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# Evaluation of Fertility Restorer Genotypes Using Multivariate Statistical Methods

F. Sohrabi[1], N. Babaeiyan Jelodar[2,*], N. Bagheri[3]

[1] M.Sc. Student, Department of Plant Biotechnology and Breeding, Faculty of Agricultural Sciences, Sari Agricultural Sciences and Natural Resources University, Sari, Iran
[2] Professor, Department of Plant Biotechnology and Breeding, Faculty of Agricultural Sciences, Sari Agricultural Sciences and Natural Resources University, Sari, Iran
[3] Assistant Professor, Department of Plant Biotechnology and Breeding, Faculty of Agricultural Sciences, Sari Agricultural Sciences and Natural Resources University, Sari, Iran

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**ABSTRACT**

In this study, eight rice fertility restorer genotypes were systematically evaluated based on a set of key morphological and agronomic characteristics. The primary aim was to identify superior restorer lines that could be effectively utilized in hybrid rice breeding programs. Multivariate statistical techniques, including principal component analysis (PCA) and cluster analysis, were applied to analyze the variation among genotypes and to elucidate the relationships among traits. The results of cluster analysis using Ward's method categorized the genotypes into three distinct groups, with the IRI 347 line, which demonstrated the highest grain yield, forming a separate cluster, highlighting its potential as an elite restorer. PCA revealed that three independent principal components collectively explained 88.75% of the total phenotypic variation. The first principal component was primarily associated with yield-related traits such as filled grains per panicle and grain weight, indicating its central role in overall productivity. The second component was strongly linked to the number of sterile spikelets, reflecting reproductive efficiency, while the third component predominantly represented plant height. These findings provide valuable insights into the genetic diversity and trait interrelationships among rice restorer lines, facilitating the selection of high-performing genotypes for hybrid seed production and contributing to the development of more productive and resilient rice cultivars.

## 1. INTRODUCTION

Hybrid breeding has revolutionized crop production by exploiting heterosis, leading to improved yield, quality, and stress resistance in various agricultural crops [1]. Central to this process is the utilization of cytoplasmic male sterility (CMS) systems, which facilitate the efficient production of hybrid seeds by inducing male sterility in one

* Corresponding Author: n.babaeiyan@umz.ac.ir
Professor, Department of Plant Biotechnology and Breeding, Faculty of Agricultural Sciences, Sari Agricultural Sciences and Natural Resources University, Sari, Iran



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parent line, while fertility restorer (Rf) genes in the other parent restore pollen fertility in the progeny [2]. The identification and evaluation of effective fertility restorer genotypes are essential for the success of hybrid programs, as they ensure stable fertility restoration and contribute to the genetic diversity needed for sustainable agriculture [3]. However, evaluating these genotypes involves analyzing multiple agronomic, morphological, and physiological traits, which often exhibit complex interrelationships and environmental influences [4].

Multivariate statistical methods, including principal component analysis (PCA), cluster analysis, and discriminant analysis, provide powerful tools for handling such multifaceted data [5]. These techniques enable the reduction of dimensionality, identification of key traits contributing to variability, and classification of genotypes into distinct groups based on similarity [6]. For instance, studies on rice restorer lines have employed multivariate analysis to elucidate relationships among morphological traits, aiding in the selection of superior genotypes for breeding [7]. Similarly, in sunflower, multivariate techniques have been used to assess genetic diversity among maintainer and restorer inbreds, revealing clusters that inform hybrid development strategies [8]. In pigeonpea, meta-analyses of quantitative trait loci (QTL) associated with fertility restoration have integrated multivariate approaches to pinpoint genomic regions linked to Rf genes, enhancing marker-assisted selection [9].

Despite advancements, challenges remain in accurately classifying fertility restorer genotypes due to genotype-by-environment interactions and the polygenic nature of fertility traits [10]. Previous research has demonstrated the efficacy of these methods in crops like rice, sunflower, and pigeonpea, but comprehensive evaluations integrating multiple traits are needed to optimize breeding efficiency [11]. This study aims to evaluate fertility restorer genotypes using multivariate statistical methods, focusing on key agronomic and fertility-related traits to identify promising lines for hybrid breeding programs.

## 2. MATERIALS AND METHODS

### 2.1. Plant Materials

In this study, eight rice fertility restorer genotypes (Table 1) were evaluated for morphological traits. Standard agronomic practices for irrigation, fertilization, and weed management were followed according to local conditions. Each genotype was planted in plots measuring 2 × 2 m with a spacing of 25 × 25 cm between plants.

**Table 1.** Names of the rice genotypes used in this study

| No. | Genotype |
|---|---|
| 1 | IRI 347 |
| 2 | MILYANG 54 |
| 3 | SUWEON 294 |
| 4 | IR 9761-19-1 |
| 5 | IR65912-90-1-6-3-2-3R |
| 6 | IR65622-151-1-2-2-2R |
| 7 | IR57301-158-1R |
| 8 | IR64724-67-2-1-2-2R |

### 2.2. Morphological Evaluation

In this study, key agronomic traits including plant height, panicle length, number of tillers per plant, total number of grains per panicle, number of filled grains per panicle, percentage of spikelet sterility, 1000-grain weight, and grain yield were measured. Morphological trait evaluations were conducted one week prior to harvest. The assessments were performed following the International Rice Research Institute (IRRI) Standard Evaluation System for rice [12].

### 2.3. Statistical Analysis

The experiments were conducted using a randomized complete block design (RCBD) with 8 genotypes and 3 replications. To determine the contribution of each trait to overall variation, reduce data dimensionality, and improve interpretation of relationships, principal component analysis (PCA) was applied. For grouping of the studied genotypes, cluster analysis was performed using Ward's method. All statistical analyses were carried out using SPSS software.

## 3. RESULTS AND DISCUSSION

### 3.1. Principal Component Analysis

Based on the results of PCA, three independent principal components explained a total of 88.75% of the overall variation in the dataset (Table 2). The higher the variance explained by a component, the greater its reliability in interpreting the total variability.

- **The first component**, which explained 55.20% of the total variance, was designated as the yield component. In this axis, all traits except plant height and number of tillers contributed to genotype differentiation. Among them, the number of filled grains per panicle and grain weight had the highest factor loadings.
- **The second component**, accounting for 19.53% of the total variance, was mainly associated with the number of unfilled grains per panicle, and was therefore termed the unfilled grain component.
- **The third component**, explaining 14.02% of the total variance, was strongly influenced by plant height, and thus named the plant height component.

In a similar study on the genetic diversity of rice fertility restorers based on morphological traits, PCA revealed that three main components accounted for 68.22% of the total variation. The first component was associated with grain filling, the second indicated the positive relationship between number of grains and panicle length, while the third reflected the relative importance of reproductive versus vegetative traits [13].

**Table 2.** Eigenvalues and variance explained by principal components in PCA of the studied traits

| Principal Component | Eigenvalue | Percentage of Variance | Cumulative Variance (%) |
|---|---|---|---|
| **1** | 7.728 | 55.204 | 55.204 |
| **2** | 2.734 | 19.526 | 74.730 |
| **3** | 1.963 | 14.021 | 88.750 |

**Table 3.** Principal components with eigenvalues greater than 1 and component loadings for each trait

| Trait | Component 1 | Component 2 | Component 3 |
|---|---|---|---|
| **Plant height** | -0.358 | 0.183 | 0.888 |
| **Tiller number** | -0.391 | -0.590 | -0.097 |
| **Panicle length** | -0.687 | -0.594 | -0.011 |
| **Filled grains per panicle** | 0.967 | -0.132 | 0.152 |
| **Unfilled grains per panicle** | -0.587 | 0.773 | 0.029 |
| **Total grains per panicle** | 0.585 | 0.674 | 0.224 |
| **Grain weight** | 0.969 | -0.092 | 0.189 |
| **Grain length** | -0.857 | -0.292 | 0.120 |
| **Grain width** | 0.547 | 0.413 | -0.703 |
| **Grain diameter** | 0.790 | 0.053 | -0.498 |
| **Spikelet sterility (%)** | -0.809 | 0.570 | -0.044 |
| **Spikelet fertility (%)** | 0.809 | -0.570 | 0.044 |
| **100-grain weight** | 0.780 | 0.009 | 0.431 |
| **Single-plant yield** | 0.915 | 0.111 | 0.329 |

### 3.2. Cluster Analysis

The cluster analysis results (Figure 1), based on 14 morphological traits and using the Ward method, classified the fertility-restoring lines into three groups. Group 1 consisted solely of the genotype IRI 347. Group 2 included the genotypes SUWEON 294, IR65912-90-1-6-3-2-3R, MILYANG 54, and IR57301-158-1R, while Group 3 contained IR 9761-19-1, IR64724-67-2-1-2-2R, and IR65622-151-1-2-2-2R. The genotype IRI 347, which exhibited the highest yield, formed a distinct group on its own. The genotypes in Group 2 displayed higher performance compared to those in Group 3.

Similar findings were reported in previous studies: Dorosty (2000) [14] and Zinali Nejad et al. (2003) [15] grouped 64 and 100 rice genotypes, respectively, into four clusters based on morphological traits using a distance criterion of 10. Additionally, Rahim Soroush et al. (2004) [16] classified 36 rice lines and cultivars into five clusters using a similar hierarchical clustering approach.

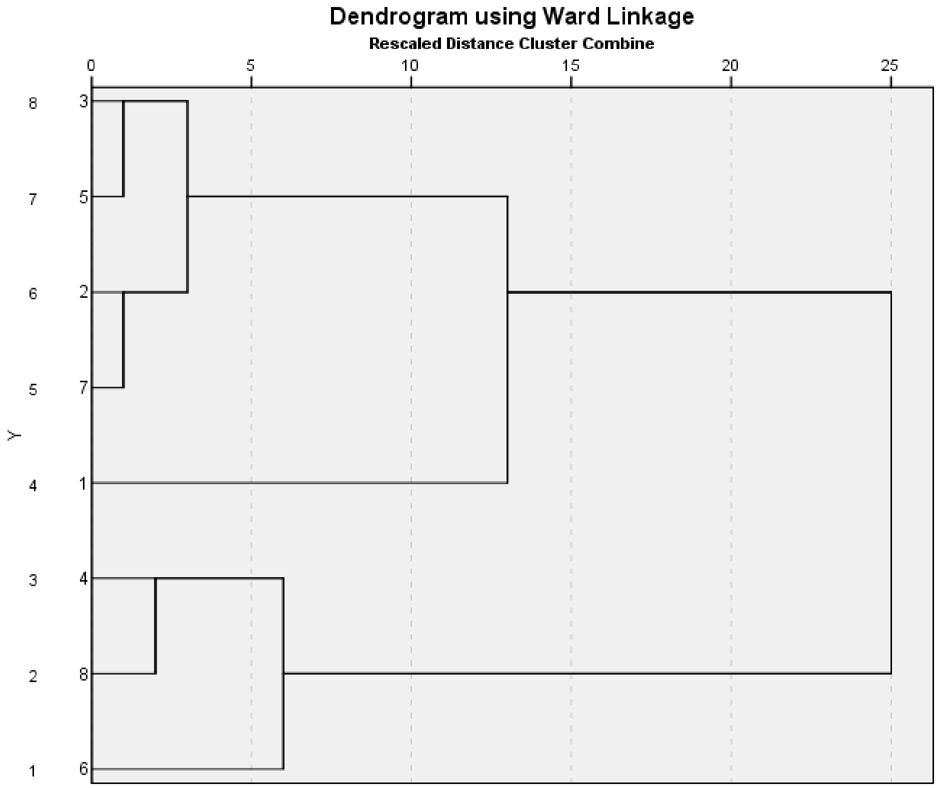


**Fig. 1.** Cluster grouping of 8 rice genotypes based on 14 agronomic traits

## 4. CONCLUSION

Based on the results of Ward's cluster analysis, the genotype IRI 347, which exhibited the highest yield, can be recommended for the development of the hybrid rice seed production process. According to the principal component analysis results, three independent main components explained 75.88% of the total variation in the data. The first component was named "yield-related traits," the second component "unfilled grains," and the third component "plant height."

### Declaration

We acknowledge that we used ChatGPT to enhance the academic writing of our manuscript while ensuring the originality and integrity of our work.

**Transparency Statement**

The data supporting this study are available upon reasonable request to the corresponding author, subject to ethical and confidentiality considerations.

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**Declaration of Interest**

The authors declare that they have no competing interests.

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