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Research Article

ASSESSMENT OF GENETIC DIVERSITY FOR ZINC DEFICIENCY TOLERANCE IN RICE (*Oryza sativa* L.) BASED ON AGRO-MORPHOLOGICAL TRAITS UNDER WATER-LOGGED CONDITION

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Abstract: Zinc deficiency is one of the important micronutrient deficiencies of rice which is widespread and causes significant yield loss in severe conditions. Evaluation of the local germplasm in target environment is a promising strategy to understand the genetic diversity for zinc deficiency tolerance and to identify potential donors. Hence, in the present study, a set of 135 accessions comprising of local landraces and improved varieties of rice were assessed for zinc deficiency tolerance under zinc deficiency in submerged conditions using zinc deficiency score and yield related morphological traits like days to fifty percent flowering, number of productive tillers, spikelet fertility percent and single plant yield. Significant variation was observed for all the traits studied. The association among the traits revealed that Zinc deficiency score is negatively correlated with plant height (-0.272**), spikelet fertility percentage (-0.358**) and single plant yield (0.197*). The hierarchical clustering analysis by UPGMA grouped 135 germplasm accessions into eight clusters and identified significant outliers such as Mapillai Samba, Senkar and Rasagadam. The landraces like Karuppu Nel and Manipur Local which recorded higher yield under zinc deficiency can be used in further studies to understand their mechanism of tolerance.

Keywords: *Zinc, Rice, Landraces, Germplasm screening, Target environment*

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Introduction

Rice (*Oryza sativa* L.) is the staple food crop for about half (nearly 3.5 billion) of the world's population and constitutes one of the four crops primarily produced in the world and is the source of sustenance and livelihood for millions of farmers in the developing countries. Approximately 850 million tons to feed 5 billion rice consumers in the world requiring at least 1.1 per cent yield increase every year [1], which urges to bridge the gap between the potential yield and the actual yield [2]. This yield gap is caused by various external factors such as drought, flooding, salinity, low soil fertility, pests and diseases which often prevent crops from reaching their true yield potential [3]. Low soil fertility is the result of deficiency of one or more nutrients essential for the growth of plants. Of the various micronutrient deficiencies in rice, Zinc deficiency is widespread [4] and it affects more than 50 per cent of the rice production worldwide [5].

Zinc is an essential micronutrient, which serves as a co-factor for more than 300 enzymes involved in the metabolism of carbohydrates, lipids, proteins, and nucleic acids [6] and in rice, its deficiency causes bronzing of leaves, stunting and mortality in severe conditions, chiefly during the seedling stage or immediately after transplanting. Though the plant may recover around 4-6 weeks after transplanting, delay in maturity and reduction in yield are unavoidable [7].

Zinc deficiency is more common in lowland rice which are cultivated in submerged condition [8], as flooding reduces the availability of Zinc to plants by changing many aspects of soil chemistry [9]. Though zinc deficiency can be temporarily overcome by zinc fertilizers, the high cost associated with its application places considerable burden on resource poor farmers [10]. Moreover, zinc fertilizers applied to paddy soils are not effectively utilized as under continuous submergence, zinc forms insoluble complexes in soil and becomes unavailable for plant uptake [11]. Hence, breeding of rice cultivars that extract or metabolize Zinc more efficiently under zinc deficiency is therefore a more promising strategy and a sustainable solution to address Zinc deficiency limitations in crop production, rather than fertilization [12]. Rice possesses a vast reserve of germplasm, which could be used to exploit the genotypic differences in Zinc uptake and tissue-use efficiency, by screening for zinc deficiency tolerance [13]. Many authors have reported the existence of noticeable genetic variation among the rice genotypes in the ability to grow under zinc deficient soils [14,15].

Since zinc deficiency is a complex trait involving various factors like soil pH, redox potential, which may require different genotypes to develop unique mechanisms to tolerate zinc deficiency [16], the local germplasm which evolved independently in various agro-climatic zones, could be screened in the target environment to identify potential donors and further to understand their tolerance mechanism. A recent study to undermine the genes involved in zinc deficiency tolerance was conducted by transcriptome profiling of extreme genotypes under zinc deficiency [17] indicating the importance of screening germplasm accessions. With this background, the study was carried out to understand the extent of genetic diversity present in the available local rice germplasm for zinc deficiency tolerance based on the morphological traits related to yield by screening them under zinc deficiency in submerged condition.

Materials and Methods

A set of 135 local germplasm including local landraces and popular varieties and few breeding lines were collected from various places of India, predominantly from Tamil Nadu and screened for zinc deficiency tolerance under zinc deficiency in submerged conditions and assessed for their performance based on the Zinc Def score, yield and yield attributing morphological traits. To study genetic diversity under zinc deficiency, the germplasm entries were raised in augmented block design with 135 test entries and 4 checks *viz*., Savulu Samba, Kotta Nel, Paiyur 1 and ADT 39 were used at Regional Research Station, Paiyur, TamilNadu, India.

Table-1 *Descriptive statistics of 135 accessions under zinc deficiency*

Variable	Min	Max	Mean (± SE)	StdDev	Skewness	Kurtosis	Shapiro-Wilks Statistics	
							W Value	Pr(<W)
ZINCS	1.00	9.00	4.70±0.22	2.52	0.2186	-1.0930*	0.91	0.0000
DFF	70.00	187.00	100.81±2.06	23.94	1.3081*	1.6275*	0.88	0.0000
PLHT	71.00	207.00	109.06±1.88	21.86	1.0417*	2.1526*	0.94	0.0000
NPT	0.0	29.67	14.71±0.36	4.20	0.5847*	0.8990*	0.97	0.0028
SFP	0.0	98.92	77.73±0.87	10.16	-1.4842*	4.9611*	0.90	0.0000
SPY	0.0	34.67	17.60±0.63	7.28	0.4000	-0.5435	0.97	0.0059

Table-2 *Correlation among the six characters for zinc deficiency under submerged conditions in 135 accessions*

	ZINCS	DFF	PLHT	NPT	SFP	SPY
ZINCS	1.000					
DFF	-0.071	1.000				
PLHT	-0.272**	0.314**	1.000			
NPT	-0.066	0.090	-0.155	1.000		
SFP	-0.358**	0.006	0.069	0.194*	1.000	
SPY	-0.197*	0.013	-0.020	0.427**	0.282**	1.000

Table-3 *Clustering of 135 germplasm accessions*

Cluster	Number of genotypes	Genotypes
Cluster1	86	ACM 0101, Dodda Byra Nel, Sivappuchithiraikar, Murugan Kar, Sembilipiriyan, Avasarasamba, Karungan, Kuzhi vedichan, Vellai Chithirai Kar, Chinna Aadukkunel, Kattanur, Kaliyan Samba, Poongar, Manavari, BAM 540, Chetty Samba, Mattai kar, Shenmolagai, TKM 11, Co 45, Ramakuruvaikar, Co48, Bharathi, TKM 2, ADT 49, Andra Sannam, Kudaivazhai, Vellaikattai, IR 64, ACM 07001, TKM 5, ADT 45, TKM 9, Bg 367-2, IR 36, TKM 6, Vellai Charai Kar, Varappu Kudaincahn, ASD 16, IR 66, Athira, JGL 1798, Columbia 2, Arupadham Kuruvai, PTB 10, Co 20, Kuliadichan, Sigapu Kuruvaikar, Njavara, ADT 37, ASD 17, IR 10, MDU 5,ADT 43, ASD 18, ADT 47, ASD 14, CB 06-563, RMD 1, Chandikar, TN 1, BAM 271, IR 42, Kakarathan, Mikuruvai, Katta Samba, Malayalathan Samba, Saranga, ADT 38, Swarna 2, Co 44, Pokkali, PB-1, Anna 4, BAM 213, Early Samba, Co 43, IR 20, PB 1460, JGL 3855, Karuthakar, Katha Nel, TRY 3, Adipu, ADT 42.
Cluster2	26	ADT 44, PY 4, ADT 46, Co 38, Co 42, Raj Shree, ADT 6, Norungan, BPT 5204, BAM 440, CR 1009, Mara Batta, Jeevan Samba, Vadakuthi Samba, Kavuni, Varakkar, Vellai Kudaivazhai, Kattu Kuthalai, Varigarudansamba, Ottadaiyan, Co 23, Salivahaiva, TKM 1, TKM 3, Manipur Local.
Cluster3	14	ASD 1, Basmathi 370, Co 13, ASD 2, Matta Kuruvai, ASD 7, ASD 8, Co 9, Veethi Vedangan, Co 46, PKM 2, PKM 1, Veerandangan, Karuppunel.
Cluster4	2	Karungan, Senkar
Cluster5	2	ASD 9, Chethunali
Cluster6	2	Co 10, T 184
Cluster7	1	Mapillai Samba
Cluster8	2	BAM 442, Co 40

The test entries were raised in 5 blocks with 27 entries in each block and all the four checks were randomly replicated in all the blocks. Seedlings were raised in the nursery bed and transplanted to zinc deficient field (Zinc content: 0.64 ppm, organic carbon: 0.52%, pH: 8.20, EC: 0.34 dS m^{-1}, Available N: 171 Kg ha^{-1}, Available P: 7.0 Kg ha^{-1}, Available K: 223 Kg ha^{-1}). The observations were recorded on five randomly selected plants in each accession. The data were recorded based on Standard Evaluation System of Rice (SES), (IRRI, 2002) [43]. Totally six characters *viz*., zinc deficiency score, days to fifty percent flowering, plant height, number of productive tillers, spikelet fertility percent and single plant yield were recorded. The zinc deficiency scoring at the scale of 0 to 9, was done based on visual observation on yellowing or browning of basal leaves, height and tillering ability of the plant.

Statistical Analysis

The observations recorded on six agro-morphological characters in 135 germplasm accessions were subjected to the statistical analyses. Basic descriptive statistics, variance components and frequency distribution were obtained using the MINITAB software version 17. Correlation analysis was performed using the statistical package SPSS 16.0 version. Cluster analysis was done using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method of hierarchical clustering technique and the accessions were grouped based on similarity matrix as implemented in Darwin software version 6 [18].

Results and Discussion

Zinc deficiency in rice, which is predominantly cultivated under submerged condition is a complex phenomenon, involving interaction of various plant and rhizospheric factors and it could not be overcome effectively by agronomic practices like application of fertilizers [19] and this emphasizes the need for a genetic solution. Screening of local germplasm and later on employing conventional breeding approach seems realistic in rice because large variation exists for total Zinc uptake and growth response under Zinc deficiency [20-22].

Assessment of variability for Zinc deficiency tolerance

In our present study we screened 135 germplasm accessions in a zinc deficient field under water-logged condition because zinc deficiency is more pronounced under submergence as it depletes oxygen, decreases redox potential and increases pH [23], which makes Zinc unavailable for plant uptake. The extent of variation present in the genotypes was observed by growth and yield related traits which directly implies the performance of genotype under zinc deficiency. We performed the screening for Zinc deficiency in the target environment (that is in zinc deficient field) because of its complexity involving various factors including soil properties and the interactions of rice plant at the level of rhizosphere with the rhizobiome, which could not be effectively represented in pot experiments conducted in the controlled conditions. Screening in target environment has been employed successfully to identify potential tolerant genotypes in various crops [24-26]. The variations for characters observed were assessed using basic descriptive statistics *viz*., mean, range, standard deviation, standard error, normality, skewness, kurtosis is presented in [Table-1]. Significantly high variation was exhibited by all the traits observed. The accessions exhibited the least to maximum zinc deficiency scores, indicating the extent of tolerance available. Days to fifty percent flowering among 135 germplasm accessions ranged from 70 to 187. Mean days to fifty percent flowering was 100.81. Minimum days to flowering was recorded by Karuppu Nel and Chethunali took maximum of 187 days to fifty percent flowering. Plant height ranged from 71.00 cm to 207.00 cm. The mean plant height was 109.06 cm. The genotype TRY3 was the shortest and Mapillai Samba was the tallest. Number of productive tillers ranged from minimum of 0.0, was recorded by 16 genotypes which recorded zinc deficiency score 9 to the maximum of 29.67 in BAM440. The spikelet fertility percent ranged from 0.0 to 98.92 with the mean of 77.73 percent. The minimum value was recorded by 16 genotypes which recorded zinc deficiency score 9 and the maximum value by Karuppu Nel. Single plant yield ranged from 0.0 g to 34.67 g with the average of 17.28 g. The lowest value was recorded by 16 genotypes which recorded zinc deficiency score 9 and highest value by Karuppu Nel.

The zinc score ranged from 1 to 9. The mean zinc score was 4.7. Nineteen genotypes which exhibited minimum score 1 and the maximum score 9 was recorded by 16 genotypes. The variation of zinc deficiency tolerance is also documented at the molecular level [27], substantiating the variation at the field level in this study.

The Normality of the values for each character studies was tested using Shapiro-Wilks test. The W value of each character is presented in [Table-1]. All the characters exhibited Pr (<W) less than 0.05, indicating absence of normal distribution. The frequency distribution for six characters across 135 rice accessions is depicted in [Fig-1]. Days to fifty percent flowering (1.31) and plant height (1.04) exhibited highly positive skewness whereas, number of productive tillers (0.59) showed moderately positively skewness. High negative skewness was observed in spikelet fertility percent (-1.48). Significant leptokurtic distribution was noted in days to fifty percent flowering, plants height, number of productive tillers and spikelet fertility percent. Significant platykurtic distribution was observed in zinc deficiency scores.

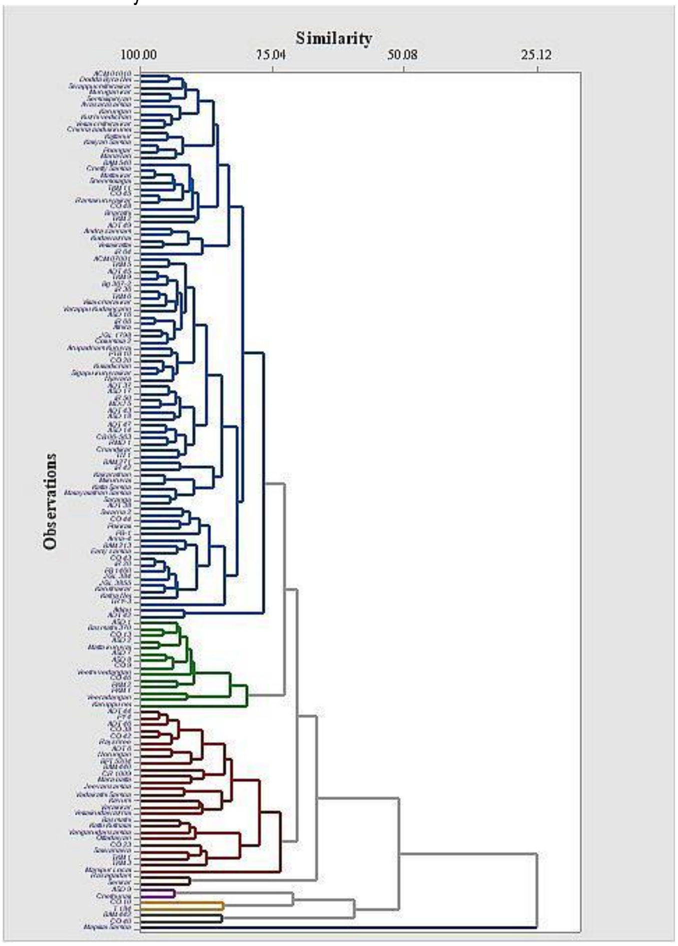

Fig-1 Frequency distribution for six characters- a. Zinc deficiency Score, b. Days to fifty percent flowering, c. Plant Height, d. Number of productive tillers, e. Spikelet Fertility percent, f. Single plant yield, across 135 rice accessions

Several studies have reported wide range of variability for zinc content in grain and tissues [28-30], whereas only few studies have recorded the performance of genotypes under zinc deficiency in field condition [31]. Agro-morphological properties of rice cultivars determine their yield potential, local agronomic suitability and ability to escape from or to tolerate biotic and abiotic stresses. They are extremely important while attempting to exploit the genetic diversity present in plant genetic resources [32,33]. It is evident from our previous study [34] that these traits proved to be able to distinguish the genotypes based on differences in their growth and yield between zinc deficient plot and zinc fertilized plot in field condition.

Zinc deficiency score was used to visually detect the extent of damage caused by zinc deficiency on the genotypes studied.

Zinc deficiency scores among the genotypes ranged from minimal score 0 to maximum score 9, indicating the varying ability of the accessions to tolerate zinc deficiency. Quijano-Guerto *et al*. (2002) classified various breeding lines as tolerant and susceptible lines based on their mean scores over years based on data obtained from independent field trials. Impa *et al*. (2013) reported that Zinc deficiency-induced leaf symptom development and severity were closely related to the Zinc supply in Agar Nutrient Solution (ANS), indicating the importance of scoring in understanding the response of the genotypes to zinc deficiency. Besides these, there are other studies where zinc deficiency score was used to assess the extent of zinc deficiency and its effect on plant growth [35,36].

Correlation of traits under zinc deficiency

Simple correlation analysis suggests that all the characters studied showed negative association with zinc deficiency scores. However, plant height (-0.27**) and spikelet fertility percent (-0.36**) exhibited highly significant negative association and single plant yield (0.20*) recorded significant negative association with zinc deficiency scores. The results are presented in [Table-2]. Single plant yield showed highly significant positive associations with number of productive tillers (0.43**) and spikelet fertility percent (0.28**).

Zinc deficiency scoring of germplasm accessions

Among the accessions, Karuppu Nel, Manipur Local, BAM 440 and IR42 exhibited lowest score coupled with high single plant yield. Karuppu Nel also exhibited maximum spikelet fertility percent. BAM 440 with zinc deficiency score 1.0 recorded maximum number of productive tillers. Chethunali took maximum days to fifty percent flowering. It also recorded low spikelet fertility percent and low single plant yield coupled with high zinc deficiency score of 7. Senkar which recorded the minimum number of productive tillers has the zinc deficiency score of 9.0. It also recorded low single plant yield. Rasgadam which recorded lowest spikelet fertility percent exhibited zinc deficiency score 9.0. Genotypes with higher zinc deficiency scores are often poor in growth, resulting in yield loss. These results re-emphasize the importance of zinc for the growth of plants.

Yakan *et al*. (2000) [37] revealed that with Zinc application, there was increase in rice yield and number of panicles by square meter and decrease in the days to maturity, spikelet sterility. It can also be inferred that these traits can be used for selection under zinc deficiency. Wissuwa *et al*. (2006) [38] has reported that plants in the unfertilized treatment plots showed typical symptoms of Zinc deficiency, such as stunting, reduction in total dry matter, high plant mortality, and severe leaf bronzing and the symptoms were indeed due to Zinc deficiency which was evident from a reduction in Zinc content per plant of more than 90 per cent relative to the fertilized control. Single plant yield under zinc deficiency in submerged condition is dependent mainly on number of productive tillers and spikelet fertility percentage. Most of the genotypes which displayed high performance under zinc deficiency such as Karuppu Nel and Manipur Local are landraces. Similar results were obtained where the landraces outperformed modern varieties of rice when screened on P-deficient soils [39,40]. The landraces and traditional varieties which are cultivated in their niche might still have the genes conferring tolerance to nutrient deficits in the soil and selected in the target environment whilst the high yielding modern varieties which are selected in the high-input conditions in research farms.

Clustering of germplasm accessions under zinc deficiency

The hierarchical cluster analysis by UPGMA grouped 135 germplasm accessions into eight clusters. The details of genotypes grouped under each cluster are presented in [Table-3.] The dendrogram is presented in [Fig-2]. The grouping of accessions into different clusters can be used to select the genotypes from different clusters to be used as parents in the breeding programs. According to Aliyu *et al*. (2000) [41], cluster analysis had the singular efficacy and ability to identify crop accessions with the highest level of similarity using the dendrogram. Cluster analysis of 135 accessions using hierarchical clustering method revealed 8 clusters. Cluster I and II were in close proximity with each other and didn't differ much in their average values. Cluster IV and V consisted of genotypes which were grouped based on similar plant height and days to fifty percent flowering.

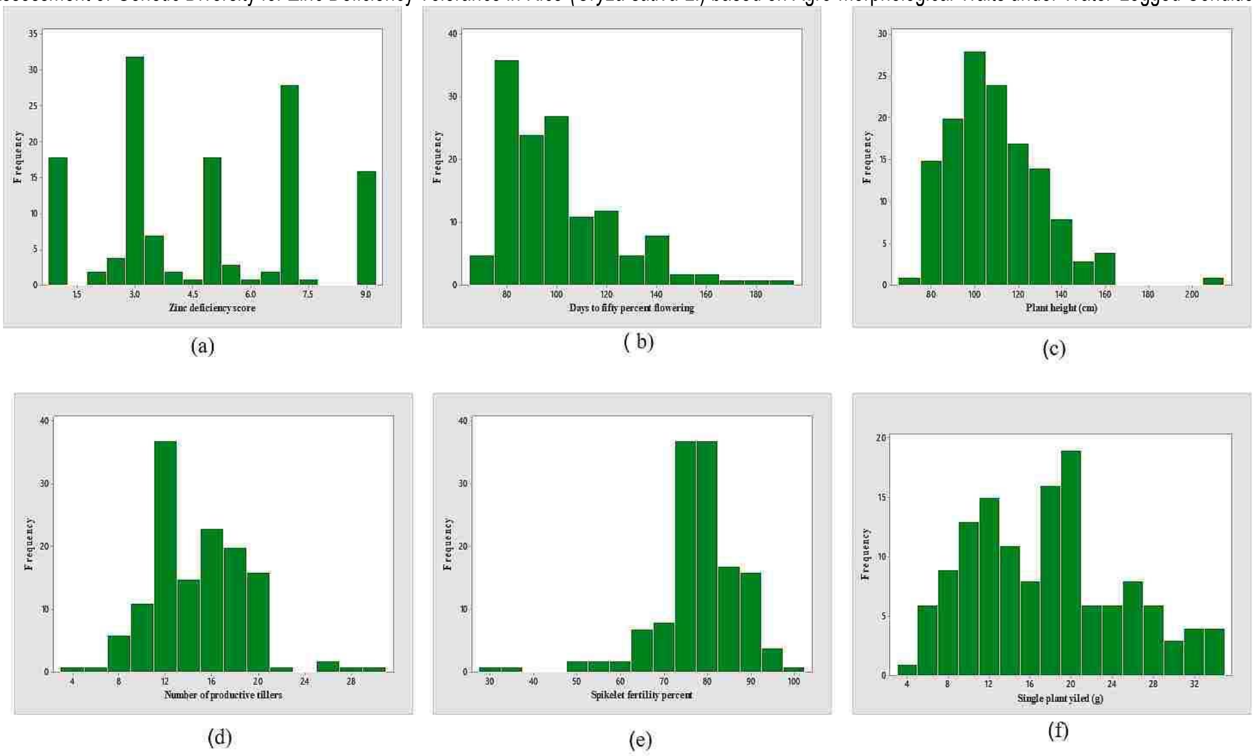

Fig-2 Dendrogram based on morphological characters in 135 rice germplasm accessions

Zinc deficiency was found to play a major role in clustering along with morphological traits. Worede *et al*. (2014) [42] had also reported clustering based on flowering, plant type and yield traits in 24 rice genotypes for 17 traits. Cluster analysis identified certain outliers and grouped them in separate clusters. The genotypes Rasagadam and Senkar which were poorest performers under zinc deficiency with lowest scores along with low productive tillers, spikelet fertility and single plant yield were grouped together in a cluster. The genotypes which recorded high score coupled with high single plant yield such as Karuppu Nel and BAM 440 are placed in the Cluster I. These extreme genotypes can be studied further to understand their mechanism of tolerance [43,44].

Conclusion

In this study, the existence of wide genetic variation among the 135 germplasm accessions for Zinc deficiency scores and the yield related morphological traits have been identified. The negative association of zinc deficiency score with single plant yield suggests that selection for zinc deficiency tolerance could be practised based on yield as a method of direct phenotyping in target environment. The cluster analysis identified the extreme genotypes including KaaruppuNel and BAM 440, which yielded high despite the zinc deficency could be used as potential donors for developing zinc deficiency tolerant rice varieties and to understand its genetic basis.

Application of research: The high performance of certain landraces identified in this study reassures the necessity to utilize local germplasm for zinc deficiency tolerance and similar soil-based problems in rice.

Research Category: Genetic Diversity

Abbreviations: ZINCS-Zinc Deficiency Score, SPF-Spikelet Fertility Percentage, DFF-Days to Fifty percent Flowering, SPY-Single Plant Yield, PLHT-Plant Height NPT-Number of Productive Tillers

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Study area / Sample Collection: Tamil Nadu Rice Research Institute, Aduthurai, 612101

Cultivar / Variety / Breed name: Rice (*Oryza sativa* L.)

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Ethical Committee Approval Number: Nil

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