



RESEARCH ARTICLE

# Comparative multimodal statistical analysis for yield stability in mutant populations of rice (*Oryza sativa* L.) var. ADT (R) 47

T Radhamani<sup>1\*</sup>, A R Priyanka<sup>2</sup>, A Shanmugam<sup>2</sup>, K Veni<sup>3</sup>, B Muralidharan<sup>4</sup>, M Umadevi<sup>5</sup>, V G Renganathan<sup>6</sup>,  
K Baghyalakshmi<sup>2</sup> & D Sassikumar<sup>7\*</sup>

<sup>1</sup>Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore 641 003, India

<sup>2</sup>Indian Council of Agricultural Research-Central Institute of Cotton Research, Regional Station, Coimbatore 641 003, India

<sup>3</sup>Anbil Dharmalingam Agricultural College and Research Institute, Tamil Nadu Agricultural University, Tiruchirapalli 620 009, India

<sup>4</sup>Horticultural College and Research Institute, Tamil Nadu Agricultural University Coimbatore 641 003, India

<sup>5</sup>Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641 003, India

<sup>6</sup>Department of Plant and Soil Science, Mississippi State University, Starkville 39762, U.S.A

<sup>7</sup>Sugarcane Research Station, Tamil Nadu Agricultural University, Cuddalore 607 001, India

\*Correspondence email - [radhamani.t@tnau.ac.in](mailto:radhamani.t@tnau.ac.in), [sassikumar@tnau.ac.in](mailto:sassikumar@tnau.ac.in)

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## Abstract

Rice is an important staple food for half of the world's population. It is a vital crop for global food security. However, the dominant cultivation of high-yielding varieties has led to significant genetic erosion, highlighting the importance of mutation breeding as a compelling alternative approach. Induced mutations have offered a single and short alternative to conventional breeding, including isolation, screening, selection and testing generation after generation. When a collection of genotypes is cultivated in diverse environments, they may not exhibit a uniform response across all settings. The effects of location, seasonal variations and their interactions significantly impact the performance and yield potential of genotypes. Consequently, it is crucial to identify stable genotypes across a broad spectrum of environments if the variety is to be recommended for wider regions. A multi-model approach employing BLUP-based simultaneous selection to assess the stability and performance of ADT (R) 47 mutant genotypes with respect to the grain yield was performed. All members of the Additive Main Effects and Multiplicative Interaction (AMMI) family were less accurate than best linear unbiased prediction (BLUP). AMMI models combine ANOVA and Principal Component Analysis (PCA) to decompose genotype-by-environment interaction (GEI). BLUP, on the other hand, provides a way to estimate the random effects in a linear mixed model. BLUP approach involved the estimation of Harmonic mean of genotypic values (HMGV) which is used to infer both yield and stability, Relative performance of genotypic values (RPGV) involved in the estimation of mean yield and genotypic adaptability, harmonic mean of the relative performance of genotypic values (HMRPGV) tangled to evaluate stability, adaptability and yield simultaneously. Comparing all the calculated indices, mutants viz., 160-39-1, 200-30-3, 250-36-1, 200-69-1 were highly stable for plant yield along with the ADT47 control variety for all the tested environments viz., Aduthurai (E<sub>1</sub>), Thanjavur (E<sub>2</sub>), Tirur (E<sub>3</sub>) and Madurai (E<sub>4</sub>).

**Keywords:** : ADT (R) 47; AMMI analysis; BLUP; rice mutagenesis; stability heat-map

## Introduction

Rice is an important staple food for half of the world's population. Genetic erosion was caused by the development of high-yielding cultivars and the extensive cultivation of these kinds. Crop agriculture is facing a significant danger from climate change as well. There is more pressure to create resistant genotypes due to unpredictable monsoons, abrupt outbreaks of pests and diseases, frequent droughts and floods, etc. Even though rice production is declining, current population projections show that Asia will need an extra  $8 \times 10^6$  t of rice annually (1). Due to the increasing number of Asian residents in western areas, there is a greater need for rice due to consumer choice and desire for rice-based cuisines. Consequently, future rice production must greatly enhance resource efficiency to

satisfy the rapidly growing demand and the specific needs of discerning customers. In the near future, rice is expected to continue to be a major component of meals for humans.

The variety ADT(R) 47, a derivative of ADT 43 × Jeeraga samba, was released from Tamil Nadu Rice Research Institute (TRRI), Aduthurai of Tamil Nadu Agricultural University during 2005. It is 90-100 cm tall with an average of 15-20 productive tillers under normal management conditions. The panicles are compact, 26-28 cm long, with approximately 250 grains/panicle and 1000 grains weigh only 13.5 g. With such slender grain, it gives an average grain yield of 6.2 t/ha with a potential yield up to 10.6 t/ha in 125 days. It is non-lodging in habit with profuse tillering response (~ 45 tillers) and ideal for System of Rice Intensification (SRI) cultivation. In spite of its advantages, the

spread of ADT(R) 47 in Tamil Nadu is very limited due to certain drawbacks like extended duration, which could not fit in the first season, just exerted panicle and hard threshability. The realization of the actual grain yield is decreased upto 15 % due to its poor threshability and retention of grains in the rachis itself at both mechanical as well as hand threshing. Hence, farmers are reluctant to cultivate this otherwise superior variety. A moderately well-exserted panicle is one of the desired traits in rice breeding. Varieties with well-exserted panicles are prone to attack by birds and those with enclosed/ just exerted panicles are prone to disease infection, discoloration, sterility of basal grains in the panicle and finally both leading to yield losses. Intermediate threshability behavior of grains at maturity is an important criterion to recover the produce without any yield loss and this is achieved by moderately well-exserted panicles. Varieties with an easy threshability nature of grains from the panicles are disadvantageous due to field losses during harvest. The shed grains will also affect the genetic purity of the subsequent rice crop. In varieties with a difficult threshability nature, grains are left out in the panicles unthreshed.

To address the above constraints, mutations are being used to upgrade the well-adapted local varieties by changing one or two main characters that hinder their productivity and keeping their quality value with the taste and texture of the variety. The idea of producing artificial mutations and utilizing them for breeding cultivars was initiated as early as 1901 by the induction of mutations for alleles which govern the heredity of quantitative characters as a promising tool for releasing new genotypes (2). Rice is a diploid species, more chances of getting genetic variability which could be available for selection in the M<sub>2</sub> generation. Gamma rays are the most commonly used radiation in rice (3). Gamma rays are known to influence plant growth and development by inducing cytological and morphogenetic changes in cells and tissues. Mutagens, including gamma rays, offered great possibilities for increasing genetic variability of quantitative traits such as yield through ionization. Ethyl Methane Sulphonate (EMS) selectively alkylates guanine bases, causing the DNA-polymerase to favor placing a thymine residue over a cytosine residue opposite to the O-6-ethyl guanine during DNA replication, which results in a random point mutation.

The level of performance of any character is a result of the genotype (G) of the cultivar, the environment in which it is grown (E) and the interaction between G and E (GEI). Genotype × Environment Interaction (GEI) exists when the responses of two genotypes to different levels of environmental stress are not consistent. GEI greatly affect the phenotype of a variety, so the stability analysis is required to characterize the performance of varieties in different environments and to help plant breeders in selecting varieties. A genotype's stability and adaptability are helpful factors when suggesting cultivars for established cropping circumstances (29).

This study aims to utilize a multi-model approach employing BLUP-based simultaneous selection to assess the stability and performance of ADT (R) 47 mutant genotypes with respect to the grain yield. The outcomes of this research will contribute valuable insights into the breeding of more productive and stable rice cultivars, ultimately supporting sustainable rice production.

## Materials and Methods

### M1 generation

The mutagenesis of the ADT(R) 47 was done using gamma irradiation and EMS treatment. For gamma irradiation, 500 well-filled seeds were exposed to gamma rays at four different doses viz., 150 Gy, 200 Gy, 250 Gy and 300 Gy in a gamma chamber (GC 1200) installed at Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore by Board of Radiation and Isotope Technology Govt. of India. The gamma source was <sup>60</sup>Co. For EMS treatment, 500 seeds presoaked in distilled water for 12 hr were soaked at different concentrations, such as 80 mM, 100 mM, 120 mM, 140 mM and 160 mM for 6 hr. The lethal dose was fixed based on probit analysis for both chemical and physical mutagens.

### M2 to M4 generation

Subsequent to the mutagenic treatment, the M<sub>1</sub> generation was advanced based on spikelet fertility and the progenies of the mutagenized plants were grown as the M<sub>2</sub> generation. Targeted selection was done among the M<sub>2</sub> mutants for spikelet threshability and panicle exertion. Selected M<sub>2</sub> progenies were advanced to M<sub>3</sub> generation and a total of 652 mutant lines (482 mutants of gamma irradiation and 170 mutants from EMS treatments) were isolated.

### M5 generation

16 mutants (Table 1) of ADT(R) 47 selected from M<sub>4</sub> generation and two short duration varieties as checks viz., ADT(R) 47 and ADT 43 were evaluated in uncontrolled macro environments viz., Aduthurai (E<sub>1</sub>), Thanjavur (E<sub>2</sub>), Tirur (E<sub>3</sub>) and Madurai (E<sub>4</sub>) for eight quantitative characters. The 16 mutants chosen for stability analysis based on earliness, moderately well-exserted panicle and intermediate panicle threshability. The evaluation was conducted for yield and component characteristics using the Best Linear Unbiased Prediction (BLUP) method. The 16 selected families were grown in a plot size of 1.8 m<sup>2</sup> per family with a spacing of 15×10 cm in randomized block design replicated thrice. Two control genotypes, ADT(R) 47 and ADT 43, were also grown along with the mutants.

### Statistical analysis

To evaluate differences between genotypes, environments (years) and GEI, grain yield was subjected to ANOVA. The AMMI statistical model is a hybrid model. It makes use of standard ANOVA procedures to separate the additive variance from the multiplicative variance (GEI) and then uses a multiplicative procedure (Principal Component Analysis-PCA) to extract the pattern from the G × E portion of the ANOVA analysis. The result is the least square analysis, which, with further graphical representation of the numerical results (Biplot analysis), often allows a straightforward interpretation of the underlying causes of G × E. The mathematical statement of the hybrid model is:

$$Y_{ge} = \mu + \alpha_g + \beta_e + \sum_{n=1}^N \lambda_n \zeta_{gn} \eta_{en} + \theta_{ge} \quad (\text{Eqn. 1})$$

G = Genotype

E = Environment

Y<sub>ge</sub> = the yield of genotype 'g' in environment 'e'

μ = the grand mean

**Table 1.** Shortlisted M<sub>5</sub> mutants based on spikelet threshability and panicle exertion

| S.No | Entries    | Days to fifty per cent flowering | Panicle exertion score | Threshability score |
|------|------------|----------------------------------|------------------------|---------------------|
| 1    | 100-16-2   | 91                               | 7                      | 2                   |
| 2    | 140-35-1   | 74                               | 7                      | 2                   |
| 3    | 160-39-1   | 95                               | 7                      | 2                   |
| 4    | 200-21-1   | 86                               | 7                      | 2                   |
| 5    | 200-26-1   | 91                               | 7                      | 2                   |
| 6    | 200-30-3   | 85                               | 7                      | 2                   |
| 7    | 200-49-1   | 81                               | 7                      | 2                   |
| 8    | 200-55-1   | 86                               | 7                      | 2                   |
| 9    | 200-69-1   | 91                               | 7                      | 2                   |
| 10   | 200-70-1   | 89                               | 7                      | 2                   |
| 11   | 200-72-1   | 83                               | 7                      | 2                   |
| 12   | 200-81-1   | 94                               | 7                      | 2                   |
| 13   | 200-146-1  | 90                               | 7                      | 2                   |
| 14   | 250-29-1   | 89                               | 7                      | 2                   |
| 15   | 250-34-1   | 87                               | 7                      | 2                   |
| 16   | 300-54-1   | 93                               | 7                      | 2                   |
| 17   | ADT (R) 47 | 96                               | 5                      | 2                   |

$a_g$  = the genotype mean deviation

$\beta_e$  = the environment mean deviation

N = the number of PCA axes (Interaction Principal Component Axis) retained in the model

$l_n$  = the eigen value for PCA axis 'n'

$\zeta_{gn}$  = the genotypes PCA scores for PCA axis 'n'

$\eta_{en}$  = the environment PCA scores for PCA axis 'n'

$\theta_{ge}$  = the residuals

The AMMI biplot is developed by placing both genotype and environment values on the x-axis and the respective PCA axis eigenvectors on the Y-axis.

#### AMMI and BLUP

Stability analysis was carried out using the software Cropstat version 2015 with eight biometrical characters viz., days to 50 per cent flowering, plant height, number of productive tillers per plant, panicle length, panicle weight, hundred grain weight, panicle exertion and yield by stability and AMMI model (4, 5). BLUP involved the estimation of HMGV (to infer both yield and stability), RPGV (to explore the mean yield and genotypic adaptability) and HMRPGV, to evaluate yield, adaptability and stability simultaneously (21).

$$HMGV_i = \frac{n}{\sum_{j=1}^n \left( \frac{1}{GV_{ij}} \right)} \quad (\text{Eqn. 2})$$

$$RPGV_i = \frac{1}{n} \left[ \frac{\left( \sum_{j=1}^n GV_{ij} \right)}{M_j} \right] \quad (\text{Eqn. 3})$$

$$HMRPGV_i = \frac{n}{\sum_{j=1}^n \left( \frac{1}{RPGV_{ij}} \right)} \quad (\text{Eqn. 4})$$

Where n is the number of crop years (n = 6); GV<sub>ij</sub> is the genetic value of ith genotype in jth year, where GV<sub>ij</sub> =  $\mu_j + g_i + ge_{ij}$ ,  $\mu_j$  is the average of jth crop year,  $g_i$  is the BLUP value of ith genotype and  $ge_{ij}$  is the BLUP value of the interaction between ith genotype and jth crop year; M<sub>j</sub> is the mean grain yield in the jth year.

#### Software used

Using the R packages (R Core Team, version 2018) "agricolae"

and "ammi stability", all of the ASTABs and correlations between the stability parameters were calculated (6, 7). Using R's "lme4" package, BLUP-based stability models such as HMGV, RPGV and HMRPGV were estimated (8).

## Results and Discussion

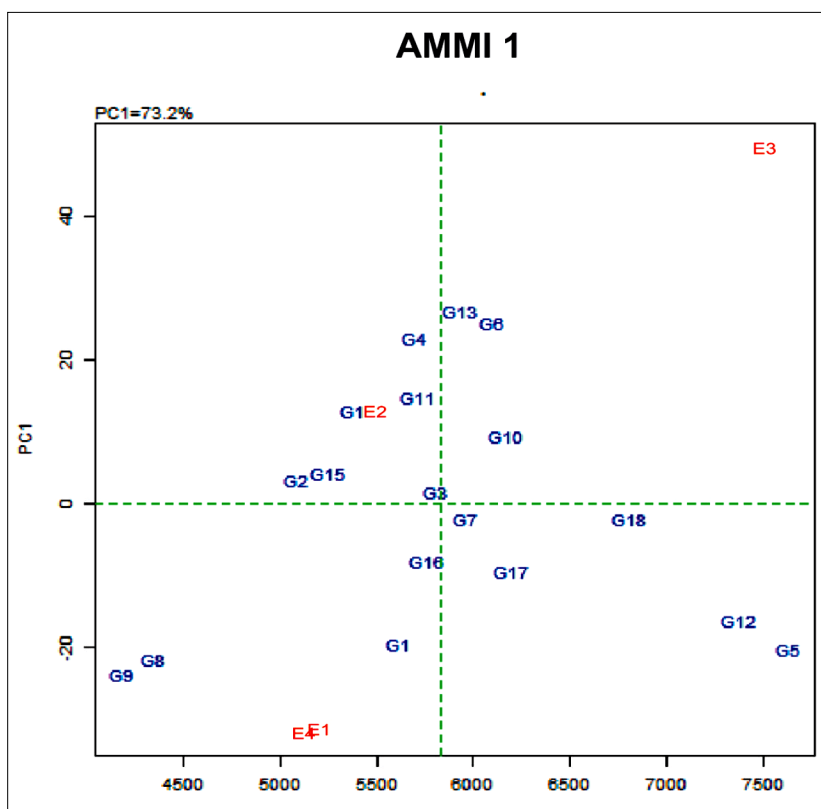
### AMMI results for single plant yield

#### AMMI 1 biplot for single plant yield

The AMMI 1 biplot for single plant yield gave a model fit 73.2 % and it clearly manifested that the environments Aduthurai, Thanjavur and Tirur had differed in both main and interaction effects. Madurai was found to possess a similar effect to Aduthurai. Aduthurai recorded 5212 kg/ha as mean, -31.25 and 9.74 as IPCA 1 and IPCA 2 scores respectively, whereas the mean, IPCA1 and IPCA2 in Thanjavur were 5496 kg/ha, 13.12 and -43.32 respectively. In Tirur, it was 7502 kg/ha, 49.82 and 23.76, respectively. In Madurai, it was 5124 kg/ha, -31.68 and 9.82 respectively. Among the environments, Tirur exhibited high interaction effects by getting plotted away from the origin in the biplot. Thanjavur had low interaction and the environments Aduthurai and Madurai indicated moderate interaction (Fig. 1).

The genotypes and environments plotted nearer to the ordinate are stable in the expression of yield across environment and a genotype or environment on the right side of the midpoint of the abscissa (axis) has higher yields than those of the left-hand side (9-11).

The mutants, viz., 200-81-1 (G13), 200-26-1 (G6) and 200-69-1 (G10), had exhibited high yield with high main (additive) effects showing positive IPCA1 score, but the mutant 200-69-1 (G10) was the overall best. The mutant 200-69-1 recorded a yield (6168 kg/ha) higher than the mean with positive IPCA1 and IPCA2. Since the environment in Thanjavur had a positive IPCA 1 score near zero and hence had small interaction effects, indicating that all the mutants performed well in this location. The mutant 160-39-1 (G3), 140-35-1 (G2) and 250-34-1 (G15) showed positive Interaction Principal Component 1 (IPCA1) score close to zero, indicating that these mutants were stable and less influenced by the environments but exhibited below average yield. The environments Madurai and Aduthurai had lower average site means and possessed negative interaction. The genotypes 100-16-2 (G1), 300-54-1 (G16) may perform better in these environments (Fig. 1).



**Fig. 1.** AMMI 1 biplot for single plant yield.

The genotypes, ADT(R) 47 (G18), 200-30-3 (G7), 160-39-1 (G3), 140-35-1(G2) and 250-34-1 (G15) had low interaction and hence they are stable. Among them, the genotypes ADT(R) 47 (G18) and 200-30-3 (G7) had high mean values and hence they are recommended for all environments. Conversely, the genotypes 200-72-1 (G12) and 200-21-1 (G5) with high mean and negative interaction are suited for highly interactive environments. Environment Tirur is highly interactive as it is plotted away from the ordinate and abscissa with high environmental mean values (Fig. 1). Similar reports were recorded in previous studies (12, 13).

#### AMMI 2 biplot for single plant yield

In the AMMI 2 biplot, the environment scores are joined to the origin by site lines. Sites with short spokes do not exert strong interactive forces. Those with long spokes exert strong interaction. The environment, Thanjavur and Tirur, had long spokes and exerted strong interaction on genotypes. Similarly, Aduthurai and Madurai had short spokes with the origin and they exert less interactive forces over genotypes.

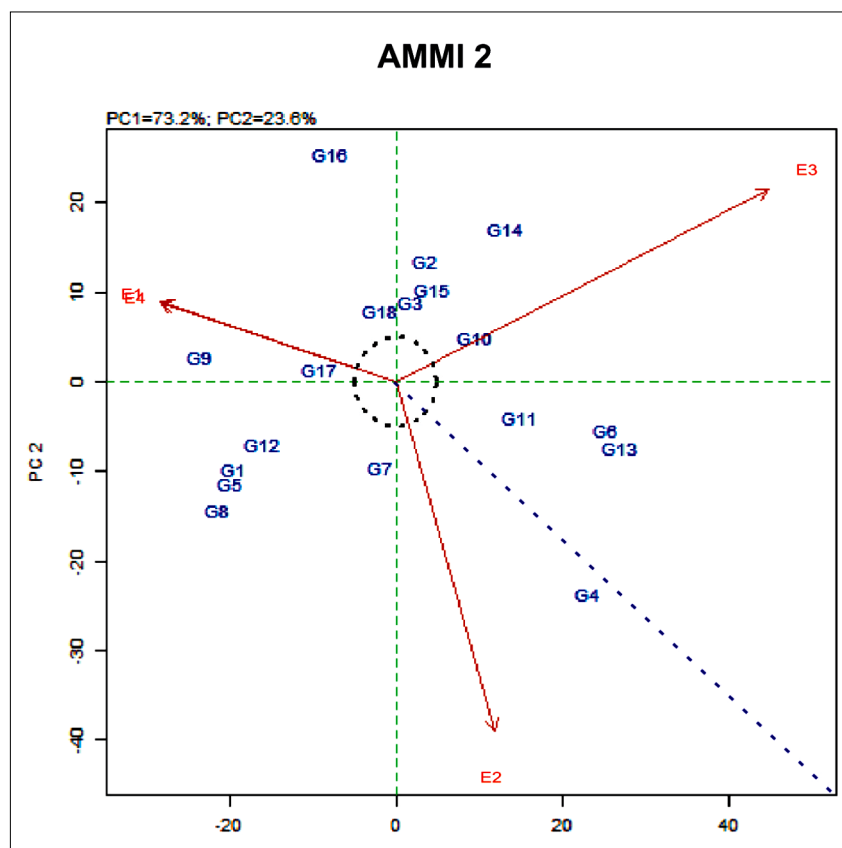
The interaction of genotypes with sites can be read directly from the biplot by projecting genotype marks on the site lines. If the projection falls onto a site line, then that genotype is positively adapted to that site. In AMMI 1 biplot, the projection line of the genotype 200-69-1 (G10) falls on the site line of environment Tirur and the genotype ADT 43 (G17) was found to have high positive interaction with Aduthurai and Madurai (Fig. 1). From AMMI 2 biplot (Fig. 2) it can be observed that, the mutant 200-69-1 had positive interaction with Tirur and had higher specific adaptation to Tirur since it had high mean values for the traits viz., number of panicles per plant (20.53), panicle length (29.37 cm), panicle weight (4.33 g) and yield (8423 kg/ha) at Tirur. On the other hand, the site line must be extended back through the origin to meet the projection and then the genotype has

negative interaction with that site. 200-146-1 (G4) fell on the extended portion of the site line, Aduthurai and Madurai. A strong negative interaction with the environment has been examined (11, 13 &14). As per AMMI analysis, the genotypes ADT (R) 47 (G18), 200-30-3 (G7), 160-39-1(G3), 140-35-1(G2) and 250-34-1 (G15) had low interaction. Among them, the genotypes ADT(R) 47 (G18) and 200-30-3 (G7) had high mean values and hence they are recommended for all environments.

#### BLUP results for grain yield

##### IPCA and WAASB/GY ratio-based stability heat-map

To improve performance and stability evaluations, experimental genotypes were rated using WAASBY index values. The WAASBY selection index combines stability and performance, allowing breeders to select genotypes based on precise breeding objectives. According to the WAASBY scores, mutants were categorized into four groups. The first (green) for plant yield comprised 100-16-2, 140-35-1, 200-70, 250-29-1, 250-36-1, 300-54-1 and for hundred grain weight, the mutant comprised 100-16-2, 250-29-1, which represents stable genotypes with optimal performance. The second (red) group encompassed genotypes for plant yield 160-39-1, 200-30-3, 200-69-1, ADT (R) 47, ADT 43, while for hundred grain weight, 14-35-1, 200-146-1, 200-49, 200-55, 250-36-1, ADT (R) 47 and ADT 43. These mutants showed unfavorable performance and instability. The third (blue) group included 200-146-1, 200-26-1, 200-49 200-55, 200-81-1 for plant yield and mutants 160-39-1, 200-21-1, 200-30-3, 200-69-1, 200-70, 200-72-1 and 200-81-1 showed good performance but instability. The fourth (black) group denotes stable performance, but unfavorable values consisted of genotypes with 200-21-1, 200-72-1 for yield, whereas mutants 200-26-1 and 300-54-1 for hundred grain weight. The WAASBY emphasizes both performance and stability, which facilitates the selection of high-yielding and stable genotypes.



**Fig. 2.** AMMI 2 biplot for single plant yield.

The Interaction Principal Components (IPCA) and weighted average of absolute Scores (WAASB) heat map portrays data in a matrix format using color-coded cells. A heatmap can rank genotypes depending on the number of PC axes used in stability analysis and WAASB index estimation. The heatmap assists in recognizing patterns or trends in genotype rankings. Genotypes that maintain consistently high rankings across principal component axes will display darker colored cells in the heatmap. Conversely, genotypes that consistently rank low will exhibit lighter-colored cells. Cells with diverse colors or transitions between light and dark colors can indicate inconsistencies or differences in ranks. Heat map revealed stable genotypes, viz. ADT47, 250-36-1, 200-30-3, 160-39-1, 140-35-1 for plant yield and 250-29-1, 200-81-1, 200-70, 200-30-3, 200-21-1 and 100-16-2 for hundred grain weight (Fig. 3). The heatmap enables researchers to evaluate how the number of PC axes affects genotype ranks. Based on this, one can identify specific PC axes where genotypes consistently rank higher.

The best linear unbiased predictor (BLUP) is a classical approach which offers the potential to improve the predictive accuracy of random effects. Plant breeders rarely use BLUP-based genotype selection. On comparison, BLUPs showed that mutants 200-21-1, 200-72-1, ADT (R) 47, ADT 43, 200-69-1, 200-26-1, 200-30-3, 200-81-1 had a higher prediction for grain yield and therefore were considered to be superior genotypes, while the mutants 200-49, 200-55 had the lowest predicted grain yield. For hundred grain weight, 300-54-1, 200-72-1, ADT 43, 200-55, 200-146-1, 250-36-1 had higher prediction (Fig. 4).

#### BLUP-based stability indices

This approach involved the estimation of Harmonic means of genotypic values (HMGV) which is used to infer both yield and stability, Relative performance of genotypic values (RPGV)

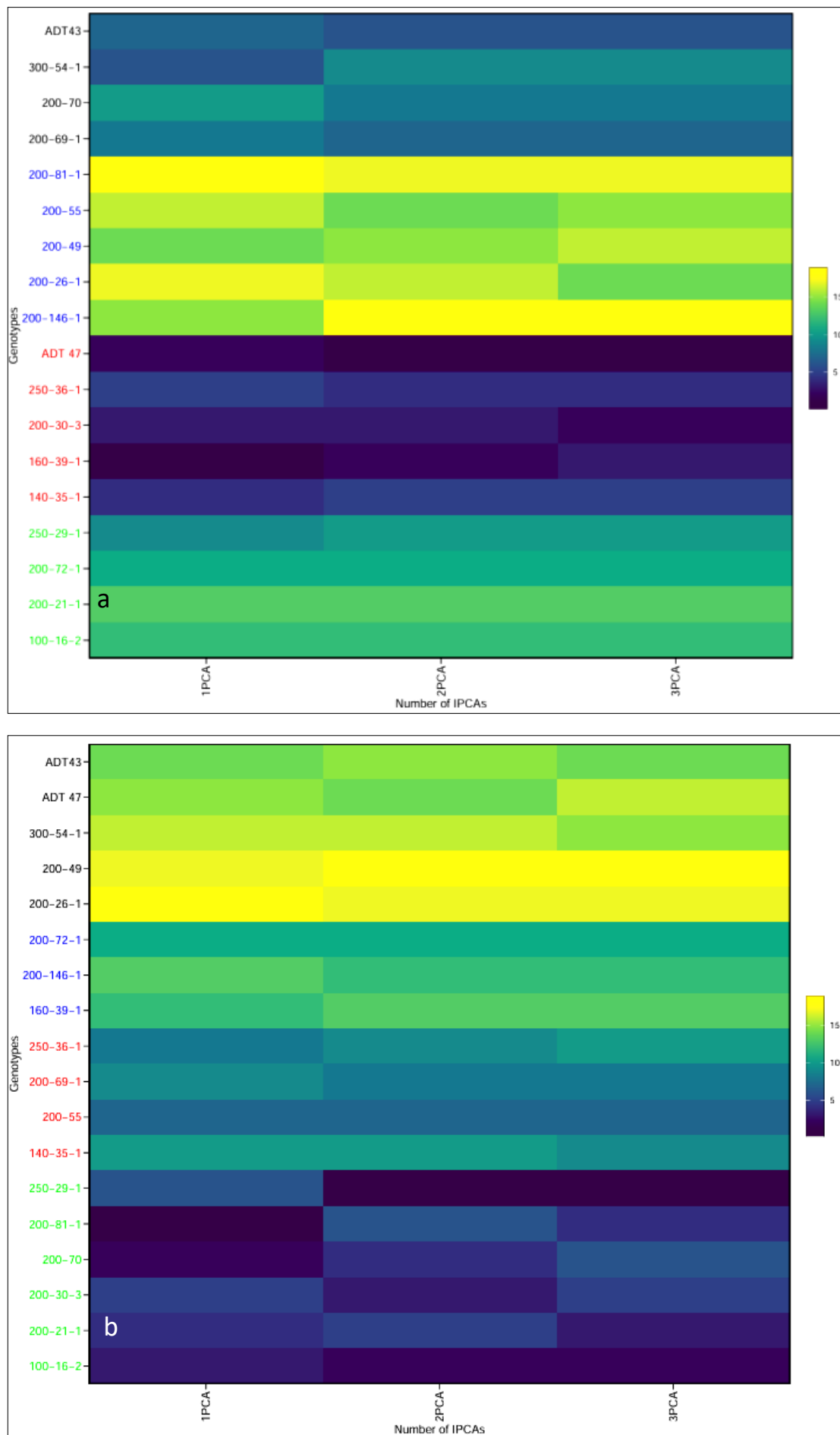
involved in the estimation of mean yield and genotypic adaptability), harmonic mean of the relative performance of genotypic values (HMRPGV) tangled to evaluate stability, adaptability and yield simultaneously. Rice mutants 200-21-1, 200-72-1, ADT (R) 47, ADT 43, 200-69-1 were identified as highly stable and yielding for grain yield and mutants 300-54-1, 200-73-1, ADT 43, 200-55, 200-146-1 were highly stable and yielding for hundred grain weight (Table 2 & 3).

#### Estimation of AMMI-based Stability indices

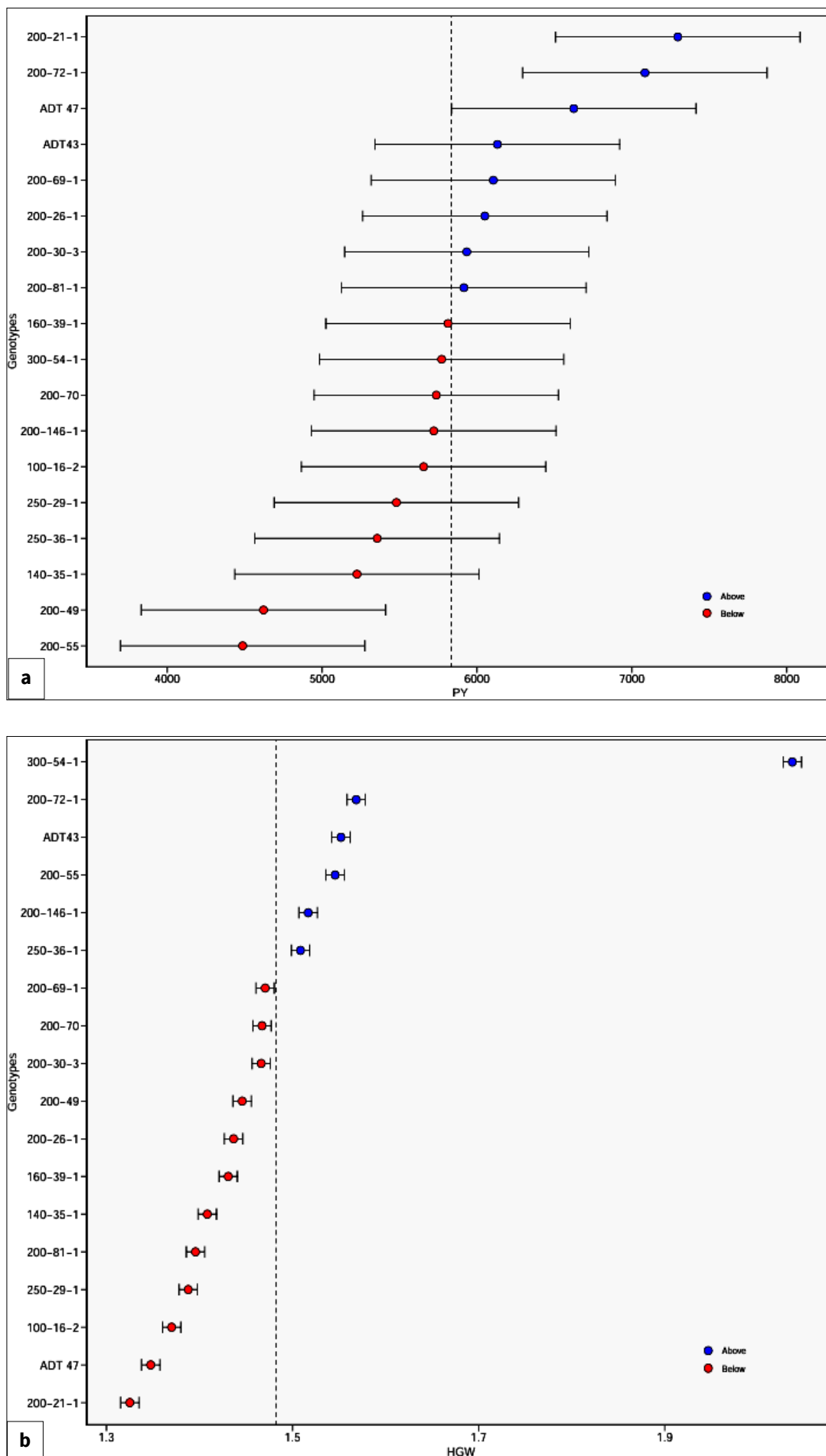
Univariate statistics such as AMMI Stability Index (ASI), AMMI stability value (ASV), AMMI-based stability parameter (ASTAB), sum across environments of the absolute value of GEI modeled by AMMI (AVAMGE), Annicchiarico's D parameter (DA), Zhang's D parameter (DZ) averages of the squared eigenvector values (EV), stability measure based on fitted AMMI model (FA), Modified AMMI Stability Index (MASI), modified AMMI stability value (MASV), sums of the absolute value of the IPC scores (SIPC) and absolute value of the relative contribution of IPCs to the interaction (Za) are used to identify the most stable genotypes based on certain parametric statistics. AMMI-based stability indices were calculated for plant yield and hundred grain weight (Supplementary Table 1 & 2). Comparing all the calculated indices, mutants ADT47, 160-39-1, 200-30-3, 250-36-1, 200-69-1 were highly stable for plant yield. Mutants 200-55, 200-69-1, 200-81-1, 250-29-1, 250-36-1 were highly stable for hundred grain weight.

#### Multi-trait stability index interpretation

The multi-trait stability index is involved in the estimation of the distance between the genotype and phenotype. It generates a factor analysis for efficient genotypic selection for mean performance and stability based on multiple traits. MTSI was calculated based on quantitative and qualitative traits. Rice



**Fig. 3.** Heatmap illustration of rice mutants along with control. Heatmap illustrates rankings of 16 rice mutants and 2 control genotypes based on utilization of IPCAs in the WAASB estimation for plant yield (a) and hundred grain weight (b).



**Fig. 4.** Best linear unbiased prediction mean values of single-plant yield (a) and hundred grain weight (b) of rice mutants.

**Table 2.** BLUP-based ranking of rice mutants for plant yield evaluated in different environments

| GEN        | Plant yield(Kg/ha) | HMGV  |      | RPGV  |      | HMRPGV |      |
|------------|--------------------|-------|------|-------|------|--------|------|
|            |                    | Score | Rank | Score | Rank | Score  | Rank |
| 100-16-2   | 5614               | 5592  | 9    | 0.976 | 11   | 0.97   | 11   |
| 140-35-1   | 5084               | 4908  | 16   | 0.871 | 16   | 0.867  | 16   |
| 160-39-1   | 5808               | 5635  | 8    | 0.993 | 9    | 0.991  | 8    |
| 200-146-1  | 5695               | 5348  | 13   | 0.967 | 13   | 0.949  | 13   |
| 200-21-1   | 7634               | 7517  | 1    | 1.31  | 1    | 1.3    | 1    |
| 200-26-1   | 6101               | 5751  | 7    | 1.03  | 6    | 1.02   | 7    |
| 200-30-3   | 5957               | 5839  | 6    | 1.02  | 7    | 1.02   | 6    |
| 200-49     | 4342               | 4386  | 17   | 0.766 | 17   | 0.762  | 17   |
| 200-55     | 4176               | 4184  | 18   | 0.737 | 18   | 0.728  | 18   |
| 200-69-1   | 6168               | 5947  | 5    | 1.05  | 5    | 1.05   | 5    |
| 200-70     | 5715               | 5470  | 12   | 0.972 | 12   | 0.968  | 12   |
| 200-72-1   | 7372               | 7259  | 2    | 1.27  | 2    | 1.26   | 2    |
| 200-81-1   | 5934               | 5565  | 10   | 1     | 8    | 0.989  | 9    |
| 250-29-1   | 5398               | 5135  | 14   | 0.917 | 14   | 0.911  | 14   |
| 250-36-1   | 5245               | 5069  | 15   | 0.898 | 15   | 0.895  | 15   |
| 300-54-1   | 5757               | 5562  | 11   | 0.987 | 10   | 0.977  | 10   |
| ADT (R) 47 | 6807               | 6632  | 3    | 1.16  | 3    | 1.16   | 3    |
| ADT 43     | 6200               | 6096  | 4    | 1.07  | 4    | 1.06   | 4    |

**Table 3.** BLUP-based ranking of rice mutants for hundred grain weight evaluated in different environments

| GEN        | Hundred grain weight (g) | HMGV  |      | RPGV  |      | HMRPGV |      |
|------------|--------------------------|-------|------|-------|------|--------|------|
|            |                          | Score | Rank | Score | Rank | Score  | Rank |
| 100-16-2   | 1.37                     | 1.37  | 16   | 0.924 | 16   | 0.924  | 16   |
| 140-35-1   | 1.41                     | 1.41  | 13   | 0.95  | 13   | 0.95   | 13   |
| 160-39-1   | 1.43                     | 1.43  | 12   | 0.965 | 12   | 0.965  | 12   |
| 200-146-1  | 1.52                     | 1.52  | 5    | 1.02  | 5    | 1.02   | 5    |
| 200-21-1   | 1.33                     | 1.33  | 18   | 0.894 | 18   | 0.894  | 18   |
| 200-26-1   | 1.44                     | 1.44  | 11   | 0.969 | 11   | 0.969  | 11   |
| 200-30-3   | 1.47                     | 1.47  | 9    | 0.989 | 9    | 0.989  | 9    |
| 200-49     | 1.45                     | 1.45  | 10   | 0.975 | 10   | 0.975  | 10   |
| 200-55     | 1.55                     | 1.55  | 4    | 1.04  | 4    | 1.04   | 4    |
| 200-69-1   | 1.47                     | 1.47  | 7    | 0.992 | 7    | 0.992  | 7    |
| 200-70     | 1.47                     | 1.47  | 8    | 0.99  | 8    | 0.99   | 8    |
| 200-72-1   | 1.57                     | 1.57  | 2    | 1.06  | 2    | 1.06   | 2    |
| 200-81-1   | 1.4                      | 1.4   | 14   | 0.942 | 14   | 0.942  | 14   |
| 250-29-1   | 1.39                     | 1.39  | 15   | 0.936 | 15   | 0.936  | 15   |
| 250-36-1   | 1.51                     | 1.51  | 6    | 1.02  | 6    | 1.02   | 6    |
| 300-54-1   | 2.04                     | 2.04  | 1    | 1.37  | 1    | 1.37   | 1    |
| ADT (R) 47 | 1.35                     | 1.35  | 17   | 0.909 | 17   | 0.909  | 17   |
| ADT43      | 1.55                     | 1.55  | 3    | 1.05  | 3    | 1.05   | 3    |

mutants were ranked from the highest to the lowest value of the MTSI. Hence, mutants with the highest value of the MTSI were in the centre and the genotypes with the lowest MTSI value were located in the outermost circle. The genotypes determined in red color dots were selected based on their MTSI values at 20 % selection intensity. The mutants 250-36-1, 200-55 and 260-39-1 were the most ideal stable lines. Favorable selection differential caused by selected genotypes.

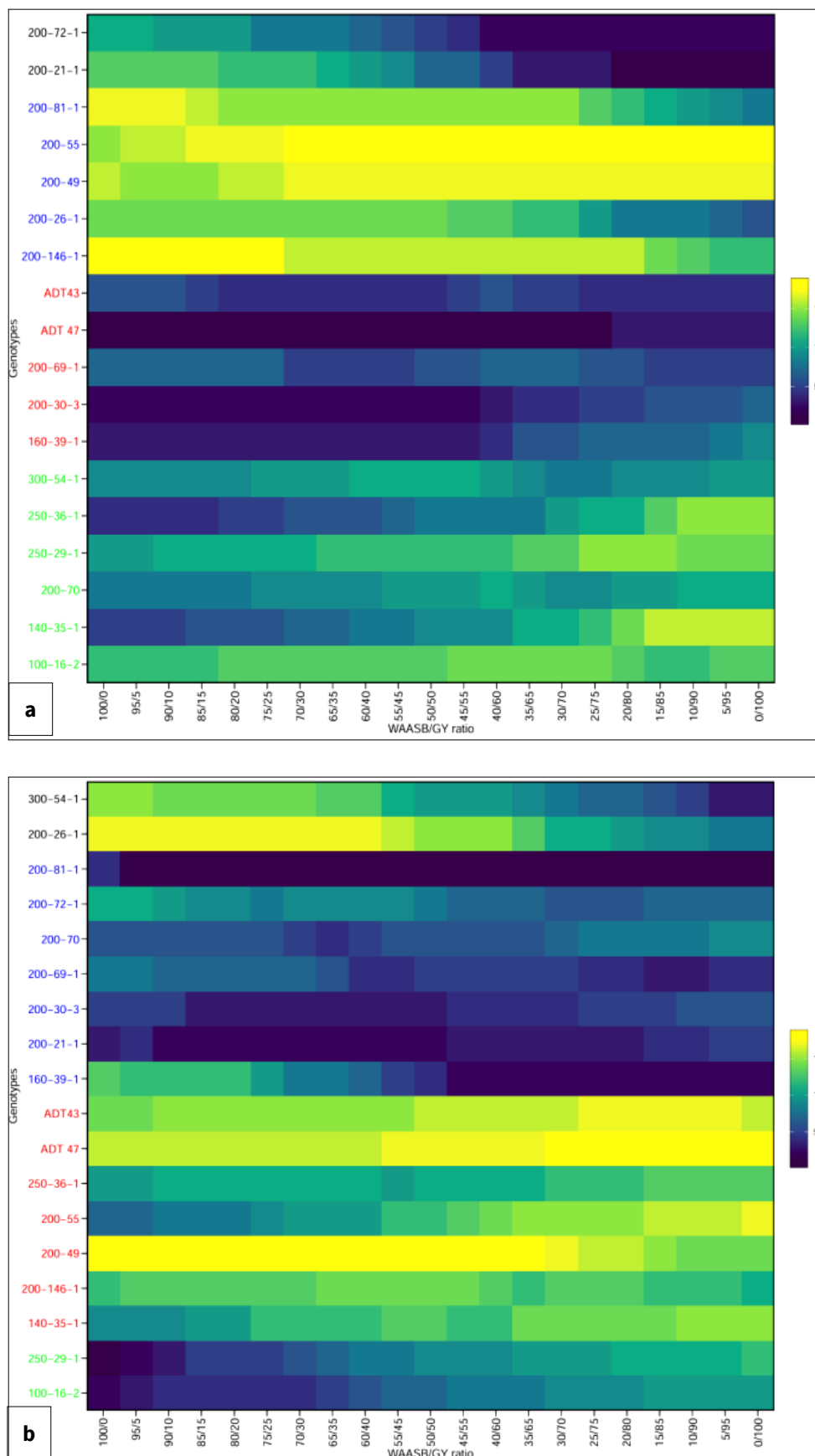
WAASBY index scores are used to enhance the accuracy of evaluation based on performance and stability. Ranking based on WAASBY may fluctuate depending on the ratio of WAASB to performance (15). When introducing a new variety, it's important to analyze both yield stability and productivity. This index combines phenotypic stability and quantitative yield, allowing for the selection of high-yielding genotypes while minimizing GEI. These indices integrate AMMI's graphical tools with BLUP's prediction accuracy to analyze stability. The WAASB index, which includes all of its principal components, provides a more reliable interpretation of stability, which leads to the selection of genotypes (16). More productive and stable oat genotypes were observed in oats (17). This helps optimize cultivar introduction programs (18). For plant yield, mutants 100-16-2, 140-35-1, 200-70, 250-29-1, 250-36-1, 300-54-1 and for hundred grain weight, mutants 100-16-2, 250-29-1, which represent stable genotypes

with optimal performance (Fig. 5). Stable rice genotypes based on WAASBY values were identified (16).

The limited maximum-likelihood (REML)/BLUP models are widely accepted for genetic evaluation in breeding, particularly in GEI investigations (19-21). Biometric techniques like HMGV, RPGV and HMRPGV reveal the unpredictability of genotypic effects and enable ranking of genotypes based on performance (19). The BLUP-based simultaneous selections, such as HMGV, RPGV and HMRPGV estimates, showed that mutants 200-21-1, 200-72-1, ADT (R) 47, ADT 43, 200-69-1 were identified as high stable and yielding for grain yield and mutants 300-54-1, 200-73-1, ADT 43, 200-55, 200-146-1 were highly high stable and yielding for hundred grain weight.

The AMMI Stability Index (ASI) and AMMI Stability Value (ASV) with two PCs, as well as the Modified AMMI Stability Index (MASI) and Modified AMMI Stability Value (MASV) with all significant PCs, were analyzed to assess genotype stability after modifications.

For selecting a stable genotype with high yields, various selection indices have been developed (22). A parametric and non-parametric genotype selection index was developed (23). These indices combine yield and stability parameters to prioritize both stability and high yield. Some argue that selection



**Fig. 5.** The rank of rice mutants based on estimating WAASB index for plant yield (a) and hundred grain weight (b).

procedures based just on yield are preferable to those that consider both stability and flexibility in one statistic. Comparing all the calculated indices, mutants ADT47, 160-39-1, 200-30-3, 250-36-1 and 200-69-1 were highly stable for plant yield. Mutants 200-55, 200-69-1, 200-81-1, 250-29-1, 250-36-1 were highly stable for hundred grain weight.

MTSI method for selecting high yield and stable genotypes in METs based on several characteristics, taking into account fixed and random influences, was proposed (24). The MTSI is calculated using factor analysis to evaluate the distance from an ideal genotype. This index allows for stable genotypes with positive selection differentials for increasing traits and negative selection differentials for decreasing traits. The MTSI Index facilitates selection for breeders and agronomists by providing opportunities for simultaneous selection based on performance and stability, even with multiple trait data. This method uses both fixed and mixed effects models. The selected genotypes resulted in beneficial selection of differentials across all characteristics. MTSI can help plant breeders select superior genotypes for many characteristics using multi-environment data (16, 25). To select stable soybean genotypes under drought and salinity stress conditions, MTIS was used (26). Five sugar beet genotypes that were stable in rhizomania disease-infected fields were identified based on MTSI data (27). These data support the effectiveness of MTSI in identifying superior genotypes. Two mung bean genotypes with high-yielding and stable resistance to MYMV were identified (28).

## Conclusion

Rice being the stable food for the people of Asia and Africa, there is a constant need for improvement of rice varieties to cater for the demand of an expanding population, changing environments and preferences. ADT (R) 47, a short duration variety to improve the major lacuna of panicle choking and poor shattering nature. This variety is high-yielding among short-duration varieties, along with good grain properties. Improvement of defects may bring out a high-yielding variety with good grain qualities, which can be grown in 3.20 lakh ha of *kuruvai* areas of Tamil Nadu to improve the state productivity. Various stability parameters, such as ASV, ASTB, AVAMGE, DA, DZ, EV and FA, considered in this study, proved that all have equal potential in the identification of stable genotypes. To ensure high stability, the BLUB method can be suggested as the best approach. To conclude, mutants viz., 160-39-1, 200-30-3, 250-36-1, 200-69-1 were highly stable for plant yield along with ADT47 control variety for all the tested environments viz., Aduthurai (E<sub>1</sub>), Thanjavur (E<sub>2</sub>), Tirur (E<sub>3</sub>) and Madurai (E<sub>4</sub>) and decisively considered as stable high-yielding genotypes for rice cultivation.

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## Authors' contributions

TR and DS carried out the investigation, project administration and drafted the manuscript. TR, ARP, AS and KV participated in the experimental analysis, methodology and data curation. KB, MU and BM conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None

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