction pattern**

**Table 1: Phase analysis report on XRD peaks-ZnO nanoparticles**

| No. | 2θ [°] | D [Å] | I/10 (Peak height) | Counts (Peak area) | Full Width Half Maxima (FHWM) |
|---|---|---|---|---|---|
| 1 | 1.81 | 48.8085 | 21.45 | 221.43 | 1.0641 |
| 2 | 12.94 | 6.8375 | 37.50 | 181.81 | 0.4998 |
| 3 | 31.81 | 2.8110 | 604.39 | 1242.62 | 0.2120 |
| 4 | 34.49 | 2.5985 | 447.81 | 975.63 | 0.2246 |
| 5 | 36.31 | 2.4720 | 1000.00 | 2199.12 | 0.2267 |
| 6 | 47.59 | 1.9092 | 183.60 | 546.32 | 0.3068 |
| 7 | 56.66 | 1.6233 | 318.54 | 812.23 | 0.2629 |
| 8 | 62.90 | 1.4764 | 264.66 | 786.35 | 0.3063 |
| 9 | 66.43 | 1.4062 | 45.97 | 130.37 | 0.2924 |
| 10 | 67.99 | 1.3777 | 235.66 | 689.05 | 0.3015 |
| 11 | 69.11 | 1.3581 | 110.10 | 331.27 | 0.3102 |
| 12 | 77.03 | 1.2370 | 30.08 | 105.49 | 0.3616 |
| 13 | 89.63 | 1.0929 | 68.27 | 242.89 | 0.3668 |
| 14 | 95.28 | 1.0424 | 72.88 | 260.01 | 0.3678 |
| 15 | 98.64 | 1.0157 | 39.49 | 141.30 | 0.3689 |
| 16 | 102.96 | 0.9845 | 23.72 | 108.37 | 0.4710 |
| 17 | 104.14 | 0.9766 | 45.28 | 180.79 | 0.4116 |
| 18 | 110.35 | 0.9383 | 31.64 | 111.67 | 0.3638 |

## Scanning electron microscopy (SEM)

The scanning electron micrographs were utilized to analyze the morphology and size of the nanoparticles, revealing distinct characteristics of the ZnO nanoparticles. Notably, ZnO nanoparticles showcased an average size falling within the range of 60-130 nm (**Figure 2**). Further examination revealed that the ZnO nanoparticles possessed a well-ordered structure with moderate to less aggregation induced by physical forces. The results indicated that these nanoparticles were in irregular shapes and sizes due to moderate aggregation. According to Alwan *et al*., (2015) and Manjunatha *et al*., (2019) in which ZnO nanoparticles produced using a sol-gel method and hydrothermal process were spherical and changed to flower-like arrangement.



**(a)** **(b)**

**Figure 2: Image of a) Scanning electron microscope and b) Transmission electron microscope (TEM) image**

## Transmission electron microscope (TEM)

The transmission electron microscopy images of the ZnO nanoparticles provided a detailed depiction and the size is determined as 45 nm. These TEM images provided conclusive evidence regarding the dimensions and shape of zinc nanoparticles (**Figure 2**). The ZnO nanoparticle exhibited a crystalline nature, with an average particle size of approximately 90 nm. The particles displayed a smooth and spherical morphology, which aligns with the previous findings by Dakhlaoui *et al*., (2009). This observation indicates that the particle morphology is predominantly spherical and uniform, though it is partly aggregated due to strong physical forces between individual particles. A similar result was found by Rajakumar *et al*., (2018) in which ZnO nanoparticles produced were spherical and hexagonal geometries.

## Fourier-transmission infrared spectrophotometer (FTIR)

The chemically synthesized zinc oxide (ZnO) nanoparticle underwent Fourier-transform infrared (FTIR) analysis, and the resulting spectral band was presented in **(Figure 3)**. The spectral image of the ZnO nanoparticle featuring distinct peaks at the following wavelengths 486, 1626, and 3450 cm$^{-1}$, respectively. The FTIR spectrogram revealed a stretching peak at 486 cm$^{-1}$ (Zn-O bonds) and other vibrational stretches was observed at 2456 (O-H bond) and 3450 cm$^{-1}$ (C=O bond) in small amounts, likely originating from atmospheric moisture and carbon dioxide ($CO_2$) absorption (Gobinath *et al*., 2021; Hasindawani *et al*., 2016; Xiong *et al*., 2006; Parthasarathi and Thilagavathi, 2011). Based on the results, it can be concluded that the precursor used in the synthesis did not significantly alter the functional composition of the ZnO nanoparticles. A similar result was observed by Kumar and Rani (2013) that the FT-IR spectra confirmed the existence of Zn-O bonding and the adsorption of surfactant molecules onto the surface of ZnO nanoparticles.

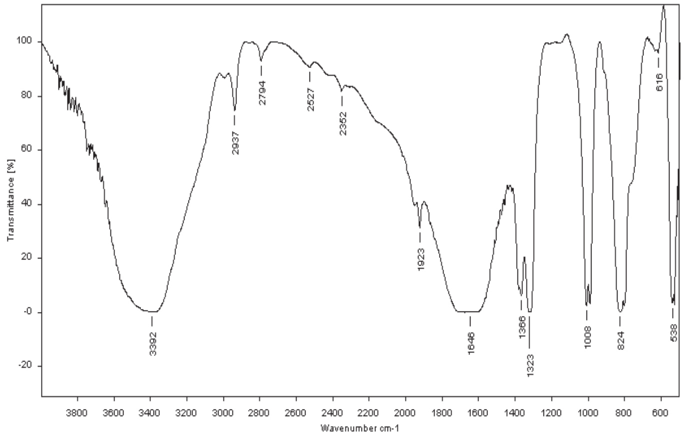


**Figure 3: Fourier Transmission Infra-red spectroscopy (FTIR) image**

## Conclusion

In the present study, XRD analysis revealed the phase purity of engineered ZnO nanoparticles and crystalline in nature. The SEM and TEM analysis revealed a flattened spherical nanoparticle with a rough surface texture. The FTIR pattern analysis exhibited distinct peaks, stretching with the narrow band confirming the presence of Zn-O bonds. However, further investigation like the use of BET and DLS techniques is required for a better delineation and understanding of the properties of ZnO particles. Based on the results, these ZnO nanoparticles can be used in a variety of fields like agriculture as nutrient supplements, solar cells, antifungal, antibacterial material and targeted delivery of nutrients in biomedical fields.

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**RESEARCH ARTICLE** https://doi.org/10.58297/SAKQ9946

# Efficacy of Fungicides on Rice Sheath Blight and Grain Discolouration Diseases in Rice

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## Abstract

Field experiments were conducted at Rice Research Station, Kerala Agricultural University, Moncompu during *kharif* 2020 & 2021 and *rabi* 2021-22 to evaluate the fungicides against sheath blight and grain discolouration. The evaluated seven fungicides were Difenoconazole 25 EC, Isoprothiolane 40 EC, Kasugamycin 3 SL, Iprobenfos 48 EC, Propineb 70 WP, Tebuconazole 25.9 EC and Thifluzamide 24 SC. The pooled analysis of three seasons data showed that Difenoconazole 25 EC @ 0.5 ml/l and Tebuconazole 25.9 EC @ 1.5 ml/l were found equally effective against the sheath blight and for grain discolouration. Highest yield (5779 kg ha$^{-1}$) was recorded by the Difenoconazole 25 EC followed by Kasugamycin 3 SL (5745 kg ha$^{-1}$), Isoprothiolane 40 EC (5514 kg ha$^{-1}$) and Thifluzamide 24 SC (5502 kg ha$^{-1}$) as against (4141 kg ha$^{-1}$) in control.

**Key words:** Rice, sheath blight, grain discolouration, fungicide.

## Introduction

In India rice (*Oryza sativa L.*) is the most important food crop occupying about more than 45 million ha. It serves as a staple food crop of more than 60 per cent of the world's population. To increase the rice production, many high yielding varieties of rice have been developed. Occurrence of diseases has completely changed with the introduction of high yielding varieties. Rice sheath blight disease caused by *Rhizoctonia solani* AG1-1A, is one of the most devasting diseases of the crop. 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 (Sathya *et al*., 2020). Before follow up the effective crop protection against sheath blight, it is important to review the published information related to pathogenicity and disease management. Research related to disease management practices has addressed the use of agronomic practices, chemical control, biological control and genetic improvement: Optimizing nitrogen fertilizer use with enough plant spacing can reduce spread of infection while smart agriculture technologies such as crop monitoring with Unmanned Aerial Systems assist in early detection and management of sheath blight disease (Pooja Singh *et al*., 2019). Fungicidal sprays have been used successfully to control the sheath blight which is the most effective for inhibiting infection lesion enlargement. Timely application of effective fungicides is essential for the better management of the disease. Systematic evaluation of commercially available fungicides from time to time is needed for evolving recommendations on chemical fungicides, so that the farmers can choose the fungicides based on the efficacy as well as cost (Ganesha Naik *et al*., 2017).

Grain discolouration is caused by complex of fungal species such as *Sarocladium oryzae, Bipolaris oryzae (Cochliobolus miyabeanus), Pyricularia grisea (Magnaporthe grisea) Curvularia lunata, Phoma sp.,* *Microdochium sp., Nigrospora sp., and Fusarium sp.* It is an important constraint for lowland and upland rice production and becoming serious concern under changing climatic conditions. Of late the disease was found to be very severe in all over the Kerala causing 5 to 10 per cent yield loss (Surendran *et al*., 2016). Use of suitable fungicide is the primary one for the effective management of the rice diseases. The present study, considering the severity of diseases and its economic importance, the field experiments were conducted using different fungicides available in the market for the control of sheath blight and grain discolouration of rice under field conditions.

## Materials and Methods

During *kharif* 2020&2021 and *Rabi* 2021-22, field experiments were conducted at Rice Research Station, Moncompu, Alappuzha under ICAR-AICRIP programme for evaluating the fungicides against location specific rice diseases *viz.,* sheath blight and grain discolouration. The trial was conducted as a part of AICRPR program. Seven commercially available fungicides were tested against sheath blight and grain discolouration. The experiments were laid out in randomized block design with 4 replications in 5x2 $m^2$ plots using the locally popular susceptible variety Uma (MO 16). The NPK fertilizers were applied as per the recommendations (90:45:45 kg $ha^{-1}$) of Kerala Agricultural University. The fungicides were applied as foliar spray at the time of booting stage for both diseases. Three sampling units of 1 $m^2$ area were fixed in each plot at random. The observations on sheath blight disease severity were recorded just before the spray and 15-20 days after the spray. Degree of severity was graded based on height of the plant portions affected by the disease and expressed as percentage of the total area as per the SES scale of rice (IRRI, 2013). Grain discolouration was measured based on the percentage of panicles and spikelets infection from 15 days before harvest. The panicle infection- percentage was calculated based on the number of panicles affected from the total number of panicles present in the sampling area. The spikelet infection percentage was recorded by counting the infected grains from each panicle.

## Results and Discussion

The results of station trial at Rice Research Station, Moncompu during *kharif* 2020 **(Table 1)** revealed that the plots treated with fungicide Thifluzamide 24

**Table 1: Influence of different fungicides on sheath blight disease severity (%) during *kharif* 2020, *kharif* 2021 and *rabi* 2021-22 (Pooled data of three seasons)**

| Sl. No. | Fungicides | Dose/ Lit | Disease Severity (%) | | | Mean |
|---|---|---|---|---|---|---|
| | | | ***Kharif* 2020** | ***Kharif* 2021** | ***Rabi* 2021-22** | |
| 1 | Difenconzole 25 EC | 0.5 ml | 25.27 (28.95) | 11.44 (19.46) | 15.34 (7.00) | 24.33(17.35) |
| 2 | Isoprothiolane 40 EC | 1.5 g | 23.05 (27.92) | 26.67 (30.95) | 19.73 (11.4) | 28.71(23.15) |
| 3 | Kasugamycin 3 SL | 2.0 g | 20.83 (26.18) | 27.95 (31.89) | 33.84 (31) | 31.54(27.54) |
| 4 | Iprobenfos 48 EC | 1.0 ml | 22.22 (27.58) | 20.93 (26.35) | 28.84 (23.3) | 29.27(24.00) |
| 5 | Propineb 70 WP | 3.0 g | 24.16 (29.20) | 25.93 (29.82) | 29.51 (24.3) | 30.98(26.53) |
| 6 | Tebuconazole 25.9 EC | 1.5 ml | 17.77 (24.88) | 25.84 (30.31) | 25.76 (18.9) | 28.66(23.12) |
| 7 | Thifluzamide 24 SC | 0.8 g | 13.61 (20.63) | 28.64 (32.34) | 31.23 (26) | 29.32(24.49) |
| 8 | Control | - | 60.55 (51.23) | 54.25 (47.50) | 49.97 (58.6) | 47.83(54.92) |
| | LSD @ 5% (P=0.05) | | 12.062 | 6.175 | 10.394 | |
| | CV (%) | | 27.700 | 13.500 | 24.128 | |

*Figures given in parentheses are arcsine transformed values

SC recorded lower sheath blight severity (13.61%) during *kharif* 2020. This was followed by molecule Tebuconazole 25.9 EC (17.77%) and Kasugamycin 3 SL (20.83%). During *kharif* 2021, the systemic fungicide Difenoconazole 25 EC (11.44%) was found superior in restricting sheath blight disease severity followed by Iprobenfos 48 EC (20.93%) and Tebuconazole 25.9 EC (25.84%). In the season *rabi* 2021-22, also the systemic fungicide Difenoconazole 25 EC (15.34%) was found superior in restricting sheath blight disease severity followed by Isoprothiolane 40EC (19.73%) and Tebuconazole 25.9EC (25.76%).

The pooled data of station trial results showed that the Difenoconazole 25 EC gave the maximum reduction in sheath blight disease severity (24.33%) followed by Tebuconazole 25.9 EC (28.66%), Isoprothiolane 40 EC (28.71%), Iprobenfos 48 EC (29.27%) and Thifluzamide 24 SC (29.32%) (**Table 1 and Figure 1**).

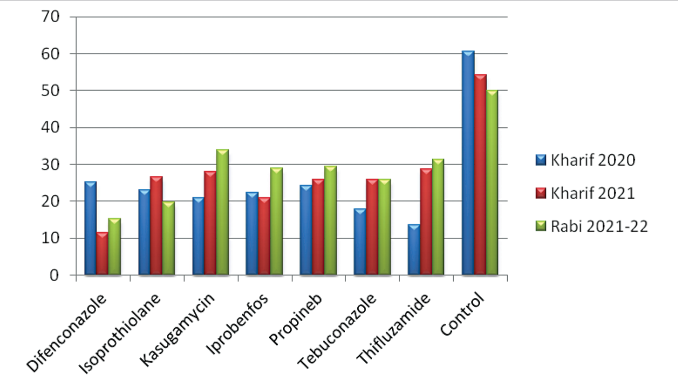

**Figure 1: Effectiveness of different fungicides on sheath blight disease severity (%)**

Neem essential oil (16.32%) showed maximum reduction in sheath blight incidence and severity when compared to lemon grass oil (17.85%) and standard check fungicide Carbendazim (18.91%) (Surendran *et al*., 2021). Triazole fungicides are also commonly used in sheath blight management. Application of other chemicals such as Flutolanil, Carbendazim, Iprobenfos, Mancozeb, Thifluzamide and Validamycin also offers effective control of this disease. The use of a single chemical with the same mode of application for a prolonged time leads to the evolution of resistance in the fungus (Uppala and Zhou, 2018). Hence, a combination chemical formulation such as Azoxystrobin 18.2% + Difenoconazole 11.4% (Bhuvaneswari and Raju, 2012; Kumar *et al.*, 2018); Propiconazole + Difenoconazole (Kandhari, 2007); Prothioconazole + Tebuconazole 240 g/kg SC (Chen *et al.*, 2021). Captan 70% + Hexaconazole 5% (Pramesh *et al*., 2017); Trifloxystrobin 25% + Tebuconazole 50% (Shahid *et al.,* 2014; Rashid *et al.,* 2020). The systemic fungicides Trifloxystrobin 25% + Tebuconazole 50 WG @ 0.4g/lit and Propiconazole 25% EC @ 1ml/lit were found to be the most effective against neck blast disease with great reduction in the per cent disease intensity and getting higher grain yield (Yadav *et al*., 2022). Surendran *et al*., (2019) reported that application of Trifloxystrobin 25% + Tebuconazole 50% WG was effectively controlled the sheath blight disease.

### Grain Discolouration

The data on grain discoloration panicles and spikelets infection indicated that fungicide Isoprothiolane 40 EC reduced disease effectively (2.90 and 7.50%) when compared with fungicides *viz.,* Difenoconazole 25 EC (4.46 and 8.39%), Tebuconazole 25.9 EC (5.25 and 10.30%) and Thifluzamide 24 SC (6.26 and 10.38%) during *kharif* 2020. During *kharif* 2021, Difenoconazole 25 EC @ 0.5ml/l was found to be effective to check the grain discoloration (4.65 and 11.35%) followed by Propineb (5.93 and 11.32%), Tebuconazole (5.93 and 12.42%) and Thifluzamide 24 SC (6.40 and 11.96%). Out of seven commercially available fungicides tested, the fungicides Tebuconazole 25.9 EC (3.06 and 1.98%), and Isoprothiolane 40 EC (3.35 and 1.95%), were found superior against the grain discolouration followed by Kasugamycin 3 SL (3.37 and 2.04%) and Thifluzamide 24 SC ( 3.51 and 1.97%) during *rabi* 2021-22.

**Table 2: Influence of different fungicides on glume discoloration panicles (%) during *kharif* 2020, *kharif* 2021 and *rabi* 2021-22 (Pooled data of three seasons)**

| Sl. No. | Fungicides | Dose/ lit | Panicles affected (%) | | | |
|---|---|---|---|---|---|---|
| | | | ***Kharif* 2020** | ***Kharif* 2021** | ***Rabi* 2021-22** | **Mean** |
| 1 | Difenconzole 25 EC | 0.5 ml | 4.46 | 4.65 | 4.4 | 2.12 |
| 2 | Isoprothiolane 40 EC | 1.5 g | 2.9 | 7.19 | 3.35 | 2.07 |
| 3 | Kasugamycin 3 SL | 2 g | 6.8 | 15.9 | 3.37 | 2.81 |
| 4 | Iprobenfos 48 EC | 1 g | 8.03 | 7.64 | 3.87 | 2.52 |
| 5 | Propineb 70 WP | 3 g | 6.45 | 5.93 | 5.36 | 2.43 |
| 6 | Tebuconazole 25.9 EC | 1.5 ml | 5.25 | 5.93 | 3.06 | 2.15 |
| 7 | Thifluzamide 24 SC | 0.8 g | 6.26 | 6.4 | 3.51 | 2.30 |
| 8 | Control | - | 10.18 | 20.34 | 6.78 | 3.43 |
| | LSD @ 5% (P=0.05) | | 0.067 | 0.044 | 0.014 | |
| | CV (%) | | 1.866 | 1.020 | 0.454 | |

*Figures given in parentheses are square root transformed values

The pooled data of three season station trials showed that the fungicides Isoprothiolane 40 EC (2.07%) and Difenoconazole 25 EC (2.12%) were found most effective in restricting discoloured grain panicle incidence followed by Tebuconazole 25.9 EC (2.15%) and Thifluzamide 24 SC (2.30%). The analysis of pooled data on panicle percentage affected showed that both Difenoconazole 25 EC and Isoprothiolane 40 EC were found equally effective than other fungicides **(Table 2 and Figure 2).**

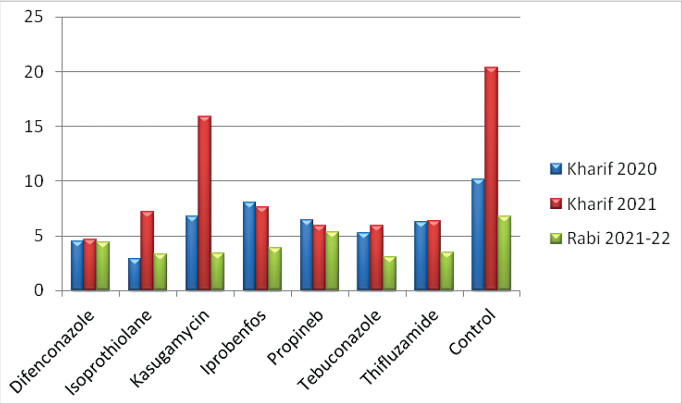

**Figure 2: Effectiveness of fungicides on grain discoloration diseases (panicles %)**

**Table 3: Influence of different fungicides on glume discolouration spikelets (%) during *kharif* 2020, *kharif* 2021 and *rabi* 2021-22 (Pooled data of three seasons)**

| Sl. No. | Fungicides | Dose/ lit | Spikelets affected (%) | | | |
|---|---|---|---|---|---|---|
| | | | ***Kharif* 2020** | ***Kharif* 2021** | ***Rabi* 2021-22** | **Mean** |
| 1 | Difenconzole 25EC | 0.5ml | 8.39 | 11.35 | 1.55 | 2.50 |
| 2 | Isoprothiolane 40 EC | 1.5g | 7.5 | 8.75 | 1.95 | 2.36 |
| 3 | Kasugamycin 3 SL | 2 g | 8.35 | 10.92 | 2.04 | 2.54 |
| 4 | Iprobenfos 48 EC | 1 g | 9.22 | 9.83 | 2.05 | 2.53 |
| 5 | Propineb 70 WP | 3 g | 8.01 | 11.32 | 2.05 | 2.54 |
| 6 | Tebuconazole 25.EC | 1.5ml | 10.3 | 12.42 | 1.98 | 2.71 |
| 7 | Thifluzamide 24 SC | 0.8 g | 10.38 | 10.26 | 1.97 | 2.69 |
| 8 | Control | - | 12.61 | 13.69 | 2.26 | 2.91 |
| | LSD @ 5% (P=0.05) | | 0.012 | 0.004 | 0.084 | |
| | CV (%) | | 0.271 | 0.085 | 4.018 | |

*Figures given in parentheses are square root transformed values

**Table 4: Influence of different fungicides on grain yield (kg ha$^{-1}$) during *kharif* 2020, *kharif* 2021 and *rabi* 2021-22 (Pooled data of three seasons)**

| Sl. No. | Fungicides | Dose/ lit | Grain yield | | | |
|---|---|---|---|---|---|---|
| | | | ***Kharif* 2020** | ***Kharif* 2021** | ***Rabi* 2021-22** | **Mean** |
| 1 | Difenconzole 25EC | 0.5ml | 3625 | 6149 | 7565 | 5779 |
| 2 | Isoprothilane 40 EC | 1.5g | 3750 | 6708 | 6085 | 5514 |
| 3 | Kasugamycin 3 SL | 2 g | 4000 | 6418 | 6818 | 5745 |
| 4 | Iprobenfos 48 EC | 1 g | 3500 | 6300 | 5798 | 5199 |
| 5 | Propineb 70 WP | 3 g | 3625 | 6160 | 5885 | 5223 |
| 6 | Tebuconazole 25.9 EC | 1.5ml | 3375 | 6172 | 6895 | 5480 |
| 7 | Thifluzamide 24 SC | 0.8 g | 3625 | 6181 | 6700 | 5502 |
| 8 | Control | - | 3125 | 3813 | 5485 | 4141 |
| | LSD @ 5% (P=0.05) | | NS | 1291.517 | NS | |
| | CV (%) | | 20.210 | 14.785 | 16.990 | |

The pooled data of three season station trials showed that both systemic fungicides *viz.,* Isoprothiolane 40 EC (2.36%) and Difenoconazole 25 EC (2.50%) were very effective in restricting discoloured grain spikelet incidence followed by Iprobenfos 48 EC (2.53%) and Kasugamycin 3 SL (2.54%).

The data on panicles and spikelet affected indicated that fungicides Difenoconazole 25 EC and Isoprothiolane 40 EC were significantly superior to all other fungicides tried **((Table 3 and Figure 3)**. Several workers have reported on the scope for controlling grain discolouration disease by application of fungicides like Edifenphos and Copper oxychloride (Govindarajan and Kannaiyan, 1982), Propiconazole (Lore *et al.*, 2007) and Captan 70% + Hexaconazole 5% (Kumar and Kumar, 2011).

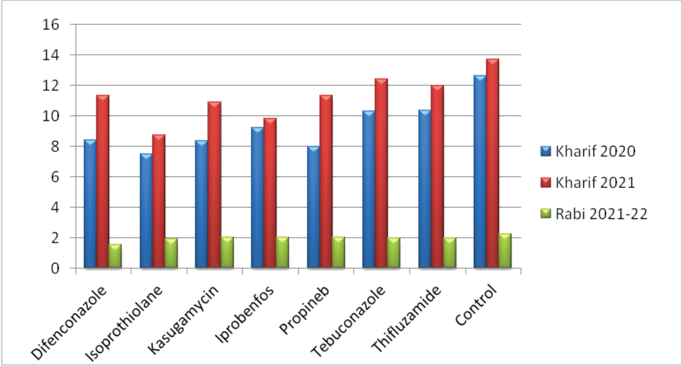

**Figure 3: Effectiveness of fungicides on grain discolouration diseases (spikelets %)**

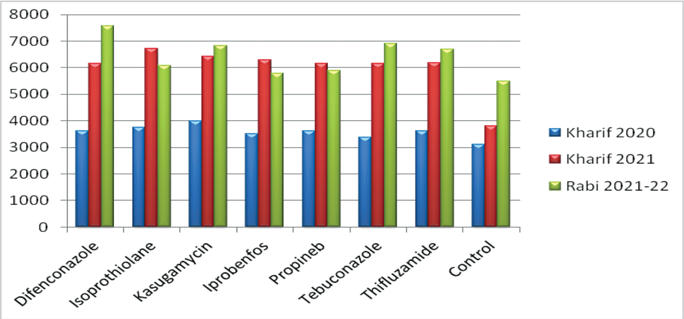

**Figure 4: Effectiveness of different fungicides on grain yield (ton/ha)**

Grain yield of each plot was recorded and expressed in kg ha$^{-1}$ at 14 per cent moisture. Significance among mean treatments was determined according to Duncan's multiple range tests (Gomez and Gomez, 1984). The maximum yield was obtained from Kasugamycin 3 SL (4000 kg ha$^{-1}$) followed by Isoprothiolane 40 EC (3750 kg ha$^{-1}$) and Thifluzamide 24 SC (3625 kg ha$^{-1}$). The control plot recorded with lowest yield of 3125 kg ha$^{-1}$ during *kharif* 2020. There was significant difference in the grain yield among the treatments in *kharif* 2021. The maximum yield was obtained from Isoprothiolane 40 EC treated plot (6708 kg ha$^{-1}$) followed by Kasugamycin 3 SL (6418 kg ha$^{-1}$) and Iprobenfos 48 EC 6300 kg ha$^{-1}$. During *Rabi* 2021-22, the highest yield was obtained from Difenoconazole 25 EC (7565 kg ha$^{-1}$) treated plot followed by Kasugamycin 3 SL (6818 kg ha$^{-1}$). The control plot recorded with lowest yield of 5485 kg ha$^{-1}$ **(Table 4 and Figure 4)**.

The pooled data of three season station trials showed that Difenoconazole 25 EC treated plot yields high (5779 kg ha$^{-1}$) followed by Kasugamycin 3 SL (5745 kg ha$^{-1}$), Isoprothiolane (5514 kg ha$^{-1}$), Thifluzamide 24 SC (5502 kg ha$^{-1}$) and Iprobenfos 48 EC (5199 kg ha$^{-1}$).

## Conclusion

It is concluded that systemic fungicides Difenoconazole 25 EC and Isoprothiolane 40 EC was found most effective against the sheath blight and grain discolouration. Thus fungicides, Difenoconazole 25 EC @ 0.5 ml/l and Isoprothiolane 40 EC @ 1.5 g/l can be recommended for the management of sheath blight and grain discoloration and improve the quality of seeds in Kuttanad region.

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RESEARCH ARTICLE https://doi.org/10.58297/NYAI9557

# Studies on Compatibility of Insecticides and Fungicides against Brown Plant Hopper and Blast in Rice

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## Abstract

Field experiments conducted for evaluation of compatibility of insecticides and fungicides against brown planthopper and blast in rice revealed that pymetrozine in combination with tricyclazole and isoprothiolane and triflumezopyrim in combination with tricyclazole and isoprothiolane were found effective against brown plant hopper and blast and recorded higher grain yields (4872 kg/ha, 4873 kg/ha, 5014 kg/ha and 5088 kg/ha) compared to untreated control (3036 kg/ha). Neither insecticides nor fungicides lost their efficacy against target pest/disease when used as tank mixtures. Insecticides, pymetrozine and triflumezopyrim are compatible with tricyclazole and isoprothiolane fungicides with nil phytotoxicity and can be safely used as tank mixtures for simultaneous management of BPH and blast in rice.

**Key words:** Brown Plant Hopper, Blast, Insecticides, Fungicides, Compatibility, Rice.

## Introduction

Rice (*Oryza sativa* L.) is an important staple food crop for more than half of the world population and accounts for more than 50 per cent of the daily calorie intake (Khush, 2005). Rice is prone to attack by several insect pests and diseases irrespective of its method of cultivation. Among the insect pests, Brown Plant Hopper (BPH), *Nilaparvata lugens* (Stal.) are considered as the major yield limiting biological constraints in all rice growing countries both in tropics and temperate regions (Krishnaiah, 2014). Both nymphs and adults of the BPH suck plant sap from phloem cells cause “hopper burn” symptoms, resulting in 10% yield loss in general, and losses exceed even up to 90% in case of severity (Seni and Naik, 2017). Rice blast caused by *Pyricularia oryzae* is the most destructive disease of rice worldwide causing significant yield losses (Kunova *et al*., 2013) and in tropical region, especially in India the disease is a serious threat to rice crop (Sireesha, 2013).

Though the incidence of BPH and blast are noticed throughout the crop growth stages, their simultaneous occurrence after primordial initiation, especially in *rabi* season necessitates application of recommended insecticides and fungicides at a time. Labour shortage coupled with increased spraying costs force the farmers to apply insecticides and fungicides as tank mixtures without any first-hand information on their compatibility, which often results improper pest control besides pest resistance and resurgence. Information on compatibility of newly recommended fungicides and insecticides as tank mix application in rice is limited. Such information is vital to achieve effective control of both BPH and Blast simultaneously.

## Materials and Methods

Field experiments were conducted to assess the physical compatibility, phytotoxicity and efficacy of insecticide and fungicide mixtures against BPH and blast at Regional Agricultural Research Station, Maruteru. The study area lies in between 16° 37’ 48” N Latitude and 81° 44’ 47” E Longitude at an altitude of 10 meters above sea level with humid to sub humid climate. Rice is an indispensable crop and grown throughout the year in two major seasons *kharif* (June-November) and *rabi* (December-March) in alluvial clay soils. Rice crop is grown under assured canal irrigation during *rabi* season.

Incidence of BPH and blast are a major concern during *rabi* season and compatibility studies were conducted for two consecutive years 2020 and 2021. Rice variety RDR-763, highly susceptible to BPH and blast was chosen for the present investigation in a randomized block design (RBD) with nine treatments replicated thrice. Transplanting and other crop husbandry operations as recommended in the package of practices of Acharya N. G. Ranga Agricultural University, Andhra Pradesh were adopted for raising the crop. Insecticidies recommended for control of BPH *viz*., Pymetrozine 50 WG @ 0.60 g/l ($T_1$); Triflumezopyrim 10SC @ 0.48 ml/l ($T_2$) and fungicides recommended for containing blast *viz*., Tricyclazole 75WP @ 0.60 g/l ($T_3$), Isoprothiolane 40 EC @ 1.5 ml/l ($T_4$) were tested alone and in combination for physical compatibility, bio-efficacy and phyto toxicity.

**Physical compatibility studies:**

Insecticide (Pymetrozine and Triflumezopyrim) and fungicide (Tricyclazole and Isoprothiolane) combinations were evaluated with jar compatibility test, where 500 ml of standard hard water (0.304 g calcium chloride and 0.139 g of magnesium chloride hexahydrate in one litre of double distilled water) was taken in a one litre jar to which one insecticide and fungicide are added in the order of Wettable powder (WP) followed by Dry flowables (DF), Flowables (F), Emulsifiable concentrates (EC) and finally Solubles designated as either soluble (S), soluble liquid (SL) or soluble concentrates (SC). Later, the volume of insecticide and fungicide mixture was made to one litre with hard water, agitated by shaking the jar and left undisturbed for 30 minutes to observe foaming and sedimentation. $p^H$ of insecticides and fungicides alone and in combinations were also recorded and designated according to Bickelhaupt (2012) **(Table 1)**.

**Table 1: Rating chart for reaction based on the value of $p^H$**

| Reaction | $P^H$ |
|---|---|
| Extremely acidic | < 4.5 |
| Very strongly acidic | 4.5 - 5.0 |
| Strongly acidic | 5.1 - 5.5 |
| Moderately acidic | 5.6 - 6.0 |
| Slightly acidic | 6.1 - 6.5 |
| Neutral | 6.6 - 7.3 |
| Slightly alkaline | 7.4 - 7.8 |
| Moderately alkaline | 7.9 - 8.4 |
| Strongly alkaline | 1.5- 9.0 |
| Very strongly alkaline | > 9.1 |

**Phytotoxicity studies:** Specific symptoms like injury to leaf tips, surface injury, necrosis, wilting, vein clearing, hyponasty and epipnasty at 1, 5 and 10 days after spray using phytotoxicity scale as prescribed by Central Insecticide Board and Registration Committee (C.I.B.R.C) were observed and per cent injury was arrived using the formula **(Table 2)**.

$$= \frac{\text{Total grade points}}{\text{Max. Grade x No. of leaves observed}} \text{ x } 100$$

**Table 2: Phytotoxicity scale of CIBRC**

| Scale | Phytotoxicity (%) |
|---|---|
| 0 | No phytotoxicity |
| 1 | 1-10 |
| 2 | 11-20 |
| 3 | 21-30 |
| 4 | 31-40 |
| 5 | 41-50 |
| 6 | 51-60 |
| 7 | 61-70 |
| 8 | 71-80 |
| 9 | 81-90 |
| 10 | 91-100 |

Bio-efficacy studies: The treatments were imposed at 60 DAT, when the population of BPH and blast disease crossed their economic threshold level. A spray fluid of 500 l/ha was used to ensure thorough coverage of the crop canopy with battery operated hand sprayer. Observations on nymphs and adults of BPH were taken directly on ten randomly selected hills per plot at one day before spray (Pre-treatment) and ten days after spray (Post-treatment). The incidence of blast was also recorded on 10 randomly selected hills one day before and ten days after treatment by using 0-9 scale of SES for Rice (IRRI, 2013) and the severity of the blast was calculated as per cent disease index (PDI) or % severity index (SI) using the formula.

$$= \frac{\text{Sum of all disease ratings}}{\text{Total number of leaves observed x maximum disease grade}} \text{ x } 100$$

**SES Scale for leaf blast**

| Score | Description |
|---|---|
| 0 | No lesions |
| 1 | Small brown specks of pinhead size without sporulating centre |
| 2 | Small roundish to slightly elongated, necrotic grey spots, about 1-2 mm in diameter with a distinct brown margin and lesions are mostly found on the lower leaves |
| 3 | Lesion type is the same as in scale 2' but significant number of lesions are on the upper leaves. |
| 4 | Typical sporulating blast lesions, 3 mm or longer, infecting less than 2% of the leaf area |
| 5 | Typical blast lesions infecting 2-10% of the leaf area |
| 6 | Blast lesions infecting 11-25% leaf area |
| 7 | Blast lesions infecting 26-50% leaf area |
| 8 | Blast lesions infecting 51-75% leaf area |
| 9 | More than 75% leaf area affected. |

Grain yield was recorded per plot leaving two border rows on all sides and expressed as kg/ha. Data on BPH population and per cent disease severity were square root and angular transformed, respectively and analyzed using ANOVA (Gomez and Gomez, 1984). The treatment means were compared by least significant difference (LSD) method.

## Results and Discussion

Physical compatibility: None of the test insecticide and fungicide mixture formed precipitation or sedimentation at 30 or 60 minutes after mixing, hence, they are physically compatible with each other. Similar observations are also made by Chander *et al*., (2020), who reported the physical compatibility of triflumezopyrim insecticide with tricyclazole and hexaconazole fungicides. The quality of water in term of pH also plays an important role in determining the efficacy of a spray fluid against target pest. All the test combinations of insecticides with fungicides recorded neutral reaction in the present study **(Table 3)**.

**Table 3: $P^H$ range of insecticides, fungicides and after their physical mixing**

| S. No. | Reaction | $P^H$ range | Pesticides |
|---|---|---|---|
| 1 | Neutral | 6.6 - 7.3 | Pymetrozine @ 0.60 g/l (6.70)<br>Triflumezopyrim @ 0.48 ml/l (6.91)<br>Tricyclazole @ 0.60 g/l (6.57)<br>Isoprothiolane @ 1.5 ml/l (6.80)<br>Pymetrozine + Tricyclazole @ 0.60 g + 0.60 g (6.91)<br>Pymetrozine + Isoprothiolane@ 0.60 g+ 1.5 ml/l (6.98)<br>Triflumezopyrim + Tricyclazole@ 0.48 ml + 0.60 g /l (7.06)<br>Triflumezopyrim + Isoprothiolane@ 0.48 ml + 1.5 ml/l (6.96) |

**Phytotoxicity:** None of the pesticide spays alone or in combination exerted phytotoxicity symptoms like injury to leaf tip, yellowing, wilting, vein clearing, necrosis, epinasty and hyponasty at 1, 5 and 10 days after spraying on rice crop.

**Bio-efficacy of pesticides:** Data presented in **(Table 4)** revealed that BPH population ranged from 125.17 to 142.33 per 10 hills before imposition of treatments and found non-significant. At ten days after spray, BPH population ranged from 24.67 to 236.33 per 10 hills among the treatments, which is statistically significant. Pymetrozine 50 WG @ 0.60 g/l, triflumezopyrim 10 SC @ 0.48 ml/l alone and their combinations with fungicides ($T_5$, $T_6$, $T_7$ and $T_8$) recorded the lower population of BPH which were at par with each other and superior to untreated check. On the other hand, treatments comprising only fungicides *viz*., tricyclazole 75 WP @ 0.60 g/l and isoprothiolane 40 EC @1.5 ml/l and untreated control registered higher BPH population. Based on per cent reduction in BPH population over control, triflumezopyrim 10 SC @ 0.48 ml/l, pymetrozine 50 WG + isoprothiolane 40 EC @ 0.60 g/l + 1.5 ml/l and triflumezopyrim 10 SC + isoprothiolane 40 EC @ 0.48 ml + 1.5 ml/l stood first, second and third best treatments by registering 89.56%, 88.65% and 87.59% reduction in BPH population over control. It was followed by triflumezopyrim 10 SC + tricyclazole 75 WP @ 0.48 ml + 0.6 g/l, pymetrozine 50 WG @ 0.60 g/l alone, pymetrozine 50 WG + tricyclazole 75 WP @ 0.60 g + 0.6 g/l with 86.11%, 85.05% and 81.95 reduction over control, respectively.

**Table 4: Efficacy of insecticide and fungicide combinations (tank mixtures) against BPH and blast in rice during *rabi* 2019-20 and *rabi* 2020-21 (Pooled)**

| Tr. No. | Treatment | Dose (g or ml/l) | BPH (No./10 hills)$^*$ | | Reduction over control (%) | Blast severity (%)$^{**}$ | | Reduction over control (%) |
|---|---|---|---|---|---|---|---|---|
| | | | PTC | 10 DAS | | PTC | 10 DAS | |
| $T_1$ | Pymetrozine 50 WG | 0.60 g/l | 134.83 (11.57) | 35.33 (5.89)$^a$ | 85.05 | 11.85 (20.03) | 16.59 (24.04)$^b$ | 3.67 |
| $T_2$ | Triflumezopyrim 10 SC | 0.48 ml/l | 129.17 (11.33) | 24.67 (4.96)$^a$ | 89.56 | 12.59 (20.73) | 16.87 (24.20)$^b$ | 2.04 |
| $T_3$ | Tricyclazole 75 WP | 0.60 g/l | 126.00 (11.02) | 233.00 (15.26)$^b$ | 1.41 | 11.85 (20.13) | 5.19 (13.15)$^a$ | 69.90 |
| $T_4$ | Isoprothiolane 40 EC | 1.5 ml/l | 139.67 (11.80) | 231.33 (15.16)$^b$ | 2.12 | 10.74 (19.07) | 5.19 (13.10)$^a$ | 69.90 |
| $T_5$ | Pymetrozine + Tricyclazole | 0.60 g + 0.60 g/l | 128.00 (11.21) | 42.67 (6.45)$^a$ | 81.95 | 11.48 (19.63) | 5.56 (13.59)$^a$ | 67.75 |
| $T_6$ | Pymetrozine + Isoprothiolane | 0.60 g+ 1.5 ml/l | 142.33 (11.87) | 26.83 (5.13)$^a$ | 88.65 | 11.11 (19.44) | 5.37 (13.31)$^a$ | 68.82 |
| $T_7$ | Triflumezopyrim + Tricyclazole | 0.48 ml + 0.60 g/l | 125.17 (11.09) | 32.83 (5.72)$^a$ | 86.11 | 11.85 (20.10) | 5.93 (13.98)$^a$ | 65.59 |
| $T_8$ | Triflumezopyrim +Isoprothiolane | 0.48 ml + 1.5 ml/l | 125.33 (11.19) | 29.33 (5.41)$^a$ | 87.59 | 11.67 (19.96) | 5.00 (12.88)$^a$ | 70.97 |
| $T_9$ | Untreated control (water spray) | - | 137.83 (11.69) | 236.33 (15.37)$^b$ | | 14.26 (22.17) | 17.22 (24.51)$^b$ | |
| **F test** | | | **NS** | **Sig.** | | **NS** | **Sig.** | |
| **CD (0.05)** | | | **-** | **1.57** | | **-** | **2.85** | |
| **CV (%)** | | | **10.70** | **10.27** | | **8.63** | **9.71** | |

*Values in the parentheses are square root transformed values; **Values in the parentheses are arc sine values; PTC- Pre-treatment count, DAS-Days after spray; NS-Non-significant; Sig.-Significant; Means followed by same letter are not significantly different by LSD method (p=0.05%).

From the above results, it is evident that the bio-efficacy of pymetrozine 50 WG and triflumezopyrim 10 SC insecticides against BPH did not adversely affect the fungicides, tricyclazole 75 WP and isoprothiolane 40 EC, as their combination treatments *i.e.*, $T_5$, $T_6$, $T_7$ and $T_8$ are equally effective as $T_1$ (Pymetrozine 50 WG) and $T_2$ (Triflumezopyrim 10 SC) against BPH. Similar observations were made by Adhikari *et al*., (2019) and Rehman *et al*., (2020), who reported the supremacy of pymetrozine 50 WG in controlling the BPH and WBPH populations in rice. Sarao and Jhansilakshmi (2019) reported triflumezopyrim 10 SC was most effective against BPH. Chander *et al*., (2020) also reported that mixing of tricyclazole 75 WP with triflumezopyrim 10 SC did not show any negative effect on efficacy of triflumezopyrim against brown plant hopper.

Similarly, fungicides controlling blast have not lost their efficacy when mixed with insecticides. Among the treatments, tricyclazole 75 WP @ 0.60 g/l ($T_3$) and isoprothiolane 40 EC @ 1.5 ml/l ($T_4$) and their combinations with insecticides ($T_5$, $T_6$, $T_7$ and $T_8$) recorded lower blast severity (5.00% to 5.93%) and significantly superior compared to control (17.22%) at ten days after spray, respectively. Based on per cent reduction in blast severity over control, triflumezopyrim 10 SC + isoprothiolane 40 EC @ 0.48 ml + 1.5 ml/l recorded highest per cent reduction (70.97%) in blast severity over control. It was followed by tricyclazole 75 WP @ 0.60 g/l, isoprothiolane 40 EC @ 1.5 ml/l, pymetrozine 50 WG + isoprothiolane 40 EC @ 0.60 g + 1.5 ml/l, pymetrozine 50 WG + tricyclazole 75 WP @ 0.60 g + 0.6 g/l, triflumezopyrim 10 SC + tricyclazole 75 WP @ 0.48 ml + 0.60 g/l with 69.90%, 69.90%, 68.82%, 67.75% and 65.59% reduction over control, respectively. These results are in agreement with the reports of earlier workers. The efficacy of isoprothiolane 40 EC (Raji and Louis, 2007) and tricyclazole 75 WP (Dar and Murtaza, 2021) in controlling the leaf and neck blast severity in rice was well documented.

The pooled data on the grain yield of two seasons, *rabi* 2019-20 and 2020-21 **(Table 5)** revealed that triflumezopyrim 10 SC + isoprothiolane 40 EC @ 0.48 ml + 1.5 ml/l recorded the highest grain yield of 5088 kg/ha with 67.59% yield increase over untreated control. It was followed by triflumezopyrim 10 SC + tricyclazole 75 WP @ 0.48 ml + 0.60 g/l (5014 kg/ha), pymetrozine 50 WG + isoprothiolane 40 EC @ 0.60 g + 1.5 ml/l (4873 kg/ha), pymetrozine 50 WG + tricyclazole 75 WP @ 0.60 g + 0.60 g/l (4872 kg/ha) with 65.14%, 60.49% and 60.48% increase in grain yield over untreated check, respectively.

**Table 5: Efficacy of insecticide and fungicide combinations (tank mixtures) on grain yield in rice**

| Tr. No. | Treatment | Dose (g or ml/l) | Grain yield (kg/ha) | | Pooled | Increase over control (%) |
|---|---|---|---|---|---|---|
| | | | ***Rabi* 2019-20** | ***Rabi* 2020-21** | | |
| $T_1$ | Pymetrozine 50 WG | 0.60 g/l | 5211$^{ab}$ | 3946 $^{b}$ | 4578$^{c}$ | 50.79 |
| $T_2$ | Triflumezopyrim 10 SC | 0.48 ml/l | 5223$^{ab}$ | 3999 $^{b}$ | 4611$^{bc}$ | 51.88 |
| $T_3$ | Tricyclazole 75 WP | 0.60 g/l | 4660$^{b}$ | 2689 $^{c}$ | 3675$^{d}$ | 21.03 |
| $T_4$ | Isoprothiolane 40 EC | 1.5 ml/l | 4719$^{b}$ | 2614 $^{c}$ | 3667$^{d}$ | 20.78 |
| $T_5$ | Pymetrozine + Tricyclazole | 0.60 g + 0.60 g/l | 5358$^{a}$ | 4386 $^{ab}$ | 4872$^{abc}$ | 60.48 |
| $T_6$ | Pymetrozine + Isoprothiolane | 0.60 g + 1.5 ml/l | 5339$^{a}$ | 4406 $^{ab}$ | 4873$^{abc}$ | 60.49 |
| $T_7$ | Triflumezopyrim + Tricyclazole | 0.48 ml + 0.60 g/l | 5377$^{a}$ | 4649 $^{a}$ | 5014$^{ab}$ | 65.14 |
| $T_8$ | Triflumezopyrim + Isoprothiolane | 0.48 ml + 1.5 ml/l | 5614$^{a}$ | 4660 $^{a}$ | 5088$^{a}$ | 67.59 |
| $T_9$ | Untreated control (water spray) | - | 3580$^{c}$ | 2493 c | 3036$^{e}$ | |
| **F test** | | | Sig. | Sig. | Sig. | |
| **CD (0.05)** | | | 592.11 | 608.25 | 426.01 | |
| **CV (%)** | | | 6.83 | 9.35 | 5.62 | |

Sig.-Significant; Means followed by same letter are not significantly different by LSD method (p=0.05%). Present studies proved that the tank mixing of insecticides (pymetrozine 50 WG and triflumezopyrim 10 SC) with fungicides (tricyclazole 75 WP and isoprothiolane 40 EC) did not cause any deleterious effect in their bio-efficacy against target pest/disease. Further, they are physically compatible and did not exert any phytotoxicty on rice. Thus, the above insecticides and fungicides can be applied as tank mixtures when BPH and blast occur simultaneously in rice.

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# Impact of Farmers' Participatory Rice (*Oryza sativa* L.) Demonstration Programme on Crop Yield and Economics Under Temperate Hill Ecology

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## Abstract

There exists a gap between the potential yield and the yield realized in farmers' field in India. Situation is no different in the Union Territory of Jammu and Kashmir. With an aim to narrow down the yield gaps Farm Science Center Kulgam-SKUAST, Kashmir conducted 63 frontline demonstrations on Shalimar Rice-4, a high yielding disease tolerant variety in the lower plains of Kashmir valley from year 2019-2021. Results of these demonstrations revealed a significant improvement in yield and higher monetary returns with the introduction of new varietyy. Average crop yield (73.8 q ha$^{-1}$) was 15.5% higher in improved practice compared to farmers' practice (63.9 q ha$^{-1}$). An extension gap of 9.9 q ha$^{-1}$ and a technology gap of 6.2 q ha$^{-1}$ were observed in the study. Net returns were Rs. 129243 ha$^{-1}$ in farmers practice against Rs.146815 ha$^{-1}$ in technology demonstrated, indicating an additional income of Rs. 17572 ha$^{-1}$ and effective gain of Rs. 14812 ha$^{-1}$ in the latter. Higher benefit: cost ratio (2.9) was registered in improved practice compared to farmers practice (2.7).

**Key Words:** Rice, Front line Demonstration, hills, yield, economics

## Introduction

Rice is staple food and also a source of employment and livelihood for majority of the populaion in India and also for the farmers associated with its cultivation in Kashmir valley situated in the North western Himalayas. Its cultivation in the valley extends from the plains having altitude 1570 meters to high hills 2180 meters above mean sea level. Like other parts of the country, rice farming has seen dramatic changes from 1960s in the Union Territory of Jammu and Kashmir. The contribution made by the State Agriculture University SKUAST-Kashmir through Mountain Research Center for Filed Crops (MRCFC), in terms of development of rice technologies is enormous. Before the availability of varieties developed and promoted by the center the productivity of tradition land races cultivated by farmers of the valley hovered between 1 to 1.5t/ha. Despite these developments, there still exists a gap between what is produced and what can be produced in the farmers' field (Mubarak and Shakoor, 2019). Use of traditional rice cultivars by farmers is one of the major cause of low productivity in farmers field in the valley (Mubarak and Sheikh, 2014). The reason farmers stick to the traditional varieties like China-1039, Jhelum and some mixtures may either be their unawareness about new ones or lack of desirable traits like early maturity, disease resistance, higher yields and good cooking quality in the varieties. In order to bridge the yield gap, it is therefore, essential that new varieties with higher yield potential and desirable traits are developed and popularized among farmers. Shalimar Rice-4 possesses resistance to major diseases and a yield potential of 10 t ha$^{-1}$. This variety has been the farmers best choice during the Participatory Varietal Selection (PVS) programme conducted by the center at farmers field (Najeeb *et al.,* 2018) as it possesses farmers' acceptable traits and can be cultivated without any fungicide or insecticide spray. In this back drop, it was required to popularize the variety in farmers participatory mode and with this aim a technology dissemination programme through frontline demonstrations in farmers' participatory mode were conducted at multi-location from 2019 to 2021.

## Materials and Methods

The study area, which included 13 villages (**Table 1**) adopted for the demonstrations, is located in the lap of Peer Panchal Himalayan Ranges between 33.62 to 33.70$^0$N latitude and 74.8 to 75.02$^0$E longitude. Soils in the demonstration plots were silty clay loam to clay loam in texture and the area possesses facility of assured irrigation round the year. Shalimar Rice-4 variety released in year 2017 was selected for the demonstrations in the farmers fields in the adopted villages against the Jhelum variety popular in the area. The variety is recommended for irrigated low lands (up to the altitude of 1700 m amsl) of the Kashmir valley due to high yielding potential in this ecology. The variety exhibits tolerant reaction to blast disease and moderately resistant reaction against major diseases under national screening trials. Farm Science Center Kulgam working under the aegis of Sher-e-Kashmir University of Agricultural Science and Technology of Kashmir in collaboration with Mountain Research Center for Field Crops (MRCFC) and the Department of Agriculture Govt. of Jammu & Kashmir, conducted 64 frontline demonstration programmes sponsored by ICAR-Agricultural Technology Application Research Institute Zone-1, over an area of 38.8 ha. from 2019 to 2021. Thirteen villages scattered across the southern part of valley were selected for the programme after consultation with the officers of Department of Agriculture and Scientists from Mountain Research Center for Field Crop (MRCFC)-Sher-e-Kashmir University of Agricultural Science and Technology of Kashmir. Each demonstration occupied 1-acre area (0.5 acre under demonstrated variety and 0.5 acre under old variety). A recommended dose of fertilizer (120: 60: 30 kg N, P and K ha$^{-1}$) was utilized in both the varieties. After transplanting of one month old seedlings at a spacing of 15 cm x 15 cm, water was impound (5 cm) until complete draining out of water at active-tillering stage (for 1st top dose of nitrogen after hand weeding) and panicle initiation stage (for 2nd top dose of nitrogen). From flowering to milk stage, a thin layer of water was maintained in the field. Alternate wetting and drying was carried out from dough stage to physiological maturity. No irrigation was given after physiological maturity. Crop was harvested in the last week of September and the grain and straw yield were recorded from each demonstration. For economics, values of both grain and paddy straw were taken into consideration, as paddy straw has economic value in the valley particularly for cattle feeding and apple packing. Additional gains, effective gains, extension gap, technology gap and technology Index were calculated by using formulae given below;

- Additional gains (₹ ha$^{-1}$) = Net returns (₹ ha$^{-1}$) from Improved practice - Net returns (₹ ha$^{-1}$) from farmers' practice
- Effective gains (₹ ha$^{-1}$) = Additional returns (₹ ha$^{-1}$) - additional costs (₹ ha$^{-1}$)
- Extension gap (q ha$^{-1}$) = Improved practice yield (q ha$^{-1}$) -farmers' practice yield (q ha$^{-1}$).
- Technology gap (q ha$^{-1}$) = Yield in demonstrated technology (q ha$^{-1}$) - Potential yield (q ha$^{-1}$).
- Technology Index (%) =

$$\frac{\text{Potential yield} - \text{Demo yield}}{\text{Potential yield}} \times 100$$

**Table 1: List of villages covered under demonstrations**

| S. No | Name of Village | S. No | Name of Village |
|---|---|---|---|
| 1 | Tarigam | 8 | Kanipora |
| 2 | Sonogam | 9 | Kujar |
| 3 | Yaripora | 10 | Brazloo |
| 4 | Home shali bough | 11 | Kaladrangh |
| 5 | Frisal | 12 | Chimegam, Chanpora |
| 6 | T N Pora | 13 | Gnosargam |
| 7 | Shurat | - | |

The data recorded on crop yield were subjected to statistical analysis through student's t -Test using excel data analysis tool and means compared at $p \leq 0.05$.

## Results and discussion

### Crop yield

Shalimar Rice-4 variety recorded an average yield of 74.5 q $ha^{-1}$ in the farmers' participatory trials conducted across Kashmir valley, which was the highest observed so far (Najeeb *et al.,* 2018). The results necessitated the promotion of this variety as fast as possible given to its yield potential and farmers acceptance. Farm science center, Kulgam took a lead and conducted Frontline Demonstration programmes on the variety along with related package of practice. Data pertaining to the yields obtained under frontline demonstrations are presented in **(Table 2)**. Improved practice registered significantly higher grain yield compared to the existing farmers practice during all the three years of study **(Tables 2 and Figures 1 to 3)**. Yield ranged between 73.2 to 74.5 q $ha^{-1}$ and 62.0 to 65.2 q $ha^{-1}$ in improved practice and farmers' practice, respectively. Improved practice gave an additional yield of 9.9 q $ha^{-1}$on an average basis. Average crop yield (73.8 q $ha^{-1}$) was 15.5% higher in improved practice compared to farmers' practice (63.9 q $ha^{-1}$). Additional yield was highest (11.2 q $ha^{-1}$) in year 2021 probably due to weather conditions suitable for the new variety. This may be attributed to the resilience of this variety to biotic and abiotic stresses and better response to farm inputs (Parray *et al.,* 2020). Similar findings were also reported by Patil *et al.,* (2018) and Mubarak and Shakoor (2019). Sharma *et al.,* (2022) also concluded that new variety has significant yield advantage over tradition one. The change in per cent increase in yield over the years indicates varying response to the environment prevailing during the respective years of study. These findings are in line with those of Singh *et al.,* (2015) and Asif *et al.,* (2017).

**Table 2: Yield of rice varieties under Frontline Demonstration programme at farmers' field**

| Year | Name of Varieties | Ecology | No. of demons. | Area under demonstra-tion (ha) | Average yield in Improved Practice (q $ha^{-1}$) | Average yield in Farmers practice (q $ha^{-1}$) | Additional yield in the improved practice | % age yield increase |
|---|---|---|---|---|---|---|---|---|
| 2019 | • Jhelum (FP)*<br>• Shalimar Rice -4(IP)** | Irrigated valley planes | 25 | 10 | 73.9 | 65.2 | 8.7 | 13.3 |
| 2020 | • Jhelum (FP)<br>• Shalimar Rice-4(IP) | Irrigated valley planes | 14 | 5.6 | 74.5 | 64.7 | 9.8 | 15.1 |
| 2021 | • Jhelum (FP)<br>• Shalimar Rice-4 (IP) | Irrigated valley planes | 25 | 23.2 | 73.2 | 62.0 | 11.2 | 18.0 |
| Total (Demos & Area) / Mean yield & % increase in yield | | | 64 | 38.8 | 73.8 | 63.9 | 9.9 | 15.5 |

*FP: Farmers' Practice **IP: Improved Practice

Figure 1: Grain yield of improved and old variety at farmers field during 2019 ($P=5.3E^{-10}$)

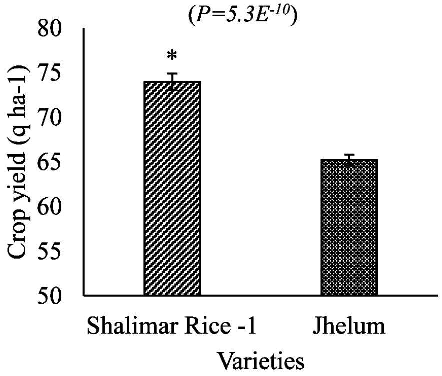


Figure 2: Grain yield of improved and old variety at farmers field during 2020 ($p$=1.85E$^{-10}$)

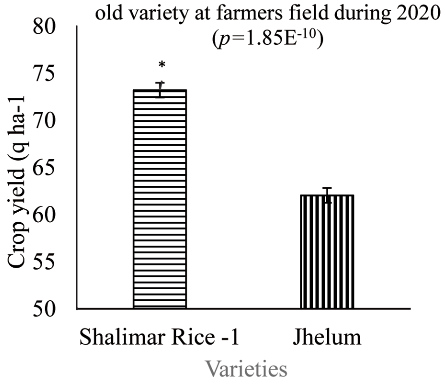
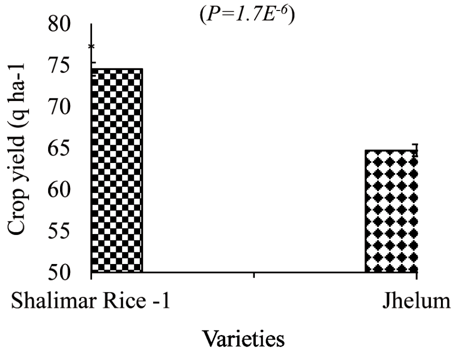


Figure 3: Grain yield of improved and old variety at farmers field during 2021 ($P=1.7E^{-6}$)



**Gap analysis**

The yield gap analysis in the present study revealed an extension gap ranging between to 8.7 to 11.2 q ha$^{-1}$ (**Table 3**). On an average the extension gap was 9.9 q ha$^{-1}$. The Technology gap varied between 5.5 to 6.8 with an average value of 6.2 pooled over the years. These results indicate that there is further scope to enhance the rice production in the valley by popularizing the latest high yielding varieties like Shalimar Rice-4. This can be achieved through collaborative efforts of extension functionaries involving farm science Centers and the department of agriculture existing in each district of the valley. Earlier Mubarak *et al.* (2013) and Sheikh *et al.* (2014) also reported similar results during their studies on demonstration of rice technologies under Kashmir conditions.

**Table 3: Gap analysis and technology Index in Frontline Demonstration on rice at farmers' field**

| Year | Name of Varieties | Ecology | Extension gap | Technology Gap | Technology Index |
|---|---|---|---|---|---|
| 2019 | • Jhelum (FP)<br>• Shalimar Rice (SR)-4 | Irrigated plains of valley | 8.7 | 6.1 | 7.625 |
| 2020 | • Jhelum (FP)<br>• Shalimar Rice (SR)-4 | Irrigated plains of valley | 9.8 | 5.5 | 6.875 |
| 2021 | • Jhelum (FP)<br>• Shalimar Rice (SR)-4 | Irrigated plains of valley | 11.2 | 6.8 | 8.5 |
| **Total** | | | **9.9** | **6.2** | 7.75 |

Similarly, variations in Technology Index were also recorded between 6.8 to 8.5% with an average value of 6.2%. Technology index is an indicator of the feasibility of the evolved technology at the farmers' fields. The lower the value of technology index more is the feasibility of the technology. Technology index ranging between 6.8 to 8.5% indicates that there is scope for further improvement in productivity of rice in Valley. These findings are in line with those of Mitra *et al.* (2014) and Singh *et al.* (2015).

**Economic impact**

Economics in terms of costs of cultivation, gross and net returns, additional returns, effective gain and B:C ratio varied during different years of study both in the improved technology and farmers practice (**Table 4**). The cost of cultivation varied from ₹ 47280 ha$^{-1}$ in year 2019 in farmers' practice to Rs.53068 ha$^{-1}$ in the improved practice in year 2020. This was due to variation in cost of inputs in different years and comparatively higher input requirement in the improved practice. On an average the cost of inputs was Rs.47647 ha$^{-1}$ and Rs.50407 ha$^{-1}$ for farmers practice and improved practice, respectively. Improved practice involved an additional cost of inputs to the tune of Rs.2760 ha$^{-1}$. Gross and net returns fluctuated during the years of study with maximum values recorded for improved practice (**Table 4**). Gross returns of Rs.176890 ha$^{-1}$ and Rs. 197222 ha$^{-1}$ were registered under farmers practice and improved practice, respectively. Net returns pooled over the years were Rs. 129243 ha$^{-1}$ in farmers practice against Rs.146815 ha$^{-1}$ in technology demonstrated, indicating an additional income of Rs. 17572 ha$^{-1}$ and effective gain of Rs. 14812 ha$^{-1}$ in the latter. Higher benefit: cost ratio (2.9) was registered in improved practice compared to farmers practice (2.7). The additional returns, effective gain and higher net returns obtained under improved practices could be due to its high yield potential under existing ecology and environmental conditions. Verma *et al.,* (2017) also concluded their study with similar results.

**Table 4: Economic Analysis of frontline demonstration programme on rice at farmers' field**

| Year | Input cost (₹ ha$^{-1}$) | | Additional cost in IP | Gross returns* ( ₹ ha$^{-1}$) | | Net returns (₹ ha$^{-1}$) | | Additional returns from IP (₹ ha$^{-1}$) | Effective Gain from IP ( ₹) | B:C ratio | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Farmers Practice (FP) | Improved Practice (IP) | | FP | IP | FP | IP | | | FP | IP |
| 2019 | 47280 | 49242 | 1962 | 176536 | 195694 | 129256 | 146452 | 17196 | 15234 | 2.7 | 3.0 |
| 2020 | 48229 | 53068 | 4839 | 180899 | 200300 | 132670 | 147232 | 14562 | 9723 | 2.8 | 2.8 |
| 2021 | 47432 | 48912 | 1480 | 173234 | 195672 | 125802 | 146760 | 20958 | 19478 | 2.6 | 3.0 |
| Average | 47647.0 | 50407.3 | 2760.3 | 176890 | 197222 | 129243 | 146815 | 17572 | 14812 | 2.7 | 2.9 |

**It also includes the returns from paddy straw owing to its demand in the valley for apple packing and as cattle fodder for lean winter season.*

## Conclusion

Rice being the staple food for the people of Kashmir Valley, will remain a top priority of agriculture research and extension. The area is shrinking day by day due to diversification into other sectors especially horticulture and non-agriculture activities, indicating that we need to get more from less land. To meet the demand, the present productivity must not only increase but sustain growth in future. So demonstration of proven technology capsules pertaining to different rice ecologies in farmers' participatory mode is vital to achieve this goal. The present study also indicates that use of new variety Shalimar Rice-4 is crucial in bridging the yield gaps and improving returns in the valley.

## Acknowledgements

This study is the outcome of Frontline Demonstration programme sponsored by ICAR-Agricultural Technology Application Research Institute Zone-1, (India). Authors acknowledge the financial support from the ICAR-ATARI and technical support from MRCFC-SKUAST-Kashmir. Authors also express their thanks to the of Department of Agriculture and the farmers associated with the programme for their cooperation.

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# DRR Dhan 65 (IET 27641) - A High Yielding Low Soil Phosphorous Tolerant and Climate Resilient Rice Variety Developed from Wild Introgression Lines

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## Abstract

The wild species of *Oryza* are an important source of genetic variability for the improvement of yield and tolerance to biotic and abiotic stresses. Phosphorus (P) is the second most important key nutrient, vital for plant growth and development at all stages. Phosphorous deficiency is one of the factors limiting rice yields and farmer's profitability so it is necessary to identify genotypes with stable yield and tolerance to P deficiency. Keeping in view the unlimited potential of wild species for yield enhancement and stress tolerance, interspecific population between *Oryza rufipogon* in the back ground of *Oryza sativa* genotype KMR3 was developed by advanced back cross breeding strategy. DRR Dhan 65 (IET 27641) (RP Bio 4919-B-B NSR86) is an interspecific wild introgression line, a derivative of KMR 3 / *O rufipogon* with short bold grains which is high yielding under both normal and low soil phosphorous conditions. It has seed to seed maturity of 130-135 days and gives an average yield of 6.5 t/ ha (under normal conditions; 60 kg/ha of P, *i.e.,* recommended dose) and 4.7 t/ ha (under low Phosphorus; 40 kg/ha of P). It was released for cultivation in Andhra Pradesh, Telangana, Karnataka, Chhattisgarh, Jharkhand and Maharashtra states through Central Sub-committee on Crop Standards, Notification and Release of Varieties for Agricultural Crops vide S.O. 4065(E) dated 31st August, 2022 [CG-DL-E-31082022-238490].

**Keywords:** Irrigated rice, low P tolerance, climate resilience, wild introgression lines.

## Introduction

Considering the unlimited potential of wild species for identification of yield enhancing genes, interspecific population between *Oryza sativa* and *Oryza rufipogon*; was developed with a primary objective to identify yield QTLs. DRR Dhan 65 (IET 27641) is a wild introgression line developed by ICAR-IIRR using parental lines a) IR 58025A, a widely used CMS line having long grain type and early maturity along with good milling and eating qualities, b) an Indian accession of *Oryza rufipogon* (IC 22015/WR120) collected from Kerala, India, which was maintained at ICAR-IIRR (erstwhile DRR) was used as a donor parent and c) KMR3, restorer line for popular high yielding hybrid KRH 2. An advanced backcross strategy was followed to develop the population. The high yielding lines in the genetic background of KMR 3 were characterized for three years and were further advanced based on single panicle selection up to $BC_3F_{10}$ and the seed was multiplied. The population was tested during four wet seasons (2014, 2015, 2016 and 2018) under normal irrigated conditions (N-100 kg/ha, P- 60 Kg/ha, K-60 kg/ha) and during two dry seasons of 2016 and 2018 under low phosphorus (Olsen P 1.8 kg/ha). NSR86 is a selection from a back cross introgression line 377-24 from this population which showed high yield under both Low P and irrigated conditions across the seasons compared to recurrent parent KMR 3. *O. rufipogon* introgressions into restorer line KMR3 helped to improve yield and tolerance to low Phosphorus in soil. The promising line, RP Bio 4919-B-B NSR86 was identified and nominated in AICRIP LPT trial-2019 after successfully performing in the 2018 pilot trial. Subsequently, the entry performed well in all the four years and released as low P tolerant rice variety DRR Dhan 65 through Central Sub-committee on Crop Standards, Notification and Release of Varieties for Agricultural Crops vide S.O. 4065(E) dated 31st August, 2022 [CG-DL-E-31082022-238490] suitable for cultivation in Andhra Pradesh, Telangana, Karnataka, Chhattisgarh, Jharkhand and Maharashtra.

DRR Dhan 65 (IET 27641), is similar to the recurrent parent, KMR 3 possessing good cooking quality with short bold grain type and higher yield. It also has resistance to leaf blast, neck blast and sheath blight. It is high yielding under both low soil phosphorus and normal irrigated condition. It recorded average grain yield advantage of +24.23% and +17.26% (in terms of weighted average) over the positive check Swarna (late duration) under 100% and 50% application of phosphorous, respectively. It also showed a yield advantage of +75.71, +22.4, +44.51 and +69.02 over Rasi (Positive check), Swarna (Positive Check), Improved Samba Mahsuri (negative check) and DRR Dhan 60 (recently released variety), respectively considering weighted mean average of *kharif* 2018, 2019, 2020 and 2021 under normal (100% of recommend dose of fertilizer (RDF) phosphorus condition. Similarly, at low phosphorus condition it yielded with +45.67, +9.91, +37.1 and, +68.91 over these checks.

IET 27641 (NSR 86) exhibited tolerant reaction to leaf blast, neck blast, sheath blight and BPH. During 2019, based on NSN 2, IET 27641 showed field tolerance to BPH at Maruteru with a DS of 3.0. During 2020, IET 27641 exhibited low over all disease score and high promising index under NSN-1 for Neck blast. IET 27641 possesses short bold grain type with high HRR (67.5%) and acceptable grain quality parameters of amylose content (25.93%), soft GC (45) and ASV (5.0) and is comparable to the recurrent parent, KMR 3 in all the grain and cooking quality parameters. Based on the grain yield efficiency index GYEI values of stable and nutrient use efficient genotypes, IET 27641 identified as the II top culture in 2020, IV top culture in 2019 in the agronomy trials. The wild introgression line NSR 86 (377-24) is identified as one of the heat tolerant line with high yield and minimum yield loss under heat stress at IIRR and also in multi-location testing conducted by AICRIP Physiology at 7 different locations all over India. IET 27641 is also having agro-morphological and grain characteristics similar to KMR 3 with enhanced stress tolerance and is very high yielding compared with parent KMR 3 and other long duration checks.

Thus, DRR Dhan 65 is high yielding with tolerance to low soil phosphorous. It has exhibited good grain quality along with tolerance to various biotic and abiotic stresses. Based on background genotyping, it has shown > 96.00% recovery of recurrent parent genome. Considering better yield performance of the variety in P deficit as well as under normal conditions and stress tolerance, grain and cooking quality traits it can replace short bold or late duration varieties like Swarna, specifically for those areas which are endemic to blast disease and/or with P deficient soils, thus significantly reducing the cost of cultivation. Therefore, DRR Dhan 65 is potential late duration climate resilient rice variety for the low-input areas of the country for making rice cultivation more economical.

DRR Dhan 65 under Normal irrigated conditions

DRR dhan 65

Grain type

DRR Dhan 65 under Low phosphorus conditions

# Rice Germplasm Registered during July-December 2023 at ICAR-National Bureau of Plant Genetic Resources, New Delhi

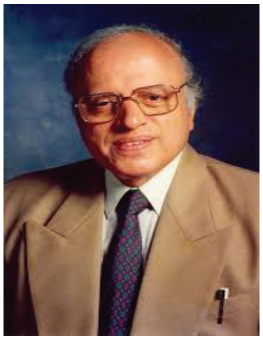
| Sl No. | Crop Name | Botanical Name | National Identity | Donor Identity | INGR No. | Novel Unique Features |
|---|---|---|---|---|---|---|
| 1 | Rice | *Oryza sativa* | IC648583 | Meghalaya Lakang; RCMR-13 | 23001 | Leaf blast resistance. Neck blast resistance. Exhibited combined resistance to leaf blast (score 2 on SES scale) and neck blast (score 1 on SES scale). |
| 2 | Rice | *Oryza sativa* | IC648978 | RP6253-MV2 (Varadhan × MTU1010/2) | 23002 | High Nitrogen Use Efficiency (NUE) under N-Low and N-50 input. |
| 3 | Rice | *Oryza sativa* | IC648592 | MSM-3, TI-3, IET-28688 | 23003 | Increased root length and root volume. Better seedling vigour index. |
| 4 | Rice | *Oryza sativa* | IC0640862 | Black Gora (IC0640862); NPM/SR4 | 23004 | Tolerant to submergence with high anaerobic germination potential. |
| 5 | Rice | *Oryza sativa* × *O. nivara* | IC648977 | RPbio4918-166S | 23005 | High photosynthetic rate. High seedling vigour. |
| 6 | Rice | *Oryza sativa* | IC648593 | IR 129477-902-121-10-1-1 | 23006 | Biotic resistance genes Xa4, BPH3, GM4, Pita. QTL markers (AG9.1, qDTY3.1, qGY6.1, qGY10.1, qNR4.1 and qNR5.1). |
| 7 | Rice | *Oryza sativa* | IC648594 | IR 129477-709-375-3-5-7 | 23007 | Biotic resistance genes GM4, Pita QTL for anaerobic germination (AG9.1). QTL markers (qDTY3.1, qDTY12.1, qGY6.1 and qNR5.1). |
| 8 | Rice | *Oryza sativa* | IC648595 | IR 129477-1629-14-1-4-2 | 23008 | Biotic resistance genes Xa4, xa5, Xa21, BPH3, Pi9, Pita. QTL markers (AG9.1, qDTY3.1, qNR5.1, qRHD1.1 and qEMM1.1). |
| 9 | Rice | *Oryza sativa* | IC648596 | IR 129477-1629-210-4-4-4 | 23009 | Biotic resistance genes xa5, Xa21, BPH3, Pita. QTL markers (AG9.1, qDTY2.1, qDTY3.1, qNR5.1, qRHD1.1 and qEMM1.1). |
| 10 | Rice | *Oryza sativa* | IC648597 | IR 129477-3343-500-36-5-1 | 23010 | Biotic resistance genes Xa4+xa5+xa13 + GM4+Pita. QTL markers (AG9.1, qDTY3.1, qRHD1.1 and qEMM11.1). |

| Sl No. | Crop Name | Botanical Name | National Identity | Donor Identity | INGR No. | Novel Unique Features |
|---|---|---|---|---|---|---|
| 11 | Rice | *Oryza sativa* | IC648598 | IR 129477-4026-249-15-1-2 | 23011 | Biotic resistance genes Xa4, Xa21, BPH3, GM4. QTL markers (AG9.1, qDTY3.1, qDTY12.1, qRHD1.1, qRHD5.1 and qEMM11.1) |
| 12 | Rice | *Oryza sativa* | IC648599 | IR 129477-4139-439-1-1-2 | 23012 | Biotic resistance genes Xa4, xa5, Xa21, Pi9, Pita. QTL markers (AG9.1, qDTY3.1, qDTY12.1 and qEMM11.1). |
| 13 | Rice | *Oryza sativa* | IC648600 | IR 129477-4197-209-2-2-2 | 23013 | Biotic resistance genes Xa4, xa5, Xa21, Pita, Pita2. QTL markers (AG9.1, qDTY3.1 and qNR5.1). |
| 14 | Rice | *Oryza sativa var. indica* | IC648601 | MTU 1184 | 23014 | Submergence tolerance. |
| 15 | Rice | *Oryza sativa var. indica* | IC648602 | MTU IJ 206-7-4-1; MTU IJ 206-7-4-1 (BM 71) | 23015 | Resistance to Brown Plant Hopper. |
| 16 | Rice | *Oryza sativa* | IC648979 | CSAR 7-9-2020<br>(IET 29356) | 23016 | Tolerance against soil sodicity. |
| 17 | Rice | *Oryza sativa* | IC646828 | SM-92; IIRR-BIO-SB-9; RP5977-BIO-SB-9 | 23065 | Tolerance to yellow stem borer. |
| 18 | Rice | *Oryza sativa* | IC650728 | IL19273, 19273, FBL 19273 | 23068 | Multiple tolerance to sheath blight, sheath rot, RTD, leaf blast and neck blast diseases. Drought tolerance-high yield under reproductive stage drought stress |
| 19 | Rice | *Oryza sativa* | IC650729 | IRGC 39111 | 23069 | Strong culm. |
| 20 | Rice | *Oryza sativa* | IC650730 | IL 19101,<br>FBL 19101,<br>FBL 19102,<br>IL 19102, RP 6614-101, RP 6614-102 | 23070 | Resistance to gall midge. Resistance to bacterial blight. Resistance to blast |
| 21 | Rice | *Oryza sativa* | IC650767 | IL 19471, IET 29834 | 23071 | Reproductive stage drought tolerance. Resistance to blast and bacterial blight. |
| 22 | Rice | *Oryza sativa* | IC635486 | MCM 109 | 23072 | Salt tolerance (EC-5 to 11.95ds/m) |

| Sl No. | Crop Name | Botanical Name | National Identity | Donor Identity | INGR No. | Novel Unique Features |
|---|---|---|---|---|---|---|
| 23 | Rice | *Oryza sativa* | IC650731 | CRR751-1-12- B-B (IET 28033) | 23073 | Tolerance to reproductive stage drought stress. Tolerance to Submergence. Resistance to blast disease. |
| 24 | Rice | *Oryza sativa* | IC650732 | IET29482 (RP6211- PR/RIL-Q181) | 23074 | High grain Zn content (28.22ppm) in polished rice grain. High Protein content (8.08%) in polished rice grain |
| 25 | Rice | *Oryza sativa* | IC650734 | IET29484 (RP6204-MB/RIL-J159) | 23075 | High grain Zn content (24.32ppm) in polished rice grain. |
| 26 | Rice | *Oryza sativa* | IC650733 | RP6257- SJ3 (Sampada× Jaya /3) | 23076 | High and stable grain yield under N-Low, N-50 and N-100 fertilizer input. High Nitrogen use Efficiency under N-Low and N-50 input. |
| 27 | Rice | *Oryza sativa* | IC650735 | RP6252-BV/RIL/1689 (CNN1) | 23077 | High and stable grain yield under N-Low, N-50 and N-100 fertilizer input. High Nitrogen Use Efficiency under N-Low and N-50 input. High nutrient (NPK) uptake and high grain yield under native sodic soil conditions (without gypsum amendment; pH 8.5 – 10.0) across field locations under AICRIP testing. |
| 28 | Rice | *Oryza sativa* | IC646727 | AC43160 | 23121 | High total anthocyanin (116.76 mg/100g). High total gammaoryzanols (86.26mg/100g). High total phenolic content (788.18 mg/100g). High total flavonoid content (221.27 mg/100g). High ABTS Activity germplasm (3163.94. AAE/g). Low phytic acid content (0.16 g/100g). |

**Source :** Head & Member-Secretary, Plant Germplasm Registration Committee **(http://www.nbpgr.ernet.in:8080/registration/InventoryofGermplasm.aspx),** ICAR-NBPGR, New Delhi.

***Dr. S.V. Sai Prasad***
**Chief Editor**



## Tributes to

# *Dr. M. S. Swaminathan*

## The father of India’s Green Revolution

Dr. MS Swaminathan born on August 7, 1925 in Kumbakonam, Tamil Nadu and completed post-graduate degree in Genetics and Plant breeding at the Indian Agricultural Research Institute (IARI), New Delhi during 1949. He earned his PhD from the University of Cambridge. After completing further studies at Wisconsin University, he turned down the offer of professorship and decided to return home. He said, “I asked myself, why I studied genetics? It was to produce enough food in India. So I came back.” He aimed to improve crop yields, promoting ecological sustainability, and empower small farmers while integrating cutting-edge technology and promoting gender equality in agriculture. His collaborative scientific efforts with Norman Borlaug, spearheading a mass movement with farmers and other scientists and backed by public policies, saved India and Pakistan from certain famine-like conditions in the 1960s that led to ‘Green revolution in India’. He developed high-yielding varieties (HYV) of wheat and rice; and later, promoted sustainable development, which he called, the ‘evergreen revolution’. He played main role in the development of the world’s first high-yielding basmati rice varieties, and also his efforts in agriculture has increased the productivity of foods like rice, wheat, gram, maize etc. He wanted to develop new and improved varieties of seeds, better farming methods, better soil and water management, and wanted to take the best of science and technology to the mostly illiterate rural masses that depended on agriculture not only for food but also for employment and income. During his tenure as Director General of the International Rice Research Institute (IRRI) in the Philippines, he developed IR-64, a rice variety that yielded up to 24% more grain than an earlier strain, IR-36. IR-64 has been grown on more than 10 million hectares worldwide and fed millions of people. This contribution made him awarded the first World Food Prize in 1987, often considered to be an agricultural Nobel Prize. Awards recognizing Swaminathan’s work include 85 honorary doctorates, the Mendel Memorial Medal in 1965, the Ramon Magsaysay Award in 1971, and the prestigious Padma Shri in 1967, Padma Bhushan in 1972, and Padma Vibhushan in 1989 and the Albert Einstein World Award of Science in 1986.

His inventions and efforts changed the agricultural system and taught us how to increase production in the same area of land. He championed technology development strategies that embodied empathy and an unwavering commitment to comprehensive progress, particularly for impoverished and food-insecure people, especially women. He recognized that genuine advancement encompassed not only science and technology but also the well-being of all, marginalized communities included. Considering his immense contributions, it is appropriate that Dr. MS Swaminathan is called the “Father of India’s Green Revolution”.

**(Dr. RM Sundaram)**
President, SARR, Hyd

# Journal of Rice Research - Author Guidelines

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

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

## General Requirement:

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

## Submission of Manuscript:

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

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

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

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

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

### Research papers

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

### Thesis

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

Book chapter

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

**Book**

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

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

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

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