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Image-Based Phenotyping of Diverse Rice Genotypes under different Nitrogen Treatments

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ABSTRACT: The current study provides a thorough investigation into the phenotypic characteristics of five rice genotypes and explores the possibility of using nano clay polymer/biopolymer composite (NCPC/NCBPC) based nitrogen fertilizers. A greenhouse experiment was conducted using five rice genotypes (Swarna, PB-1, Pusa-44, MTU-1010 and Nagina-22) employing five nitrogen treatments (T1-control (without N fertilizer), T2- 100% RDF of N through urea, T3- 75% RDF of N through urea loaded NCPC, T4- 75% RDF of N through urea loaded NCBPC-I (NCBPC prepared with wheat flour), T5- 75% RDF of N through urea loaded NCBPC-II (NCBPC prepared with maida)). Advanced phenomic techniques, utilizing non-invasive sensors and computer platforms, were employed for precise phenotyping. The results reveal significant variations in plant height, leaf area, and convex hull area across different genotypes and nitrogen treatments. Notably, NCPC treatment showed superior performance, followed by NCBPC-II, NCBPC-I and urea, highlighting its potential to improve plant growth. The study highlights the importance of understanding morphological traits for the development of nitrogen-efficient rice varieties and the potential of nano clay polymer based nitrogen fertilizers.

Keywords: Nitrogen use efficiency, Genotype, Phenotype, Polymer, Sensors.

INTRODUCTION

Rice is one of the staple food crops for roughly 50% of the world's population. In order to meet the demands of the expanding global population, rice production must increase by 70% by the year 2050. After wheat, it is the cereal that is consumed the most widely worldwide (Kumar *et al*., 2016). More than 65% of Indians eat rice as a staple food, and it produces about 40% of all food grains, playing a crucial role in the food and livelihood security of families (Pathak *et al*., 2020). Because of improved rice varieties, more sophisticated cultivation methods, and higher fertilizer inputs, rice yield has steadily increased despite the dire situation of increasing populations and decreasing cultivated area contradictions (Liu *et al*., 2013). Because nitrogen (N) is a macronutrient that is crucial for plant development and growth and plays a role in cell structure and energy metabolism, the variation in N uptake by rice has been extensively studied, but variations in the morphology of rice plants that may contribute to this variation are not fully understood (Li *et al*., 2017). It is a difficult task to manage fertilizer N as it's a mobile nutrient in soil-plant system, so that a variety of techniques are used, both individually and collectively. Firstly, the use of nitrogen efficient rice varieties could reduce excessive N input without sacrificing yields. However, the plant traits associated with N-efficient rice varieties have not been fully defined or comprehensively explored (Zhu *et al*., 2022). Second aspect to increase N use efficiency is the development of efficient nitrogen fertilizers. The application of nano clay polymer composites is an option which serves a reservoir of loaded nutrient as well as water. These composites enhance the use efficiency of applied N fertilizers by holding N for extended periods (Liang and Liu 2007). However, in rice, the relations between these efficient N fertilizer sources and N efficient genotypes have yet to be identified.

While nitrogen uptake in rice has been extensively studied, the influence of plant morphology on this process remains a subject of exploration. An understanding of the shoot and root traits associated with high nitrogen absorption and utilization is very important in the development of N-efficient varieties in crop breeding programs (Xin *et al*., 2022) and to study the effect of N-efficient fertilizers. Therefore, in present study we tried to explore this area using computer platforms and non-invasive sensors in Phenomics facility. Phenomics is an evolving trans-discipline comprising the methodical study of phenotypes which uses different type of sensors simultaneously, to develop efficient data which can be interpreted for plant's various inter related properties (Pratap *et al*., 2019). Phenomics uses advanced computer platforms and non-invasive sensors for non-destructive and preciously phenotyping a large number of genomes.

MATERIAL AND METHODS

A. Details of greenhouse experiment

Surface soil (0–15 cm, Typic Haplustepts) was collected from Research farm of Indian Agricultural Research Institute (IARI), New Delhi for greenhouse experiment studies. The experiment was conducted using five rice genotypes *ie.*, Swarna, Pusa Basmati-1 (PB-1), Pusa-44, MTU-1010 and Nagina-22 at Nanaji Deshmukh Plant Phenomics Centre, IARI, New Delhi. The experiment was conducted during Kharif 2020 with a natural day light of 12h and a photoperiod of 14h. The temperature, inside the green house was maintained 32°C at day and 28°C at night. The mean light intensity during the experiment was 1500 µmol m^{-2} s^{-1} at plant level. Total five different treatments were selected for evaluation under pot culture experiment using completely randomized design. Treatments combination were - T1- control (without N fertilizer), T2- 100% RDF of N through urea, T3- 75% RDF of N through urea loaded NCPC, T4- 75% RDF of N through urea loaded NCBPC-I (NCBPC prepared with wheat flour), T5- 75% RDF of N through urea loaded NCBPC-II (NCBPC prepared with maida). Nano clay polymer/biopolymer composites (NCPCs/NCBPCs) were synthesized by polymerization reaction of partially neutralized acrylic acid, acrylamide, and bentonite clay (10 wt.%) with varying concentration of starch *viz.*, wheat flour and Maida at 20% by weight (Saurabh *et al*., 2019).

B. Image acquisition and image analysis

Platform imaging in the phenomics facility was carried out at regular intervals. The imaging was carried out using a Scan analyzer 3D imaging system (Lemna Tec GmbH, Aachen, Germany) (Golzarian *et al*., 2011). The RGB colour images each of 4384*6576 (28 mega pixel) were taken from the top (TV) of the plant and the images were processed using Lemna Grid Software (Lemna Tec GmbH, Aachen, Germany). Plant leaf area was estimated by processing the images taken under visible light spectrum. The RGB images particularly pixels above mean horizontal line were used to determine plant height. The convex hull area was calculated at different stages of plant for all the treatments.

C. Statistical analysis

The data obtained from the experiment was subjected to analysis of variance appropriate to the experimental design (two factor-completely randomized designs) as given by Gomez and Gomez (1984). All the data were statistically analyzed following computer package OPSTAT.

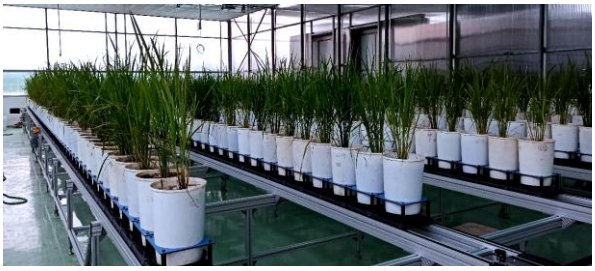

Fig. 1. Experimental conditions.

RESULTS AND DISCUSSION

A. Plant height

At the earliest stage of growth, all chosen treatments had plants that were noticeably taller than controls while remaining at par with each other (Table 1). The NCPC treatment had the highest recorded plant height at the final stage (94.4 cm), significantly higher than all other treatments. Treatments NCBPC-II (90.9 cm), NCBPC-I (90.1 cm) and urea (88.0 cm) were found statistically at par with each other. The use of NCPC or NCBPC regulate the release of urea and retain N in the soil for longer periods of time (Saurabh *et al*., 2021) which provides more opportunity to plant to take N from soil which may be attributed to increased overall plant growth. Higher N absorption and utilization, increase the photosynthetic activity, so that N is quickly assimilated by rice plants to accelerate their growth (Sui *et al*., 2013). The highest plant height (58.1 cm) among the varieties at the first growth stage was observed in N-22, which was significantly higher than all other genotypes. PB-1 and MTU-1010, Swarna and Pusa-44, were found to be statistically at par at this point. At the final stage (70 DAT), N-22 recorded the significantly highest height ((97.4 cm) followed by MTU-1010. In terms of plant height, Pusa-44 and PB-1 were found to be comparable. Swarna was recorded with significantly lowest plant height (74.7 cm) among all the selected genotypes. Interaction among varieties and treatments was recorded insignificant at all growth stages. Overall increase in plant height is the combined effect of genotype (Zou *et al*., 2005) and N treatment (Gawdiya *et al*., 2023).

B. Plant leaf area

The statistical analysis after image processing revealed that, at first growth stage all the treatments were found significantly higher over control but at par with each other in terms of leaf area. At final growth stage (70 DAT) highest leaf area was recorded under NCPC treatment (Table 2). The treatment NCBPC-II was significantly higher than urea but at par with NCBPC-I treatment. Among varieties at first growth stage significantly highest leaf area was recorded in PB-1 and this trend was continuing till 70 DAT. Our results were in agreement with Gawadiya *et al*. (2023), where they reported improved overall agro-morphological performance including leaf area index and crop biomass.

Varieties Pusa-44, N-22 and MTU-1010 were found statistically at par with respect to leaf area at first growth stage. At 70DATsignificantly maximum leaf area was recorded with PB-1 followed by MTU-1010, Pusa-44, Swarna and N-22. It might be due to genetic makeup of variety. Knops and Reinhart (2000) also studied effect of nitrogen fertilization on leaf area of different grass species and reported increased specific leaf surface area with increasing level of nitrogen fertilization.

C. Convex hull area

At first stage the NCPC treatment was recorded with significantly higher convex hull area followed by NCBPC-II treatment and the trend continues till 40 DAT stage (Table 3). At final growth stage significantly highest hull area was obtained in NCPC treatment followed by NCBPC-II treatment. Convex hull is the area of smallest convex polygon to enclose plant canopy. For a bounded subset of the plane, the convex hull may be visualized as the shape enclosed by a rubber band stretched around the subset (Vishal *et al*., 2020). Among varieties, at 70 DAT stage statistically higher hull area was obtained in PB-1 followed by N-22. The lowest hull area was recorded in Pusa-44 which was statistically at par with Swarna. Swarna was statistically at par with MTU-1010 in terms of convex hull area. Rakshitha (2019) also studied the effect of nitrogen fertilization on convex hull area of rice and reported that nitrogen application through NCPC is recorded with high convex hull area as compared to conventional N sources (UAN and Urea).

Table 1: Effect of different nitrogen fertilizer treatments on plant height (cm) of five rice genotypes at different growth stages.

	30 DAT	40 DAT	55 DAT	70 DAT
Treatment				
Control	44.6	51.5	70.1	77.2
Urea	51.0	63.2	78.3	88.0
NCPC	53.8	67.9	83.7	94.4
NCBPC I	52.4	65.7	80.0	90.1
NCBPC II	52.3	66.5	81.0	90.9
SEm (±)	1.18	1.17	1.72	1.09
CD (5%)	3.36	3.33	4.89	3.10
Variety				
Swarna	45.0	51.2	65.9	74.7
Pusa-44	47.4	59.2	77.8	86.8
PB-1	51.6	63.0	79.9	89.0
MTU 1010	52.1	61.1	79.3	92.7
Nagina-22	58.1	80.3	90.1	97.4
SEm (±)	1.18	1.17	1.72	1.09
CD (5%)	3.36	3.33	4.89	3.10

*DAT = Days after transplanting

Table 2: Effect of different nitrogen fertilizer treatments on visible leaf area (pixels/plant) of five rice genotypes at different growth stages.

	30 DAT	40 DAT	55 DAT	70 DAT
Treatment				
Control	49691	58425	102069	123233
Urea	72212	78325	135680	174445
NCPC	76558	86576	158578	198907
NCBPC I	74153	82414	146256	179168
NCBPC II	75191	84640	148296	184025
SEm (±)	1572	1703	2483	3361
CD (5%)	4479	4852	7074	9575
Variety				
Swarna	62931	70547	136453	178158
Pusa-44	67660	74826	122432	165144
PB-1	82928	91856	172439	190004
MTU 1010	68164	78667	129235	168766
Nagina-22	66122	74485	130321	157706
SEm (±)	1572	1703	2483	3361
CD (5%)	4479	4852	7074	9575

Table 3: Effect of different nitrogen fertilizer treatments on visible convex hull area (pixels/plant) of five rice genotypes at different growth stages.

	30 DAT	40 DAT	55 DAT	70 DAT
Treatment				
Control	318730	402624	787916	1017724
Urea	422873	547956	1047583	1296350
NCPC	523154	628977	1286737	1652448
NCBPC I	465798	566380	1126731	1419522
NCBPC II	493769	613151	1185172	1515877
SEm (±)	7613	1703	17626	27310
CD (5%)	21690	30096	50215	77805
Variety				
Swarna	391813	431203	946187	1321314
Pusa-44	347115	464398	881567	1264040
PB-1	512948	719949	1319642	1523561
MTU 1010	459231	575135	1113239	1350035
Nagina-22	513217	568404	1173503	1442971
SEm (±)	7613	1703	17626	27310
CD (5%)	21690	30096	50215	77805

CONCLUSIONS

The plant height, leaf surface area and convex hull of a plant is an inherent trait for image-based phenotyping which can be the basis for screening of rice genotypes and monitoring the development of rice plants. This study also delves into the potential of clay polymer composites as slow release nitrogen fertilizers where NCPC/NCBPC based nitrogen fertilizers better performed over the conventional urea fertilizer in terms of plant growth and development. This study holds great promise for future advancements in sustainable agriculture, offering the potential for more efficient, high-yielding rice varieties and development of more efficient nitrogen fertilizers that can address global food security challenges.

FUTURE SCOPE

The current investigation provides insights into the short-term effects of Nano clay biopolymer fertilizers on plant growth. However, field studies are still needed. The identification of key morphological characteristics associated with superior nitrogen use efficient genotypes of various field crops beyond rice is needed for targeted breeding programs, potentially revolutionizing agricultural production on a global scale.

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Conflict of Interest. None.

REFERENCES

Gawdiya, S., Kumar, D., Shivay, Y. S., Bhatia, A., Mehrotra, S., Chandra, M. S. and Sutton, M. A. (2023). Field-Based Evaluation of Rice Genotypes for Enhanced Growth, Yield Attributes, Yield and Grain Yield Efficiency Index in Irrigated Lowlands of the Indo-Gangetic Plains. *Sustainability*, *15*(11), 8793.

Golzarian, M. R., Frick, R. A., Rajendran, K., Berger, B., Roy, S., Tester, M. and Lun, D. S. (2011). Accurate inference of shoot biomass from high-throughput images of cereal plants. *Plant methods*, *7*, 1-11.

Gomez, K. A., and Gomez, A. A. (1984). Statistical Procedures for Agricultural Research. John Wiley & Sons.

Knops, J. M. and Reinhart, K. (2000). Specific leaf area along a nitrogen fertilization gradient. *The American Midland Naturalist*, *144*(2), 265-272.

Kumar, S., Dhandapani, R., Sahoo, R. N. and Viswanathan, C. (2016). Phenomics: Unlocking the hidden genetic variation for breaking the barriers in yield and stress tolerance rice. *Indian Journal Plant Physiology, 21*(4), 409–419.

Li, H., Hu, B. and Chu, C. (2017). Nitrogen use efficiency in crops: Lessons from Arabidopsis and rice. *J. Exp. Bot.*, *68*, 2477–2488.

Liang, R. and Liu, M. (2007). Preparation of poly (acrylic acid-co-acrylamide)/kaolin and release kinetics of urea from it. *Journal of Applied Polymer Science, 106*(5), 3007-3015.

Liu, X., Zhang, Y., Han, W., Tang, A., Shen, J., Cui, Z., Vitousek, P., Erisman, J.W., Coulding, K. and Christie, P. (2013). Enhanced nitrogen deposition over China. *Nature*, *494*, 459–462.

Pathak, H., Tripathi, R., Jambhulkar, N. N., Bisen, J. P. and Panda, B. B. (2020). Eco-regional based Rice Farming for Enhancing Productivity, Profitability and Sustainability. *NRRI Research Bulletin*, 22; pp 28.

Pratap, A., Gupta, S., Nair, R. M., Gupta, S. K., Schafleitner, R., Basu, P. S. and Mishra, A. K. (2019). Using Plant Phenomics to Exploit the Gains of Genomics. *Agronomy*, *9*(3), 126.

Rakshitha, K. R. (2019). Effect of nitrogen applied through nano clay polymer composites on phenotypic changes and nitrogen use efficiency in elite rice genotypes. M.Sc. thesis. Division of Soil Science and Agricultural Chemistry ICAR-Indian Agricultural Research Institute New Delhi.

Saurabh, K., Manjaiah, K.M., Datta, S. C., Thekkumpurath, A. S. and Kumar, R. (2019). Nanoclay polymer composites loaded with urea and nitrification inhibitors for controlling nitrification in soil. *Archives of Agronomy and Soil Science, 65*(4), 478-491.

Saurabh, K., Manjaiah, K.M., Datta, S.C., Biswas, D.R., Bhatia, A., Bandyopadhay, K. K. and Tomar, R. (2021). Mitigating nitrous oxide emission using nanoclay-polymer composites in rice-wheat cropping system. *Archives of Agronomy and Soil Science*, *67*(4), 459-473.

Sui, B., Feng, X., Guangli, T., Hu, X., Shen, Q. and Guo, S. (2013). Optimizing nitrogen supply increases rice yield and nitrogen use efficiency by regulating yield formation factors. *Field Crops Res*., *150*, 99–107.

Vishal, M. K., Tamboli, D., Patil, A., Saluja, R., Banerjee, B., Sethi, A. and Adinarayana, J. (2020). Image-based phenotyping of diverse rice (*Oryza Sativa* L.) genotypes. *arXiv preprint arXiv:2004.02498*.

Xin, W., Zhang, L., Zhang, W., Gao, J., Yi, J., Zhen, X. and Chen, L. (2022). Morphological and Physiological Characteristics of Rice Cultivars with Higher Yield and Nitrogen Use Efficiency at various Nitrogen Rates. *Agronomy*, *12*(2), 358.

Zhu, K. Y., Yan, J. Q., Yong, S. H. E. N., Zhang, W. Y., Xu, Y. J., Wang, Z. Q., and Yang, J. C. (2022). Deciphering the morpho–physiological traits for high yield potential in nitrogen efficient varieties (NEVs): *A japonica* rice case study. *Journal of Integrative Agriculture*, *21*(4), 947-963.

Zou, J., Chen, Z., Zhang, S., Zhang, W., Jiang, G., Zhao, X., Zhaim, W., Pan, X. and Zhu, L. (2005). Characterizations and fine mapping of a mutant gene for high tillering and dwarf in rice (*Oryza sativa* L.). *Planta*, *222*, 604–612.

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