*Biological Forum – An International Journal* (SI-AAEBSSD-2021) 13(3b): 201-204(2021)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

# Molecular Characterization and Phenotypic Validation of Rice Genotypes for Nutrient Use Efficiency

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*(Received 01 July 2021, Accepted 01 October, 2021)*
*(Published by Research Trend, Website: www.researchtrend.net)*

**ABSTRACT: Enhancement of rice productivity with low inputs not only reduces cost of cultivation but also improves soil and human health. Nitrogen and phosphorous are important macro nutrients of chemical fertilizers. Breeding of nutrient use efficient genotypes is complex influenced by soil and environmental parameters .Use of molecular markers is one of the best strategies for breeding programmes and selection of high yielding nutrient use efficient genotypes. Present study aimed to screen 350 rice genotypes using Nitrogen use efficiency (NUE) and Phosphorous uptake (*Pup1*) QTL linked markers. Allelic variation in expression of positive alleles for both Nitrogen and phosphorous use efficiency was observed. Only 13.14% of genotypes (Swarna, MTU 1061, Swarnasub1, Non-abokara, MTU 1229, MTU 1190, MTU 1226) were identified as nutrient use efficient for both Nitrogen and Phosphorous after molecular characterization and phenotypic validation. The future research has to be focused on breeding nutrient use efficient genotypes by novel approaches.**

**Keywords:** Rice, Molecular characterization, NUE, PUE, phenotypic validation

## INTRODUCTION

Rice production has increased globally and nationally by three folds which inturn raised cost of cultivation by utilization of excess fertilizers and pesticides. Excess fertilizer application not only enhances cost of cultivation but also contributing to climate change by emission of nitrous oxide (Tian *et al*., 2020) and deteriorating soil health. In India, 29% of fertilizers were consumed for rice cultivation in 2014-15 (Patrick *et al.,* 2017). Identification and development of nutrient use efficient genotypesare essential to realize higher yields with low inputs. The inherent nutrient management of efficient genotypes without yield penaltyis inevitable for climate resilience in case of extreme events of floods, drought and salinity (Ali *et al*., 2018). Besides curtail in cost of cultivation, nutrient use efficient genotypes can improve soil health by minimizing use of chemical fertilizers. Breeding and identification of nutrient use efficient genotypes is limited by complexity of heterogeneous nature of soil and physiochemical properties. Exploitation of genetic variability using biotechnological tools is one of the strategies for breeding nutrient use efficient varieties of rice (Ali *et al*., 2018; Neeraja *et al*., 2019; Chithrameenal *et al*., 2018; Sajid *et al*., 2021).

Molecular markers are powerful tools to detect presence of nutrient use efficient alleles for Nitrogen and phosphorous. Use of molecular markers linked to QTLs of Low Nitrogen (Shi-kai *et al.*, 2012), Nitrogen use efficiency (NUE) genes GS1 and AspAt3m (Liu *et al*., 2016) would helpin identification of nutrient use efficient genotypes. Identified QTLs for yield traits under low nitrogen by screening 800 rice genotypes under low and recommended nitrogen for two consecutive seasons for use in marker assisted breeding for Nitrogen use efficiency (Neeraja *et al*., 2019). Thirty four N responsive genes associated with NUE would help in marker assisted breeding (Narendra *et al*., 2021).

Genotypes performing well under p deficient soils can be identified using Phosphorous uptake (*Pup1*) gene linked markers on chromosome 12 (Chin *et al*., 2009) and the *Phosphorus uptake 1* (*Pup1*) major QTL for tolerance of P deficiency is an enhancer of root growth (Gamuyao *et al.*, 2012). Eleven P tolerant genotypes under p deficient soils were identified out of 168 genotypes by screening using *Pup1*gene linked markers (Sompong *et al.*, 2019), three genotypes with high p uptake were identified out of 31 genotypes (Sarkar *et al*., 2011) and Phosphorous deficiency tolerant genotypes were identified out of 30 genotypes (Aluwihare *et al*., 2018). Important candidate mechanism for phosphorous use efficiency was detected (Suresh *et al*., 2021) and phosphorous starvation tolerance gene OsPSTOL1 gene was incorporated into genetic stocks ASD 16 and ADT 43 through marker assisted breeding (Chithrameenal *et al*., 2018). Hence present study aimed to identify nutrient use efficient genotypes using molecular markers linked to Nitrogen and Phosphoroususe efficiency QTLs.

## MATERIALS AND METHODS

Forty eight rice genotypes were screened for two years during wet season of 2015 and 2016 for Nitrogen and phosphorous use efficiency in augmented design using Swarna (MTU 7029) and Indra (MTU 1061) as check varieties in four blocks at Regional Agricultural Research Station (RARS), Maruteru of Acharya NG Ranga Agricultural University (ANGRAU) and pooled data subjected to analysis using PB tools. These genotypes were characterized using nitrogen and phosphorous use efficiency QTL linked markers.

Four SSR markers linked to QTLs for nitrogen use efficiency RM 1111 on chromosome 8 (Shi-kai *et al.*, 2012), RM 5639, RM 5748 on chromosome 6, RM 3628 on chromosome 3 (Liu *et al*., 2016) and four INDEL markers linked to Phosphorous uptake (*Pup1*) *viz*., Pup1K52, Pup1 K48, Pup1K42, Pup1K46 were used (Chin *et al*., 2009) for molecular characterization of nutrient use efficient genotypes. Amplicon sizes of180bp of SSR marker RM 1111linked to QTLs of nitrogen use efficiency (Shi-kai *et al.*, 2012), RM5639 (166bp), RM3628 (125bp), RM 5748(166bp) related to Nitrogen use efficiency genes GS1 and Asp At 3m (Liu *et al*., 2016) were used as positive alleles for identification of nitrogen use efficient genotypes. Presence of *Pup1* alleles were detected usingPup1K52 (505bp), Pup1 K48 (847bp), Pup1 K42 (918bp) and Pup1 K46 (523bp).

Genomic DNA, PCR amplification, electrophoresis and gel documentation was performed as per Girija Rani *et al*., 2019. These markers are validated with co-segregation pattern of phenotypic data of NUE and PUE during 2015 and 2016.One hundred eleven ANGRAU rice varieties were genotyped and selected 10 genotypes were subjected to phenotypic validation in Randomized complete block design in three replications for Nitrogen and Phosphorous use efficiency in 2017. During the year 2018, large number of advanced elite rice lines (192 No) were screened using nitrogen and phosphorus use efficiency QTL linked markers. All the phenotypic validation was carried out at control without N and with 100% N for Nitrogen use efficiency, control without P and 100% P of recommended dose of fertilizers (90:60:60 N: P: K kg/ha).

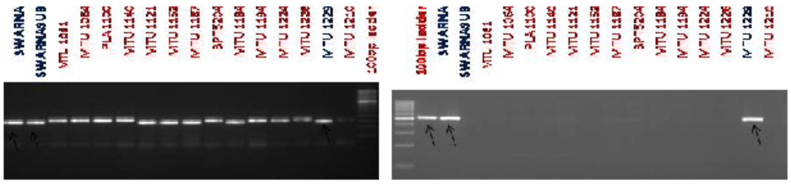
## RESULTS AND DISCUSSION

Nineteen genotypes expressed higher values than the mean NUE of 29.2%co-segregating for one positive alleleout of four markers screened among 48 genotypes under phenotypic evaluation in 2015 and 2016 (Table 1, Fig 1). Only one genotype MTU 2077 showed higher phenotypic value than mean nitrogen use efficiency without any alleles. Rice genotype MTU 1064 exhibited 53.76% of NUE with 0.75frequency of positive alleles and check variety Swarna expressed 1.00 frequency of positive alleles with phenotypic NUE 27.49 % and another check MTU 1061 with 25.69% NUE and 0.25 allele frequency.

Swarna variety showed*Pup1*allele frequency of 1.00 with phenotypic PUE value 15.41, higher than mean 12.65. Other rice genotypes Swarnasub1, MTU 1061 and Nonabokara expressed *Pup1* allele frequencies 0.75, 0.25, 0.5 respectively with higher phenotypic value of PUE than the mean. Thirteen genotypes expressed higher PUE values than mean with 0% *Pup1* alleles indicating other genes are contributing for PUE in these genotypes. Earlier workers (Sarkar *et al.,* 2011; Sompong *et al.,* 2019; Aluwihare *et al.*, 2018) also found similar pattern of allelic expression for *Pup1*.

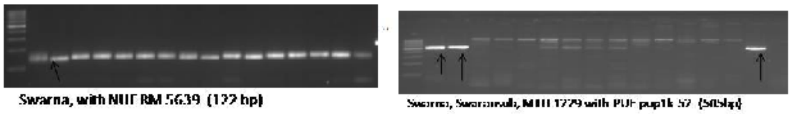
**Table 1: Summary of best nitrogen and phosphorous use efficient genotypes identified in 2015 and 2016.**

| Sr.No. | Genotypes | NUE % | Frequency of NUE positive alleles | PUE % | Frequency of NUE positive alleles |
|---|---|---|---|---|---|
| 1. | Sambasub 1 | 49.95 | 0.25 | 24.83 | 0 |
| 2. | Swarnasub1 | 46.92 | 0.75 | 18.97 | 0.75 |
| 3. | MTU 1166 | 32.93 | 0.50 | 16.79 | 0 |
| 4. | MTU1112 | 29.27 | 0.75 | 11.44 | 0 |
| 5. | MTU 1064 | 53.76 | 0.75 | 21.24 | 0 |
| 6. | PLA1100 | 29.95 | 0.50 | 10.58 | 0 |
| 7. | HHZ11DT10SAL1SUB1 | 37.05 | 0.50 | 20.66 | 0 |
| 8. | MTU 2077 | 32.77 | 0 | 12.6 | 0 |
| 9. | ISUKARVALU | 34.85 | 0.25 | 14.69 | 0 |
| 10. | NONABOKARA | 41.92 | 0.50 | 12.66 | 0.50 |
| 11. | MTU1001 | 40.96 | 0.25 | 19.01 | 0 |
| 12. | MTU5293 | 38.44 | 0.25 | 22.4 | 0 |
| 13. | MTU4870 | 35.33 | 0.50 | 17.26 | 0 |
| 14. | RGL1880 | 42.72 | 0.50 | 18.06 | 0 |
| 15. | RGL2537 | 32.03 | 0.50 | 16.65 | 0 |
| 16. | CR2594-5-1-1-1-1 | 30.17 | 0.25 | 16.19 | 0 |
| 17. | Swarna (check 1) | 27.49 | 1.00 | 15.41 | 1.00 |
| 18. | MTU 1061 (check2) | 25.69 | 0.75 | 15.1 | 0.25 |
|  | Mean | 29.2 |  | 12.65 |  |
|  | SEm | 10.88 |  | 3.11 |  |



Swarna, Swaransub, MTU 1229 with NUE RM 1111

Swarna, Swaransub, MTU 1229 with PUE pup1k46 (523bp)



Swarna, with NUE RM 5639 (132 bp)

Swarna, Swaransub, MTU 1229 with PUE pup1k 52 (505bp)

**Fig. 1.** Molecular characterization of rice genotypes with NUE and Pup1 linked markers.

Swarna, MTU 1061, Swarnasub1, Nonabokara expressed positive alleles for both nitrogen and phosphorous use efficiency with phenotypic confirmation. Twelve rice genotypes MTU 1166, MTU 1112, MTU 1064, PLA 1100, HHZ11DT10SAL1SUB1, Isukaravalu, MTU 1001,MTU5293, MTU 4870, RGL 1880, RGL 2537, CR 2594-5-1-1-1-1 have higher phenotypic values for both nitrogen and phosphorous use efficiency and genotypic confirmation only for nitrogen. This indicates genes other than *Pup1* region are contributing for PUE. This indicated molecular markers selected for nitrogen and phosphorous use efficiency are useful in identification of nutrient genotypes and there is variation in expression of number of positive alleles. However, very low positive alleles were detected among genotypes for phosphorous uptake. Large number of genotypes can be screened using molecular markers and short listed positive genotypes can be used for phenotypic confirmation. Allelic variation and lower level of expression of NUE and *Pup1* in the rice genotypes might be due to skewed selection towards yield, disease resistance and quality over past few decades (Ali *et al*., 2018; Sajid *et al.*, 2021)

In 2017, eighteen genotypes ( MTU 1031, MTU 1190, MTU 1229, MTU 1226, BPT 3291, BPT 2270, BCP1, BCP2, NLR 28523, NLR 27999, NLR 20084, NLR 201014, NLR 3238, NLR 3513, NDLR 7) expressing at least one positive allele for both nitrogen and phosphorous use efficiencywere identified out of 111 rice varieties screened using molecular markers. Seventeen genotypes expressed 2 or more positive alleles for nitrogen use efficiency and 18 genotypes exhibited 2 or more positive alleles for phosphorous uptake (*Pup1*). Three genotypes were confirmed as nutrient use efficient forboth nitrogen and phosphorous by phenotypic validation. Rice genotype MTU1229 expressed higher values and MTU 1190 showed on par values with nutrient use efficient checks varieties Swarna and Indra and MTU 1226 showed relatively lower values (Table 2). This implies allelic expression is specific to genotype and there is no common type of marker expression. Neerja *et al*., 2019 also found that there is no commonality of marker and phenotypic character of nitrogen use efficiency.

**Table 2: Summary of phenotypic and molecular characterization for selected genotypes in 2017.**

| Sr. No. | Genotypes | NUE % | Frequency of NUE positive alleles | PUE % | Frequency of PUE positive alleles |
|---|---|---|---|---|---|
| 1. | MTU-1231 | 51.27 | 0.25 | 18.56 | 0 |
| 2. | MTU-1190 | 54.60 | 0.25 | 22.59 | 0.50 |
| 3. | MTU-1226 | 42.29 | 0.25 | 24.81 | 0.25 |
| 4. | MTU-1184 | 49.40 | 0.50 | 24.68 | 0 |
| 5. | MTU-1210 | 63.82 | 0.25 | 31.46 | 0 |
| 6. | MTU-1187 | 56.31 | 0.50 | 28.58 | 0 |
| 7. | MTU-1194 | 52.55 | 0.25 | 28.31 | 0 |
| 8. | MTU-1229 | 62.63 | 0.50 | 32.39 | 1.00 |
| 9. | MTU-7029 (check) | 57.86 | 1.00 | 25.94 | 1.00 |
| 10. | MTU-1061 (chek) | 55.86 | 0.75 | 28.36 | 0.25 |
|  | Mean | 54.98 |  | 25.99 |  |
|  | SEm | 2.52 |  | 1.5 |  |

Among 192 elite rice lines screened in 2018, twenty four elite rice lines MTU 1290, NLR 3561, MTU 2513-24-2-2, C101A51, BPT 3111, RM 144-9-1-1-1, BPT 2795,Prathika, Varalu, Dhanrasi, AC39397, AC 39416A, NLR 3186, DST 46-43-1, MCM 129, 4910, 4914, 4802, 4803, 4806, 4807, 4809, 4819 and 4822 expressed at least one positive allele for both nitrogen and phosphorous use efficiency. Another 12 genotypes were identified as nitrogen use efficient with 2 or more positive alleles. Twenty four genotypes expressed positive alleles for 2 or more linked markers of *Pup1*.

Nitrogen and phosphorous use efficiency are complex characters controlled by several minor genes besides major genes. Present study revealed that use of molecular markers will be useful in detecting nutrient use efficient genotypes. Our results are in confirmation with earlier workers (Sarkar *et al*., 2011; Sompong *et al.*, 2019; Aluwihare *et al*., 2018) who alsofound lower number of rice genotypes tolerant to P deficiency.

Mega rice variety Swarna expressed all positive alleles for both Nitrogen and Phosphorous use efficiency linked markers followed by Swarna derived lines MTU 1229, MTU 1190, Swarnasub1 having positive alleles for both Nitrogen and phosphorous. This implies that genes contributing for nitrogen and phosphorous use efficiency were inherited from Swarna. Wider adaptation of mega rice variety Swarna which was released three decades back by RARS, Maruteru over various rice ecologies might be due to its nutrient use efficiency (Ali *et al*., 2018).

## CONCLUSION

Out of 350 genotypes screened, only 13.71% genotypes showed at least two positive alleles for Nitrogen and 16.85% for phosphorous. Genotypes possessing one positive allele for both NUE and *Pup1* are 13.14%. Lower per cent of allelic expression for nitrogen use efficiency and phosphorous uptake genes were noticed in the present study. Identification of nutrient use efficient genotypes using molecular markers would reduce time, resources on phenotypic evaluation. Shortlisted nutrient efficient genotypes further can be validated by phenotypic confirmation for release as variety or to use as genetic stock. This indicated that there is need to frame breeding strategies for development of nutrient use efficient genotypes by identification of genetic sources using molecular markers as phenotyping is cumbersome. The identified nutrient genotypes can be used in future for the development of high yielding rice varieties with nutrient use efficiency by adopting modern breeding techniques and also molecular markers can be used for selection of existing genotypes for nutrient use efficiency.

**Acknowledgements.** We acknowledge Acharya NG ranga Agricultural University for supporting above research work

**Conflict of interest**. None.

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