



RESEARCH ARTICLE

# Estimation of genetic variability and association studies for yield in the *OsPSTOL1* gene introgressed F<sub>4</sub> in rice (*Oryza sativa* L.) for phosphorus deficiency tolerance

Aananthi N<sup>1\*</sup>, S K Mohamed Yaseen<sup>2</sup>, M Arumugam Pillai<sup>2</sup>, D Shoba<sup>3</sup>, K Manikandan<sup>4</sup>, V Nirubana<sup>1</sup>, J Ramalingam<sup>5</sup> & G Raiza Christina<sup>1</sup>

<sup>1</sup>Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai 625 104, Tamil Nadu, India

<sup>2</sup>Department of Plant Breeding and Genetics, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

<sup>3</sup>Department of Plant Breeding and Genetics, Agricultural Research Station, Tamil Nadu Agricultural University, Kovilpatti 628 502, Tamil Nadu, India

<sup>4</sup>Department of Soil Science and Agricultural Chemistry, VOC Agricultural College and Research Institute, Tamil Nadu Agricultural University, Killikulam 621 712, Tamil Nadu, India

<sup>5</sup>Department of Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

\*Correspondence email - [aananthi.n@tnau.ac.in](mailto:aananthi.n@tnau.ac.in)

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## Abstract

In India, nearly 85 % of the soils are phosphorus-deficient, significantly limiting rice production. Yield improvement based solely on phenotypic selection is challenging due to its polygenic control. This study aimed to identify superior segregants with high yield potential and phosphorus deficiency tolerance. During the *Rabi* season of 2020, an F<sub>4</sub> population derived from the cross Anna (R) 4 × IR 64 Pup1 was evaluated under a non-replicated trial at the Agricultural College and Research Institute, Killikulam. Observations were recorded for eight biometrical traits. A high magnitude of variability was observed among the genotypes, as indicated by high mean values and a wide range for all studied traits. Phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV), signifying environmental influence, while high heritability was observed for most traits. Traits such as the number of tillers per plant, number of productive tillers per plant, number of filled grains per panicle, phosphorus content in shoot, hundred-seed weight and single-plant yield exhibited high heritability coupled with high genetic advance as a percentage of the mean, indicating the predominance of additive gene action, thus making selection effective. Correlation analysis revealed that days to 50 % flowering, number of tillers per plant and panicle length had a strong positive association with grain yield per plant, suggesting their significance in yield improvement. Path analysis indicated that panicle length (0.3012) had the highest positive direct effect on grain yield per plant, followed by phosphorus content in shoot (0.2366), number of productive tillers per plant (0.1965), hundred-seed weight (0.1623) and number of filled grains per panicle (0.1423), while acid phosphatase (-0.172) and number of tillers per plant (-0.0923) had negative direct effects. Based on the overall evaluation, eleven superior families (AI-17, AI-15, AI-19, AI-16, AI-84, AI-64, AI-53, AI-71, AI-82, AI-48 and AI-11) were identified for further breeding programs aimed at improving phosphorus starvation tolerance in rice.

**Keywords:** genetic variability; phosphorus deficiency; tolerance; yield improvement

## Introduction

Rice (*Oryza sativa* L.) is one of the most important and widely grown staple food crop in the world. It is the primary source of food for millions of the human population, providing enormous nutritional and economic security. Global rice demand in 2050 is expected to be around 584 MT or lower (1). To meet the requirements of the ever-increasing population and to ensure food security for the future, enhanced food production is essential. Rice production is challenged by several biotic and abiotic stresses, which decrease the productivity and, in turn, reduce the economic levels of the farmers. Further, the high cost

of cultivation also reduces the income level for farmers since the farmer has to purchase all inputs in retail, especially the phosphatic fertilisers. Among the abiotic stresses, drought, salinity, submergence and nutrient deficiency are the major ones influencing crop yields drastically. P is one of the major nutrients necessary for the growth and development of rice crop as it is required for enzymes, nucleic acids, cell membranes and other metabolic activities (2). Insufficient soil phosphorus is the major challenge affecting crop growth and development in the initial stages, which may be due to its low solubility and high fixation. Therefore, the rice plant exhibits stunted growth, lesser biomass, reduced grain yield, late flowering and maturity (3). Although the

addition of phosphate fertilizers can alleviate this problem, the high cost of fertilizers discourages small and marginal farmers from rice cultivation and also increases the burden of imports (4). Thus, improving phosphorus efficiency in crops could play a critical role in encouraging the sustainability of agro ecosystems and developing cultivars with greater ability to grow well and yield in low soil P conditions is an imperative goal in contemporary plant breeding (5, 6).

Genetic variability serves as the vital strength for plant breeders wishing to improve plant performance. This variability within a population is due to genetic diversity, environmental effects and the interaction between genetics and the environment. Genetic variability in crop germplasm aids in precisely selecting high-yielding, well-adapted genotypes which can be utilised either introduced variety or as parents in breeding programs. Understanding the magnitude of genetic variability present in the gene pool of a crop species is highly essential to start a wise plant breeding program. Development of phosphorus-efficient cultivars depends on genetic variability for low phosphorus tolerance aimed to bring improvements in the plant-breeding program, which reveals differences at the genomic level for the level of phosphorus efficiency (6). Thus, it is crucial to investigate the genetic variability among the rice genotypes for low phosphorus tolerance by screening under low P stress conditions. The present study was therefore aimed at assessing the genetic variability of yield and its attributing traits of rice genotypes in low P conditions.

## Materials and Methods

The study was conducted during the 2019 *Rabi* season at the experimental field of the Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Killikulam. The experimental material consisted of  $F_4$  progenies (Fig. 1) derived from the cross Anna (R) 4  $\times$  IR 64 Pup1, obtained from the Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Madurai. The parental line Anna (R) 4 is a drought-tolerant variety susceptible to phosphorus starvation, while IR 64 Pup1 is a phosphorus deficiency-tolerant line carrying the *OsPSTOL1* gene. The  $F_4$  progenies, along with their parental lines, were raised in a non-replicated trial. A total of 71 families were planted with row-to-row spacing of 20 cm and plant-to-plant spacing of 10 cm. Each family consisted of four rows with 15

plants per row and standard agronomic practices were followed to ensure optimal growth and yield. Biometrical observations were recorded for eleven traits, including days to 50 % flowering, plant height, number of tillers and productive tillers per plant, panicle length, filled grains per panicle, hundred-grain weight, P content in shoots, length-to-breadth ratio, acid phosphatase activity and grain yield per plant. Data were collected from ten randomly selected plants per family and analysed using standard statistical methods. The analysis included the calculation of mean, variance, genotypic and phenotypic coefficients of variation, heritability, genetic advance and correlation between grain yield and component traits (7-10).

## Results and Discussion

### Variability parameters

The estimates of phenotypic and genotypic coefficients of variation are crucial for assessing the influence of environmental factors on various traits (11). Understanding the nature and extent of phenotypic and genotypic variability in a crop is fundamental for developing effective breeding strategies (12). In this study, the PCV was consistently higher than the corresponding GCV for all traits examined. Research indicates that environmental factors played a role in trait expression (13). However, a narrow difference between PCV and GCV suggested that the number of unfilled grains per panicle, number of filled grains per panicle, number of grains per panicle and flag leaf area were relatively stable and less affected by environmental fluctuations (14). The estimates of phenotypic variance, genotypic variance, PCV, GCV, heritability and genetic advance as a percentage of the mean for the eleven quantitative traits analysed in the Anna (R) 4  $\times$  IR 64 Pup1 cross are summarised in Table 1. In the  $F_4$  generation of crosses, high PCV and GCV were observed for traits such as the number of filled grains per panicle and hundred-seed weight, indicating significant genetic variation within the population. This suggests strong potential for improvement through simple selection methods (14-16). Moderate PCV and GCV were recorded for traits including the number of tillers per plant, number of productive tillers per plant, phosphorus content in shoots and grain yield per plant. This reflects moderate variability, highlighting a good scope for genetic improvement, which may require one or two generations of selfing to achieve. Similar results have been reported for the number of tillers and



**Fig. 1.** Field view of  $F_4$  generation of Anna (R) 4  $\times$  IR 64 Pup1.

**Table 1.** Estimates of genetic parameters for yield and its attributing traits in F<sub>4</sub> Population of Anna (R) 4 × IR 64 Pup1

| S. No | Characters      | Coefficient of variation |               | Heritability broad sense (%) | Genetic advance as per cent mean (%) | Skewness | Kurtosis |
|-------|-----------------|--------------------------|---------------|------------------------------|--------------------------------------|----------|----------|
|       |                 | Phenotypic (%)           | Genotypic (%) |                              |                                      |          |          |
| 1.    | <b>DFF</b>      | 3.50                     | 3.07          | 76.74                        | 5.53                                 | 1.38     | 5.13     |
| 2.    | <b>PH</b>       | 5.70                     | 5.59          | 96.43                        | 11.32                                | 0.22     | -0.45    |
| 3.    | <b>NTPP</b>     | 11.73                    | 11.08         | 89.32                        | 21.58                                | 0.09     | -0.15    |
| 4.    | <b>NPTP</b>     | 16.15                    | 15.54         | 92.48                        | 30.78                                | 0.67     | 0.39     |
| 5.    | <b>PL</b>       | 7.51                     | 6.88          | 84.05                        | 13.00                                | 0.46     | 0.49     |
| 6.    | <b>NFGPP</b>    | 31.00                    | 30.25         | 95.26                        | 60.83                                | -0.32    | 0.31     |
| 7.    | <b>100 SW</b>   | 33.38                    | 30.17         | 92.44                        | 58.78                                | -0.32    | -0.38    |
| 8.    | <b>LB RATIO</b> | 6.69                     | 6.53          | 95.25                        | 13.13                                | 0.04     | -0.12    |
| 9.    | <b>AP</b>       | 9.90                     | 5.15          | 67.08                        | 5.52                                 | 0.53     | -0.15    |
| 10.   | <b>SP</b>       | 11.12                    | 10.75         | 93.36                        | 21.39                                | 0.26     | 0.31     |
| 11.   | <b>GY</b>       | 18.02                    | 17.53         | 94.61                        | 35.13                                | -0.42    | 0.37     |

DFF - Days to 50 % flowering (days), PH - Plant height (cm), NTPP - No. of tillers per plant, NPTP - No. of productive tillers per plant, PL - Panicle length (cm), NFGPP - No. of filled grains per panicle, 100 SW - Hundred seed weight (g), LB ratio - Length breadth ratio, AP - Acid phosphatase (molar paranitro phenol released per min per mg of fresh weight), SP - Phosphorus content in shoot (mg/g shoot weight), GY - Grain yield per plant (g).

productive tillers per plant and grain yield per plant (17, 18). Conversely, traits such as days to 50 % flowering, plant height, panicle length, length-to-breadth ratio and acid phosphatase exhibited low PCV and GCV, indicating limited variability (19-22). The observed low variability is attributed to the rapid reduction in heterozygous progenies and a corresponding increase in homozygous progenies in the F<sub>4</sub> generation. Consequently, selection for these traits is expected to be less effective due to the limited genetic variability. Similar observations have been reported for plant height, days to 50 % flowering and kernel length-to-breadth ratio and panicle length (23-26).

Heritability estimates are essential for understanding a population's response to selection, as they indicate the proportion of heritable variation and emphasize its importance in breeding programs. These estimates are critical in identifying elite genotypes (27). When combined with genetic gain, heritability becomes even more effective in selecting superior individuals within a population (26). In the F<sub>4</sub> generation of the cross, traits such as the number of tillers per plant, number of productive tillers per plant, number of filled grains per panicle, hundred-seed weight, phosphorus content in shoots and grain yield per plant exhibited high heritability along with high genetic advance as a percentage of the mean. This indicates the predominance of additive gene action, suggesting that simple selection would be effective in developing desirable genotypes. Research observed similar findings from the results for the number of tillers per plant, number of productive tillers per plant, number of filled grains per panicle, phosphorus content in shoots and grain yield per plant and hundred-seed weight, plant height and the number of grains per panicle (28-34). Research indicates that similar trends for grains per panicle were observed (35). Traits such as plant height, panicle length and length-to-breadth ratio displayed high heritability coupled with moderate genetic advance as a percentage of the mean, indicating the potential for further improvement through simple selection (14, 27). On the other hand, traits such as days to 50 % flowering and acid phosphatase had shown high heritability but low genetic advance as a percentage of the mean. This suggests that selection for these traits would likely be ineffective (36).

The number of filled grains per panicle, hundred seed weight and single plant yield showed negative skewness under this study, indicating duplicate gene effects, while other characters

exhibited positive skewness, suggesting complementary gene action. Days to 50 % flowering, number of productive tillers per plant, panicle length, number of filled grains per panicle and single plant yield showed negative kurtosis, indicating gene interactions, while other traits showed an absence of gene interaction. Research indicates that panicle length and number of filled grains per panicle showed positive kurtosis in rice (37).

Grain yield is a complex trait influenced by multiple contributing factors. Understanding the relationships between grain yield, biometrical traits and phosphorus-related characteristics can enhance selection efficiency in breeding programs. This study examined genotypic correlation coefficients for yield-contributing and phosphorus-associated traits in the F<sub>4</sub> generation of a cross. The results indicated a significant positive correlation between grain yield per plant and several key traits, including plant height, the number of productive tillers per plant, the number of filled grains per panicle, hundred-seed weight and phosphorus content in the shoot. Results observe a similar observation from their studies for plant height, productive tillers, filled grains per panicle and both hundred-seed weight and phosphorus content in the shoot (38-41). Additionally, traits such as days to 50 % flowering, the number of tillers per plant and panicle length also exhibited positive associations with grain yield. These results corroborate earlier reports for days to 50 % flowering and panicle length and number of tillers per plant (42, 43). The significant correlations observed in this study suggest that simultaneous improvement of these traits could effectively enhance grain yield per plant, providing valuable insights for breeding programs focused on yield optimisation.

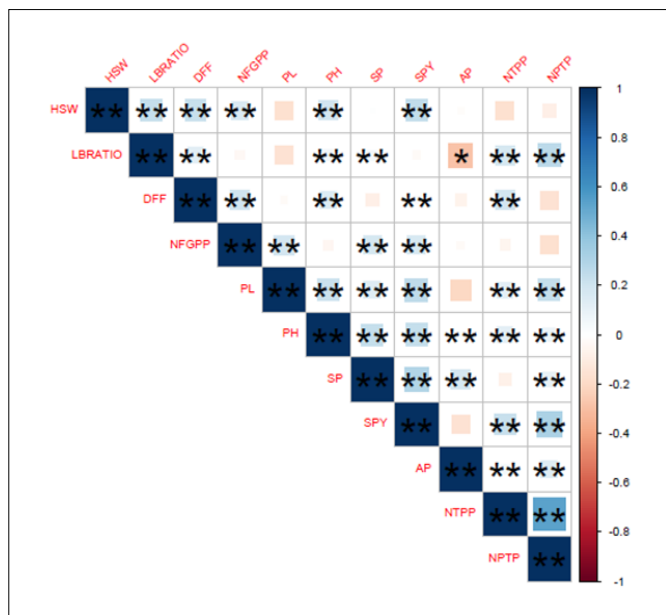
### Correlation Analysis

Understanding the relationships between yield-related traits and phosphorus-associated characteristics is crucial for enhancing selection efficiency in breeding programs. In the F<sub>4</sub> generation of a cross, phosphorus content in the shoot, the number of tillers per plant, productive tillers per plant and plant height exhibited a strong positive correlation with acid phosphatase. Notably, phosphorus content in the shoot showed a significant association with grain yield (Table 2 & Fig. 2), plant height, acid phosphatase, filled grains per panicle, panicle length, productive tillers and the length-breadth ratio (44). Conversely, days to 50 % flowering demonstrated a negative correlation with shoot phosphorus content (45, 46). Productive tillers per plant exhibited positive

**Table 2.** Correlation coefficient among yield components in F<sub>4</sub> derivatives of Anna (R) 4 × IR64 Pup1

| Traits  | DFF | PH       | NTPP     | NPTP     | PL       | NFGPP    | 100SW    | LBRATIO  | AP       | SP       | GY       |
|---------|-----|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| DFF     | 1   | 0.1444** | 0.1808** | -0.159   | -0.0246  | 0.1900** | 0.2278** | 0.1253** | -0.0625  | -0.0804  | 0.0255** |
| PH      |     | 1        | 0.1164** | 0.0756** | 0.2134** | -0.0451  | 0.1831** | 0.0554** | 0.0125** | 0.2314** | 0.2356** |
| NTPP    |     |          | 1        | 0.5315** | 0.1181** | -0.052   | -0.1666  | 0.1778** | 0.0488** | -0.0716  | 0.2265** |
| NPTP    |     |          |          | 1        | 0.2334** | -0.1636  | -0.0896  | 0.2637** | 0.1329** | 0.0902** | 0.3140** |
| PL      |     |          |          |          | 1        | 0.1862** | -0.1654  | -0.1575  | -0.2062  | 0.1363** | 0.2545** |
| NFGPP   |     |          |          |          |          | 1        | 0.1573** | -0.0379  | -0.0272  | 0.1716** | 0.1636** |
| 100SW   |     |          |          |          |          |          | 1        | 0.2311** | -0.0179  | 0.006    | 0.2450** |
| LBRATIO |     |          |          |          |          |          |          | 1        | -0.2850* | 0.0464** | -0.0297  |
| AP      |     |          |          |          |          |          |          |          | 1        | 0.1781** | -0.1595  |
| SP      |     |          |          |          |          |          |          |          |          | 1        | 0.2920** |
| GY      |     |          |          |          |          |          |          |          |          |          | 1        |

\*\* Significance at 1 % level and \* Significance at 5 % level. DFF- Days to 50 % flowering (days), PH – Plant height (cm), NTPP- No. of tillers per plant, NPTP - No. of productive tillers per plant, PL - Panicle length (cm), NFGPP - No. of filled grains per panicle, 100 SW - Hundred seed weight (g), LB ratio - Length breadth ratio, AP - Acid Phosphatase (molar paranitrophenol released per min per mg of fresh weight), SP - Phosphorus content in shoot (mg/g shoot weight), GY – Grain yield per plant (g).

**Fig. 2.** Correlation coefficient among yield components in F<sub>4</sub> derivatives of Anna (R) 4 × IR 64 Pup1.

\*\* Significance at 1 % level & \* Significance at 5 % level

correlations with tillers per plant, grain yield, panicle length, the length-breadth ratio, acid phosphatase, shoot phosphorus content and plant height (47, 48). Additionally, filled grains per panicle showed a positive correlation with days to 50 % flowering, panicle length, shoot phosphorus content and grain yield (14). Association analysis identified grain yield per plant, productive tillers, filled grains per panicle, acid phosphatase and shoot phosphorus content as key traits for breeding programs aimed at improving both grain yield and phosphorus starvation tolerance. These findings provide valuable insights into selecting desirable traits for the development of high-yielding, phosphorus-efficient genotypes.

### Path analysis

Path analysis for 71 F<sub>4</sub> families of Anna (R) 4 × IR 64 Pup1 revealed that panicle length (0.3012) had the highest positive direct effect on grain yield per plant (Table 3 & Fig. 3), followed by P content in shoot (0.2366), number of productive tillers per plant (0.1965), hundred seed weight (0.1623) and number of filled grains per panicle (0.1423). In contrast, acid phosphatase (-0.172) and the number of tillers per plant (-0.0923) exhibited negative direct effects. Indirect effects varied among traits, with days to 50 %

**Table 3.** Direct and indirect effects of different traits on yield in F<sub>3</sub> derivatives of Anna (R) 4 × IR64 Pup1

| Traits  | DFF    | PH     | NTPP   | NPTP   | PL     | NFGPP  | 100SW   | LBRATIO | AP     | SP     | GY       |
|---------|--------|--------|--------|--------|--------|--------|---------|---------|--------|--------|----------|
| DFF     | -0.136 | -0.005 | 0.0173 | -0.022 | 0.009  | 0.0227 | 0.0738  | 0.0056  | 0.0061 | -0.029 | -0.0589  |
| PH      | -0.001 | 0.370  | -0.001 | -0.005 | -0.011 | 0.0005 | 0.0487  | -0.0048 | -0.012 | 0.0396 | 0.4238** |
| NTPP    | -0.005 | 0.002  | -0.115 | 0.097  | -0.001 | 0.0278 | -0.0388 | 0.0005  | -0.003 | 0.0251 | -0.0107  |
| NPTP    | -0.006 | -0.007 | 0.148  | 0.2584 | -0.000 | 0.0303 | -0.0329 | 0.0015  | 0.0013 | 0.0018 | 0.3941** |
| PL      | -0.008 | 0.0478 | -0.005 | 0.0007 | -0.039 | 0.0118 | -0.0176 | -0.0023 | -0.007 | 0.0226 | 0.0024   |
| NFGPP   | 0.0025 | 0.0003 | -0.009 | 0.0109 | -0.001 | 0.3301 | 0.0121  | 0.0044  | 0.0103 | 0.0067 | 0.3662** |
| 100SW   | 0.0123 | 0.038  | 0.0204 | -0.017 | 0.0032 | 0.0182 | 0.2186  | 0.0045  | -0.010 | 0.0206 | 0.3077** |
| LBRATIO | 0.0047 | -0.019 | -0.001 | 0.0041 | 0.0021 | 0.0334 | 0.0226  | 0.0433  | -0.010 | -0.008 | 0.0708   |
| AP      | -0.001 | 0.017  | -0.002 | -0.001 | -0.002 | -0.028 | 0.0185  | 0.0037  | -0.121 | -0.016 | -0.1355  |
| SP      | -0.005 | 0.0315 | -0.013 | 0.001  | -0.004 | 0.0103 | 0.021   | -0.0018 | 0.0096 | 0.2146 | 0.2636*  |

DFF- Days to 50 % flowering (days), PH - Plant height (cm), NTPP- No. of tillers per plant, NPTP - No. of productive tillers per plant, PL - Panicle length (cm), NFGPP - No. of filled grains per panicle, 100 SW - Hundred seed weight (g), LB ratio - Length breadth ratio, AP - Acid Phosphatase (molar paranitrophenol released per min per mg of fresh weight), SP - Phosphorus content in shoot (mg/g shoot weight), GY - Grain yield per plant (g).



flowering showing a negligible contribution through number of tillers per plant (0.0221) and P content in shoot (0.019), while plant height had a slight positive influence via panicle length (0.0568) and P content in shoot (0.0548). Number of tillers per plant positively affected yield through productive tillers (0.0513) and filled grains per panicle (0.015), whereas productive tillers contributed positively via tillers per plant (0.065) and P content in shoot (0.0213). Panicle length exhibited a minor positive effect through P content in shoot (0.0323) and productive tillers (0.0225), but negatively influenced yield through acid phosphatase (-0.0200). Similarly, the number of filled grains per panicle had contributed positively through P content in the shoot (0.0406) but negatively through panicle length (-0.0432). Hundred seed weight showed a minor positive effect via tillers per plant (0.0081) but negatively impacted yield via panicle length (-0.0829). The residual effect (0.302) indicated that the selected traits adequately explained the variability in grain yield. These findings highlight the importance of panicle length, phosphorus content and productive tillers in yield improvement. The positive impact of P content in the shoot had suggested a potential role of phosphorus in nutrient efficiency, while the negative influence of acid phosphatase might indicate its association with stress conditions rather than direct yield enhancement. The observed direct and indirect effects align with previous studies supporting the significance of panicle length and productive tillers in grain yield determination (49, 50).

The study identified *AI-17* as a superior genotype for grain yield and phosphorus starvation tolerance. Traits such as productive tillers per plant, filled grains per panicle, grain yield per plant, phosphorus content in shoot and acid phosphatase exhibited high heritability, indicating their potential for selection in breeding programs. Path and correlation analysis confirmed the importance of productive tillers, filled grains per panicle, acid phosphatase and phosphorus content in shoot as major yield-contributing traits. The identified families hold promise for further breeding to develop phosphorus-efficient rice varieties.

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**Conflict of interest:** The Authors declare no conflict of interest

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