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Article

Keywords: Rice restorer lines, grain quality, BLUP, multi-trait selection, MGIDI

Posted Date: November 24th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11599404/v1

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Additional Declarations: No competing interests reported.

Multi-environment, multi-trait selection of elite grain quality restorers from iso-cytoplasmic and tropical *japonica*-derived rice lines for hybrid rice breeding

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Abstract

Restorer lines determine the grain quality of hybrid rice, yet their quality is seldom evaluated across environments with several traits considered at once. We evaluated 240 restorer lines (198 iso-cytoplasmic and 42 tropical *japonica*-derived) for twelve grain quality traits in three environments. A mixed model showed that genotype was the largest source of variance for eleven traits (34.3-90.0% of the total), and the between-environment genetic correlation was 0.87 or higher for seven traits and lowest for elongation ratio (0.54). Best linear unbiased

predictions were used to rank the lines with four multi-trait indices (MGIDI, MTSI, MTMPS and FAI-BLUP), each selecting 36 lines (at 15% selection intensity). The indices selected 64 different lines, and 14 of them (eleven iso-cytoplasmic and three tropical *japonica*-derived) were selected by all four; twelve of these were long slender grain types. MGIDI and FAI-BLUP ranked the lines almost identically ($\rho = 0.99$), and MTSI and MTMPS, which include stability, selected more stable lines. The two backgrounds differed for elongation ratio, alkali spreading value, head rice recovery and kernel length, but not for amylose content or gel consistency. The 14 consensus lines are candidates for testing heterosis and combining ability.

Keywords: Rice restorer lines; grain quality; BLUP; multi-trait selection; MGIDI

Introduction

Rice (*Oryza sativa* L.) is the primary staple food for more than half of the world's population and contributes substantially to global food security, particularly in Asia, where it provides a major proportion of the daily caloric intake.[1,2] While previous breeding efforts primarily focused on increasing grain yield to meet food demand, current rice improvement programs increasingly emphasise grain quality because consumer preference and market value are now strongly influenced by cooking, eating, and processing characteristics, in addition to productivity.[3,4] Modern rice markets demand cultivars possessing desirable grain appearance, superior milling recovery, favourable cooking quality, and acceptable sensory attributes together with high and stable yield performance. Consumer preferences vary across geographical regions; however, long- to medium-slender grains with a favourable cooking texture, intermediate amylose content, and desirable gelatinisation temperature characteristics are generally preferred across major rice-consuming regions .[5,6]

Hybrid rice technology has emerged as one of the most effective strategies for enhancing rice productivity through the exploitation of heterosis and has substantially contributed to yield improvements in several rice-growing regions.[7,8] However, despite considerable advances in hybrid development, the widespread adoption of hybrid rice has frequently been constrained

by inferior grain and cooking qualities relative to elite inbred cultivars.[3,7] Consequently, the simultaneous improvement of grain yield and quality has become one of the major objectives of modern hybrid rice breeding programs.

Restorer lines constitute one of the most important components of hybrid rice breeding because, in addition to restoring fertility in cytoplasmic male sterile systems, they contribute substantially to the grain quality, adaptation, and yield performance of the resulting hybrids.[7] However, identifying superior restorer lines remains challenging because breeders must simultaneously improve productivity, grain quality, adaptation, and maturity characteristics. In many breeding programs, restorer selection has historically focused primarily on fertility restoration and agronomic performance, while grain quality parameters are frequently evaluated at later stages of selection. Such sequential approaches may result in the advancement of high-yielding lines with suboptimal quality characteristics, thereby reducing consumer acceptance and the commercial success of hybrid cultivars.[3,9]

An additional challenge in hybrid rice improvement is the relatively narrow genetic base of elite restorer germplasms. *Indica*-derived restorers have historically dominated hybrid rice breeding because of their strong fertility restoration ability and favourable grain quality characteristics. However, prolonged dependence on a limited set of elite restorers has reduced genetic diversity and restricted opportunities to broaden the breeding pool .[10] In contrast, tropical *japonica* germplasm possesses broader environmental adaptability, favourable agronomic characteristics, and desirable yield-associated traits, but its utilisation in restorer breeding has remained limited because of comparatively inferior grain quality attributes and weaker restoration behaviour .[11] Introgression between *indica* and tropical *japonica* backgrounds provides an important opportunity to combine the favourable grain quality characteristics of indica with the broader adaptation and productivity potential of tropical

japonica, while simultaneously expanding the genetic diversity available to hybrid breeding programs.

Grain quality is a complex quantitative trait governed by multiple components, including milling quality, grain appearance, cooking behaviour, and physicochemical properties of starch. Traits such as hulling percentage (HP), milling percentage (MP), head rice recovery (HRR), kernel dimensions, amylose content (AC), gel consistency (GC), and alkali spreading value (ASV, an indicator of gelatinisation temperature) collectively determine consumer preferences and market value.[5,12] These traits are frequently controlled by multiple genes and influenced by both genetic and environmental factors, leading to complex interactions among the quality components.[13,14] Several of these traits also have an optimum rather than a simple 'more is better' direction, for example a medium-to-long slender kernel and intermediate amylose content, so that improving one trait can move another away from its market target.[3,4,6]

Single-trait selection is therefore often inadequate for identifying superior restorer lines, because improvement in one trait may occur at the expense of another.[15] Multi-trait selection indices address this by combining several traits into one criterion. The multi-trait genotype–ideotype distance index (MGIDI) uses factor analysis to group correlated traits and ranks genotypes by their distance from a defined ideotype, without the need for economic weights and without the problems that multicollinearity causes in classical linear indices.[16] For multi-environment trials, the multi-trait stability index (MTSI) adds a stability component, the weighted average of absolute scores (WAASB) derived from best linear unbiased predictions (BLUP), to the same factor-analytic distance, so that lines are chosen for both mean performance and stability.[17,18] The multi-trait mean performance and stability index (MTMPS) follows the same logic but allows other stability statistics, such as Wricke's ecovalence,[18,19] and the FAI-BLUP index ranks lines by their relative distance from an ideotype and an anti-

ideotype.[20] However, information on the comparative performance of these indices for grain quality improvement in rice restorer lines is limited.

In the present investigation, a diverse restorer population comprising tropical japonica × indica-derived and iso-cytoplasmic restorer lines was evaluated across three environments. Multi-environment evaluation is particularly important because quality traits, such as head rice recovery, kernel dimensions, amylose content, alkali spreading value, and gel consistency, are substantially influenced by environmental conditions and genotype × environment interactions (GEI).[12,21] When GEI is present, rankings based on values pooled over environments can differ from the ranking within each environment,[22,23] so the share of variance due to genotypes needs to be supported by the between-environment genetic correlation. The yield and yield-related traits of the same panel have been reported previously;[24] the present study therefore concentrates on twelve grain quality traits. The aims were: (i) to partition the variance of the twelve quality traits into genotype, GEI and residual components using a linear mixed model, and to estimate the between-environment genetic correlation of each trait; (ii) to classify the restorer lines into the standard International Rice Research Institute (IRRI) quality classes; (iii) to define a quality ideotype from these classes and to identify the best lines with four multi-trait indices (MGIDI, MTSI, MTMPS and FAI-BLUP) applied to BLUPs; and (iv) to identify the lines selected by all four indices and to describe the strengths and weaknesses of each index's selection, as a basis for choosing restorers for hybrid rice breeding programmes.

Results

Genetic variability, environmental influence and stability of the quality traits

Twelve grain quality traits were analysed: HP, MP, HRR, kernel length (KLBC) and breadth (KBBC) before cooking, length–breadth ratio (LB), kernel length (KLAC) and breadth (KBAC) after cooking, elongation ratio (ER), AC, GC and ASV. The combined analysis of

variance showed highly significant genotype effects ($P < 0.001$) for all twelve grain quality traits (Table 1). The GEI was significant ($P < 0.001$) for every trait except KLBC. The environment effect, tested against replicate within environment, was significant for KLBC, KBBC and LB ($P < 0.001$), ASV ($P < 0.01$) and KLAC ($P < 0.05$), but not for the other seven traits.

In the mixed model, genotype was the largest source of variance for eleven of the twelve traits, from 34.3% for KBAC to 90.0% for HRR; for KBAC the residual (54.5%) was larger. The genotypic share was above 80% for HRR (90.0%), KLBC (88.9%), LB (85.0%), GC (83.8%), MP (83.1%) and AC (81.4%) (Table 1). The GEI variance was zero for KLBC and between 4.7% and 12.3% for LB, HRR, GC, ASV, KBAC, MP and AC, but between 21.5% and 38.0% for KBBC, HP, KLAC and ER.

The between-environment genetic correlation (rB) was 0.87 or higher for seven traits (KLBC 1.00, LB 0.95, HRR 0.94, GC 0.90, MP 0.88, AC 0.87 and ASV 0.87), which means that the lines kept nearly the same order in the three environments for these traits. It was lower for HP (0.74), KBAC (0.75), KBBC (0.69), KLAC (0.69) and especially ER (0.54), for which the ranking of the lines depended more on the environment.

The environment means differed most between Aduthurai (E2) and the other two environments. In E2 the kernels were shorter and less slender (KLBC 5.56 mm vs 6.25 mm in E1 and 6.06 mm in E3; LB 2.71 vs 3.46 and 3.38), the cooked kernels were shorter (KLAC 7.24 mm vs 9.31 and 9.28 mm), ER was lower (1.30 vs 1.49 and 1.54), AC was lower (14.02% vs 16.36% and 16.12%), ASV was lower (3.37 vs 4.56 and 4.55) and GC was higher (54.86 mm vs 42.96 and 43.07 mm). The means of HP, MP and HRR differed between environments by less than 3.5%.

Distribution of the traits and standard quality classes

148 The coefficient of variation (CV) of the genotype means ranged from 4.12% for HP to 34.96%
149 for ASV (Table 1). It was above 10% for HRR (10.72%), KBBC (10.76%), LB (14.54%), AC
150 (19.27%), GC (32.70%) and ASV (34.96%), and lowest for HP, MP (5.43%) and ER (6.72%).
151 GC ranged from 22.94 to 79.19 mm (mean 46.96 mm), ASV from 1.03 to 6.50 (mean 4.16),
152 AC from 8.43% to 24.09% (mean 15.50%), LB from 1.94 to 5.35 (mean 3.18) and KLBC from
153 4.68 to 8.60 mm (mean 5.96 mm). The means of HP (80.74%), MP (73.16%) and HRR
154 (63.41%) were in the upper part of their ranges (Fig. 1A–L).

155 Based on the IRRI kernel dimension standards, the 240 restorer lines were classified into five
156 grain-type categories. Long slender grains constituted the largest group, with 102 genotypes
157 (42.50%), followed by short slender with 63 genotypes (26.25%), medium slender with 53
158 genotypes (22.08%), short bold with 12 genotypes (5.00%), and long bold with 10 genotypes
159 (4.17%). Overall, the slender grain types (long, medium and short slender) accounted for
160 90.83% of the evaluated restorers, whereas bold grain types represented only 9.17% of the
161 restorers (Fig. 2A,B).

162 According to the IRRI classification, AC was skewed toward the lower classes, with most
163 genotypes (80.42%) classified under the low amylose class (AC < 20%), followed by 42
164 genotypes (17.50%) categorized as intermediate AC (20-25%), and only five genotypes
165 (2.08%) exhibiting high AC (>25%) (Fig. 2C,D). In contrast, the genotypes showed a balanced
166 yet variable distribution for GC such as, 99 genotypes (41.25%) were classified as hard gel
167 (GC < 40 mm), 82 genotypes (34.17%) as medium gel (41-60 mm), and 59 genotypes (24.58%)
168 as soft gel (> 61 mm) (Fig. 2E,F). Furthermore, the evaluation of genotypes for ASV according
169 to the IRRI classification highlighted broad diversity in cooking temperature characteristics
170 (Fig. 2G,H). Specifically, 69 genotypes (28.75%) displayed low ASV scores (1-3), which
171 correspond to a high GT, while the largest group of 92 genotypes (38.33%) exhibited

intermediate ASV scores (4-5) aligned with an intermediate GT. The remaining 79 genotypes (32.92%) fell into the high ASV category (6-7), indicating a low GT.

Differences between the iso-cytoplasmic and tropical japonica-derived lines

After correction for multiple testing, the two backgrounds differed for four of the twelve traits (Fig. 1M). The tropical japonica-derived lines had a higher ER (1.471 vs 1.440; FDR-adjusted P = 0.004) and a lower ASV (3.54 vs 4.30; P = 0.004), a lower HRR (60.95% vs 63.93%; P = 0.045) and a shorter KLBC (5.81 vs 5.99 mm; P = 0.044) than the iso-cytoplasmic lines. The backgrounds did not differ for HP, MP, KBBC, LB, KLAC, KBAC, AC and GC (adjusted P ≥ 0.22).

Relationships among the traits

Of the 66 pairs of traits, 25 were correlated after correction for multiple testing (P < 0.05), 12 positively and 13 negatively (Fig. 3). The strongest correlations were between LB and KBBC (r = -0.77), KLBC and LB (0.75), HP and MP (0.71), KLBC and KLAC (0.64), MP and HRR (0.59) and LB and KLAC (0.55). The three milling traits were positively correlated with each other (HP–MP 0.71, MP-HRR 0.59, HP–HRR 0.24), but HP and MP were not correlated with the kernel dimension traits and HRR was weakly and negatively correlated with LB (-0.23) and KLAC (-0.19) and positively with KBBC (0.21). ER was positively correlated with KLAC (0.44) and negatively with KLBC (-0.40) and LB (-0.23). AC was negatively correlated with GC (-0.48) and positively with ASV (0.31).

Factor structure of the four indices

Factor analysis of the twelve rescaled traits retained five factors for MGIDI and FAI-BLUP (73.7% of the variance) and for MTSI (70.5%), and six factors for MTMPS (80.4%) (Table 2 and Supplementary Tables S3–S5). In MGIDI and FAI-BLUP the factors were grain shape (LB and KBBC; FA1), milling quality (MP, HP and HRR; FA2), eating quality (AC and GC; FA3),

cooked kernel length (KLAC and KLBC; FA4) and elongation (ER and KLBC; FA5). MTSI and MTMPS gave the same shape and milling factors, and in MTSI KLAC and ER formed one factor, while AC, ASV and GC formed another (Supplementary Tables S3 and S4).

Lines selected by the four indices and the consensus restorers

The partition of the variance of each trait and its rB, which entered the indices, are summarised in Fig. 4a. Each index selected 36 lines (15%), and together they selected 64 different lines (Fig. 4b). Fourteen lines were selected by all four indices, seven by three, 24 by two and 19 by one index only (11 by MTSI only, five by MTMPS only, two by FAI-BLUP only and one by MGIDI only). MGIDI and FAI-BLUP had 34 lines in common, MTSI and MTMPS 25, MGIDI and MTMPS 21, MTMPS and FAI-BLUP 20, MGIDI and MTSI 15, and MTSI and FAI-BLUP 14. The ranks of all 240 lines agreed almost completely between MGIDI and FAI-BLUP (Spearman $\rho = 0.99$) and closely between MTSI and MTMPS ($\rho = 0.91$), which are the two pairs that share the same type of information (the BLUP alone, or the BLUP plus a stability statistic); the correlation between the other pairs was 0.55–0.70. The rankings of the top 60 lines of MGIDI, MTSI and FAI-BLUP are shown in Fig. 4c, e and g. The lines common to each combination of indices are listed in Table 3.

The 14 consensus lines were G-211, G-20, G-212, G-3, G-28, G-210, G-26, G-11, G-94, G-100, G-54, G-10, G-1 and G-183 (Table 3). Eleven of them were iso-cytoplasmic lines and three (G-210, G-211 and G-212) were tropical japonica-derived, which is similar to the share of this background in the panel (21.4% vs 17.5%; Fisher's exact test, $P = 0.72$). Twelve of the 14 lines were long slender and two (G-28 and G-100) were short slender. Thirteen had low amylose and only G-210 was in the intermediate class. Nine lines had a hard gel, four a medium gel and one (G-20) a soft gel, and five had an ASV of 6–7. Compared with the mean of the panel, the consensus lines had a longer kernel (KLBC 6.27 vs 5.96 mm), a higher L/B (3.63 vs

3.18), a longer cooked kernel (KLAC 9.30 vs 8.61 mm), a lower GC (39.71 vs 46.96 mm), a higher ASV (4.87 vs 4.16) and a higher AC (17.82% vs 15.50%), whereas HP, MP, HRR and ER were close to the panel means. None of the consensus lines was among the eight yield-stable lines reported previously for this panel;[24] two of those eight lines, G-121 and G-23, were selected by MTSI and MTMPS (the two indices that include stability) but not by MGIDI or FAI-BLUP.

Stability and standardised performance of the selected lines

The WAASB of the lines was zero for KLBC, for which the GEI variance was zero, and was largest for GC (up to 23.89) and for HRR, MP and HP (up to 5.43, 4.92 and 4.86; Supplementary Fig. S3). Averaged over the twelve traits, the consensus lines were slightly more stable than the panel (mean rank 0.44 for WAASB and 0.45 for ecovalence, against 0.50 for the panel), and G-28 was the most stable of them (0.33). The lines selected by MTSI (0.42) and MTMPS (0.46), which include a stability statistic, were more stable than the lines selected by MGIDI and FAI-BLUP (0.51 for both), which did not differ from the panel mean.

On the Z-score scale, the 14 consensus lines were on average above the panel for LB (+1.02), KLAC (+1.12), AC (+0.83), KLBC (+0.65), ER (+0.54) and ASV (+0.52), and below it for KBBC (−0.77), KBAC (−0.79) and GC (−0.50), which are the desired directions; their means for HP (+0.28), MP (+0.08) and HRR (+0.08) were close to the panel means (Fig. 5). Individual lines were weaker for milling quality: the HRR of G-1 (46.6%) and G-10 (53.0%) was 2.5 and 1.6 standard deviations (SD) below the panel mean, and the MP of G-183 was 1.3 SD below it.

The yield-related traits of the 14 consensus lines, from the same trials, are also shown in Fig. 5. On average the consensus lines did not differ from the panel for days to 50% flowering (DFF; 98.2 vs 98.2 days), productive tillers per plant (PTN; 11.4 vs 11.4) and filled grains per panicle

(FG; 164.0 vs 161.4), and were slightly lower for panicle length (PL; 25.1 vs 25.4), thousand-seed weight (TSW; 21.2 vs 21.5 g) and seed yield per plant (SYP; 21.9 vs 22.3 g; mean Z-score -0.60). Ten of the 14 lines had a SYP below the panel mean, and four ranked in the better half of the panel for SYP: G-11 (rank 43 of 240), G-211 (83), G-26 (99) and G-100 (112).

Strengths and weaknesses of the selected lines

In the lines selected by MGIDI, two factors contributed more to the distance from the ideotype than expected if all factors had contributed equally (20%): cooked kernel length (FA4, 34.2%) and milling quality (FA2, 32.8%). Grain shape (FA1, 3.0%) and eating quality (FA3, 12.9%) were the strengths (Fig. 4d; Table 2). FAI-BLUP gave almost the same profile (FA4 33.6%, FA2 32.7%, FA1 2.4%; Fig. 4h; Supplementary Table S5). In MTSI the weaknesses were milling quality (FA2, 37.1%), the AC–ASV–GC factor (FA4, 27.2%) and the KLAC–ER factor (FA3, 23.8%), and the strengths were grain shape (4.2%) and KBAC–KLBC (FA5, 7.7%) (Fig. 4f; Supplementary Table S3). In MTMPS (expected 16.7%) the weaknesses were the ER–KLAC factor (FA2, 38.4%) and milling quality (FA3, 27.1%), and grain shape was again the main strength (FA1, 1.6%) (Supplementary Table S4). In all four selections, milling quality and cooked kernel length or elongation were therefore the factors that remained farthest from the ideotype, and grain shape was the factor that the selected lines came closest to.

Discussion

This study used best linear unbiased predictions (BLUPs) from a multi-environment mixed model to rank 240 restorer lines for twelve grain quality traits, and compared four multi-trait indices for selecting the lines. The results are discussed in relation to the genetic control and stability of the traits, the differences between the two genetic backgrounds, the relationships among the traits, and the agreement between the indices.

Genotype was the largest source of variance for eleven of the twelve traits, and the entry-mean heritability ranged from 0.73 to 0.98, so the BLUPs are a reliable basis for ranking the lines. The between-environment genetic correlation (rB) showed how far the ranking depended on the environment. It was 0.87 or higher for KLBC, LB, HRR, GC, MP, AC and ASV, which means that these traits can be selected with confidence from a limited number of environments. The high rB of these traits is consistent with strong genetic control: AC and GC are strongly influenced by allelic variation at the Wx, SSIIa and ALK loci,[13,25–27] although no molecular analysis was made in the present study. In contrast, rB was low for ER (0.54), KLAC and KBBC (0.69) and HP (0.74), so the ranking of the lines for these traits changed from one environment to another. Cooked kernel traits are known to respond to temperature during grain filling,[28] but the present data do not identify the cause of the lower KLAC, ER and L/B in the Aduthurai environment. KLBC had no GEI variance although the environment mean differed, which means that the environment shifted all lines by the same amount. The high heritability and stability of kernel length is consistent with its control by a few major genes such as GS3.[29] The significant GEI for most traits is consistent with the influence of pre-harvest conditions on rice quality.[30,31] Selection for ER and KLAC therefore needs evaluation in more than one environment.

The CV was high for GC (32.7%) and ASV (35.0%) and low for HP, MP and ER (4.1–6.7%), so the greatest diversity of the panel was in the starch-related traits that determine eating and cooking quality, which are regulated by a complex network of starch synthesis genes.[13,26] The wide variation in GC and ASV agrees with the variability reported for grain quality traits in rice germplasm.[32] Most lines were slender grain types (90.83%), as expected for restorers bred for the grain type that is preferred in the main rice markets.[3,6,33] The classes of GC and ASV were spread over the whole range, which gives scope to develop hybrids for different consumer groups.[4,21]

The tropical japonica-derived lines had a higher ER and a lower ASV than the iso-cytoplasmic lines, and a lower HRR and a shorter kernel, but did not differ for AC and GC, the traits that are most closely related to the eating quality of cooked rice.[4,9] The lower HRR is consistent with the weaker milling quality that is often reported for tropical japonica germplasm,[11] and it is a trait to be improved in further crosses. Three of the 14 consensus lines were tropical japonica-derived (21.4%, against 17.5% of the panel), and the share did not differ from that in the panel, so this background can provide restorers that meet the same multi-trait standard as the iso-cytoplasmic lines. With only three such lines this finding should be regarded as indicative.

The correlations between the traits explain the structure of the four indices. HP, MP and HRR were positively correlated, and HP and MP were not correlated with the kernel dimension traits ($|r| \leq 0.11$), so milling quality can be selected independently of grain shape. The strong correlations among KLBC, LB, KBBC and KLAC show that the raw kernel dimensions largely determine the cooked kernel dimensions, and the negative correlation of ER with KLBC follows from the fact that ER is the ratio of the cooked to the raw kernel length: a longer raw kernel gives a lower ratio for the same cooked length. AC was correlated with GC and ASV, which agrees with the view that the eating quality of rice cannot be described by a single trait.[9,34] These groups of correlated traits correspond to the factors extracted for the indices (shape, milling, eating quality, cooked kernel length and elongation). The weak negative correlation of HRR with LB and KLAC may partly explain why milling quality was the factor that stayed farthest from the ideotype in all four selections: lines selected for slender and elongating kernels may be weaker for head rice recovery.

The four indices selected 64 different lines, and 14 lines were selected by all four. MGIDI and FAI-BLUP, which use only the BLUPs, shared 34 lines and ranked the lines almost identically ($\rho = 0.99$), and MTSI and MTMPS, which add a stability statistic, shared 25 lines ($\rho = 0.91$).

The main difference between the indices was therefore whether stability was included, and not the choice of the factor analysis, as MGIDI[16] and MTSI[17,18] have the same structure. Including stability had a visible effect: the lines selected by MTSI and MTMPS were more stable than the panel, whereas the lines selected by MGIDI and FAI-BLUP were not. Because 19 lines were selected by only one index, the choice of index changes the list of lines, and the 14 consensus lines are the most robust choice, as they were selected whether or not stability was included. None of them was among the eight yield-stable lines reported previously for the same panel,[24] and G-121 and G-23 were selected only by the indices that include stability. Only four of the 14 lines ranked in the better half of the panel for seed yield per plant (Fig. 5), so high quality and high yield do not coincide in most of these lines, and the quality-based list needs to be combined with the yield data in the next stage of selection.

The study has limitations. The trials covered three environments, with one season each and two replicates per environment. The ideotype rules (KLBC of 6.0 mm or more, L/B of 3.0 or more and AC of 20–25%) were defined for this breeding objective, and a different target would give a different set of lines. The consensus lines were mostly low in amylose (13 of 14), although their mean AC (17.82%) was higher than the panel mean (15.50%) and therefore closer to the target class of 20–25%; they were selected for their long slender kernels, hard gel and high ASV. Because AC had a high heritability (0.94) and a high between-environment genetic correlation (0.87), it can be improved in later crosses. The yield traits were used only to describe the 14 consensus lines (Fig. 5) and were not part of the indices, and no molecular or combining ability data were included. The consensus lines should therefore be validated in additional environments and seasons, for their yield, for combining ability with the cytoplasmic male sterile lines, and for the quality of the hybrids that they produce.

Conclusion

Genotype was the main source of variation for the grain quality traits of the 240 restorer lines, and the ranking of the lines was stable across the three environments for seven of the twelve traits. Elongation ratio and cooked kernel length depended most on the environment, so selection for these traits should be based on evaluation in more than one environment. Four multi-trait selection indices applied to best linear unbiased predictions selected different sets of lines, and the 14 lines selected by all four indices, eleven iso-cytoplasmic and three tropical japonica-derived, are the most promising candidates. The presence of tropical *japonica*-derived lines among them indicates that this background can provide restorers that meet the same multi-trait standard as the iso-cytoplasmic lines. These 14 lines are candidates for testing heterosis and combining ability. Their evaluation in further environments and seasons is needed before they are used in hybrid breeding.

Materials and Methods

Plant material and multi-environment trials

A panel of 240 diverse rice restorer lines was used in the current study to characterise the grain and cooking quality parameters of potential rice restorers. The panel consisted of 198 iso-cytoplasmic (ISO) restorer lines derived from popular rice hybrids grown in India through generation advancement, followed by selection for desirable agronomic traits and spikelet fertility ,[35] and 42 tropical *japonica*/*indica* (TRJ)-derived lines developed by crossing Pusa 44 (a popular non-aromatic indica rice variety) with 29 diverse tropical *japonica* lines sourced from IRRI.[36] All materials used in this study were developed at the Division of Genetics, Indian Council of Agricultural Research - Indian Agricultural Research Institute (ICAR-IARI), New Delhi, India. The 240 diverse restorers used in this study were designated as G, followed by a unique serial number (Table S1).

The test panel was evaluated at three different locations, namely, the Division of Genetics, ICAR-IARI, New Delhi, and the IARI-Collaborative Outstation Research Centre (CORC), Rakhra, Punjab, during *kharif* 2022, and at IARI-Rice Breeding and Genetics Research Centre (RBGRC), Aduthurai, Tamil Nadu, during the *rabi* season of 2022–23. The three test environments were coded as E_1 (New Delhi, *kharif* 2022), E_3 (Rakhra, *kharif* 2022), and E_2 (Aduthurai, *rabi* 2022–23) (Figure S1B). The dates of sowing, transplanting, and pH of the soils are listed in Table S2. The monthly means of the maximum and minimum temperatures and cumulative rainfall during the crop growing period are summarised in (Figure S1A) to characterise the environmental conditions prevailing at each test environment.

The experiment was conducted using an alpha lattice design with two replications, each consisting of incomplete blocks with entries randomised within the blocks. At all sites, the nursery was raised on elevated beds for 21 days before being transplanted into a well-puddled field (Table S2). Crop was planted at 15 cm spacing between plants and 20 cm between rows in a 1 m^2 plot and was grown under fully irrigated conditions with the recommended dose of fertilisers (RDF) applied as per local recommendation across all environments. To minimise border effects, observations were recorded from plants in the central area of each plot, excluding border plants.

Grain quality traits were determined in the laboratory on seed bulked from each plot at harvest, which gave 1,440 plot-level records for each trait (240 lines × 3 environments × 2 replicates, with no missing values). The yield and yield-related traits of the same panel and trials (DFF, PL, PTN, TSW, FG and SYP) were reported previously.[24] They were not part of the indices, and their BLUPs for the consensus lines are shown in Fig. 5.

Grain physical characteristics

To understand the distribution of grain quality attributes among the evaluated restorers, they were classified into standard quality categories based on the internationally accepted IRRI classification system.[37] The classification of grain shape, AC, GC, and ASV facilitated the assessment of variability in cooking, eating, and market-preferred quality traits within the restorer population.

Milling characteristics

The harvested grains were threshed, cleaned, and dried to a moisture content of 14%. The 250 g paddy samples were dehusked using a hand-operated paddy Palm Dehusker, and the brown rice thus obtained was weighed and polished in a rice polisher (Mini Lab Rice Polisher Model K-710, Krishi International). The polishing time was adjusted such that the polishing percentage was approximately 5% to obtain milled rice. To separate broken grains defined as those less than 75% of the original grain length after milling, polished grains were passed through a rice grader with different grooves (mm). Full and ¾ size grains were weighed and considered as HRR. The proportions of hulled, milled, and head rice derived from the paddy sample were calculated using the following equations:[28,38]

$$Hulling\ percentage\ (HP) = \frac{\text{Weight of dehusked rice}}{\text{Wieight of paddy}} \times 100$$

$$Milling\ percentage\ (MP) = \frac{\text{Weight of polished kernel}}{\text{Wieight of paddy}} \times 100$$

$$Head\ rice\ recovery\ (HRR) = \frac{\text{Weight of whole polished grains}}{\text{Wieight of paddy}} \times 100$$

Grain dimensions

Ten polished whole kernels per restorer were lined up end-to-end and placed under a 3x photo-magnifier to measure the average kernel length before cooking (KLBC). In addition, the kernel breadth before cooking (KBBC) was measured by aligning the kernels sidewise. The average length and width of 10 kernels in millimetres (mm) were recorded for further analysis. Based on their length, rice cultivars were classified by IRRI[37] as extra-long (>7.5 mm), long (6.61–7.5 mm), medium (5.51–6.60 mm), and short (<5.5 mm). The grain shape was determined based on the length-breadth ratio (L/B), which was calculated as follows:

$$L/W = \frac{\text{L (mm)}}{\text{B (mm)}}$$

Where L, the average length of milled rice (mm); and W, the average breadth of milled rice (mm). Based on the L/B ratio, rice grains were further classified into slender (>3.0), medium (2.1–3.0), bold (1.1–2.0), and round (<1.1) grain types.[37]

Cooking characters

Elongation ratio (ER)

The length (L_0) and breadth (B_0) of five milled rice grains were measured under a photo enlarger with a magnification of 3× on a graph paper. For cooking, ten individual milled kernels of the sample along with checks were taken separately in long labelled test tubes and pre-soaked in 5 ml of distilled water for 30 min. The tubes were then placed in a water bath maintained at boiling temperature for 8 - 9 min. After cooking, the tubes were removed and cooled under running water for 2 min. The cooked kernels were removed from the tubes, and excess water was removed using blotting paper. The length (L_1) and breadth (B_1) of five cooked kernels were measured under a photo enlarger with a magnification of 3× on a graph paper. For each of L_0, B_0, L_1, and B_1, the mean of the five grains was calculated as follows: The ER was calculated using the following equation:[33]

$$Elongation\ ratio = \frac{\text{Kernel length after cooking (mm)}}{\text{Kernel length before cooking (mm)}} \times 100$$

Physico-chemical characteristics

Amylose content (AC)

AC was estimated using the modified method described previously[39] (Figure S2A). Milled rice flour (100 mg) was weighed in a 100 ml volumetric flask. Then, 1 ml of 95% ethanol and 9 ml of 1N NaOH were added. The suspension was heated in a boiling water bath for 10 min to gelatinise the starch. The mixture was then cooled to room temperature for 1 h. The samples were diluted to a volume of 100 ml with distilled H_2O. Then, 2 ml of iodine solution (0.2% I2, 2% KI) was added to 5 ml of the starch solution. The solution was made up to 100 ml with distilled H_2O, shaken well, and allowed to stand for 20 min in the dark. Spectrophotometric determination of the absorbance of the formed colour was determined at 620 nm. Calibration was performed by measuring the absorbance of a standard potato amylose sample supplied by Sigma (Sigma-Aldrich, St. Louis, MO, USA; Product No. A0512-1G), and a calibration curve was constructed to obtain the percentage of AC in our samples. Based on the AC, rice cultivars were divided into five amylose groups: waxy/glutinous (1–2%), very low (2–9%), low (9–20%), intermediate (20–25%), and high (>25%).

Gel consistency (GC)

GC of the rice samples was determined using the method described previously[40] (Figure S2C). Rice flour (100 mg) from each genotype was weighed and placed in a 20 mL (1.6 cm × 15 cm) slim borosilicate glass test tube. A total volume of 0.2 ml of 95% ethanol containing 0.25% thymol blue was added to each tube and mixed sufficiently to prevent clumping of the rice flour. The mixture was vortexed gently, and 2 ml of 0.2 N KOH was added and vortexed again. Each tube was covered with glass marbles to prevent steam loss and reflux of the samples and

heated in a vigorously boiling water bath at 90-100°C for 8 min. The tubes were removed from the water bath, cooled to room temperature for 5 min, vortexed, and then transferred to an ice bath (0-2°C) for 20 min. The tubes were wiped off moisture and then laid horizontally on a graph paper on a flat laboratory bench for 1 h. The total length of the blue gel was measured from the bottom of the test tube to the end of the gel in millimetres (Figure S2C). Genotypes were grouped into three classes based on the length of the gel: Hard (length of gel <40 mm), Medium (length of gel 41–60 mm), and Soft (length of gel >61 mm).

Alkali spreading values (ASV)

Six whole milled rice grains were placed in duplicate Petri dishes containing 10 ml of 1.7% KOH solution. The samples were then incubated at 30°C for 23 h, following the protocol described previously[41] (Figure S2B). After incubation, the degree of endosperm disintegration was visually assessed and scored according to a seven-point scale. The ASV score was subsequently used as an indirect indicator of gelatinisation temperature (GT), wherein ASV scores of 1–3 indicated a high gelatinisation temperature (>75°C), scores of 4–5 represented an intermediate gelatinisation temperature (70–75°C), and scores of 6–7 denoted a low gelatinisation temperature (<70°C).[42]

Statistical analysis

Descriptive statistics and classification

Descriptive statistics (mean, minimum, maximum, range, SD, standard error (SE = SD/√n) and CV = 100 × SD/mean) were calculated for each trait from the means of the 240 genotypes across the three environments and two replicates (n = 240). The 240 lines were assigned to the IRRI classes for kernel length, kernel shape, amylose content, gel consistency and alkali spreading value[37,42] as described above, using the genotype means.

Combined analysis of variance

Phenotypic observations recorded in the three environments were subjected to a combined analysis of variance for each trait to test the effects of environment, genotype and GEI. Replicates were treated as nested within environments, because the replicates are physically separate in each environment. The mean square of environment was tested against replicate within environment, genotype against the GEI mean square, and GEI and replicate against the residual mean square. The contribution of each source to the total sum of squares was also calculated.

Linear mixed model, variance components and between-environment genetic correlation

For each trait the plot-level data were analysed with the model $y_{ijk} = \mu + E_j + R_{k(j)} + G_i + (GE)_{ij} + e_{ijk}$, where E_j is the fixed effect of environment j, $R_{k(j)}$ is the fixed effect of replicate k nested within environment j, G_i is the random effect of genotype i, $(GE)_{ij}$ is the random genotype × environment interaction and e_{ijk} is the residual. No incomplete-block term was fitted because grain quality traits were measured in the laboratory on seed bulked per plot. Variance components (σ^2G, σ^2GE and σ^2e) were estimated by restricted maximum likelihood with the lme4 package.[43] The significance of σ^2G and σ^2GE was tested by likelihood-ratio tests between nested models, with the P value halved because a variance is tested on the boundary of its parameter space.[44]

The share of each component in the total variance was calculated as

$\sigma^2/(\sigma^2_G + \sigma^2_{GE} + \sigma^2_e)$.

The genetic correlation between environments was estimated as

$rB = \sigma^2_G/(\sigma^2_G + \sigma^2_{GE})$,

the correlation between the genotypic values of the same lines in two environments when the genotypic variance is the same in all environments.[45,46] A value of rB close to 1 means that the

ranking of the lines is the same in every environment, and a low value means that the lines are re-ranked.

Best linear unbiased predictions and stability statistics

The BLUP of each genotype across environments was calculated from the same model as the overall mean plus the predicted genotype effect, which shrinks each estimate according to its reliability.[47] For stability, the BLUPs of the genotype × environment effects were arranged in a genotype × environment matrix and decomposed by singular value decomposition, and WAASB was calculated from the first two interaction principal component axes, weighted by the share of the interaction variance that each axis explains.[17] Wricke's ecovalence[19] was calculated from the genotype × environment means as the squared deviation of each genotype–environment cell from additivity.

Ideotype and selection rules

The ideotype was defined from the breeding objective for restorers. HP, MP, HRR, KLAC, ER and ASV were to be increased, and KBBC, KBAC and GC (hard gel) were to be decreased. For the three traits for which a class and not a direction is preferred, the value entered in the indices was the distance of the BLUP outside the preferred class, which is zero inside the class and is minimised: KLBC of 6.0 mm or more, LB of 3.0 or more (slender grain[37]) and AC of 20-25% (intermediate amylose[37]).

Correlation between traits

Pearson correlation coefficients were calculated between the BLUPs of the twelve traits and P values were adjusted for multiple testing by the Benjamini–Hochberg false discovery rate.[48]

Multi-trait selection indices

Four multi-trait indices were calculated from the BLUPs with the metan package,[49] following the published procedures. For each trait, the value (or the distance outside the class) was rescaled to 0–100, where 100 is the ideotype. The correlation matrix of the twelve rescaled traits was subjected to factor analysis (principal-component extraction, retention of factors with eigenvalue > 1, varimax rotation), and factor scores were calculated for each line. MGIDI[16] is the Euclidean distance between the factor scores of a line and those of the ideotype (100 for every trait); a smaller distance is better. MTSI[17,18] was calculated in the same way from a matrix in which each trait was the mean of the rescaled BLUP and the rescaled WAASB (a smaller WAASB is better), so that mean performance and stability had equal weight. MTMPS was calculated as MTSI with Wricke's ecovalence in place of WAASB. For FAI-BLUP,[20] factor scores were calculated from the BLUPs, and the ideotype and the anti-ideotype were the best and the worst BLUP of each trait; lines were ranked by FAI = (1/dI)/((1/dI) + (1/dA)), where dI and dA are the distances to the ideotype and to the anti-ideotype, and a larger value is better. All traits had the same weight, and the selection intensity was 15% (36 of the 240 lines) for each index as suggested previously[16].

Convergence of the indices and strengths and weaknesses

The sets of 36 lines selected by the four indices were compared with a Venn diagram, and the lines selected by all four indices were taken as consensus lines. The agreement between the indices was also measured by the Spearman rank correlation of the ranks of all 240 lines. The strengths and weaknesses of each selection were described by the contribution (%) of each factor to the squared distance between the selected lines and the ideotype. A factor that contributed more than the share expected if all factors contributed equally (100 divided by the number of factors) was considered a weakness of the selected lines, and a factor that contributed less was considered a strength.[16]

Stability and standardised performance of the selected lines

The stability of the selected lines was compared with that of the panel by the rank of each line for WAASB and for ecovalence within each trait, expressed as a proportion of 240 (0 = most stable, 1 = least stable) and averaged over the twelve traits. For the heat map of the consensus lines (Fig. 5), the BLUPs of each trait were converted to Z-scores (mean 0 and SD 1 over the 240 lines). The BLUPs of the yield-related traits were obtained from the same model with an additional random effect of block within environment and replicate.

Comparison of the two backgrounds

The iso-cytoplasmic (n = 198) and the tropical japonica-derived (n = 42) lines were compared for the BLUPs of the twelve traits with Welch's t-test, and P values were adjusted for multiple testing by the Benjamini–Hochberg false discovery rate.[48] The share of tropical japonica-derived lines among the consensus lines was compared with their share in the panel by Fisher's exact test.

Software

All analyses were carried out in R 4.3.3.[50] Mixed models were fitted with lme4 1.1.35.1 and the figures were drawn with ggplot2, patchwork, ragg and sf. MGIDI, MTSI, MTMPS and FAI-BLUP were calculated with the metan package[49].

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

CRediT authorship contribution statement

SR: Investigation, Validation, Visualization, Writing – original draft. **VS:** Writing – review & editing, Data curation, Methodology. **SN:** Methodology, Formal analysis, Writing – original

draft. **SK:** Resources, Software, Writing – review & editing, **BT:** Software, Validation, Writing– review & editing, **RE, HB, DSB:** Software, Validation, Writing – review & editing. **KV:** Software, Validation, Writing – review & editing. **BKD:** Methodology, Writing – review & editing. **DS, Sonu, FTMB:** Data curation, Writing – review & editing. **SS:** Software, Writing – original draft. **PJ:** Methodology, Writing – review & editing. **AS:** Writing – review & editing, Conceptualization. **PB:** Conceptualization, Supervision, Writing – review & editing.

Funding

The authors declare that no specific funding was received for this research. The first author (SR) received an ICAR Senior Research Fellowship (SRF) from the Indian Council of Agricultural Research, New Delhi, India, for personal expenditure; this fellowship was not provided for the research.

Acknowledgments

The first author (SR) gratefully acknowledges the Indian Council of Agricultural Research (ICAR), New Delhi, India, for providing the ICAR-SRF fellowship. The first author also gratefully acknowledges the NAHEP–CAAST Discovery Centre, ICAR–Indian Agricultural Research Institute (IARI), New Delhi, India, for providing facilities for grain quality assessment.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Supplementary material

The Supplementary material for this article is given in separate file

AI-assisted figure preparation statement:

Figures illustrating the experimental workflow were prepared using SciSpace and subsequently reviewed and edited by the authors for scientific accuracy and presentation (Figure S1 and S2).

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714 Figure 1. Violin plots showing the phenotypic distribution of 12 grain quality traits among 240 rice restorer lines.
715 (A) Hulling percentage (HP, %), (B) Milling percentage (MP, %), (C) Head rice recovery (HRR, %), (D) Kernel
716 length before cooking (KLBC, mm), (E) Kernel breadth before cooking (KBBC, mm), (F) Length-breadth ratio
717 (LB), (G) Kernel length after cooking (KLAC, mm), (H) Kernel breadth after cooking (KBAC, mm), (I)
718 Elongation ratio (ER), (J) Amylose content (AC, %), (K) Gel consistency (GC, mm), (L) Alkali spreading value
719 (ASV). Each violin shows the kernel density distribution of the trait; the embedded boxplot indicates the median,
720 interquartile range, and outliers; n = 240 genotypes. (M) Comparison of the 12 grain quality traits between iso-
721 cytoplasmic (ISO, n = 198) and indica × tropical japonica-derived (TRJ, n = 42) restorer backgrounds. Boxplots
722 show the median and interquartile range; individual points represent the BLUPs of the lines. Significance was
723 assessed by Welch's t-test with Benjamini-Hochberg FDR correction across the 12 traits (ns, not significant; *p <
724 0.05, **p < 0.01).

725 Figure 2. Representative photographs illustrating grain quality trait determination among rice restorer lines. (A)
726 Variation in grain morphology among representative restorer lines (G-5, G-30, G-35, G-154, G-204, G-105),

shown as paddy (rough rice, top row), brown rice (middle row), and milled rice (bottom row), illustrating diversity in kernel length and shape. (B) Number of genotypes in each grain type category (long bold, long slender, medium slender, short bold and short slender, based on IRRI kernel dimension standards). (C) Amylose content (AC) estimation by the iodine-colorimetric method; deepening blue-black coloration corresponds to increasing amylose content (Std, standard reference; G-5, G-35, G-44, G-72, G-85, G-96, representative restorer lines). (D) Amylose content (AC) classes (low, intermediate, high). (E) Gel consistency (GC) classification based on cold gel flow length in test tubes, categorized as hard, medium, or soft gel (IR64, check variety; G-205, G-60, G-101, G-107, G-126, G-70, G-155, representative restorer lines). (F) Gel consistency (GC) classes (hard, medium, soft). (G) Alkali spreading value (ASV) assessment based on the extent of grain disintegration and spreading in potassium hydroxide (KOH) solution (TB, Taraori Basmati and PB1, Pusa Basmati 1; parental checks; remaining dishes, restorer lines). (H) Alkali spreading value (ASV) classes (low, intermediate, high). Values above each bar indicate the number and percentage of genotypes in each class.

Figure 3. Pearson correlation matrix of the best linear unbiased predictions (BLUPs) of twelve grain quality traits in 240 rice restorer lines. The traits are ordered by hierarchical clustering. The numbers are the correlation coefficients and the colour scale shows their sign and size. Stars show significance after Benjamini–Hochberg correction (*P < 0.05, **P < 0.01, ***P < 0.001). HP, hulling percentage; MP, milling percentage; HRR, head rice recovery; KLBC and KBBC, kernel length and breadth before cooking; LB, length–breadth ratio; KLAC and KBAC, kernel length and breadth after cooking; ER, elongation ratio; AC, amylose content; GC, gel consistency; ASV, alkali spreading value.

Figure 4. Variance components and multi-trait selection of the restorer lines. (a) Share of genotype, genotype × environment (G × E) and residual variance in the total variance of each trait, with the between-environment genetic correlation (rB) on the right. (b) Venn diagram of the 36 lines selected by each of the four indices (MGIDI, multi-trait genotype–ideotype distance index; MTSI, multi-trait stability index; MTMPS, multi-trait mean performance and stability index; FAI-BLUP, factor analysis and ideotype-design index based on best linear unbiased predictions); 14 lines were selected by all four. (c, e, g) Ranking of the top 60 lines by MGIDI, MTSI and FAI-BLUP (MTMPS is not shown); red bars are the lines within the 15% selection (36 lines) and blue bars are lines that were not selected. (d, f, h) Strengths and weaknesses of the 36 lines selected by MGIDI, MTSI and FAI-BLUP, shown as the contribution (%) of each factor (FA, factor; FA1–FA5) to the distance of each selected line from the ideotype; the dashed circle is the contribution expected if all factors contributed equally. HP, hulling percentage; MP, milling percentage; HRR, head rice recovery; KLBC and KBBC, kernel length and breadth before cooking; LB, length–breadth ratio; KLAC and KBAC, kernel length and breadth after cooking; ER, elongation ratio; AC, amylose content; GC, gel consistency; ASV, alkali spreading value.

Figure 5. Heat map of the Z-scores of the best linear unbiased predictions (BLUPs) of the 14 restorer lines selected by all four indices, for the twelve grain quality traits (left) and six yield-related traits (right). The Z-score was calculated for each trait over the 240 lines (mean 0, SD 1), and the lines are ordered by their rank in the multi-trait genotype–ideotype distance index (MGIDI). DFF, days to 50% flowering; PL, panicle length; PTN, productive tillers per plant; TSW, thousand-seed weight; FG, filled grains per panicle; SYP, seed yield per plant. Quality trait abbreviations as in Figure 3.

Table 1 Combined analysis of variance, variance components and descriptive statistics for twelve grain quality traits evaluated across three environments in 240 rice restorer lines

Source / Statistic	df	HP	MP	HRR	KLBC	KBBC	LB	KLAC	KBAC	ER	AC	GC	ASV
Combined analysis of variance (mean squares)													
Replication (ENV)	3	2470***	1990***	1720***	0.406***	0.0101	0.150***	49.1***	0.905***	1.58***	2880***	20400***	5.05***
Environment (ENV)	2	449	427	582	62.0***	10.6***	81.4***	680*	7.75	7.67	796	22500	226**
Genotypes (GEN)	239	66.3***	94.8***	277***	1.49***	0.254***	1.28***	3.09***	0.322***	0.0566***	53.5***	1410***	12.7***
GEN:ENV	478	7.88***	5.08***	7.67***	0.0296	0.0515***	0.0477***	0.444***	0.0875***	0.0147***	3.18***	69.5***	0.864***
Error	717	1.19	1.01	2.36	0.031	0.021	0.025	0.042	0.062	0.003	0.65	17.20	0.30
Variance components (REML)													
σ^2_G		9.74***	15.0***	44.9***	0.244***	0.0338***	0.206***	0.442***	0.0391***	0.00698***	8.39***	224***	1.97***
$\sigma^2_{G\times E}$		3.35***	2.03***	2.66***	0.00	0.016***	0.015***	0.201***	0.013***	0.00597***	1.27***	26.2***	0.282***
σ^2_e		1.19	1.01	2.36	0.0306	0.0213	0.0248	0.0420	0.0620	0.00277	0.644	17.2	0.300
G (% of total variance)		68.2	83.1	90.0	88.9	48.2	85.0	64.5	34.3	44.4	81.4	83.8	77.2
G×E (% of total variance)		23.5	11.3	5.3	0.0	21.5	4.7	29.4	11.2	38.0	12.3	9.8	11.0
Residual (% of total variance)		8.3	5.6	4.7	11.1	30.3	10.3	6.1	54.5	17.6	6.2	6.4	11.7
rB		0.74	0.88	0.94	1.00	0.69	0.95	0.69	0.75	0.54	0.87	0.90	0.87
Descriptive statistics (240 genotype means)													
Mean		80.74	73.16	63.41	5.96	1.91	3.18	8.61	2.72	1.44	15.50	46.96	4.16
Minimum		43.48	39.46	35.17	4.68	1.33	1.94	4.46	2.00	0.83	8.43	22.94	1.03
Maximum		89.42	88.14	76.06	8.60	3.43	5.35	11.09	3.58	1.82	24.09	79.19	6.50
Range		45.93	48.69	40.89	3.92	2.11	3.40	6.63	1.58	0.99	15.65	56.25	5.47
SD		3.32	3.97	6.80	0.50	0.21	0.46	0.72	0.23	0.10	2.99	15.36	1.46
SE		0.21	0.26	0.44	0.03	0.01	0.03	0.05	0.01	0.01	0.19	0.99	0.09
CV (%)		4.12	5.43	10.72	8.38	10.76	14.54	8.34	8.51	6.72	19.27	32.70	24.96

*** = P < 0.001, ** = P < 0.01 and * = P < 0.05; no star = not significant. The analysis of variance is on plot-level data (1,440 records per trait) with replicate nested in environment: environment was tested against replicate within environment, genotype against GEN:ENV, and GEN:ENV and replicate against error. df, degrees of freedom; ENV, environment; GEN, genotype; GEN:ENV, genotype × environment interaction. Variance components were estimated by restricted maximum likelihood (REML): σ^2G, genotypic variance; σ^2G×E, genotype × environment (G×E) interaction variance; σ^2e, residual variance (stars: likelihood-ratio test of σ^2G and σ^2G×E, boundary-corrected). G, G×E and Residual (%) are the shares of σ^2G, σ^2G×E and σ^2e in the total variance; rB = σ^2G/(σ^2G + σ^2G×E) is the genetic correlation between environments. Mean, minimum, maximum, range, SD, SE and CV are from the 240 genotype means. HP – Hulling percentage (%); MP – Milling percentage (%); HRR – Head rice recovery (%); KLBC – Kernel length before cooking (mm); KBBC – Kernel breadth before cooking (mm); KLAC – Kernel length after cooking (mm); KBAC – Kernel breadth after cooking (mm); LB – Kernel length / Kernel breadth; ER – Elongation ratio; AC – Amylose Content (%); GC – Gel Consistency (mm); ASV – Alkali Spreading Value; SD = Standard deviation; SE = Standard error; CV = Coefficient of variation (%).

Table 2 Factor analysis of the twelve rescaled grain quality traits in the multi-trait genotype-ideotype distance index (MGIDI) for 240 rice restorer lines evaluated in three environments.

VAR	FA1	FA2	FA3	FA4	FA5	Communality	Uniquenesses
LB	**-0.83**	0.04	0.03	-0.29	0.35	0.89	0.11
KBBC	**-0.82**	0.10	-0.12	-0.13	0.02	0.72	0.28
MP	0.01	**-0.95**	0.05	-0.00	-0.01	0.90	0.10
HP	-0.07	**-0.81**	0.14	-0.09	-0.15	0.72	0.28
HRR	0.32	**-0.68**	-0.16	0.08	0.21	0.64	0.36
AC	-0.00	0.09	**-0.84**	-0.11	0.02	0.72	0.28
GC	-0.09	-0.04	**-0.81**	0.21	-0.09	0.72	0.28
ASV	**0.49**	0.08	-0.49	-0.30	0.31	0.67	0.33
KLAC	-0.17	0.07	0.16	**-0.89**	-0.19	0.88	0.12
KLBC	-0.33	0.02	0.21	**-0.63**	0.55	0.85	0.15
KBAC	-0.04	-0.06	-0.09	**-0.45**	-0.06	0.22	0.78
ER	0.12	-0.01	0.01	-0.32	**-0.88**	0.89	0.11
Mean communality						0.74	
Eigenvalues	1.87	2.06	1.75	1.75	1.41		
Variance (%)	15.6	17.2	14.6	14.6	11.8		
Cumulative variance (%)	15.6	32.8	47.3	61.9	73.7		
Contribution to the distance of the selected lines (%)	3.0	32.8	12.9	34.2	17.1		

VAR: traits. HP, hulling percentage; MP, milling percentage; HRR, head rice recovery; KLBC and KBBC, kernel length and breadth before cooking; LB, length–breadth ratio; KLAC and KBAC, kernel length and breadth after cooking; ER, elongation ratio; AC, amylose content; GC, gel consistency; ASV, alkali spreading value. FA1-FA5, rotated factors (varimax); the highest loading of each trait is in bold. Eigenvalues are the sums of squared loadings of each rotated factor. The last row is the contribution (%) of each factor to the squared distance of the 36 selected lines from the ideotype. MGIDI, multi-trait genotype-ideotype distance index. BLUP, best linear unbiased prediction. The traits were rescaled (0–100) before the factor analysis.

Table 3 List of common rice restorer lines identified by the different multi-trait selection indices (MGIDI, multi-trait genotype–ideotype distance index; MTSI, multi-trait stability index; MTMPS, multi-trait mean performance and stability index; FAI-BLUP, factor analysis and ideotype-design index based on best linear unbiased predictions; selection intensity 15%, 36 of 240 lines per index).

SN	Methods	Count	Name of the Genotypes
1	MGIDI and FAI-BLUP	34	G-1, G-3, G-4, G-6, G-10, G-11, G-20, G-26, G-28, G-38, G-54, G-62, G-69, G-82, G-83, G-84, G-85, G-94, G-95, G-100, G-101, G-103, G-110, G-182, G-183, G-193, G-194, G-198, G-202, G-210, G-211, G-212, G-215, G-219
2	MGIDI and MTSI	15	G-1, G-3, G-10, G-11, G-20, G-26, G-28, G-54, G-94, G-100, G-106, G-183, G-210, G-211, G-212
3	MGIDI and MTMPS	21	G-1, G-3, G-4, G-10, G-11, G-20, G-26, G-28, G-54, G-62, G-94, G-95, G-100, G-106, G-182, G-183, G-194, G-210, G-211, G-212, G-215
4	FAI-BLUP and MTSI	14	G-1, G-3, G-10, G-11, G-20, G-26, G-28, G-54, G-94, G-100, G-183, G-210, G-211, G-212
5	FAI-BLUP and MTMPS	20	G-1, G-3, G-4, G-10, G-11, G-20, G-26, G-28, G-54, G-62, G-94, G-95, G-100, G-182, G-183, G-194, G-210, G-211, G-212, G-215
6	MTSI and MTMPS	25	G-1, G-3, G-10, G-11, G-20, G-23, G-26, G-28, G-54, G-58, G-60, G-65, G-90, G-94, G-100, G-106, G-108, G-121, G-125, G-127, G-183, G-199, G-210, G-211, G-212
7	MGIDI, MTSI, MTMPS and FAI-BLUP	14	G-1, G-3, G-10, G-11, G-20, G-26, G-28, G-54, G-94, G-100, G-183, G-210, G-211, G-212

SN, serial number. Count is the number of lines selected by both (or all four) indices; the lines selected by all four indices are included in each of the pairs. Genotypes are given by their G-codes.

811 **Table S1 A set of 240 diverse restorers utilized in the study and their pedigree**

SN	Lines	Pedigree	SN	Lines	Pedigree	SN	Lines	Pedigree	SN	Lines	Pedigree
1	G-1	CMH-1	61	G-61	CMH-63	121	G-121	CMH-124	181	G-181	MLT-44
2	G-2	CMH-2	62	G-62	CMH-64	122	G-122	CMH-125	182	G-182	MLT-45
3	G-3	CMH-3	63	G-63	CMH-65	123	G-123	CMH-126	183	G-183	MLT-46
4	G-4	CMH-4	64	G-64	CMH-66	124	G-124	CMH-127	184	G-184	MLT-47
5	G-5	CMH-5	65	G-65	CMH-67	125	G-125	CMH-128	185	G-185	MLT-48
6	G-6	CMH-6	66	G-66	CMH-68	126	G-126	CMH-129	186	G-186	MLT-49
7	G-7	CMH-7	67	G-67	CMH-69	127	G-127	CMH-130	187	G-187	MLT-50
8	G-8	CMH-8	68	G-68	CMH-70	128	G-128	CMH-131	188	G-188	MLT-51
9	G-9	CMH-9	69	G-69	CMH-71	129	G-129	CMH-132	189	G-189	MLT-52
10	G-10	CMH-10	70	G-70	CMH-72	130	G-130	CMH-133	190	G-190	MLT-53
11	G-11	CMH-11	71	G-71	CMH-73	131	G-131	CMH-134	191	G-191	MLT-54
12	G-12	CMH-12	72	G-72	CMH-74	132	G-132	CMH-135	192	G-192	MLT-55
13	G-13	CMH-13	73	G-73	CMH-75	133	G-133	CMH-136	193	G-193	MLT-56
14	G-14	CMH-14	74	G-74	CMH-76	134	G-134	CMH-137	194	G-194	MLT-57
15	G-15	CMH-15	75	G-75	CMH-77	135	G-135	CMH-138	195	G-195	MLT-58
16	G-16	CMH-16	76	G-76	CMH-78	136	G-136	CMH-139	196	G-196	MLT-59
17	G-17	CMH-17	77	G-77	CMH-79	137	G-137	CMH-140	197	G-197	MLT-60
18	G-18	CMH-18	78	G-78	CMH-80	138	G-138	MLT-1	198	G-198	MLT-61
19	G-19	CMH-19	79	G-79	CMH-81	139	G-139	MLT-2	199	G-199	PP-1
20	G-20	CMH-20	80	G-80	CMH-82	140	G-140	MLT-3	200	G-200	PP-2
21	G-21	CMH-21	81	G-81	CMH-83	141	G-141	MLT-4	201	G-201	PP-3
22	G-22	CMH-22	82	G-82	CMH-84	142	G-142	MLT-5	202	G-202	PP-4
23	G-23	CMH-23	83	G-83	CMH-85	143	G-143	MLT-6	203	G-203	PP-5
24	G-24	CMH-24	84	G-84	CMH-86	144	G-144	MLT-7	204	G-204	PP-7
25	G-25	CMH-25	85	G-85	CMH-87	145	G-145	MLT-8	205	G-205	PP-8
26	G-26	CMH-26	86	G-86	CMH-88	146	G-146	MLT-9	206	G-206	PP-9
27	G-27	CMH-27	87	G-87	CMH-89	147	G-147	MLT-10	207	G-207	PP-13
28	G-28	CMH-28	88	G-88	CMH-90	148	G-148	MLT-11	208	G-208	PP-14
29	G-29	CMH-31	89	G-89	CMH-91	149	G-149	MLT-12	209	G-209	PP-15
30	G-30	CMH-32	90	G-90	CMH-92	150	G-150	MLT-13	210	G-210	PP-17
31	G-31	CMH-33	91	G-91	CMH-93	151	G-151	MLT-14	211	G-211	PP-18

32	G-32	CMH-34	92	G-92	CMH-94	152	G-152	MLT-15	212	G-212	PP-19
33	G-33	CMH-35	93	G-93	CMH-95	153	G-153	MLT-16	213	G-213	PP-20
34	G-34	CMH-36	94	G-94	CMH-96	154	G-154	MLT-17	214	G-214	PP-21
35	G-35	CMH-37	95	G-95	CMH-97	155	G-155	MLT-18	215	G-215	PP-22
36	G-36	CMH-38	96	G-96	CMH-98	156	G-156	MLT-19	216	G-216	PP-23
37	G-37	CMH-39	97	G-97	CMH-99	157	G-157	MLT-20	217	G-217	PP-24
38	G-38	CMH-40	98	G-98	CMH-100	158	G-158	MLT-21	218	G-218	PP-25
39	G-39	CMH-41	99	G-99	CMH-101	159	G-159	MLT-22	219	G-219	PP-27
40	G-40	CMH-42	100	G-100	CMH-103	160	G-160	MLT-23	220	G-220	PP-28
41	G-41	CMH-43	101	G-101	CMH-104	161	G-161	MLT-24	221	G-221	PP-29
42	G-42	CMH-44	102	G-102	CMH-105	162	G-162	MLT-25	222	G-222	PP-31
43	G-43	CMH-45	103	G-103	CMH-106	163	G-163	MLT-26	223	G-223	PP-32
44	G-44	CMH-46	104	G-104	CMH-107	164	G-164	MLT-27	224	G-224	PP-33
45	G-45	CMH-47	105	G-105	CMH-108	165	G-165	MLT-28	225	G-225	PP-34
46	G-46	CMH-48	106	G-106	CMH-109	166	G-166	MLT-29	226	G-226	PP-35
47	G-47	CMH-49	107	G-107	CMH-110	167	G-167	MLT-30	227	G-227	PP-36
48	G-48	CMH-50	108	G-108	CMH-111	168	G-168	MLT-31	228	G-228	PP-37
49	G-49	CMH-51	109	G-109	CMH-112	169	G-169	MLT-32	229	G-229	PP-38
50	G-50	CMH-52	110	G-110	CMH-113	170	G-170	MLT-33	230	G-230	PP-39
51	G-51	CMH-53	111	G-111	CMH-114	171	G-171	MLT-34	231	G-231	PP-40
52	G-52	CMH-54	112	G-112	CMH-115	172	G-172	MLT-35	232	G-232	PP-41
53	G-53	CMH-55	113	G-113	CMH-116	173	G-173	MLT-36	233	G-233	PP-42
54	G-54	CMH-56	114	G-114	CMH-117	174	G-174	MLT-37	234	G-234	PP-43
55	G-55	CMH-57	115	G-115	CMH-118	175	G-175	MLT-38	235	G-235	PP-45
56	G-56	CMH-58	116	G-116	CMH-119	176	G-176	MLT-39	236	G-236	PP-46
57	G-57	CMH-59	117	G-117	CMH-120	177	G-177	MLT-40	237	G-237	PP-47
58	G-58	CMH-60	118	G-118	CMH-121	178	G-178	MLT-41	238	G-238	PP-48
59	G-59	CMH-61	119	G-119	CMH-122	179	G-179	MLT-42	239	G-239	PP-51
60	G-60	CMH-62	120	G-120	CMH-123	180	G-180	MLT-43	240	G-240	PP-53

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Table S2 Description of experimental locations and crop establishment details across the three study environments

Location	Soil type	pH	Date of sowing	Date of Planting
Delhi	Sandy loam	7.2-7.5	06-06-2022	27-06-2022
Rakhra	Sandy loam	7.8	10-06-2022	01-07-2022
Aduthurai	Alluvial clay	7.4-7.5	20-12-2022	10-01-2023

Table S3 Factor analysis of the twelve rescaled grain quality traits in the multi-trait stability index (MTSI) for 240 rice restorer lines evaluated in three environments.

VAR	FA1	FA2	FA3	FA4	FA5	Communality	Uniquenesses
LB	**-0.94**	-0.05	0.08	0.04	-0.10	0.90	0.10
KBBC	**-0.86**	-0.07	0.01	-0.03	0.14	0.76	0.24
MP	-0.14	**-0.88**	0.02	0.13	0.06	0.82	0.18
HP	-0.08	**-0.75**	0.03	0.23	-0.08	0.63	0.37
HRR	0.13	**-0.73**	-0.03	-0.29	0.00	0.64	0.36
KLAC	-0.18	0.00	**0.93**	0.00	-0.17	0.93	0.07
ER	0.05	-0.04	**0.92**	-0.02	0.15	0.88	0.12
AC	-0.09	0.11	0.08	**-0.81**	-0.08	0.70	0.30
ASV	0.25	0.00	0.12	**-0.66**	-0.02	0.51	0.49
GC	-0.12	-0.01	-0.28	**-0.55**	0.13	0.41	0.59
KBAC	0.19	-0.05	-0.09	-0.05	**-0.76**	0.63	0.37
KLBC	-0.44	0.06	0.19	0.08	**-0.64**	0.66	0.34
Mean communality						0.70	
Eigenvalues	2.02	1.91	1.86	1.57	1.11		
Variance (%)	16.8	15.9	15.5	13.1	9.2		
Cumulative variance (%)	16.8	32.7	48.2	61.3	70.5		
Contribution to the distance of the selected lines (%)	4.2	37.1	23.8	27.2	7.7		

VAR, trait. HP, hulling percentage; MP, milling percentage; HRR, head rice recovery; KLBC and KBBC, kernel length and breadth before cooking; LB, length–breadth ratio; KLAC and KBAC, kernel length and breadth after cooking; ER, elongation ratio; AC, amylose content; GC, gel consistency; ASV, alkali spreading value. FA1–FA5, rotated factors (varimax); the highest loading of each trait is in bold. Communality is the sum of squared loadings of a trait and uniqueness is 1 − communality. Eigenvalues are the sums of squared loadings of each rotated factor. The last row is the contribution (%) of each factor to the squared distance of the 36 selected lines from the ideotype. MTSI, multi-trait stability index; BLUP, best linear unbiased prediction; WAASB, weighted average of absolute scores. In MTSI each trait is the mean of the rescaled BLUP and the rescaled WAASB.

Table S4 Factor analysis of the twelve rescaled grain quality traits in the multi-trait mean performance and stability index (MTMPS) for 240 rice restorer lines evaluated in three environments.

VAR	FA1	FA2	FA3	FA4	FA5	FA6	Communality	Uniquenesses
LB	**-0.94**	-0.02	-0.15	-0.04	-0.13	-0.06	0.92	0.08
KBBC	**-0.76**	0.01	-0.27	-0.21	-0.23	-0.12	0.76	0.24
KLBC	**-0.67**	-0.10	0.17	0.37	0.37	0.20	0.80	0.20
ER	0.17	**-0.94**	-0.09	-0.03	-0.06	-0.06	0.93	0.07
KLAC	-0.29	**-0.87**	0.04	0.16	0.16	0.08	0.91	0.09
MP	-0.13	-0.04	**-0.89**	0.04	0.01	-0.04	0.82	0.18
HP	-0.21	-0.07	**-0.80**	0.02	-0.17	0.11	0.74	0.26
HRR	0.18	0.13	**-0.62**	0.03	0.56	-0.04	0.76	0.24
GC	0.00	0.12	-0.00	**-0.80**	-0.03	-0.02	0.66	0.34
AC	-0.10	-0.06	0.13	**-0.72**	0.41	0.11	0.72	0.28
ASV	0.10	-0.09	0.07	-0.17	**0.79**	-0.07	0.68	0.32
KBAC	0.06	0.00	-0.04	-0.04	-0.06	**0.97**	0.95	0.05
Mean communality							0.80	
Eigenvalues	2.13	1.71	1.99	1.41	1.37	1.04		
Variance (%)	17.7	14.3	16.6	11.7	11.4	8.7		
Cumulative variance (%)	17.7	32.0	48.6	60.3	71.7	80.4		
Contribution to the distance of the selected lines (%)	1.6	38.4	27.1	13.1	7.0	12.9		

VAR, trait. HP, hulling percentage; MP, milling percentage; HRR, head rice recovery; KLBC and KBBC, kernel length and breadth before cooking; LB, length–breadth ratio; KLAC and KBAC, kernel length and breadth after cooking; ER, elongation ratio; AC, amylose content; GC, gel consistency; ASV, alkali spreading value. FA1–FA6, rotated factors (varimax); the highest loading of each trait is in bold. Communality is the sum of squared loadings of a trait and uniqueness is 1 − communality. Eigenvalues are the sums of squared loadings of each rotated factor. The last row is the contribution (%) of each factor to the squared distance of the 36 selected lines from the ideotype. MTMPS, multi-trait mean performance and stability index; BLUP, best linear unbiased prediction. In MTMPS each trait is the mean of the rescaled BLUP and the rescaled Wricke's ecovalence (a stability statistic, lower values mean more stable).

Table S5 Factor analysis of the twelve grain quality traits in the FAI-BLUP index for 240 rice restorer lines evaluated in three environments.

VAR	FA1	FA2	FA3	FA4	FA5	Communality	Uniquenesses
LB	**-0.83**	-0.04	0.03	0.29	0.35	0.89	0.11
KBBC	**-0.82**	-0.10	-0.12	0.13	0.02	0.72	0.28
MP	-0.01	**-0.95**	-0.05	-0.00	0.01	0.90	0.10
HP	0.07	**-0.81**	-0.14	-0.09	0.15	0.72	0.28
HRR	-0.32	**-0.68**	0.16	0.08	-0.21	0.64	0.36
AC	-0.00	-0.09	**-0.84**	0.11	0.02	0.72	0.28
GC	-0.09	0.04	**-0.81**	-0.21	-0.09	0.72	0.28
ASV	**-0.49**	0.08	0.49	-0.30	-0.31	0.67	0.33
KLAC	0.17	0.07	-0.16	**-0.89**	0.19	0.88	0.12
KLBC	-0.33	-0.02	0.21	**0.63**	0.55	0.85	0.15
KBAC	-0.04	0.06	-0.09	**0.45**	-0.06	0.22	0.78
ER	-0.12	-0.01	-0.01	-0.32	**0.88**	0.89	0.11
Mean communality						0.74	
Eigenvalues	1.87	2.06	1.75	1.75	1.41		
Variance (%)	15.6	17.2	14.6	14.6	11.8		
Cumulative variance (%)	15.6	32.8	47.3	61.9	73.7		
Contribution to the distance of the selected lines (%)	2.4	32.7	14.3	33.6	17.0		

VAR, trait. HP, hulling percentage; MP, milling percentage; HRR, head rice recovery; KLBC and KBBC, kernel length and breadth before cooking; LB, length–breadth ratio; KLAC and KBAC, kernel length and breadth after cooking; ER, elongation ratio; AC, amylose content; GC, gel consistency; ASV, alkali spreading value. FA1–FA5, rotated factors (varimax); the highest loading of each trait is in bold. Communality is the sum of squared loadings of a trait and uniqueness is 1 − communality. Eigenvalues are the sums of squared loadings of each rotated factor. The last row is the contribution (%) of each factor to the squared distance of the 36 selected lines from the ideotype. FAI-BLUP, factor analysis and ideotype-design index based on BLUPs; BLUP, best linear unbiased prediction. FAI-BLUP uses the BLUPs of the traits without rescaling.

Supplementary figures

Figure S1. Experimental sites and prevailing weather conditions during the crop growth period. (A) Monthly maximum temperature, minimum temperature, and rainfall recorded at Delhi (2022), Rakhra, Punjab (2022), and Aduthurai, Tamil Nadu (2023). (B) Map of India showing the geographic location, coordinates, and elevation of the three multi-location evaluation sites: Punjab (30°22'N, 76°15'E; 253 m), Delhi (28°38'N, 77°10'E; 224 m), and Tamil Nadu (11°00'N, 79°28'E; 23 m).

Figure S2. Schematic representation of the analytical protocols used for grain quality trait determination. (A) Amylose content (AC) estimation by the iodine-colorimetric method,[39] showing sample preparation, alkali digestion, heating, reagent addition, serial dilution, dark incubation, and absorbance measurement at 620 nm. (B) Alkali spreading value (ASV) determination, showing kernel preparation, treatment with 1.7% KOH, incubation at 30°C for 23 h, and visual scoring of grain spreading on a 1-7 scale (1 = least spread, 7 = most spread). (C) Gel consistency (GC) determination[40], showing flour weighing, alkali digestion, heating, cooling, ice-bath treatment, and measurement of gel flow length on graph paper after 1 h.

Figure S3. Mean performance and stability of the 240 restorer lines for the twelve traits. The horizontal axis is the BLUP across environments and the vertical axis is the weighted average of absolute scores (WAASB; lower values mean more stable lines). The 14 lines selected by all four indices are highlighted. Abbreviations as in Figure 3.

Figures

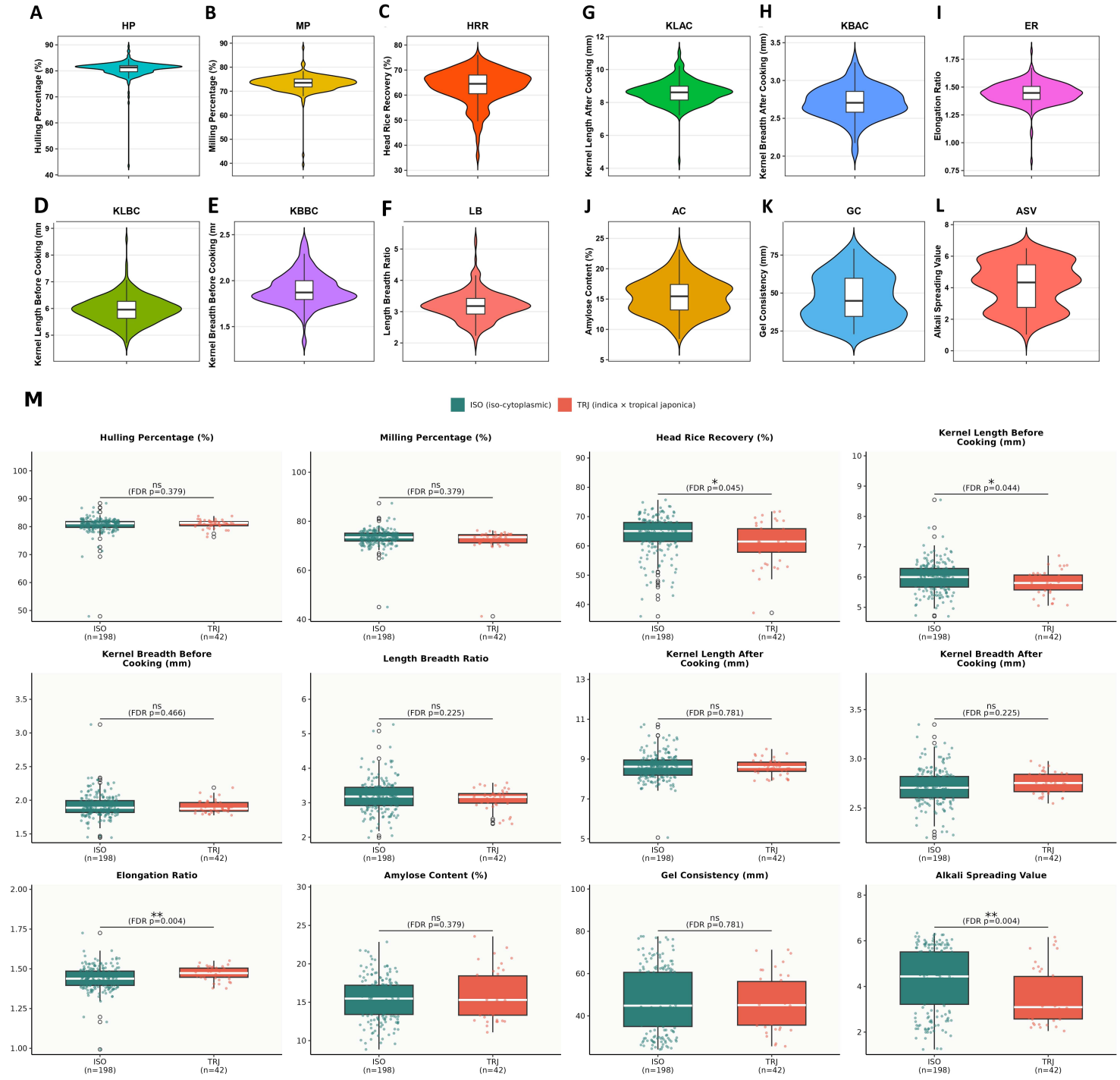


Figure 1

Violin plots showing the phenotypic distribution of 12 grain quality traits among 240 rice restorer lines. (A) Hulling percentage (HP, %), (B) Milling percentage (MP, %), (C) Head rice recovery (HRR, %), (D) Kernel length before cooking (KLBC, mm), (E) Kernel breadth before cooking (KBBC, mm), (F) Length-breadth ratio (LB), (G) Kernel length after cooking (KLAC, mm), (H) Kernel breadth after cooking (KBAC, mm), (I) Elongation ratio (ER), (J) Amylose content (AC, %), (K) Gel consistency (GC, mm), (L) Alkali spreading

value (ASV). Each violin shows the kernel density distribution of the trait; the embedded boxplot indicates the median, interquartile range, and outliers; n = 240 genotypes. (M) Comparison of the 12 grain quality traits between iso-cytoplasmic (ISO, n = 198) and indica × tropical japonica-derived (TRJ, n = 42) restorer backgrounds. Boxplots show the median and interquartile range; individual points represent the BLUPs of the lines. Significance was assessed by Welch's t-test with Benjamini-Hochberg FDR correction across the 12 traits (ns, not significant; *p < 0.05, **p < 0.01).

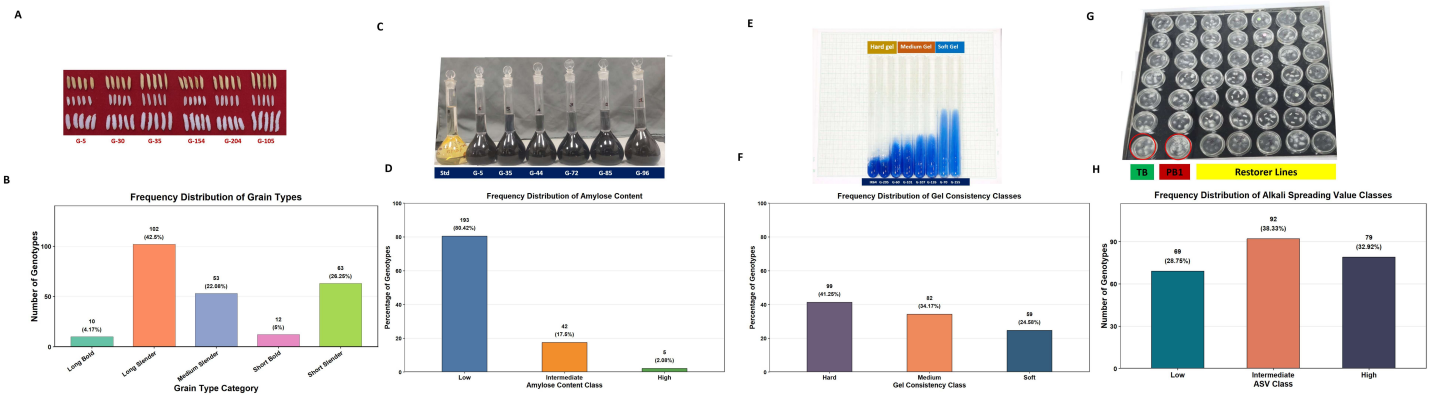


Figure 2

Representative photographs illustrating grain quality trait determination among rice restorer lines. (A) Variation in grain morphology among representative restorer lines (G-5, G-30, G-35, G-154, G-204, G-105), shown as paddy (rough rice, top row), brown rice (middle row), and milled rice (bottom row), illustrating diversity in kernel length and shape. (B) Number of genotypes in each grain type category (long bold, long slender, medium slender, short bold and short slender, based on IRRI kernel dimension standards). (C) Amylose content (AC) estimation by the iodine-colorimetric method; deepening blue-black coloration corresponds to increasing amylose content (Std, standard reference; G-5, G-35, G-44, G-72, G-85, G-96, representative restorer lines). (D) Amylose content (AC) classes (low, intermediate, high). (E) Gel consistency (GC) classification based on cold gel flow length in test tubes, categorized as hard, medium, or soft gel (IR64, check variety; G-205, G-60, G-101, G-107, G-126, G-70, G-155, representative restorer lines). (F) Gel consistency (GC) classes (hard, medium, soft). (G) Alkali spreading value (ASV) assessment based on the extent of grain disintegration and spreading in potassium hydroxide (KOH) solution (TB, Taraori Basmati and PB1, Pusa Basmati 1; parental checks; remaining dishes, restorer lines). (H) Alkali spreading value (ASV) classes (low, intermediate, high). Values above each bar indicate the number and percentage of genotypes in each class.

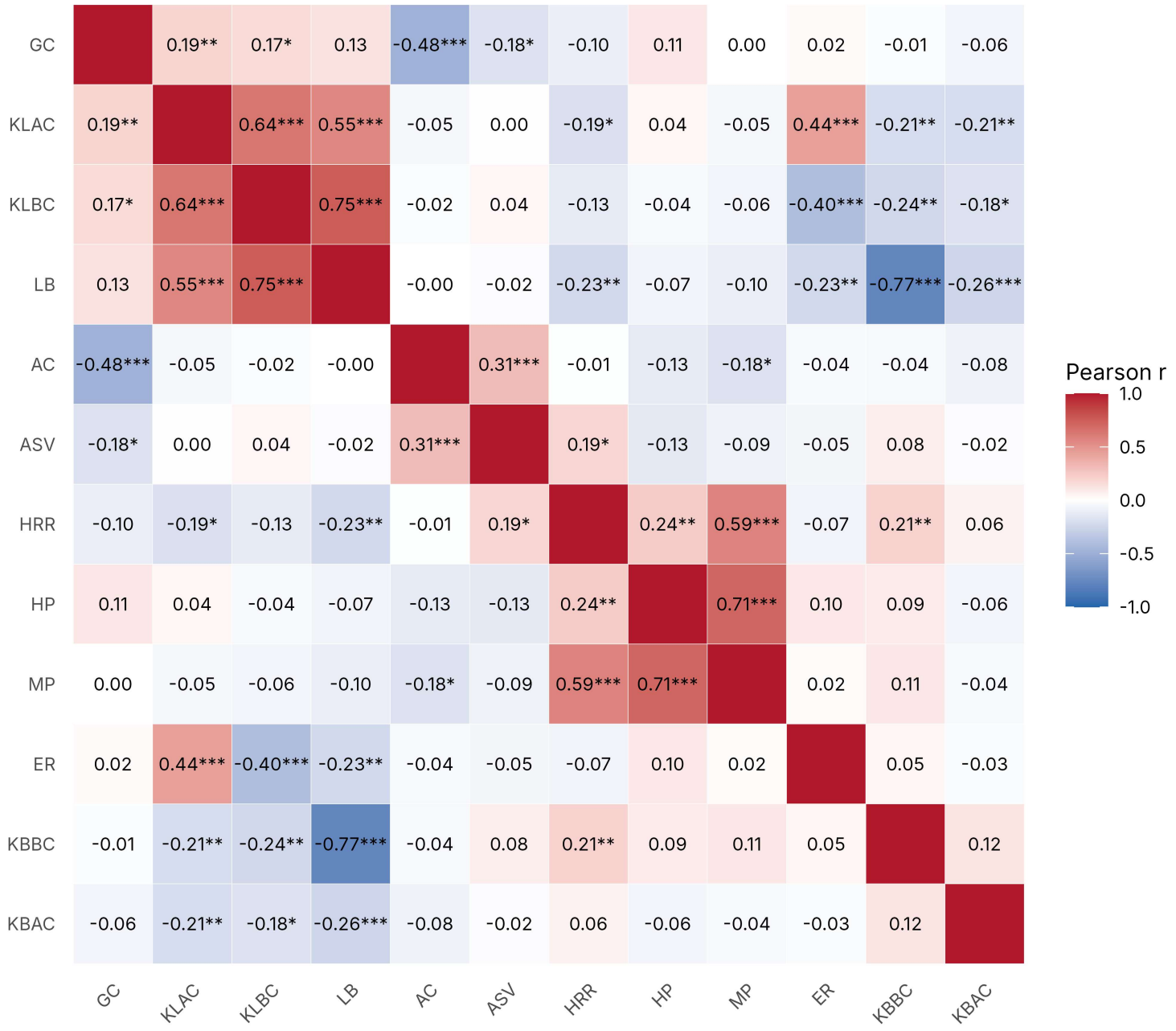
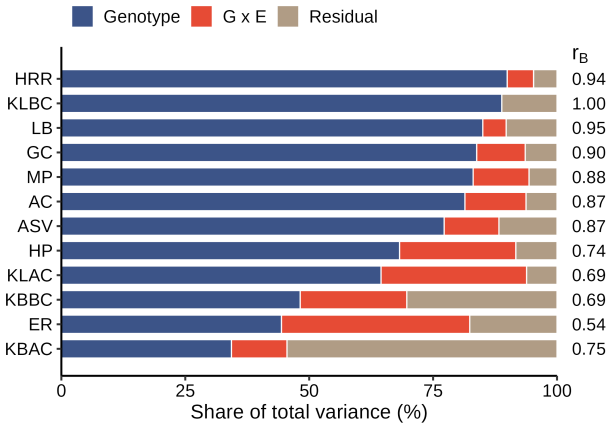
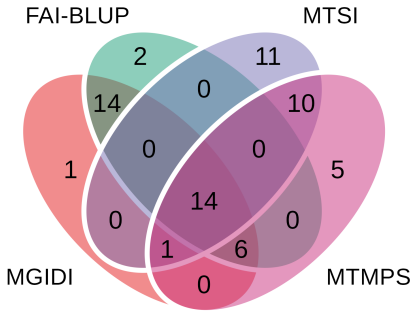


Figure 3

Pearson correlation matrix of the best linear unbiased predictions (BLUPs) of twelve grain quality traits in 240 rice restorer lines. The traits are ordered by hierarchical clustering. The numbers are the correlation coefficients and the colour scale shows their sign and size. Stars show significance after Benjamini–Hochberg correction (*P < 0.05, **P < 0.01, ***P < 0.001). HP, hulling percentage; MP, milling percentage; HRR, head rice recovery; KLBC and KBBC, kernel length and breadth before cooking; LB, length–breadth ratio; KLAC and KBAC, kernel length and breadth after cooking; ER, elongation ratio; AC, amylose content; GC, gel consistency; ASV, alkali spreading value.



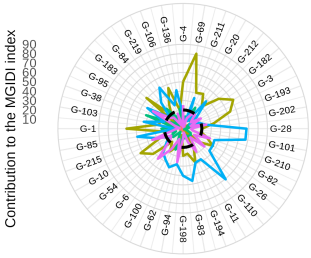
(a) Variance partition and genetic correlation



(b) Lines selected by the four indices



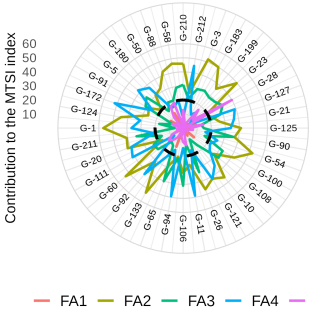
(c) MGIDI ranking



(d) MGIDI strengths and weaknesses



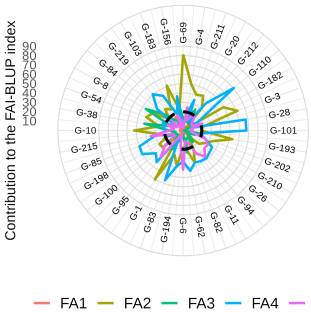
(e) MTSI ranking



(f) MTSI strengths and weaknesses



(g) FAI-BLUP ranking



(h) FAI-BLUP strengths and weaknesses

Figure 4

Variance components and multi-trait selection of the restorer lines. (a) Share of genotype, genotype × environment (G × E) and residual variance in the total variance of each trait, with the between-environment genetic correlation (rB) on the right. (b) Venn diagram of the 36 lines selected by each of the four indices (MGIDI, multi-trait genotype–ideotype distance index; MTSI, multi-trait stability index; MTMPS, multi-trait mean performance and stability index; FAI-BLUP, factor analysis and ideotype-design

index based on best linear unbiased predictions); 14 lines were selected by all four. (c, e, g) Ranking of the top 60 lines by MGIDI, MTSI and FAI-BLUP (MTMPS is not shown); red bars are the lines within the 15% selection (36 lines) and blue bars are lines that were not selected. (d, f, h) Strengths and weaknesses of the 36 lines selected by MGIDI, MTSI and FAI-BLUP, shown as the contribution (%) of each factor (FA, factor; FA1–FA5) to the distance of each selected line from the ideotype; the dashed circle is the contribution expected if all factors contributed equally. HP, hulling percentage; MP, milling percentage; HRR, head rice recovery; KLBC and KBBC, kernel length and breadth before cooking; LB, length–breadth ratio; KLAC and KBAC, kernel length and breadth after cooking; ER, elongation ratio; AC, amylose content; GC, gel consistency; ASV, alkali spreading value.

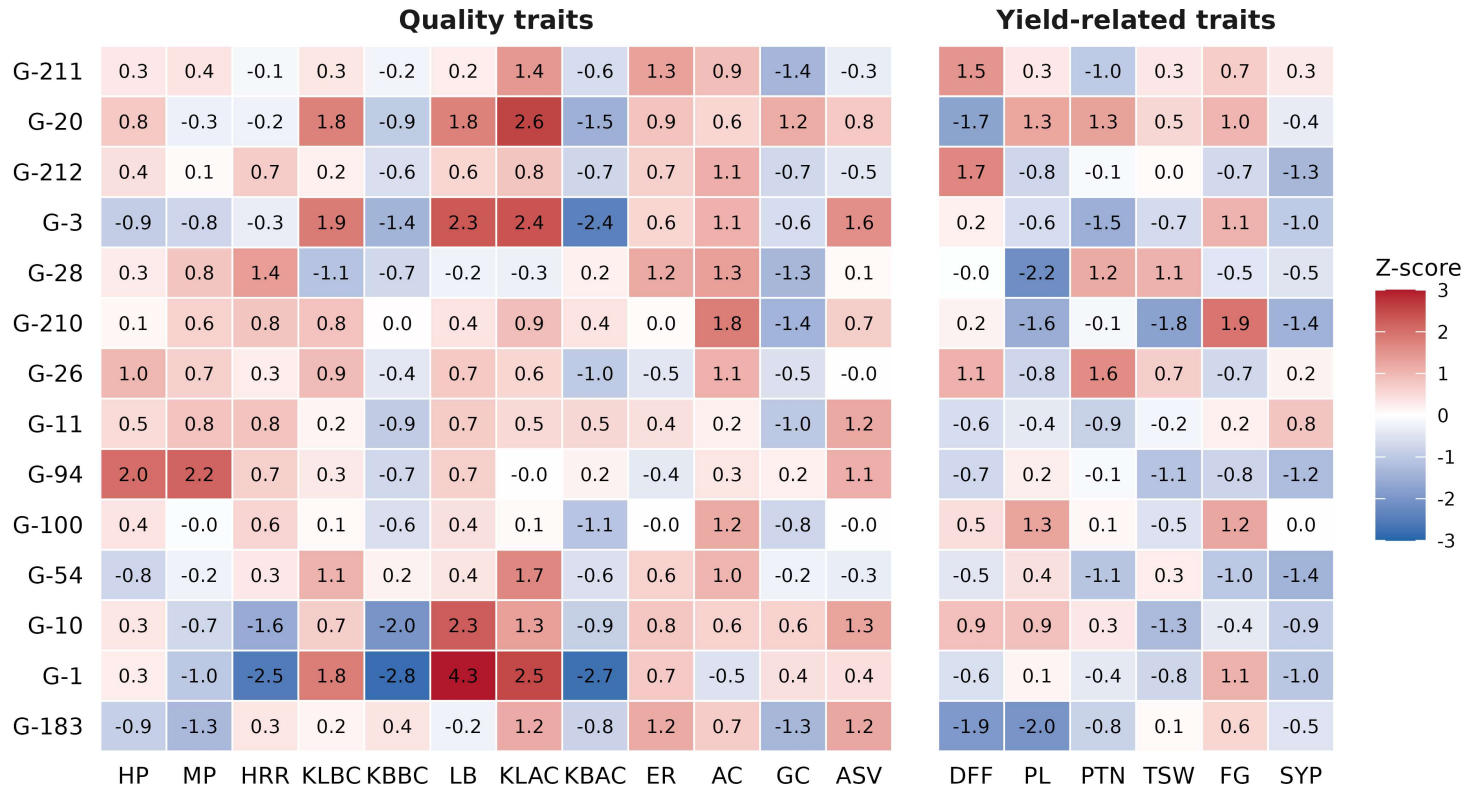


Figure 5

Heat map of the Z-scores of the best linear unbiased predictions (BLUPs) of the 14 restorer lines selected by all four indices, for the twelve grain quality traits (left) and six yield-related traits (right). The Z-score was calculated for each trait over the 240 lines (mean 0, SD 1), and the lines are ordered by their rank in the multi-trait genotype–ideotype distance index (MGIDI). DFF, days to 50% flowering; PL, panicle length; PTN, productive tillers per plant; TSW, thousand-seed weight; FG, filled grains per panicle; SYP, seed yield per plant. Quality trait abbreviations as in Figure 3.

Supplementary Files

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- S1.png
- S2.png
- S3.png