Analyzing genetic variations for head rice recovery under heat stress in rice (Oryza sativa L.)

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Abstract
High temperature induces yield losses in rice, by affecting pollination, fertilization and also by affecting grain quality. High temperature stress coinciding with the grain filling period affects starch granule compaction thereby leading to reduced head rice recovery (HRR). The broken grains significantly reduce the price of rice by 50% in the market. Hence, the development of rice varieties that exhibit a lower reduction in grain quality coupled with higher head rice recovery under high temperature stress has become a major mandate in rice breeding programs. The present study was undertaken to survey the genetic variation for head rice recovery in rice under both normal (wet season) and high temperature (dry season) conditions. Evaluation of 50 diverse rice germplasm lines across different temperature regimes during grain filling viz. mean temperature of 30.8°C (wet season; May to Sep-Oct) and 35.8°C (Dry season 2017; January to May) and 36.2°C (Dry season 2018; Nov-Dec to May) identified huge genetic variation for HRR in rice. During the wet season, HRR ranged between 20.6 per cent and 90.9 per cent. During summer, rice genotypes exhibited a significant reduction in HRR from 3.6 to 82.7 per cent. Stability analysis revealed that the rice genotypes viz. CO 39, ChiemChanh, CO 18, Guan-Yin-Tsan, IR36, Teqing, ARC 10818 and Cimarron exhibited stable head rice recovery across all seasons.

Keywords: Rice, heat stress, stability, head rice recovery

INTRODUCTION
Rice (Oryza sativa L.) serves as a staple food for more than 50 per cent of the Asian population (Bishwajit et al., 2013). Rice is also a major source of income for many small and marginal farmers in Asia and Africa. Even though rice production has increased by several folds after the Green Revolution (1960s) and the introduction of hybrids (1980s), it has to be doubled by 2050 to meet the requirements of the growing population. But, yield plateau, diminishing natural resources, changing climate and increasing pests and diseases are posing serious threats to increasing rice productivity and thereby reaching the goal of doubling rice production by 2050 (Ray et al., 2013)

Rice is particularly vulnerable to heat stress (>35 °C), especially during the gametogenesis (Jagadish et al., 2013) and flowering (Prasad et al., 2006; Jagadish et al., 2015) stages. Climate change is likely to have a negative impact on the world’s rice output in the coming years. Global climatic
estimates show an increased frequency of heat spikes and warmer nights (IPCC, 2013), posing additional threats. According to climate simulations, by 2030, 16% of rice-growing land will be exposed to temperatures at least 5°C above the crucial threshold during the reproductive phase (Gourdji et al., 2013).

Rice is sensitive to high temperature stress at almost all the stages of its growth and development. Heat stress will have negative impact on spikelet fertility and also grain quality if it coincides with flowering and grain-filling stages. High temperature (HT) stress at the ripening phase affects the grain quality and head rice recovery. Head rice recovery (HRR) is defined as the proportion of paddy rice that retains 75% of its length after milling. For a new rice variety to be accepted and adopted by farmers, it should satisfy the consumer requirements of minimum 55 per cent HRR or above. Hence, HRR is a crucial attribute by which new varieties are selected for release (Lapis et al., 2019). Previously, Dalvi et al. (2007), Panwar et al. (2008), Waghmode and Mehta (2011), Padmavathi et al. (2013), Radhamani et al. (2017), Parimala et al. (2019) and Chandrashekhar et al. (2020) reported the existence of G x E interaction for quality traits in rice and yield related traits. Development of rice varieties exhibiting a lesser reduction in HRR during HT stress necessitates measuring the genetic variation of HRR in rice under both normal and HT conditions.

In the present study, HRR was estimated in a set of 50 diverse rice genotypes grown under contrasting temperature regimes during the grain filling stage. Genetic parameters namely, heritability, genetic advance and stability were estimated to identify rice genotypes exhibiting stable HRR across varying temperatures.

MATERIALS AND METHODS
The field trials were conducted at Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore, India (11°N, 77°E and 426.7m above MSL) across three different seasons viz. kharif (WS 2016) and summer (DS 2017 and DS 2018) (Table 1) involving 50 diverse rice accessions (Table 2).

Table 1. Details of season, harvesting time and mean temperature during grain filling stage

| Season               | Growing season          | Mean temperature during grain filling stage (°C) | Temperature range (°C) |
|----------------------|-------------------------|-------------------------------------------------|------------------------|
| Wet season (WS 2016) | May - October 2016      | 30.58                                           | 27.5 – 31.9            |
| Dry season 2017 (DS 2017) | January - May 2017 | 34.97                                           | 31.7 - 35.8            |
| Dry season 2018 (DS 2018) | November - May 2018 | 34.70                                           | 31.6 – 36.3            |

The head rice recovery analysis was adapted from Singh et al. (2000). About 100 to 150 g of rough rice was taken and milled rice was obtained by dehulling or dehusking with a rice sheller (Rice Polishing Machine LTJM-2099, Garg Instrumentation, Haryana). Milled rice kernels were separated into head rice and broken kernel fractions with different sized separator/ sieves. Full kernel and ¾ size kernels were considered as head rice and weighed for calculating HRR percentage. Head rice recovery percentage was calculated as (DRR, 2014).

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\text{Head rice recovery (\%)} = \frac{\text{Weight of full kernel}}{\text{Weight of rough rice}} \times 100
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To assess the genetic diversity of HRR, descriptive statistics and frequency distribution were calculated using TNAUSTAT software (Manivannan, 2014). Stability analysis (AMMI model) was performed using the HRR data from all three seasons to identify stable genotypes.

RESULTS AND DISCUSSION
During the wet season of 2016, the maximum day temperature during the grain filling period ranged between 27.5°C and 31.9°C with a mean of 30.58°C. HRR ranged between 20.6 and 90.9 per cent with an average of 62.8 per cent (Table 3). In contrary, the maximum day temperature during the dry season was ranged between 31.7 and 35.8°C with an average of 34.97°C (Summer 2017) and 31.6 to 36.3°C with an average of 34.7°C (Summer 2018). This increased maximum day temperature had a significant effect on the HRR. During Summer, 2017, head rice recovery ranged from 4.3 to 88.1 per cent with an average of 37.4 per cent and during Summer, 2018, the HRR ranged from 2.8 to 88.4 per cent with an average of 33.56 per cent. From these results, it is clearly evident that a rise in temperature of >4°C during summer seasons over the wet season drastically reduced the average HRR.

To analyze the genetic variations for head rice recovery, the data from all three seasons to identify stable genotypes.
Table 2. Head rice recovery (%) and Interaction principal component analysis (IPCA) parameters of AMMI model for HRR across different seasons

| G. No. | Genotype       | Country of origin | WS 2016 | DS 2017 | DS 2018 | AMMI results |
|--------|----------------|-------------------|---------|---------|---------|--------------|
|        |                |                   |         |         |         | HRR (%)      |
| G1     | IR64-21        | Philippines       | 58.7    | 46.5    | 44.2    | 49.8*        |
| G2     | Minhui 63      | China             | 80.9    | 10.5    | 7.6     | 33           |
| G3     | Shan-Huang-Zhan-2 | China        | 83.3    | 11.4    | 15.9    | 36.8         |
| G4     | CHITRAJ (DA 23):IRGC 6208-1 | Bangladesh | 48.8    | 10.4    | 30.0    | 29.7         |
| G5     | CO39:IRGC 51231-1 | India         | 78.5    | 46.8    | 31.8    | 52.3*        |
| G6     | OF ABRIL:IRGC 50463-1 | Brazil       | 55.5    | 22.5    | 20.3    | 32.8         |
| G7     | FANDRAPOTSY:IRGC 10984-1 | Madagascar | 38.8    | 32.2    | 30.9    | 34.0         |
| G8     | GIES5:IRGC 8231-1 | Vietnam      | 30.3    | 27.4    | 26.7    | 27.9         |
| G9     | JC92:IRGC 9176-1 | India         | 72.4    | 32.6    | 35.4    | 46.8         |
| G10    | LAL AMAN:IRGC 46202-1 | India      | 54.1    | 21.5    | 20.4    | 32           |
| G11    | MADAEL:IRGC 7722-1 | Sri Lanka    | 55.7    | 4.9     | 3.6     | 21.4         |
| G12    | MAKALIOKA 34:IRGC 6087-1 | Madagascar | 54.4    | 34.4    | 35.8    | 41.5         |
| G13    | G05:IRGC 34393-1 | South Korea    | 35.0    | 17.7    | 17.1    | 23.2         |
| G14    | MTU9:IRGC 7919-1 | India         | 59.2    | 40.3    | 38.0    | 46.8         |
| G15    | PATIK:IRGC 43530-1 | Indonesia    | 71.7    | 58.4    | 22.6    | 50.9*        |
| G16    | PIN KAO:IRGC 5803-1 | Thailand    | 80.1    | 72.7    | 68.8    | 73.9*        |
| G17    | RTS4:IRGC 8177-1 | Vietnam       | 33.5    | 26.6    | 25.3    | 28.5         |
| G18    | VANDANA:IRGC 117398-1 | India      | 50.5    | 41.0    | 36.5    | 42.6         |
| G19    | RTS14 | Vietnam         | 55.2    | 36.8    | 20.7    | 37.6         |
| G20    | AI-CHIAO-HONG | China         | 55.5    | 17.3    | 16.4    | 28.8         |
| G21    | BINULAWAN | Philippines     | 84.1    | 82.7    | 77.9    | 81.6*        |
| G22    | CHANG CH' SANG HSU TAO | China      | 54.3    | 25.0    | 20.6    | 33.3         |
| G23    | CHIEM CHANH | Vietnam        | 70.6    | 49.6    | 47.0    | 55.7*        |
| G24    | CO18 | India          | 78.5    | 42.8    | 40.8    | 54.1*        |
| G25    | GUAN-YIN-TSAN | China         | 85.4    | 64.6    | 51.6    | 67.2*        |
| G26    | IR36 | Philippines    | 82      | 49.4    | 44.7    | 56.7*        |
| G27    | KUM-MIN-TSIEH-HUNAN | China      | 54.1    | 19.3    | 46.1    | 39.8         |
| G28    | DRYZICA LLANOS 5 | Colombia     | 77.3    | 22.2    | 18.7    | 39.4         |
| G29    | PAO TOU HUNG | China         | 53.1    | 23.1    | 19.6    | 31.9         |
| G30    | PAPPAKU | China         | 72.1    | 69.4    | 60.1    | 67.2*        |
| G31    | PEH-KUH-TSAO-TU | Taiwan       | 80.3    | 76.7    | 73.1    | 76.7*        |
| G32    | TEQING | China         | 67.2    | 41.9    | 43.6    | 50.9*        |
| G33    | TKM6 | India          | 87.7    | 24.9    | 23.6    | 45.4         |
| G34    | 17733:02-005:IRGC 51080-1 | Sri Lanka | 71.5    | 59.3    | 13.3    | 48.1         |
| G35    | 849:IRGC 5970-1 | Madagascar    | 20.8    | 14.2    | 11.1    | 15.4         |
| G36    | AGAMI M 1::IRGC 4158-1 | Egypt       | 84.2    | 73.2    | 38.1    | 65.1*        |
| G37    | AI LAN KE 1110:IRGC 67034-1 | China   | 56.2    | 39.6    | 37.6    | 44.5         |
| G38    | ARC 10818::IRGC 21079-1 | India     | 73.8    | 53.3    | 50.4    | 59.2*        |
| G39    | BADA DHAN::IRGC 26540-1 | Bangladesh | 48.8    | 22.2    | 40.4    | 37.2         |
| G40    | BALGALA GURMATIA::IRGC 61074-1 | India | 80.4    | 35.7    | 34.3    | 50.1*        |
| G41    | BANDOUROU::IRGC 15980-1 | Senegal    | 57.8    | 38.6    | 36.4    | 44.3         |
| G42    | BIRAIN 360::IRGC 6650-1 | Bangladesh | 70.1    | 59.9    | 56.8    | 62.3*        |
| G43    | BYAT KYAR::IRGC 33004-1 | Burma       | 61.6    | 24.9    | 23.5    | 36.7         |
| G44    | CHI TOU HUANG 1::IRGC 51280-1 | China     | 53.8    | 39.2    | 37.3    | 43.4         |
| G45    | CHINA 98-45-1::IRGC 1598-1 | China       | 71.3    | 62.4    | 59.4    | 64.4*        |
| G46    | CIMARRON::IRGC 116967-1 | Venezuela   | 67.2    | 42.4    | 38.0    | 49.2*        |
| G47    | BANDHUKH5:IRGC 6050-1 | Bangladesh | 70.3    | 26.8    | 25.4    | 40.9         |
| G48    | DA NUO (ZHAN)::IRGC 72024-1 | China       | 31.4    | 11.4    | 10.8    | 17.8         |
| G49    | DENG DENG QI::IRGC 72671-1 | China       | 53.7    | 26.2    | 14.8    | 31.6         |
| G50    | E 5168::IRGC 68021-1 | China       | 69.5    | 36.9    | 34.9    | 47.1         |

**Significant at 5% level**

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Bhaskaran and Sebastian (2017) had earlier reported that a 4.4°C increase in day temperature resulted in a 24.9 per cent reduction in HRR. Similarly, it was reported that high air temperature during grain filling significantly reduced the HRR (Truong et al., 2012; Liu et al., 2013; Abayawickrama et al., 2017). Frequency distribution analysis clearly indicated a greater variability for HRR across three seasons (Fig. 1). During WS 2016, 10 genotypes recorded more than 80 per cent head rice recovery with a maximum HRR of 90.9 per cent. However, during DS 2017, only one genotype registered a HRR of more than 80 per cent and during DS 2018, none of the entries showed head rice recovery of more than 80 per cent. This indicates that high temperature during the grain filling stage had a significant negative effect on the percentage of head rice recovered.

Genetic variability parameters such as genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h²) and genetic advance as percentage of the mean (GAM) were estimated for all the 50 rice accessions. High PCV and GCV values were observed during all three seasons (Table 3). PCV was higher than their corresponding GCV, which signifies the influence of environmental interaction. Higher GCV and PCV values indicated that the traits are genetically controlled and amenable for selection. This also indicated that head rice recovery percentage could be improved through hybridization and selection (Bisne et al., 2009). The results showed a higher heritability and high GAM for head rice recovery during all three seasons. Previous studies also indicated that HRR exhibited a higher heritability with high GAM (Singh et al., 2021). Similarly, Devi et al. (2016), Nirmaladevi et al. (2015) and Subudhi et al. (2011) also observed high heritability for HRR.

Genotype × Environment (G × E) interaction is a major problem in the study of quantitative traits. Hence, the identification of stable genotypes over a wide range of environments is an important but challenging task for breeders. The AMMI analysis of variance revealed highly significant variance due to genotypes and environments for HRR percentage. Variance due to genotype × environment interactions was significant for HRR percentage (Table 4). The G × E interaction was again partitioned into two, IPCA 1 and IPCA 2 axes without any residual value. Both the IPCA scores representing the interaction pattern were significant for HRR percentage. The significance of two IPCA scores suggested the presence of a complex, multidimensional variation in genotypes by environment data.

![Fig. 1. Frequency distribution of head rice recovery (%) during three different seasons](image)

Table 3. Descriptive statistics for head rice recovery percentage across different seasons

| Parameters     | WS 2016 | DS 2017 | DS 2018 |
|----------------|---------|---------|---------|
|                | Temp (°C) | HRR (%) | Temp (°C) | HRR (%) | Temp (°C) | HRR (%) |
| Grand Mean     | 30.58    | 62.8    | 34.97    | 37.4    | 34.7    | 33.56    |
| S.E.           | 0.15     | 3.2     | 0.13     | 3.1     | 0.19    | 3.02     |
| Range          | 27.5 - 31.9 | 20.6 - 90.9 | 31.7 - 35.8 | 4.3 - 88.1 | 31.6 - 36.3 | 2.8 - 88.4 |
| CD(5%)         | -        | 8.9     | -        | 8.7     | -       | 8.46     |
| CV(%)          | -        | 8.8     | -        | 14.4    | -       | 15.60    |
| PCV(%)         | -        | 26.9    | -        | 53.1    | -       | 52.01    |
| GCV(%)         | -        | 25.4    | -        | 51.1    | -       | 49.62    |
| h²(%)          | -        | 89.4    | -        | 92.6    | -       | 91.01    |
| GAM(%)         | -        | 49.4    | -        | 101.4   | -       | 97.51    |

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Among the two AMMI components, the first IPCA (73.2%) explained most of the proportion of genotype x environment interaction than the second IPCA (26.8%). AMMI with two interaction principal component axes together explained 100 per cent of G x E interaction.

The AMMI 1 biplot for HRR clearly indicated that the three environments differed in both main and interaction effects. A total of eight genotypes viz. CO39 (G5), ChiemChanh (G23), CO18 (G24), Guan-Yin-Tsan (G25), IR36 (G26), Teqing (G32), ARC 10818 (G38) and Cimarron (G46) showed IPCA 1 score close to zero with high main effects (Table 2). This indicated that the above mentioned lines were stable and had general adaptability overall seasons for HRR.

Among the three seasons studied, environment 1 (WS 2016) with temperatures ranged from 27.5 - 31.9°C had increased head rice recovery (62.84%) as compared to the other two seasons (DS 2017, DS 2018) with temperatures ranged from 31.7 - 35.8°C (37.4% HRR) and 31.6 - 36.3°C (33.5% HRR), respectively (Table 2). This indicates high temperature stress plays a major role in grain quality, especially head rice recovery. Among the 50 lines studied, seven lines viz. CO39, (G5) Patik (G15), CO18 (G24), Guan-Yin-Tsan (G25), IR36 (G26), Agami M 1 (G36) and Cimarron (G46) exhibited high main effect with positive IPCA 1 score near to origin. Since the environment E1 had a positive IPCA 1 score, it had a positive interaction with these genotypes and environment E1 can be considered as the favorable environment for the selected genotypes (Fig. 2). On the other hand, three lines, Chiem Chanh (G23), Teqing (G32) and ARC 10818 (G38) had a high main effect with negative IPCA 1 score far from origin. The environments E2 and E3 had negative IPCA 1 scores and therefore E2 and E3 can be considered as favorable environments for these three genotypes.

The IPCA 1 component accounted for 73.2 per cent of G x E interaction, while IPCA 2 accounted for 26.8 per cent in AMMI 2 biplot indicated that this model fit 100 per cent. A total of 31 genotypes viz., Chitraj (G4) (DA 23), CO 39 (G5), De abril (G6), JC92 (G9), LalAman (G10), Makalioka 34 (G12), Milyang 23 (G13), MTU9 (G14), Patik (G15), RTS14 (G17), Al-Chiao-Hong (G20), ChangCh’sangHsu Tao (G22), ChiemChanh (G23), CO18 (G24), Guan-Yin-Tsan (G25), IR36 (G26), Kun-Min-Tsief-Hunan (G27), PaoTou Hung (G29), Teqing (G32), 17/79/02-005 (G34), Agami M 1 (G36), AlLanKe1110 (G37), ARC 10818 (G38), BadaDhan (G39), Bandiourou (G41), ByatKyar (G43), ChiTou Huang 1 (G44), Cimarron (G46), DaNuO (ZHAN) (G48), DengDeng OI (G49) and E 5168 (G50) were positioned close to the origin for IPCA 1 and IPCA 2 scores (Fig 3). This showed minimal interaction of these genotypes with environments. The remaining lines were scattered away from the origin in the biplot revealing that the genotypes were more sensitive to environmental interactive forces. Among the three environments, E1 (wet season, 2016) is the less interacting environment for head rice recovery and would be adjudged the best season for improved HRR as compared to the other two dry seasons of 2017 and 2018. Among these 31 lines, ten lines viz. CO 39 (G5), Patik (G15), ChiemChanh (G23), CO18 (G24), Guan-Yin-Tsan (G25), IR 36 (G26), Teqing (G32), Agami M 1 (G36), ARC 10818 (G38) and Cimarron (G46) exhibited high significant mean values for HRR percentage.

The "which-won-where" biplot was been constructed to identify the best performing genotypes for HRR for each season. The polygon view of the GGE biplot is used for visualization of the best performing genotypes for a specific environments (Das et al., 2018). Polygon is constructed by joining the genotypes far away from the biplot origin to contain all the genotypes inside the polygon. Genotypes positioned in the vertices of the polygon are the best performer or poor performer in one or more environments (Yan and Tinker, 2006). From the results (Fig. 4), it is evident that the rice genotypes viz. CO 39 (G5), Patik (G15), ChiemChanh (G23), CO 18 (G24), Guan-Yin-Tsan (G25), IR36 (G26), Teqing (G32), Agami M 1 (G36), ARC 10818 (G38) and Cimarron (G46) are better performing genotypes in terms of HRR for the environment E1 (wet season 2016). Furthermore, the genotypes viz. IR 64-21 (G1), PinkKaeo (G16), Binulawan (G21), Pappaku (G30), Peh-Kuh-Tsao-Tu (G31), Birain 360 (G42) and China 98-45-1 (G45) are good performers for HRR in environments E2 (dry season 2017) and E3 (dry season 2018).
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Fig. 2. AMMI 1 model for HRR showing mean of genotypes and seasons against their IPCA1 scores

Fig. 3. AMMI 2 model for head rice recovery showing IPCA1 vs IPCA2 scores of rice genotypes across seasons
The "which-won-where" biplot was constructed to identify the best performing genotypes for HRR for each season. The polygon view of the GGE biplot is used for visualization of the best performing genotypes for a specific environment (Das et al., 2018). Polygon is constructed by joining the genotypes far away from the biplot origin to contain all the genotypes inside the polygon. Genotypes positioned in the vertices of the polygon are the best performer or poor performer in one or more environments (Yan and Tinker, 2006).

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Fig. 4. "Which-won-where" biplot of rice genotypes across three different seasons

Fig. 5. GGE biplot for head rice recovery in rice

In GGE biplot analysis, E2 (DS 2017) exhibited a positive correlation with E3 (DS 2018) as the E2 environment vector made an acute angle with the E3 environment vector while E2 and E3 environment vectors formed approximately a right angle with E1 (WS 2016) indicating independent nature of E1 (wet season) and E2, E3 (dry seasons) environments. This is consistent with the high degree of variability in the observed head rice recovery of genotypes which might be attributed to contrasting agroclimatic conditions of these seasons and the presence of a high degree of cross-over interactions due to higher G x E interactions.

By considering AMMI 1, AMMI 2, and GGE biplot results, eight genotypes viz. CO 39 (G4), ChiemChanh (G23), CO 18 (G24), Guan -Yin-Tsan (G25), IR36 (G26), Teqing (G32), ARC 10818 (G38) and Cimarron (G46) were found to exhibit stable HRR and general adaptability overall environments. These genotypes with superior mean values of HRR and less interaction with the environment identified by the AMMI model could be used in breeding programs to develop high temperature tolerant rice varieties with improved HRR.

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The interrelationship between the environments is depicted in Fig. 5. The cosine of the angle between two environmental vectors in a GGE biplot reflects the correlation between them (Yan and Tinker, 2006). The acute angle between two environmental vectors indicates a positive correlation between the concerned test environments while the obtuse angle indicates a negative correlation between them. In the environment-vector view of the GGE biplot, concentric circles help in the visualization of the magnitude of length of the environment vectors, which is proportional to the standard deviation within the respective environment (Das et al., 2018).

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