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RESEARCH ARTICLE

Participatory Plant Breeding to develop biofortified upland rice for marginal environments

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Abstract
In the Highlands of Madagascar, where rice is the main staple food, explosive demographic growth has driven the need for the development of upland rice. In that context, a Participatory Plant Breeding (PPB) program conducted by the FOFIFA-Cirad partnership, aims to develop upland rice varieties adapted to farmers' needs, with superior agronomic performances, and with high grain zinc concentration. In the area, where ferralitic soils with N and P deficiencies prevail, limited fertilizer usage persists due to elevated costs, and upland rice varieties must adapt to these low fertility environments. Thus, this paper aims to identify the adequate selection conditions and methods that allow combining the above-mentioned criteria for selection. So, 56 rice breeding lines, including high-zinc genotypes, were evaluated in field trials with contrasting fertility conditions. A relative selection efficiency analysis demonstrated that selection for yield should be done in moderate fertility environments, while selection for grain zinc concentration could be done across a diverse range of conditions. Through participatory evaluations, we identified that, for this case, grain appreciation was the most important character for deciding whether to select a line, followed by productivity and earliness. We also noted that farmers were more willing to accept a variety if it had higher grain zinc concentration. Finally, we proposed a selection index that combines agronomic, farmers' and nutritional criteria, with the purpose of selecting lines that fulfill the expectations on these areas. Overall, this paper proposes an adapted methodology for the combination of PPB and biofortification in marginal environments.

Keywords: upland rice; biofortification; G × E interactions; participatory plant breeding; relative selection efficiency; selection index

Introduction
In Madagascar, rice (*Oryza sativa* L.) is by far the main staple food, with an estimated yearly consumption of 115 kg per person (Nikiema *et al.*, 2023). The Central Highlands region (1300–2000 masl) faces unique challenges stemming from high population density and decreasing farm sizes per household. While rice cultivation has traditionally occurred in irrigated paddies, there has been a significant expansion of strictly rainfed upland rice cultivation to meet the growing demand. This process has been largely driven by a national breeding program, jointly

conducted by the Malagasy National Research Center for Rural Development (FOFIFA) and the French Agricultural Research Center for International Development (CIRAD), which delivers varieties specifically tailored to the conditions of the Highlands (Breumier *et al.*, 2018; Raboin *et al.*, 2013). Since 2007, the program extended its operations from the Highlands to the mid altitude regions (900–1300 masl), operating mainly in the Mid-West area of the Vakinankaratra region, a less densely populated area where the culture of upland rice is rather recent and expanding.

In this region, most soils are desaturated ferralitic with important P deficiency and N depletion (Rabeharisoa *et al.*, 2012; Naudin *et al.*, 2019; Raminoarison *et al.*, 2020), and farmers' use of inputs, such as mineral fertilizers, is limited mainly due to affordability and accessibility (Minten *et al.*, 2007). Delivering varieties for marginal and low-input farms is challenging, especially when breeding is conducted on high-input research stations, because genotype performance often differs significantly between these contrasting conditions, particularly for traits with strong Genotype–Environment Interactions (GEI) such as grain yield (Atlin *et al.*, 2001). A decentralised plant breeding scheme, which includes testing and selecting materials from early generations in farmers' fields, could be a viable option to maximise genetic gain and deliver adapted genotypes (Bänziger and Cooper, 2001; Ceccarelli and Grando, 2007). However, logistical complexities, including transportation costs, limited seed quantity in early breeding stages, and the implementation of complex experimental designs, may restrain this possibility, especially for small breeding programs with limited resources (Desclaux *et al.*, 2012; Mangione *et al.*, 2006). Therefore, a practical alternative is to modify the research station conditions to mimic those found in real farming environments, for example, by reducing or eliminating mineral fertilizer use (Leiser *et al.*, 2014; Rattunde *et al.*, 2016).

Decentralised plant breeding schemes often align with the participatory plant breeding (PPB) principles, involving farmers throughout the breeding process, from ideotype conception to the evaluation of segregating and elite lines (Ceccarelli and Grando, 2007). Besides direct benefits like increased variety adoption and farmer empowerment (Sperling *et al.*, 1993; Ceccarelli, 2015), this approach fosters mutual understanding of breeding objectives and selection criteria, as farmers' priorities may differ from those of breeders (vom Brocke *et al.*, 2010). Yet, PPB principles can also be applied in non-decentralised contexts, including the evaluation of segregating materials on research stations (Rattunde *et al.*, 2016; Trouche *et al.*, 2012), and the FOFIFA-Cirad breeding program has embraced this approach.

While the primary focus of this program is improving yield stability and potential, there is also an urgent need to address nutritional quality. In Madagascar, heavy reliance on rice has led to severe micronutrient deficiencies, and the Central Highlands experience one of the world's highest rates of child stunting and underweight, at 52% (INSTAT, 2021). Among other causes, this has been particularly attributed to zinc (Zn) deficiency (Nikiema *et al.*, 2023), which is linked to several physiological disorders, such as growth retardation, cognitive impairment, and immune dysfunction (Wessels *et al.*, 2017; Majumder *et al.*, 2019).

Chronic malnutritions, including zinc deficiency, should be addressed through integrated strategies, primarily focusing on dietary diversification (van Ginkel and Cherfas, 2023). This approach is particularly effective in rural areas where increased production diversity can lead to improved diets (Bechoff *et al.*, 2023). Paradoxically, the Vakinankaratra region, despite being one of Madagascar's largest producers of fruits, vegetables, and milk, also suffers one of the highest rates of chronic malnutrition (Rakotomanana *et al.*, 2020). In such contexts, where cultural and economic factors make local populations highly vulnerable to nutritional risks, biofortification through plant breeding offers a complementary, cost-effective, and mid-term strategy against chronic malnutrition (Malézieux *et al.*, 2024; Wissuwa *et al.*, 2008).

Important genetic variability for grain zinc concentration has been found in rice, ranging from 8 ppm to 58 ppm, but the majority of globally cultivated varieties have concentrations of around 16 ppm in brown rice (Graham *et al.*, 1999). So, in countries with high rice consumption such as

Madagascar, delivering rice varieties with a grain zinc concentration of at least 28 ppm could fulfil up to 80% of the estimated average requirement for children between 4 and 6 years (Bouis and Saltzman, 2017). Rice zinc biofortification efforts have been conducted in other countries like India (Naik *et al.*, 2020) and Colombia (Baertschi *et al.*, 2021). In Madagascar, this strategy has been recently adopted for lowland rice (Rakotondramanana *et al.*, 2024) and upland rice, the latter by the FOFIFA-CIRAD PPB program.

To effectively incorporate this trait into a participatory breeding program, it is essential to employ tailored methodologies for collecting, analysing, and integrating varied information from different sources (Trouche *et al.*, 2012; Ceccarelli, 2015). Therefore, the current study seeks to establish an approach that integrates farmers' preferences for selecting new varieties, alongside the assessment of zinc concentration and the evaluation of agronomic performances across a range of soil fertility conditions. Our objectives were: (1) to dissect the GEI associated to the traits of interest, in order to determine the adequate environmental selection conditions, (2) to better understand and use farmers' priority criteria, and (3) to develop a Selection Index (SI) combining agronomic data, zinc values and farmers' varietal priority criteria. For this purpose, a set of advanced upland rice lines from the FOFIFA-CIRAD breeding program along with breeding lines from the International Center for Tropical Agriculture (CIAT, now called the 'Alliance Bioversity International CIAT') zinc biofortification program were tested and evaluated with farmers though a gradient of fertility conditions in the Mid-West area of Vakinankaratra, Madagascar.

Materials and methods

Plant material and experimental design

A total of 56 rice genotypes, mostly from the tropical japonica group, were evaluated in this study. Among those, 41 represent advanced F_6 and F_7 breeding lines from the local FOFIFA-CIRAD pedigree breeding program, also called SCRID program (*Systèmes de Culture et Riziculture Durable*). These lines had been selected during previous years on a research station under conventional conditions. There were also 11 biofortified (BF) high zinc lines from the CIAT-CIRAD upland recurrent selection program in Colombia. Two locally popular varieties (Nerica 4 and FOFIFA 182), as well as two worldwide known varieties (Azucena and IR64) were added as controls (Table S1). FOFIFA 182 was only tested during the second year.

These genotypes were cultivated over two consecutive seasons (2019–2020 and 2020–2021, hereafter referred to as year 1 and year 2) in different fields managed by FOFIFA at Ivory (19° 33'26.6 S, 46° 24'42.6 E; 900 masl), a village in the Mid-West area of Vakinankaratra, Madagascar. All trials were conducted during the main growing season (November to April). For each year, two independent fields with different fertility management strategies were used, resulting in a total of four environments (Table 1). The management options included either zero fertilizer, fertilization using cattle manure at 5t ha^{-1}, or a combined fertilization scheme, which consisted of 5t ha^{-1} cattle manure at sowing, 120 kg ha^{-1} mineral fertilizer (11N-22P-16K) at sowing and 80 kg ha^{-1} of urea in two applications, one at booting and one at flowering stage. All treatments, except the combined fertilization scheme, intend to mimic farmer field conditions, which mainly rely on organic matter input and rotation with legume crops (Razafimahatratra *et al.*, 2017).

All four trials followed an alpha-lattice design with three repetitions; each of the eight blocks held six test genotypes and Nerica 4 as a check. Plot dimensions varied across the environments (Table 1), with plant spacings being constant at 0.2 m between rows and hills. A total of six grains were sown per hill and no thinning was conducted. All agronomic management was done manually and followed common practices in the region; this included plowing for field preparation, chemical treatment for soil insects and weeding when needed. These trials represent, within the breeding pipeline, the first replicated field trials for evaluating quantitative and qualitative traits to be used as selection criteria.

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Table 1. Description of trial environments used for the evaluation of 56 upland rice genotypes in the mid-west region of Vakinankaratra during the 2019–2020 (year 1) and 2020–2021 (year 2) growing seasons

Year	Environment	Historical field management characterisation	Fertilization during trials	Elementary plot dimensions (m)
1	HFM	High Fertility and Mineral Fertilizer: Combined organic and mineral fertilizer in rotation with soy bean.	5t ha^{-1} cattle manure 120 kg ha^{-1} NPK 80 kg ha^{-1} Urea	2.4 × 1.4
	LFZ	Low Fertility and Zero Fertilizer: Manure application of 5T ha^{-1} in rotation with ground nut since 2012.	None	3.2 × 1.2
2	LNO	Low Nitrogen and Organic Fertilizer: No mineral N fertilizer but additional mineral P and K application during 2015–2018.	5t ha^{-1} cattle manure	2 × 1.2
	LFO	Low Fertility and Organic Fertilizer: Field in fallow for 5 years, then used as low fertility field for upland rice for two years in rotation with Bambara groundnut.	5t ha^{-1} cattle manure	2 × 1.4

Evaluation of selection criteria

The following morphologic traits were observed on five random hills per plot: plant height (PH), panicle length (PL), number of tillers (NT), and number of panicles (NP). We also determined days to 50% flowering (DTF) at the plot level. Total dry grain yield (GY) was measured per plot and extrapolated to kg per hectare. The percentage of spikelet fertility (FERT), which represents the ratio of filled grains over total grain number, and thousand-grain weight (TGW) were also calculated. Due to a marked abundance of the parasitic weed *Striga asiatica* at two locations (LNO and LFO), an incidence score was recorded using a visual scale ranging from 1 to 5 per plot. A score of 1 indicated no damaged plants, while a score of 5 represented more than 40% of rice plants being damaged.

A participatory evaluation of the 56 genotypes was conducted during maturity in the second year; evaluations on the first-year trials were cancelled due to Covid-19 restrictions. A total of 24 farmers (10 women and 14 men) participated, all of which are part of a group involved in the upland rice breeding program for more than five years. The participatory evaluation method described by vom Brocke *et al.* (2010) was adapted to the local context for our selection program. This method entails a group discussion in order to identify and define the relevant evaluation criteria for selecting a new variety, followed by the evaluation on each genotype by the members of the group. The exercise was conducted in five small groups of four to five farmers (two groups of women and three of men) and using a preference score on a 1 to 4 scale (4 = 'most appreciated', 3 = 'appreciated', 2 = 'acceptable', and 1 = 'rejected'). Each small group was accompanied by a researcher or a technician that encouraged discussions among farmers within the groups. The assessment was conducted within a single replication of the LNO trial, which was selected due to its alignment with farmers' conditions and consistent plant stand uniformity.

Prior to the exercise, participants were informed of the nutritional value of zinc, its potential impact on health for young generations and the new breeding objective to increase zinc concentration in rice grains. In order to have a first appraisal of farmers' considerations on biofortification, the evaluation included a preference voting exercises for two scenarios: a case of a hypothetical high zinc value on the breeding lines, and a case of not considering potential zinc values. For each scenario, each group indicated a consensual approval or refusal of whether the variety should be kept in the program for further testing.

The grains from five random hills in each plot were collected for the evaluation of zinc concentration. The paddy grains were hulled into brown rice using a manual Teflon dehuller and were then prepared using the analytical methods referenced in Wheal *et al.* (2011). Samples from

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the first-year field trials were analysed at Flinders University using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Second-year samples were analysed trough energy dispersive X-ray fluorescence spectrometry (X-supreme 8000, Oxford Instruments, Shanghai, CN) available at the CIAT-Harvest Plus Nutritional Laboratory.

For a better characterisation of the trial environments, total daily rainfall was measured with an automatic meteorological station (CIMEL, Paris, France). Soil samples were also taken at 6 random points per field, at depths between 0 and 20 cm and they were analysed at the Laboratoire des RadioIsotopes from the University of Antananarivo, Madagascar.

Estimation of heritability and BLUPs

All statistical analyses were performed using R through the RStudio platform (RStudio Team, 2023). In a first step, variance components and best linear unbiased predictors (BLUP) for quantitative agronomic traits were estimated per genotype according to the underlying alpha design for each environment using a mixed model:

$$y_{ijk} = \mu + r_j + b_k(rj) + \alpha_i + \varepsilon_{ijk}$$

Where y_{ijk} is the observed value of the ith genotype in the kth block of the jth replication; μ is the overall mean; r is the fixed replication effect; b is the random block effect nested within each replication; α_i is the random genotype effect; and ε_{ijk} is the random error. The Nerica 4 plots used as check in each block were only phenotyped for GY and DTF. Thus, the models for these two traits included the check value within the block as a fixed effect.

In order to extract overall BLUPs and to quantify GEI, a combined multi environment analysis was performed by using the previous model, but adding the e fixed effect for environments, nesting the replication effect within the environment and adding a random genotype × environment (α_i e_l) effect. The models were fitted using the *lme4* package (Bates *et al.*, 2015). Significance of the random effects was tested via the Satterthwaite's degrees of freedom method (Kuznetsova *et al.*, 2017). Based on these models, and in order to obtain a measure for precision, broad-sense heritability (H^2) on a genotype-difference basis was calculated for each trait in single environments and for the combined multi environment analysis, following Piepho and Möhring (2007) and Schmidt *et al.* (2019).

Genotype × environment effects

To further investigate GEI for GY and zinc concentration, correlation coefficients were calculated between single-environment BLUPs for the same genotypes. Additionally, the stability of all breeding lines was analysed using the Additive Main effects and Multiplicative Interaction (AMMI) model of the *metan* R package (Olivoto and Lúcio, 2020). This model was chosen because of its straightforward and easy to interpret graphical representation (Gauch *et al.*, 2008). In this case, the block, the repetition, the environment, the genotype, and the GEI are all taken as fixed effects.

Estimation of relative efficiency of selection

In an effort to identify which of the available selection environments might be more suitable for selecting genotypes that better adapt to farmers' conditions and preferences, the relative selection efficiency (RSE) was calculated between the four selection environments differing in fertility conditions and management. For that purpose, the same multi environment mixed model described above was used, but an unstructured variance-covariance matrix was imposed, where the diagonal of corresponds to the genetic variance at each environment, and the off-diagonal represents the genetic covariance between every pair of environments (van Eeuwijk *et al.*, 2021).

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This model was fitted using the *nlme* package in R (Pinheiro and Bates, 2022). Genetic variances for each environment, $\sigma_e$and $\sigma_{e'}$as well as covariances between pairs of environments ($\sigma_{ee'}$)were used to calculate the genetic correlations ($r_{ee'}$) between two environments as follows:

$$r_{ee'} = \sigma_{ee'} / \sqrt{\sigma_e * \sigma_{e'}}$$

For the calculation of the RSE, every environment was considered once as a possible 'selection' environment and the other three environments were taken separately as possible 'target' environments, as follows:

$$RSE = r_{ee'} \times H^2{}_e / H^2{}_{e'}$$

Where e is the 'selection' environment and e' is the 'target' environment.

Analysing farmers' preferences and variety acceptance

The preference scores obtained from farmer evaluations were first analysed using an ANOVA as recommended by vom Brocke (2010), in order to detect gender, group and genotype effects for trait appreciation. In addition, a logarithmic binomial model was constructed in order to determine which of the farmer's evaluation criteria could better predict the variety acceptance or rejection:

$$A_{ij} = t_{1\ ij} + t_{2\ ij} + t_{3\ ij} + t_{4\ ij} + \varepsilon_{ij}$$

Where A is the acceptance/rejection of the ith genotype by the jth group, t_1 to t_4 are the evaluated traits; and ε represents the error. This model was also fitted with the *lme4* package (Bates *et al.*, 2015). In order to characterise the relationship between measured agronomic observations and farmers' trait evaluations, a pairwise Pearson correlation test was carried out for each pair of traits. For this purpose, only the data obtained from the third replicate of the LNO trial were used, as the plots of this replication were the ones evaluated by farmers. This analysis was restricted to the SCRID lines, in order to avoid data structure biases, which were caused by marked phenotypic differences observed between the SCRID lines and the imported BF lines, as the latter had later maturity and lower yields. Pearson correlations were also computed between the measured traits at each environment, and this analysis was also conducted exclusively using SCRID lines due to the same reasons.

Selection index

Following previous experiences on using SIs considering farmers' preferences and breeders' observations (Trouche *et al.*, 2012; Annicchiarico *et al.*, 2019), a weighted SI for the breeding lines was elaborated. The index was based on the most relevant agronomic traits and farmer evaluation scores, using the formula:

$$I_i = W_1 V_{i1} + W_2 V_{i2} \ldots. + W_{tn} V_{in}$$

Where I is the Index value for the ith individual, W_1 is the weight assigned to the trait 1, and V_{i1} is the standardised phenotypic value of a given trait for the ith genotype. Standardisation was done via z-score normalisation.

Results

Agronomic traits

The crop conditions and the performance of measured agronomic traits varied notably among environments. The total rainfall was higher in year 2 than in year 1, and the soil analysis showed strong differences in phosphorus and organic matter among the different environments, but all

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soils were strongly acidic and had poor content of bases (Table S2). For GY results, means ranged from over 4000 kg ha^{-1} for the high fertility and mineral fertilizer (HFM) environment to less than 1000 kg ha^{-1} for the low fertility and organic fertilizer (LFO) site (Table 2). Broad-sense heritability (H^2) for yield under HFM conditions was the highest (0.70) compared to the other environments, with the lowest under LFO conditions (0.19). The HFM environment presented the highest mean values for PH and PL, as well as the lowest mean for days to flowering (*i.e.* shortest growing cycle of plants). Heritability values were also highest in the HFM trial for these three variables.

Grain zinc concentration also varied among environments, with an environmental mean range from 22.8 (LFO) to 28.7 ppm (HFM) in brown rice. Broad-sense heritability for this trait was generally high, and highest under LFO conditions (0.90). Striga infestation was only noted for two environments (LFO and LNO) and the high variability under natural infestation may account for the low heritabilities for this variable (Table 2).

The variance decomposition at the multi-environment trials shows that, for GY and spikelet fertility, the variance due to GEI was at least three times larger than the variance due to the genotypic effect, resulting in a relatively low overall H^2 of 0.31 and 0.40 (Table 3). For all other traits, expect the Striga Index, genetic variance components exceeded those for GEI, with corresponding H^2 values ranging between 0.72 and 0.93.

GEI analysis

For GY, the correlation of single-environment genotype BLUPs indicate moderate but significant correlations between all environments expect for HFM, which had no significant correlation with any other environment (Table 4). For grain zinc concentration, on the other hand, correlations are significant across all environments, with slightly higher correlation coefficients among the low fertility environments (LFO, LNO, and LFZ).

In the AMMI analysis, 65.8% and 54.0 % of the multiplicative interaction effect for GY and grain zinc concentration are captured by the PC1, the primary mode of interaction variation (Figure 1). Both bi-plots expose a similar pattern, where the environmental vector of the HFM is opposing the other three environments. Figure 1a shows that genotypes with the highest mean GY closely align with the HFM vector, suggesting their elevated responsiveness to this high yielding environment. On the other hand, the bi-plot for zinc concentration shows a rather scattered distribution of genotype points, with less marked specific responsiveness to the environments (Figure 1b). The genotypes with the highest mean for zinc concentration, such as S12 and Azucena, are relatively stable as they are centrally aligned in PC1. Moreover, some breeding lines do exhibit a large positive interaction with HFM, such as S39 and S38, although their mean zinc concentration are lower than the population mean.

Relative selection efficiency

The RSE for GY in the proposed scenarios ranged from −0.67 to 2.52, indicating strong implications of indirect selection depending on the choice of the selection environment (Table 5). An RSE value higher than 1 indicates that selection in the that environment is efficient for the target. So, selecting on HFM is only efficient if the target environment is LFZ, where heritability is relatively low. But it is inefficient when aiming for other environments such as LNO and LFO, as indicated by the low RSE values. The highest RSE for yield is seen when the selection environment is LNO and the target environment is LFO, due to both their high genetic correlation and the low H^2 at LFO. The year effect might be also very important in this case, as both of these treatments were conducted on the same year.

As heritabilities and genetic correlations for zinc concentration are relatively high in every environment (Table 2), RSE values stay below one in all cases. However, the environment that has

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Table 2. Environmental means and heritabilities (H^2) for eight agronomic traits observed in four rice breeding field trials during 2019/20 and 2020/21

Trait	Environment	Mean	Std. Dev.	H^2
Grain yield (kg ha^{-1})	LFZ	1700	611	0.48
	HFM	4247	1139	0.70
	LNO	2746	830	0.49
	LFO	668	327	0.19
Grain zinc concentration (ppm)	LFZ	27.1	3.3	0.80
	HFM	28.7	3.4	0.75
	LNO	23.7	3.2	0.83
	LFO	22.8	2.6	0.90
Plant height (cm)	LFZ	84.9	9.9	0.77
	HFM	102.1	9.5	0.89
	LNO	92.6	11.3	0.83
	LFO	63.2	9.8	0.58
Days to 50% Flowering	LFZ	92.7	4.9	0.89
	HFM	86.8	6.7	0.95
	LNO	87.7	7.8	0.83
	LFO	96.1	5.6	0.69
Panicle Length (cm)	LFZ	18.3	1.8	0.68
	HFM	21.0	1.6	0.74
	LNO	18.8	1.6	0.67
	LFO	15.2	1.9	0.48
Thousand Grain Weight (g)	LFZ	25.0	2.9	0.97
	HFM	25.1	3.0	0.95
	LNO	25.8	2.7	0.94
	LFO	23.7	3.0	0.87
Panicles (Nbr m^{-2})	LFZ	33.2	7.19	0.23
	HFM	59.5	12.7	0.41
	LNO	41.6	9.2	0.62
	LFO	25.0	6.3	0.54
Spikelet fertility (%)	LFZ	90.9	5.6	0.74
	HFM	80.8	11.2	0.77
	LNO	84.6	7.8	0.64
	LFO	90.0	7.6	0.48
Striga Index	LFZ	NA	NA	NA
	HFM	NA	NA	NA
	LNO	2.70	1.37	0.00
	LFO	3.25	1.04	0.26

NA: Not available.

the highest mean of RSE across the spectrum of environments is LFO, as the RSE values are 1.00, 0.76, and 0.91 for LFZ, HFM, and LNO, respectively.

Farmer trait preferences and appreciation of varieties

In group discussions, farmers mentioned the various different factors that they take into account for variety appreciation, but ultimately identified four main evaluation criteria for breeding lines: (i) productivity, (ii) earliness, (iii) plant height and (iv) grain appreciation. Even though striga resistance is also one of farmers' most mentioned criteria, it was not included in their evaluations, as its occurrence in the trials was too heterogeneous to be used as a discriminative variable.

The discussions held during the evaluation indicated farmers' priorities and the complex nature of the four selected criteria. For the participating farmers, productivity is linked to high tillering capacity and long panicles with short ramifications to enable a high grain density. They also pay special attention to the quantity of non-filled grains. Panicles that are bending down at maturity are preferred as they indicate good panicle and grain filling. Earliness is a criterion linked to local adaptation, *ie*. varieties adapted to the current rainfall pattern. Very short cycle varieties are also

Table 3. Percentage of the total variance (%VAR) and broad sense heritability (H^2) for eight agronomic traits observed in all the field trials through the two growing seasons (2019/20 and 2021/22)

Trait	Variance component	% VAR	H^2	Trait	Variance component	% VAR	H^2
Grain yield	Genotype	5.8	0.31	Grain zinc concentration	Genotype	37.2**	0.83
	G × E	28.6**			G × E	14.9**	
	Repetition	2.6**			Repetition	4.5**	
	Residual	62.8			Residual	43.4	
Plant height	Genotype	37.4**	0.83	1000-grain weight	Genotype	66.9**	0.93
	G × E	14.6**			G × E	14.0**	
	Repetition	1.8*			Repetition	1.1*	
	Residual	46.2			Residual	18.0	
Days to Flowering	Genotype	57.4**	0.92	Percentage of spikelet fertility	Genotype	9.0*	0.40
	G × E	8.1**			G × E	31.0**	
	Repetition	0.5			Repetition	0.5	
	Residual	34.0			Residual	59.4	
Panicle Length	Genotype	22.6**	0.72	Striga Index	Genotype	2.0	0.14
	G × E	13.4**			G × E	0.0	
	Repetition	1.1			Repetition	26.2**	
	Residual	62.9			Residual	71.8	
Number of Panicles per m^2	Genotype	16.5**	0.67				
	G × E	7.1					
	Repetition	5.1**					
	Residual	71.3					

*Significant at $p > 0.05$.
**Significant at $p > 0.01$.

Table 4. Correlation coefficients of single-environment BLUP values for grain yield and grain zinc concentration of 56 rice breeding lines across four trial environments

	Grain Yield			Grain Zinc concentration		
Environment	LFO	LNO	LFZ	LFO	LNO	LFZ
LNO	0.42**	–	–	0.65**	–	–
LFZ	0.42**	0.31*	–	0.69**	0.78**	–
HFM	0.12	0.19	0.19	0.43**	0.41**	0.50**

*Significant at $p < 0.05$.
**Significant at $p < 0.01$.

appreciated as they reduce the lean period. Plants whose stems and/or leaves stay green during (early) maturity are in general preferred. These farmers associate taller plants to higher productivity, but the ideal PH is a compromise between shorter plants to prevent plant lodging and longer stems to ease labour during harvest and threshing; in general, a PH of 90–100 cm is preferred. Also, grains that are semi-long, of mainly white pericarp colour with pale or golden hulls, are preferred.

ANOVA results for farmers' scores of the four selection criteria show a significant and strong genotype effect (Table S3). The analysis also revealed no gender effect and a small effect of the group for earliness, grain appreciation, and PH. The percentages of accepted breeding lines per group varied between 53% and 87% (Table 6). This percentage increased on average by 11% under the assumption that the varieties had a high zinc concentration, however this rate varied markedly among the different groups from 1.8%, all the way to 32.1% (Table 6). Finally, the results from the logistic regression (Table S4) indicate that the grain appreciation ($p < 0.001$) was by far the most important criteria in defining the acceptance or rejection of any given variety. Earliness ($p = 0.002$)

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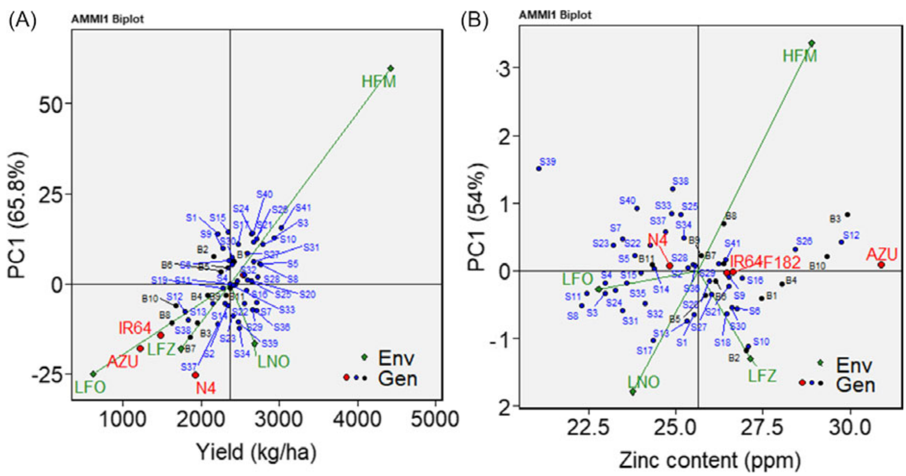

Figure 1. AMMI-1 Bi-Plots for grain yield (kg ha^{-1}) and grain zinc concentration (ppm) across the four trial environments in a panel of rice breeding lines.

and productivity ($p = 0.011$) have also a significant influence, whereas PH ($p = 0.279$) was not as important.

Correlations among measured agronomic traits and farmers' criteria

Correlation analysis between agronomic traits indicate that no single correlation between two traits is significant at all the four environments (Figure 2). Significant positive correlations between PH and GY, as well as between PH and PL, were however detected in all three environments without mineral fertilizer use. As expected, in all four environments, GY and grain zinc concentration were negatively correlated, although this was only significant for HFM and LNO. Additionally, at LFO, grain zinc concentration showed a significant negative correlation with PL. In the HFM environment, there was a significant positive correlation between grain zinc and days to flowering.

Three measured agronomic traits stand out as having the highest correlations with most of farmer evaluations: GY, PH, and Striga Index (Figure 3). While GY and PH are positively correlated with farmers' evaluation criteria, Striga Index has a negative relationship. PL is moderately correlated with PH appreciation and grain appreciation, whereas the zinc concentration is negatively correlated with PH appreciation and grain appreciation. Another strong (and expected) correlation between farmers' criteria and measured traits was found between days to flowering and the appreciation of earliness by farmers, with an R^2 of –0.47.

Selection index

The traits to build a SI were selected by the breeder in charge of the program and consisted in three most discriminating farmers' evaluation criteria (earliness, productivity and grain appreciation) as well as two measured agronomic traits (yield and zinc concentration). For the measured traits, the BLUP values from the multi-environmental model were used, but the data from the HFM trial environment were excluded due to the low correlations between environments previously mentioned. Different weight assignments were tested, but assigning the same weight (1) to the five variables gave the best adjustment between actual farmers/breeder's visual selection and the selection based on the index (data not shown). A visual representation of this index is shown in Figure 4. By setting a selection pressure of 70% on this index, a threshold value is defined in order

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Table 5. Relative selection efficiency for yield and grain zinc concentration between every pair of environments for yield and grain zinc concentration within a panel of rice breeding lines

		Target environment							
		Grain yield				Grain zinc concentration			
		LFZ	HFM	LNO	LFO	LFZ	HFM	LNO	LFO
Selection Environment	LFZ	–	0.63	0.42	0.56	–	0.62	0.90	0.79
	HFM	1.34	–	0.05	−0.67	0.55	–	0.40	0.53
	LNO	0.43	0.03	–	2.52	0.97	0.49	–	0.78
	LFO	0.09	−0.05	0.38	–	1.00	0.76	0.91	–

Table 6. Percentage of accepted genotypes ($n = 56$) of rice by farmer group, without (%AG) and with (%AGZ) the assumption that they had high zinc content, divided by farmers group and gender

Farmer-group	Gender	% AG	% AGZ	% Increase in acceptance
I	Men	87.5	89.3	1.8
II	Men	53.5	58.9	5.3
III	Men	58.9	91.1	32.1
IV	Women	58.9	73.2	14.3
V	Women	64.3	67.9	3.6
Mean		64.6	76.1	11.4

to select the best overall performing lines, for subsequent testing according to the breeding pipeline. Nonetheless, this exercise was conducted after the actual selection process, as it was as a joint reflection exercise. This allowed the opportunity to compare the results of using this index with those obtained by the breeders using their usual methodology.

Discussion

This paper addresses the challenges faced by breeding programs that operate in marginal environments characterised by complex GEI. Breeders in contexts like these often face the longstanding and well documented dilemma of making a compromise between selecting under high input, high yielding, and high heritability environments, or opting for low yielding environments that better resemble farmers' conditions, but which could be more heterogenous and thus offer less heritability (Ceccarelli, 1989; Atlin *et al.*, 2001; Dawson *et al.*, 2008). In order to address this dilemma in the framework of a small national breeding program, the present study exploited the use of various on-station selection environments designed to represent a spectrum of fertility conditions.

Our results confirm important cross-over interactions for GY and low genetic correlations between the high fertility environment (HFM) and the moderate to low fertility environments, as has been seen before in various other contexts (Stagnari *et al.*, 2013; Le Campion *et al.*, 2014; Petitti *et al.*, 2022). The low RSE values observed in most cases when selecting for yield under high fertility conditions affirm the choice of a breeding strategy that prioritises environments that closely resemble farmers' fields. The only exception for this trend was the case of the relatively high RSE values between the HFM and the LFZ environments, which result from a moderate correlation between environments (both were conducted on the same year) and a very low heritability in the second site. For future trials, special attention must be given to phosphorus; although our treatments primarily modulated nitrogen levels, the site with the lowest yields and heritabilities (LFO) also had extremely low phosphorus levels, which manure inputs did not sufficiently compensate for.

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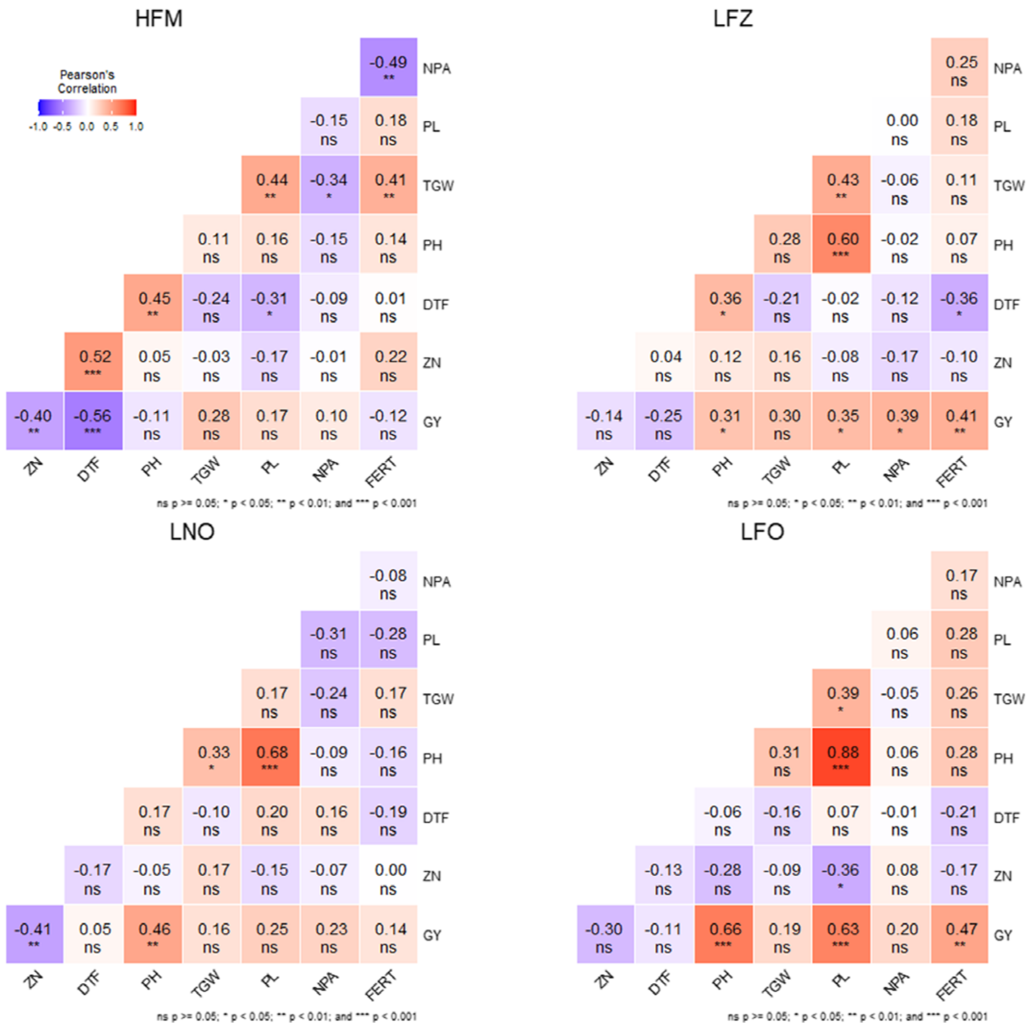

Figure 2. Environment-wise phenotypic correlations for BLUPs of the main agronomic traits measured on 41 SCRID rice breeding lines. GY = Grain Yield, ZN = Grain Zinc Concentration, DTF = Days to Flowering, PH = Plant Height, TGW = Thousand Grains Weight, PL = Panicle Length, NPA = Number of Panicles per m^2, FERT = Percentage of Spikelet Fertility. The symbols ***, **, and * indicate significance at $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively.

For the case of zinc, preceding research in rice biofortification has identified different situations of GEI for grain zinc concentration, from very small to large interactions (Inabangan-Asilo *et al.*, 2019; Naik *et al.*, 2020; Rakotondramanana *et al.*, 2024; Wissuwa *et al.*, 2008). Our results show that heritability was relatively high for most environments, that GEI was modest and no *cross-over* effects were found. This is in line with observations of Wissuwa *et al.* (2008), who observed that genotype rankings were consistent across a range of soils with varying fertility conditions. Overall, based on the GEI and RSE results, selection for grain zinc concentration in any single environment is feasible. When adaptation to farmers' fields with low fertility is a priority, a simultaneous selection for zinc concentration and GY is more effective in moderate fertility environments like LFZ and LNO, rather than in the extremely high or low yielding environments, such as HFM or LFO, respectively.

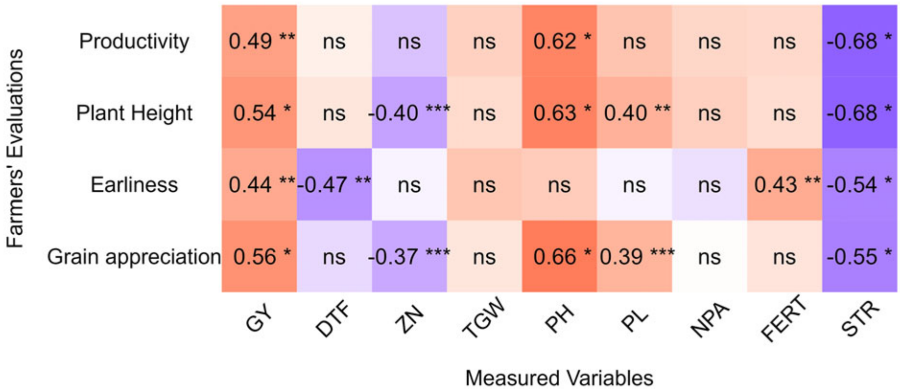

Figure 3. Correlation between farmer's criteria and agronomic traits measured on 41 SCRID breeding lines. GY = Grain Yield, ZN = Grain Zinc Concentration. DTF = Days to Flowering, PH = Plant Height, TGW = Thousand Grains Weight, PL = Panicle Length, NPA = Number of Panicles per m^2, FERT = Percentage of Spikelet Fertility, STR = Striga Index. The symbols ***, **, and * indicate significance at $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively.

Regarding farmers' evaluations, they effectively distinguished between genotypes, regardless of participants' gender or group composition. The most influential factor in their decision-making process was grain appreciation, followed by earliness and productivity. Autfray *et al.* (2022) analysed farmer preferences of upland rice in the same region, and identified GY, and striga tolerance, as the most important traits for farmers. The importance given to this last trait in a given evaluation can be highly dependent on the varietal diversity presented to the farmers and the spatial distribution of striga infection, especially under natural infection as occurred in the LNO and LFO field trials. This makes the evaluation and selection for striga tolerance specifically difficult as also concluded by Haussmann *et al.* (2000).

On the other hand, grain appreciation is a trait that frequently emerges in PPB evaluations, but it is often overlooked by breeders in favour of yield (Asfaw *et al.*, 2012). This trait is also challenging to use as a selection variable because it is hard to quantify and heavily relies on individual assessments. Small-scale rice farmers prioritise quality due to their use of harvested rice for personal consumption, or the potential for selling high-quality rice at premium prices (Joshi *et al.*, 2007; Shiratori *et al.*, 2023). Based on these two scenarios, Autfray *et al.*, (2022) identified two quality-defining trends for farmers in our target area: when selling, grain weight and size can be a discriminating factor because of more favourable yields after decortication/dehulling, whereas for personal consumption, a 'sweet' taste is a crucial quality criterion (but hard to determine on field). So, in our evaluation, farmers primarily appreciated grain based on its large size, heavy weight, white pericarp, and golden hull colour. Among these traits, there was a strong negative selection against pericarp colour, with red rice being largely discarded. However, while grain appreciation was particularly significant in this context, it's important to note that its relevance (and that of other traits) may shift depending on the diversity of lines presented to the farmers. For instance, if grain types were more homogeneous and yield differences more pronounced, the latter might become the more relevant trait.

Farmers also often tend to give particular importance to earliness, a trend observed in previous PPB experiences in different countries and crops. However, the definition and perception of this trait can be influenced by various factors during evaluation, such as the timing within the season, specific environmental conditions like drought, or by individual plant type preferences (vom Brocke *et al.*, 2010; Trouche *et al.*, 2012; Petitti *et al.*, 2022). Our results confirm other studies on participatory variety evaluation, showing that farmers' evaluation of traits does not align precisely with the conventional definition of a singular specific agronomic trait such as yield or earliness.

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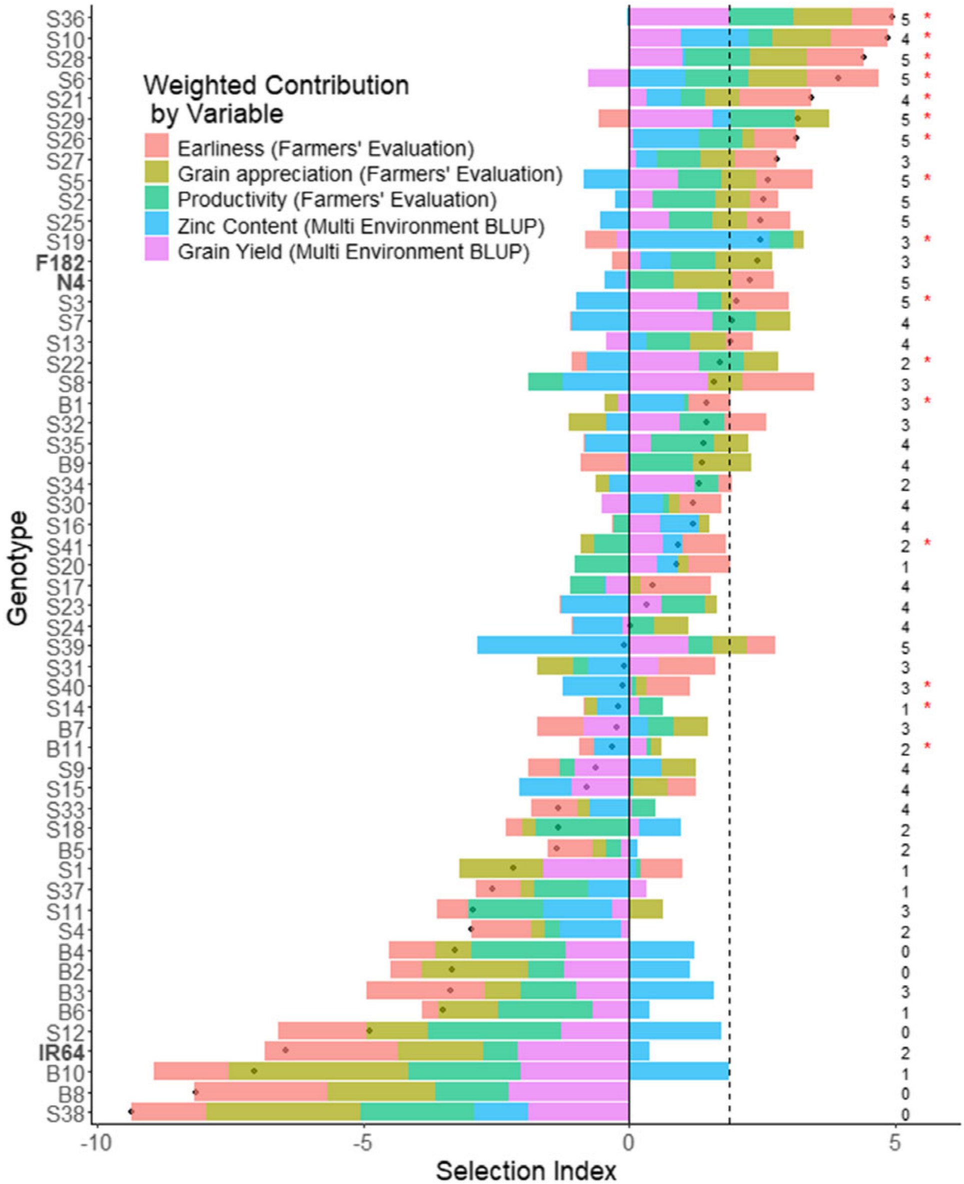

Figure 4. Visual representation of the Selection Index used to select among rice breeding lines, through a combination of both measured traits and farmer evaluation criteria. The black dots mark the Index value for each genotype and the coloured bars indicate the contribution of each trait to this index value. The dashed line represents the top 30% threshold. The numbers on the right represent the number of farmer groups who accepted the breeding line as a cultivable variety. The red stars mark the lines selected by the breeder.

Instead, farmers tend to evaluate multiple traits simultaneously depending on agro-ecological context and household needs. For instance, when they assess an early genotype, they not only consider the number of days to maturity but also place significance on the yield and the quality of grains achieved within that time frame (vom Brocke *et al.*, 2010; Weltzien and Christinck, 2009). Consequently, there are limited correlations between farmer evaluations and objectively measured traits, as demonstrated by the weak -but significant- correlation between earliness (as assessed by

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farmers) and days to flowering in our dataset (Figure 3). This explains why PPB programs may not produce varieties with exceptionally extreme phenotypes, but they often achieve a favourable compromise between yield and earliness (Witcombe *et al.*, 2006; Trouche *et al.*, 2012).

Additionally, a limitation of our evaluations is the fact that farmers only evaluated one environment and one repetition per genotype, confounding thus genotype, repetition and environment effects. Nonetheless, when the sample size is large (56 lines in this case) it is rather complicated and time-consuming to evaluate several repetitions with farmers. This raises the question of the specific trial conditions and whether the results would have been the same if evaluations were conducted directly at actual farmers' fields instead of a low fertility research station. This not only prompts the question of specific trial conditions but also suggests potential solutions. For instance, a comprehensive understanding of farmer field conditions and meticulous selection of the most representative repetition could enhance result reliability. Similarly, increasing farmers' awareness of objectives, coupled with detailed explanations of field conditions, as we have done, may contribute to improved evaluations.

Our study also explored the possibility of incorporating biofortification as a desirable trait and its potential influence on farmers' variety preferences. This is especially important, as biofortification may involve significant trade-offs. Our observations reveal that grain zinc concentration was negatively correlated with earliness and productivity, as reported in previous studies (Gao *et al.*, 2012; Inabangan-Asilo *et al.*, 2019). These negative relationships between traits highlight the importance of developing suitable methods to minimise compromises, using a well-designed SI and potentially selecting outliers from the correlation (i.e., early, high yielding, and high zinc genotypes).

In our study, we asked farmers whether they would select or not a given variety if it had a higher nutrient content, and there was a greater willingness to accept certain varieties if they were to be BF (Table 6). However, recognising the methodological limitation in our approach, additional research is required to comprehend the compromises that farmers are willing to make, considering yield, earliness, or other criteria, in their pursuit of obtaining and producing zinc BF varieties. It is crucial to consider gender as a significant variable in this process, as previous experiences in biofortification, such as those at CIAT in Colombia, have shown that women tend to prioritise high nutritional value over high yield, while men often do the opposite (Arora and García, 2009). In their program, consumer sensory studies were done in order to demonstrate that BF rice could match the sensory qualities of the varieties already consumed locally (Woods *et al.*, 2020); eventually, this should also eventually be tested in our context. Furthermore, research has demonstrated that involving farmers in the process is crucial for increasing the adoption of BF crop varieties (Samuel *et al.*, 2024). Altogether, this novel aspect warrants further investigation to better understand the implications and impact of biofortification in PPB programs, as well as for the diet and health of farmers and local populations (Bechoff *et al.*, 2023).

Based on our findings, we propose using a SI as a simple and transparent way to combine farmers' evaluations with conventional agronomic traits and grain nutritional quality. This basic SI can easily be adapted in function of the trial conditions, the breeding panel and the traits involved in the selection process. This makes it a flexible and useful tool for selection decision, especially in early stages when the number of lines tested is large and preliminary screening must be done (Sharma and Duveiller, 2006). When comparing the varieties selected by the index to those previously selected by the breeders, we observe that the top 7 varieties identified by the index were also chosen by the breeder. However, beyond this, the breeder's selection does not align closely with the index, which can be attributed to several factors. Most importantly, conventional selection may be influenced by extreme values (in terms of yield, zinc content, or other traits), whereas the index suggests genotypes with a more balanced profile.

Of course, the decisions regarding the construction of the index might also heavily impact its alignment with the breeder's usual selection. Here, we assigned equal weights to all variables for simplicity, but different weights could be assigned according to farmers preferences or economic criteria, or to properly address correlations between traits (Ceron-Rojas *et al.*, 2015). Issues might occur regarding the environmental sensitivity of the index, as the input data should be carefully chosen to represent the target environment. In our case, we used farmers' evaluation from only one repetition at the LNO site and multi-environment BLUPS for GY and zinc, excluding the HFM. We sustain that integrating these two data types allows us to give more weight to environments that closely resemble farmers' fields, such as the one used for farmers' evaluations. Previous studies on PPB have shown that farmers' evaluations show relatively low susceptibility to GEI, as they are somehow capable of seeing the potential and stability of the genotype beyond the given evaluation environment (Annicchiarico *et al.*, 2019).

Conclusions

This research addresses the interactions between genotypes, environments, and human preferences in the context of a participatory breeding program focusing on zinc biofortification. By examining the outcomes of selecting across a range of environments, we effectively demonstrated that neither the highest (HFM) nor the lowest (LFO) yielding environments were suitable for selecting biofortified upland rice lines that adapt to the conditions of farmers in the Highlands of Madagascar. This was particularly evident in the assessment of yield, where significant crossover interactions were observed between the high input environment and all the low-input fields. Environments with moderate yields, where mineral fertilizer was not used in order to mimic farmers' conditions, revealed the best results in terms of RSE. This relation was not as important when evaluating grain zinc content, a trait characterised by higher heritability and stability.

By incorporating participatory evaluations, we integrated farmers' perspectives, enabling the development of varieties that perform well and align with local contexts and consumer preferences. Including a cryptic trait like nutritional value in a participatory breeding program presents challenges, such as the potential impact on genetic gain for productivity. To address this, we devised a SI that integrates biofortification, agronomic performance, and farmer preferences, allowing us to choose from 56 breeding lines. To our knowledge, this is the first published work to apply such a comprehensive SI.

By carefully selecting breeding environments, incorporating farmer preferences, and addressing nutrient deficiencies, small-scale breeding programs in marginal conditions can significantly contribute to the overall well-being of local communities and foster a more resilient agricultural and food sector.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0014479724000218

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Competing interests. The authors declare none.

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