

1 Genetic Gains in IRRI's Rice Salinity Breeding and Elite Panel Development as a Future 2 Breeding Resource

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27

28 **Abstract**

29 Genetic gain is a crucial parameter to check the breeding program's success and help optimize
30 future breeding strategies for enhanced genetic gains. In this work, IRRI's historical data from
31 the Philippines and Bangladesh of the salinity breeding program was used to estimate the genetic
32 gains and identify the best lines based on higher breeding values for yield as a future genetic
33 resource. Two-stage mixed-model approach accounting for experimental design factors and
34 pedigrees was adopted to obtain the breeding values for yield and estimate genetic trends under
35 the salinity conditions. A positive genetic trend of 0.1% per annum with a yield advantage of
36 1.52 kg/ha for the Philippines and 0.31% per annum with a yield advantage of 14.02 kg/ha for
37 Bangladesh datasets was observed. For the released varieties, genetic gain was 0.12% per annum
38 with a yield advantage of 2.2 kg/ha/year and 0.14% per annum with a yield advantage of 5.9
39 kg/ha/year, respectively. Further, based on higher breeding values for grain yield, a core set of
40 the top 145 genotypes with higher breeding values of >2400 kg/ha in the Philippines and >3500
41 kg/ha in Bangladesh with a selection accuracy >0.4 were selected for formulating the elite
42 breeding panel as a future breeding resource. Conclusively, higher genetic gains are pivotal in
43 IRRI's rice salinity breeding program, which requires a holistic breeding approach with a major
44 paradigm shift in breeding strategies to enhance genetic gains.

45 **Keywords:** Rice, Historical Data, Pedigrees, Salinity, Genetic trends

46 **Key Message**

47 Estimating genetic gains and formulating a future salinity elite breeding panel for rice pave the
48 way for developing better high-yielding salinity tolerant lines with enhanced genetic gains.

49 **Introduction**

50 Rice (*Oryza Sativa L.*) is a major staple food crop, particularly in Asia, Latin America, and
51 Africa. Rice is the most sensitive to soil salinity (EC above four dS/m). Millions of hectares of
52 land in South Asia, Southeast Asia, and Africa are adopted for rice cultivation but have lower
53 yields due to salinity stress effects (Smajgl et al. 2015). The future rice food security heavily

54 depends on the rapid development of high-yielding salinity tolerant lines with much better
55 adaptation to the changing climatic scenarios.

56 The salinity breeding program at IRRI has been at the forefront of developing salt-tolerant rice
57 varieties utilizing various donor lines and landraces following conventional breeding approaches
58 (Negrao et al. 2011). In the last 2-3 decades, immense efforts have been made at IRRI to develop
59 high-yielding salt-tolerance rice varieties through conventional and molecular breeding
60 approaches. The salinity breeding program at IRRI was further boosted by the STRASA (Stress
61 Tolerant Rice for Africa and South Asia) Project launched in the year 2005 and continued up to
62 2019 (<https://strasa.irri.org/variety-releases/salinity-iron-toxicity>) and Green Super Rice (GSR)
63 projects (2006-2016). The progression of these projects led to the identification of new donor
64 lines for vegetative and reproductive stage tolerance and the dissemination of more than 50
65 varieties for cultivation in saline coastal, saline, and irrigated areas/ecosystems
66 (<https://strasa.irri.org/variety-releases/salinity-iron-toxicity>, Ali et al. 2012, Singh et al. 2021).
67 The varieties developed through these projects offer great potential for cultivating them in saline
68 environments to increase rice production.

69 In the future, rice production will be immensely limited by extreme environmental conditions of
70 salinity which would exacerbate due to climate change (Liu et al. 2020). For example, in the
71 coastal regions of South and Southeast Asia, a source of 65% of global rice production, an
72 increase in flood and salt intrusion has been found due to direct consequences of climate change
73 (Radanielson et al. 2018). In 2050, the human population will reach 10 billion, and the demand
74 for rice production will increase by 87% (Solis et al. 2020, Rawat et al. 2022). Due to the limited
75 resources and less availability of land in the future, meeting the future rice demands will be a
76 daunting challenge. Moreover, future rice production will only be met with heavy reliance on
77 irrigation water (Liu et al. 2020). However, dependency on irrigation water for rice production
78 comes with an additional cost of land salinization, and the level of dissolved salts in irrigation
79 water has significantly increased in the past 20 years (Liu et al. 2020). Thus, global rice food
80 security mainly depends on plant breeders to develop high-yielding, salinity-tolerant lines with
81 broader adaptation to the above climatic changes. To develop better salinity-tolerant lines with
82 wider adoption and achieve the required food demands in challenging conditions, it is critical to
83 check the progress of the existing salinity breeding program and assess where we stand and how

84 we can move forward for better improvement and enhanced genetic gains. Genetic gain is an
85 important parameter to check the progress of the breeding program and measure its efficiency.
86 The breeding program's achieved rate of genetic gain will immensely help guide future breeding
87 strategies and help allocate resources and rapid development of varieties for enhanced genetic
88 gains. Genetic gains under salinity environments at the global level in rice have never been
89 estimated.

90 Thus, this study was undertaken to accomplish two primary objectives: (i) estimating the genetic
91 trends in the IRRI's salinity breeding program using the data from IRRI, Philippines, and
92 Bangladesh, and (ii) identifying top-performing genotypes based on high grain yield breeding
93 values as future breeding resources.

94 **Materials and methods**

95 **Breeding materials and experimental details**

96 For this work, the historical datasets from salinity breeding trials conducted at various locations
97 in Philippines from 2008 to 2019 (12 years) and Bangladesh from 2005- 2014 (10 years) were
98 used. The major traits of focus were grain yield (kg/ha) and days to flowering. The Bangladesh
99 trials were undertaken in the districts Satkhira (22.7185° N, 89.0705° E), Ghazipur (25.5878° N,
100 83.5783° E), and Rajshahi (24.3745° N, 88.6042° E). The trials were organized twice a year, in
101 two season's dry and wet seasons in the Philippines and Aman and Boro in Bangladesh. The
102 genotypes were staggered based on their maturity groups; early, medium, and late to synchronize
103 appropriate stress imposition. The genotypes were screened for tolerance to salinity stress across
104 trials starting tillering onwards or at the reproductive stage. The genotypes were planted in
105 customized saline micro plots for imposing salinity stress, and the standard protocol was used to
106 screen for the salinity stress. The experimental designs in the trials conducted in the Philippines
107 varied across years from randomized complete block design (RCBD), row-column design,
108 augmented RCBD, and alpha-lattice; however, all the trials conducted in Bangladesh were
109 organized in RCBD.

110 **Pre-processing and Quality Check of the Data**

111 The breeding values were estimated yearly, taking season and location combinations as a single
112 trial or environment. The historical datasets retrieved were subjected to pre-processing and

113 quality checks to ensure high-quality trials and phenotypes are retained for the downstream
114 analysis and estimating the breeding values and genetic gains. The data pre-processing was done
115 per the procedure detailed in Khanna et al. 2022a and Hussain et al. 2022. Trials with unexpected
116 phenotypic values, high missing data points (>20%), missing replications, and/or design errors
117 were filtered. After filtering, the trials were subjected to quality checks by removing the extreme
118 data points and outliers using the Bonferroni-Holm test for studentized residuals (Bernal-
119 Vasquez et al. 2016; Philipp et al. 2019). After pre-processing and quality check, the dataset
120 consisted of 86 trials with 16,251 phenotypic data points with 4993 unique genotypes from IRRI
121 HQ Philippine data sets. For Bangladesh, 110 trials possessing 3097 data points with 600 unique
122 genotypes were retained. The details of the trials conducted across the two countries are outlined
123 in Supplementary Table 1.

124 **Retrieval of pedigrees and crossing strategies**

125 The pedigree data consisting of the parent's and grandparent's information on the 4,993
126 genotypes was utilized for substituting the pedigree-based-relationship matrix in the Philippines
127 dataset only. Pedigree information was not available for the data sets from Bangladesh. The
128 information of grandparents up to ten generations, along with the crossing strategy employed for
129 each genotype, was retrieved from the state of art repository, B4R (Breeding 4 Results (B4R),
130 2021, <https://b4r.irri.org>) and IRRI genealogy management system (McLaren et al. 2005; Collard
131 et al. 2019) with their customized R scripts. The genotypes were bred across the years employing
132 various breeding strategies, single, double, three-way, complex crosses, and backcrosses based
133 on their breeding objectives. The pedigree information of the genotypes for Bangladesh was
134 unavailable and hence was not accounted for in estimating the breeding values.

135 **Statistical modeling**

136 Due to different experimental designs across the trials and to account for the specific
137 experimental design factors, the two-stage approach of mixed model analysis (Piepho et al. 2008;
138 Piepho et al. 2012; Smith and Cullis 2018) was used. The two-stage approach also reduces the
139 time and computational burden of analyzing huge datasets (Smith et al. 2005). In the first stage,
140 adjusted means or best linear unbiased estimates (BLUEs) per year for each genotype were
141 extracted for grain yield. The mixed model consists of genotypes as fixed effects with
142 replications and seasons as random effects. Days to flowering (DTF) was used as a covariate in

143 the model to reduce error owing due to the difference in the synchronization of flowering and to
144 ensure the selections for tolerant genotypes would be across different maturity groups. The
145 strategy of covariance adjustment of DTF would significantly reduce variance due to
146 differentiation in flowering time among the genotypes in the analysis (Moreno-Amores et al.
147 2020; Juma et al. 2021; Khanna et al. 2021a, b). The model used in the first stage of analysis is
148 given below:

149
$$y_{ijkl} = \mu + g_i + r_j + s_k + \varepsilon_{ijkl} \quad (1)$$

150 where, y_{ijkl} represents BLUEs or phenotypic observation for the traits, this is with respect to
151 each datapoint or genotype classified as the i th observation in j th replication, and k th season, μ is
152 the overall mean, g_i is the fixed effect of i th genotype, r_j is the random effect of j th replication in
153 each trial, s_k is the random effect for season and ε_{ijkl} is the residual error. The random effects
154 were independently and identically distributed (IID).

155 In the second stage, the BLUEs estimated from the first stage were weighted and used as a
156 response variable (Damesa et al. 2017, Khanna et al. 2022a). The weights were estimated by
157 calculating the inverse of the squared standard errors (Mohring and Piepho 2009), which
158 minimized the heterogeneous error variance. In this stage, a relationship matrix based on the
159 pedigrees was fitted to account for the genetic covariances among the genotypes for reliable
160 estimates of breeding values. The same model was used for the Bangladesh data set without
161 fitting the pedigree matrix to extract the BLUPs. The model fitted in the second stage is as
162 follows:

$$y_{ij} = \mu + g_i + y_{ej} + \varepsilon_{ij} \quad (2)$$

163 where y_{ij} is the BLUE values weighted by the standard errors for i th observation in j th year, μ is
164 the overall mean, g_i is the breeding value of i th genotype with $g_i \sim N(0, A\sigma_g^2)$ where σ_g^2 is the
165 genetic variance and A is the additive genetic pedigree relationship matrix, y_{ej} is the random
166 effect of year, and ε_{ij} is the residual error, with $\varepsilon_{ij} \sim N(0, R\sigma_\varepsilon^2)$, where R is the identity error
167 covariance matrix and σ_ε^2 is the error variance. The reliability values (Isik et al. 2017) of the
168 breeding values with respect to each genotype were calculated using the equation mentioned
169 below:

$$r = 1 - \frac{PEV}{\sigma_g^2} \quad (3)$$

170

171 Heritability for the yield was estimated using the method suggested by Cullis et al. 2006 and
172 Piepho and Mohring 2007 using model 1 with genotypes as a random effect. This approach is
173 useful when the data is highly unbalanced, with uncommon genotypes screened across the years
174 and seasons. The following equation was used to calculate the heritability for trials per year:

175

$$H^2 = 1 - \frac{\bar{V}_{BLUP}}{2\sigma_g^2} \quad (4)$$

176

177 All the analysis was done using the ASReml-R package (Butler et al. 2017) in the R software (R
178 Core Team, 2020). The pedigree-based relationship matrix (A-matrix) was constructed using the
179 R package AGHMatrix (Amadeu et al. 2016).

180 **Estimation of the genetic trends**

181 For the IRRI-HQ data, the genetic gains were estimated by regressing each genotype's breeding
182 values over the year of origin or the year when the cross was attempted for each genotype. The
183 year of origin for each genotype record was extracted using the customized R scripts from the
184 genealogy management system IRRI (McLaren et al. 2005; Collard et al. 2019). However, in the
185 case of Bangladesh, the genetic trends were estimated by regressing the BLUPs over the year of
186 testing for each genotype. To estimate the gains only with released varieties, a similar strategy
187 was followed by regressing each genotype's breeding values or BLUPs over the year of release
188 for each of the two countries. Additionally, genetic gain trends were plotted using the non-linear
189 approach of *loess* (Local weighted regression) to check the short-term and long-term genetic
190 trends in the salinity breeding program at IRRI, Philippines, and BRRI, Bangladesh data sets.

191 **Formulation of elite breeding panel**

192 Breeding values or BLUPs for grain yield obtained from the second-stage analysis were used to
193 formulate the salinity breeding panel as a future genetic resource. Based on the higher breeding
194 values of >2300 kg/h in the Philippines and BLUPs >3550 kg/h in Bangladesh and prediction

195 accuracy of >0.4, 145 genotypes were selected as a part of the breeding panel. For the Philippine
196 IRRI-HQ data, genetic similarity between the selected lines and in comparison, to the whole
197 historical line collection was assessed using the relationship matrix based on pedigrees. The
198 diversity and similarity of the lines over the complete set of 4993 genotypes from the historical
199 salinity dataset was visualized through the bi-plot graph. The variables for the bi-plot were
200 obtained through the principal component analysis (PCA) performed using the function
201 *princomp* in R software on the A-matrix or pedigree matrix.

202 **Genetic Trends of released lines**

203 From the IRRI-HQ data in the Philippines, 17 IRRI-released saline tolerant varieties were
204 utilized to estimate the genetic gains. Similarly, the gains were estimated using 12 released
205 salinity-tolerant varieties in Bangladesh. The breeding values from the Philippine data set and
206 BLUPs from Bangladesh data were regressed to their year of release for estimating the genetic
207 gains. Also, to further understand the breeding program's growth, recently nominated 25 IRRI
208 varieties across nine countries for the year 2021-22 were compared for their breeding values.

209 Superior performing 12 genotypes comprising seven nominated varieties and four selected
210 varieties from the historical core panel possessing higher breeding values and salinity tolerance
211 were tested for stability using their grain yield performances in 5 environments, *viz.*, in the year
212 2018 at IRRI, Philippines, and Ajuy Iloilo during the wet season: in the year 2019 at IRRI
213 Philippines during dry and wet seasons and at Ajuy Iloilo during the wet season. Stability
214 analysis was performed using R software's GGE Biplot GUI package (Frutos et al. 2014). The
215 percentages of GGE explained by the top two PC axes were estimated for ranking genotypes
216 based on their relative performance and ranking genotypes relative to the ideal genotype. The
217 analysis was based on a Tester-centered (G+GE) table without scaling and with row metric
218 preserving.

219 **Results**

220 **Description of historical salinity datasets**

221 A high difference in the mean values for grain yield (kg/ha) was observed in the Philippine and
222 Bangladesh data sets (Fig. 1 a, b). For the DTF in the Philippines dataset, two maturity
223 categories of early and medium were found, with 98% (DTF: 66-109 days) of genotypes falling

224 under early and 2% (DTF: 110-124 days) genotypes under medium maturity groups. However, in
225 the Bangladesh dataset, the genotypes were found to fit into all three maturity groups, with 66%
226 of genotypes possessing DTF values between 70-109 days, 27% of genotypes having DTF
227 ranges between 110-124 days, and 7% of the genotypes were the late category with DTF ranges
228 between 124-135 days respectively.

229 The heritability based on BLUP differences for grain yield was estimated season-wise for each
230 year. The heritability estimates ranged between 0.20-0.89 for the trials from 2008-2019 in the
231 Philippines dataset (Fig. 1c). Similarly, in the historical dataset from Bangladesh, the heritability
232 ranges were between 0.17-0.95 (Fig. 1c). The heritability values were very low in few of the
233 seasons due to the environmental/season/year influence of genotypes to the salinity stress
234 conditions, which would, in turn, affect the grain yields (Rauf et al. 2012). This could be due to
235 different stress levels and environmental conditions across the years, as the priority objective of
236 the stress trials would be to impart higher salinity stress for identifying tolerant genotypes.

237 **Historical data connectivity**

238 The connectivity in the historical dataset is a major parameter affecting the estimates of genetic
239 gains. The current study had apt connectivity in the data across growing seasons and years. In the
240 Philippine data, the pedigree matrix was included in the second stage of analysis to build the
241 connectivity (Khanna et al. 2022a) and get a reliable estimation of the breeding values and hence
242 genetic gains (Fig. 1d). Additionally, the suitable connectivity in the data can be attributed to the
243 common saline tolerant (FL478, Pokkali-8558) and susceptible checks and varieties (IR29, IRRI
244 104, IRRI 165, etc.,) used in the breeding trials across seasons and years. Alongside, the
245 connectivity was maintained as the tolerant genotypes/varieties were evaluated in subsequent
246 years to reconfirm their stable tolerance across years (Fig. 2a, b). It was also observed that there
247 was connectivity amongst the trials across seasons in each year in both countries (Supplementary
248 Fig 1a, b,c,d)

249 **Crossing strategy across salinity historical breeding program**

250 It is crucial to decipher the crossing strategy or the breeding scheme adopted by the breeders
251 during this period and associate it with the genetic gain trends. The crossing strategy used was
252 extracted from the B4R database. From 2000 to 2016, the breeders performed single, double,
253 three-way, complex, and backcrosses (Fig. 2 c, d). It was found that during the initial years, 2000

254 to 2005, most of the crosses were single and backcrosses, most of which, until 2012, included
255 double, three-way, and complex cross combinations. However, post-2012, the era when IRRI
256 was rephrasing from marker-assisted backcross breeding to complex/multiple crossing strategies
257 for developing various stress-tolerant breeding varieties, the complex crossing strategy was more
258 highlighted along with three-way and backcrosses (Fig. 2c). The unique 4, 993 genotypes from
259 the historical breeding trials dataset of Philippines had a broad background with differing parents
260 and cross combinations and can also be classified into 770 families based on their diverse
261 parental crosses, amongst which 108 families were derived from backcrosses; 451 from single
262 cross combinations; 49 from double cross combinations; 152 from three-way crosses; 13 from
263 complex crosses, two from induced mutations procedures and remaining were landraces, and
264 remaining 5 were accessions and donors from the gene bank. However, no clear association was
265 found between the crossing strategy and change in the genetic gain trends. After 2012, more
266 fluctuations were observed with genetic trends because the breeding program focused on
267 complex/multiple crossing strategies to develop multiple stress-tolerant breeding lines with a
268 limited focus on using population improvement strategy to enhance the yield.

269 **Estimation of breeding values and genetic trends**

270 The breeding values ranged between 1326.06-4720.35 kg/ha in the Philippines dataset and
271 3587.27-5829.77 kg/ha in the Bangladesh dataset (Supplementary Fig. 2a, b). The genetic gain at
272 IRRI, Philippines, between 2008 and 2019 was 0.1% per annum, with a yield advantage of 1.53
273 kg/ha/year. The gain was estimated by regressing the year of origin or year of crossing for each
274 of the genotypes spanning from 2000 to 2016, tested in the breeding trials across the years 2008
275 to 2019 (Fig. 3a). In Bangladesh, the genetic gain was 0.31% per annum with a yield advantage
276 of 14.02 kg/ha per annum (Fig. 3b). Both in IRRI Philippines and BRRI, Bangladesh data sets,
277 linear and non-linear genetic trends were plotted to check the fluctuations in genetic trends over
278 the years (Fig. 3a, b). The genetic trends were estimated for the released varieties in the
279 Philippines and Bangladesh. The genetic gain was 0.12% per annum with a yield advantage of
280 2.2 kg/ha/year as estimated for the released varieties at IRRI, Philippines (Fig. 3c). The gain was
281 0.14% per annum with a yield advantage of 5.9 kg/ha/year (Fig. 3d).

282 **Development of an elite breeding panel**

283 The genotypes with higher breeding values of >2400 kg/ha in the Philippines and >3500 kg/ha in
284 Bangladesh and having selection accuracy >0.4 were selected for formulating the elite breeding
285 panel. The top 145 genotypes were selected as a future breeding resource for the elite core panel.
286 The criteria for selecting the elite lines, their breeding values, cross combinations, and crossing
287 strategies employed are given in Supplementary Table 2. We also accessed the kinship of the
288 lines from IRRI-HQ Philippine data using the pedigree relationship matrix (Fig. 4a). The
289 genotypes selected for the elite core panel represent the whole data collection and cover the
290 diversity of the entire collection very well. The genotypes selected for the elite panel not only
291 possess a high breeding value for yield but harbor salinity-tolerant landraces, including Sadri,
292 Pokkali, and Cheriviruppu; elite breeding genotypes like NERICA (New Rice for Africa), which
293 are early maturing (<100 days); tolerant to major stresses of Africa; AT401, variety can
294 withstand coastal saline environments; Zinc fortified genotypes (IR68144; BR7840-54-3-1);
295 Zinc efficient donor parents (IR55179). The panel additionally possesses genotypes with superior
296 characters, including genotypes with superior yields under DSR conditions (Supplementary
297 Table 3), zinc-efficient genotypes with superior breeding values, iron toxicity tolerant genotypes,
298 and coastal and acid saline tolerant genotypes (Supplementary Table 2).

299 **Comparison of breeding values in IRRI released, nominated, and core panel lines**

300 The breeding values for the IRRI-released varieties were compared with the saline tolerant
301 checks and varieties for estimating the gains obtained in the released varieties. The comparative
302 analysis would help to comprehend the genetic progress and identify superior genotypes across
303 the years. The breeding values of the released varieties were superior to the popular checks for
304 both countries. In the Philippines dataset, the released global salinity varieties IRRI 147, IRRI
305 207 and IRRI 172, IRRI 170, and IRRI 198 depicted superior breeding values of 2493.84 kg/ha,
306 2261.63 kg/ha, 2210.60 kg/ha, 2198.40 kg/ha and 2089.28 compared to other salinity tolerant
307 varieties and popular checks A69-1 and FL478 with the breeding value of 2200 kg/ha and
308 2086.74 kg/ha respectively. Amongst these superior-performing varieties, IRRI 207 and IRRI
309 198 were recently released in the years 2018 and 2016 (Fig. 2c). In the Bangladesh dataset, all
310 the genotypes included for estimating the gain were superior performing to salinity tolerant
311 check FL478. Amongst all, BRRI Dhan 47, BRRI Dhan 61, BRRI Dhan 69, and BRRI Dhan 67
312 depicted superior breeding values of 5829.77 kg/ha, 5720.48 kg/ha, 5568.19 kg/ha and 5110.41
313 kg/ha respectively, to the salinity tolerant check FL478 (BINA Dhan 8) and Pokkali with the

314 breeding values of 4071.16 kg/ha and 4021.65 kg/ha respectively (Fig. 2d). Amongst these
315 varieties BRRI Dhan 61, BRRI Dhan 69 and BRRI Dhan 67 were released in the years 2013 and
316 2014 respectively.

317 Alongside the released varieties across years, 25 IRRI-bred varieties nominated for eight
318 countries in 2021-22 were also compared for their breeding values. The breeding values of these
319 varieties ranged between 2025.80 to 2920.67 kg/h (Supplementary Fig. 3). Overall, IR121094-B-
320 B-AJY3-2-B (IR18T1021) nominated for release in Thailand depicted the highest breeding value
321 of 2920.67 kg/h followed by IR121188-28-1-CMU2-2-B (IR18T1015), nominated for release in
322 the Philippines with a breeding value of 2718.15 kg/h. Interestingly, the latter possesses higher
323 breeding value compared with the till date released varieties utilized for estimating gains for
324 2008-2019 and amongst other nominated varieties for the year 2021-22 for the Philippines.
325 Additionally, IR121188-28-1-CMU2-2-B was also found to be the most stable variety based on
326 the relative performance when ranked amongst the other varieties, including IRRI 147, which
327 possessed the highest breeding value amongst the date-released varieties for the Philippines, and
328 was found the most preferred variety residing along relative to the ideal genotype as shown by
329 the arrow in the Supplementary Fig. 4.

330 Further, succeeding IR117676-318-1-1-1 has been nominated for Sri Lanka, which possesses a
331 breeding value of 2640.39 kg/h and ranked fourth for stability amongst the tested varieties,
332 followed by IR112462-B-25-2-1-1 (IR16T1631) nominated for Bangladesh and Lao PDR with a
333 breeding value of 2625.29 kg/h. IR16T1009, nominated for Thailand, also depicted a breeding
334 value of 2514.73 kg/h. This was followed by IR63307-4B-4-3, nominated for Indonesia,
335 Vietnam, and Sri Lanka with a breeding value of 2493.843 kg/h, bred using a soma clonal
336 variant of Pokkali. Another variety, IR117839-22-15-B-CMU10-1-B, nominated for release in
337 the Philippines and Vietnam, possessed a breeding value of 2479.29 kg/h and was the second
338 most stable genotype amongst the tested varieties in the five environments.

339 In the selected panel, 8% of the genotypes comprise IRRI-bred varieties that can withstand
340 salinity stress in hand with superior performance under DSR conditions with yields ranging
341 between 2195-4758 kg/h (Supplementary Table 3). Regarding including the genotypes from
342 current year nominations, 12% of the panel comprises recently nominated varieties with the
343 highest breeding values. In all eight superior breeding value harboring genotypes of the

344 nominations *viz.*, IR121094-B-B-AJY3-2-B (2920.67), IR121188-28-1-CMU2-2-B (2718.15),
345 IR117676-318-1-1-1 (2640.39), IR112462-B-25-2-1-1 (2625.29), IR16T1009 (2514.73),
346 IR63307-4B-4-3 (2493.843), IR117839-22-15-B-CMU10-1-B (2479.29), IR117749-B-B-
347 CMU6-1-B (2379.65) was part of the core breeding panel (Supplementary Table 2). Another 3%
348 of the panel formulates zinc & iron bio-fortified genotypes with superior breeding values of
349 >2400 kg/ha for being future-ready for upscaling salinity tolerance and additional characteristics.

350 **Discussion**

351 We demonstrated the genetic trends in IRRI's rice salinity breeding program by leveraging
352 historical data and pedigree information. Besides genetic gain estimates, top-performing
353 genotypes based on high breeding values for grain yield were also identified as a future elite
354 breeding resource. Availability of the pedigree information from the IRRI-HQ data was crucial
355 to fit and use in the second stage of analysis for reliable estimation of the breeding values to help
356 in the identification of accurate genotypes for the development of the elite panel and accurate
357 estimation of genetic gains (Rutkoski et al. 2019a). As these lines have already been bred under
358 saline environments, they are not only tolerant to salinity but possess high breeding values for
359 yield, making them readily available as a future genetic resource for the population
360 improvement-based breeding strategy, further enhancing the genetic gains in the salinity
361 breeding program.

362 **Genetic gain estimations**

363 One of the main goals of the breeding program is to achieve higher and constant rates of increase
364 in genetic gain while maintaining genetic diversity. Genetic gain estimations are crucial to
365 assess the success and growth of the breeding programs through an increase in the mean
366 population breeding values over the years of selection (Ramstein et.al. 2019, Rutkoski et al.,
367 2019b). This work is the first report demonstrating the genetic gains in the rice salinity breeding
368 program and assessing its progress in terms of genetic gains. Positive genetic gains were
369 obtained in IRRI-Philippines and BRRI, Bangladesh. However, the rate of genetic gain was just
370 0.33% in Bangladesh, and 0.13% in the Philippines, which is much lower than the required rate
371 of genetic gain in rice is 1.5% or above (Khanna et al. 2022a, Li et al. 2018). To achieve the
372 necessary rates of genetic gains in the IRRI's rice salinity breeding program, a major tweaking in

373 the breeder's equation (Cobb et al., 2019, Merrick et al. 2022) through modernization and
374 optimization is highly required. Further, major paradigm shifts are required in rice salinity
375 breeding to deliver high salinity tolerant lines with higher genetic gains. A highly focused
376 population improvement with systematic pre-breeding efforts is needed to deliver constant and
377 higher rates of genetic gains in IRRI's salinity breeding program.

378 **Core panel formulation for identifying elite genotypes**

379 A set of high-performance, elite breeding lines with salinity tolerance is highly required to
380 unlock the potential of cultivation in saline soils with enhanced genetic gains. The conventional
381 salinity breeding at IRRI has mainly focused on crossing the non-elite (salinity tolerant
382 traditional donors/ landraces) to the high-yielding elite breeding lines to develop the high-
383 yielding elite salinity tolerant lines. Further, since the inception of molecular breeding, the main
384 focus has been on the introgression or pyramiding of salinity-tolerant QTLs in elite backgrounds
385 (Singh et al. 2021). The rice breeders have used different crossing strategies (Figure 2 a, b, c)
386 single, complex, double, and backcrosses to integrate these QTLs into the elite genetic
387 backgrounds and develop the new breeding lines. Diverse materials, including landraces and
388 donors, have been extensively used to diversify the gene pool and develop climate-resilient
389 salinity varieties (Singh et al. 2021, Sandhu et al. 2021, Yadav et al. 2021). The genotypes used
390 in this study represent the breadth of the diversity of IRRI's salinity breeding program through
391 which several high-yielding salinity tolerant varieties have been released (Singh et al. 2021).
392 This breeding resource represents an essential source of elite genetic variation that can be
393 leveraged to extract the diverse genotypes with high breeding values for grain yield and salinity
394 tolerance as a future genetic resource. To this end, an effort was made to develop the
395 representative set of the elite pool from this historical collection based on high breeding value
396 and genetic divergence. The developed elite pool can be readily used in a rapid, recurrent
397 selection-based breeding strategy to quickly re-cycle the lines for enhanced genetic gains.
398 Recurrent selection is critical to increase the frequency of favorable additive alleles of grain yield
399 and enhance genetic gains (Khanna et al. 2022a, Juma et al. 2021).

400 For example, the elite pool identified in this work has released lines like BRRI Dhan 55, BRRI
401 Dhan 47, BRRI Dhan 67, IRRI 185, IRRI 235, and IRRI 147, which showed high breeding
402 values for the grain yield (Fig. 3; Supplementary Table 2). Specifically, the released variety

403 IRRI 147 for the Philippines, also released as BRRI Dhan 47 in Bangladesh, depicted the highest
404 breeding values amongst the released varieties. The variety harbors a unique characteristic of
405 erect plant architecture as its leaf angle falls between 5°-20° (BRRI annual report 2018-2019).
406 The erect plant architecture renders higher photosynthetic abilities by impacting the source and
407 sink organs, making it crucial to identify genotypes with superior “ideotypes,” which can
408 significantly enhance yield, productivity, and gains (Chang et al. 2020). Additionally, genotypes
409 IR58443-6B-10-3, IR16T1110, IR16T1086, IR16T1661, and IR16T1018 were also included in
410 the breeding pool, and these genotypes have shown to have high performance under the direct-
411 seeded conditions (DSR) along with salinity tolerance (IRRI personal communication). The elite
412 pool consists of 8 genotypes from the freshly nominated 25 varieties for eight countries in
413 2021-22. These genotypes revealed high salinity tolerance and superior grain yield, which
414 provide ample evidence that conscious efforts are being made to develop the high-yielding lines
415 under saline environments to achieve desired genetic gains.

416 Interestingly, the nominated variety IR63307-4B-4-3 for three countries, Indonesia, Vietnam,
417 and Sri Lanka, has been bred by crossing IR 51511-B-B-34-B/TCCP 266-2-49-B-B-3 using a
418 single cross-breeding strategy. The donor parent used here, TCCP 266-2-49-B-B-3, is a soma
419 clonal variant of Pokkali with superior characteristics, including semi-dwarf plant type with
420 white pericarp, medium consistency of grain type and possesses high yield potential along with
421 vigorous growth without lodging. All eight superior breeding values harboring nominations
422 genotypes *viz.*, IR121094-B-B-AJY3-2-B, IR121188-28-1-CMU2-2-B, IR117676-318-1-1-1,
423 IR112462-B-25-2-1-1, IR16T1009, IR63307-4B-4-3, IR117839-22-15-B-CMU10-1-B, and
424 IR117749-B-B-CMU6-1-B is part of the elite panel. Conclusively, the selected lines for the elite
425 pool development don't have only high breeding values for yield but possess a tolerance under
426 salinity stress. The genotypes will be a great source of readily available variation to use in
427 recurrent selection and further recombine and reshuffle in it to create an additional novel source
428 of variation for grain yield and enhance the genetic gains. Consequently, a systematically
429 designed holistic breeding approach was implemented with multi-location trial evaluations of the
430 newly identified panel with significant emphasis on economically targeted traits considering the
431 target population of the environment (TPE), plant architecture, and yield component traits
432 (Yadav et al. 2021, Chang et al. 2020) laterally with prediction based breeding strategies would

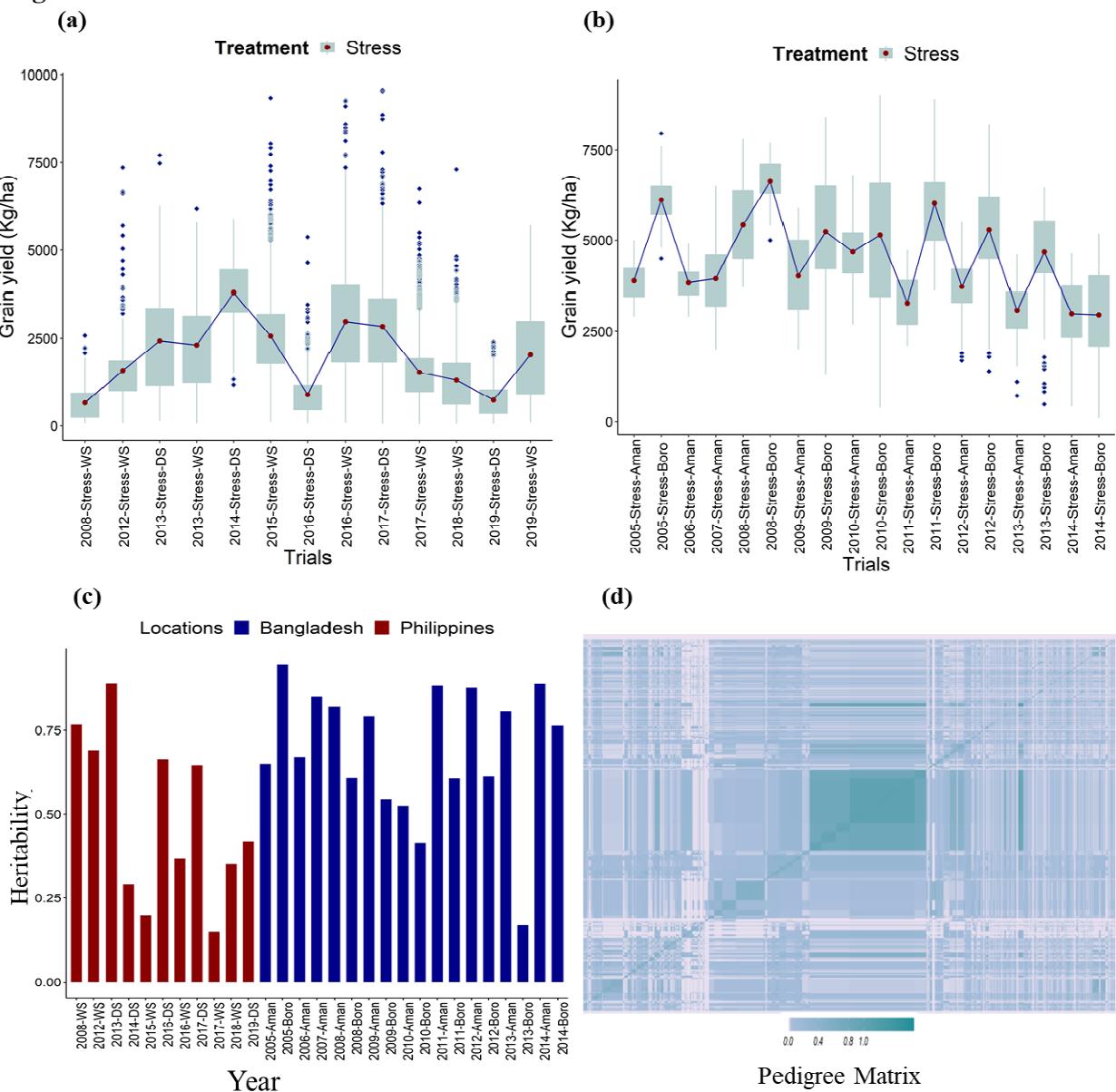
433 help in sustainably improving and enhancing genetic gains in the future rice salinity breeding
434 program at IRRI (Reynolds et al. 2011; Yadav et al. 2021, Gerard et al. 2020).

435 **Conclusions**

436 The current rate of genetic gains observed in the salinity breeding program is comparatively
437 lower than the required rates of 1.5% or above. The rate of genetic gain in rice will increase to
438 2.5% or above in 2050. To deliver higher rates of genetic gains in the salinity breeding of IRRI, a
439 holistic and systematic breeding effort with the integration of modern tools and technologies is
440 required. A population improvement breeding strategy based on an elite x elite scheme with the
441 integration of genomic selection is highly required. The elite breeding pool identified in this
442 study would be the most potent and readily available genetic resource to drive the population
443 improvement-based breeding strategy in IRRI's salinity breeding program.

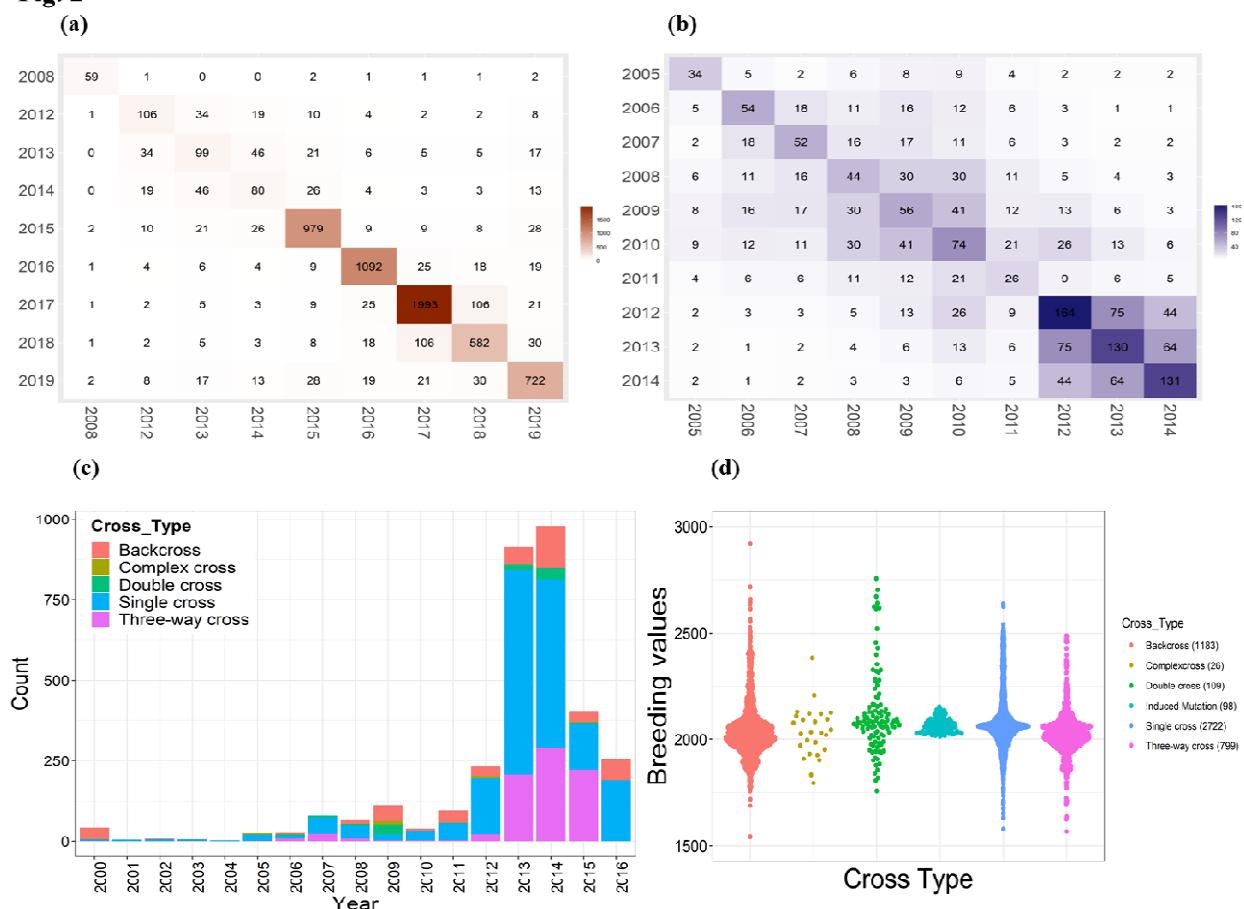
444 **Figures**

Fig. 1



445
446 Boxplots depicting the ranges of raw grain yields (kg/ha) across historical breeding trials for salinity stress from **(a)**
447 2008-2019 at the Philippines, **(b)** 2005-2014 in Bangladesh. The grain yields have been shown season-wise **(a)** DS
448 and WS, symbolize dry and wet seasons for the mentioned respective years at the Philippines; likewise in **(b)** aman
449 and boro, represent the trial seasons in Bangladesh respective to mentioned years. **(c)** The bar plot shows the
450 heritability of the trials at IRRI, Philippines, from 2008 to 2019, as depicted by the red bars, and the heritability of
451 the trials conducted in Bangladesh between 2005 to 2014, as depicted by the blue bars. The WS and DS represent
452 the wet and dry seasons in the Philippines. Similarly, aman and boro are two seasons in which trials were conducted
453 in Bangladesh **(d)** Pedigree matrix depicting pedigree-based relationship based on 4,993 unique genotypes bred
454 across 12 years at IRRI, Philippines. The range is represented by blue and green color, As the color scale progresses
455 from light blue to through sea green in the off diagonals to dark cyan in the diagonals, the genetic similarity
456 increases amongst the genotypes scaling 0.4 to 0.8 to 1. The higher the genetic similarity the higher the score.

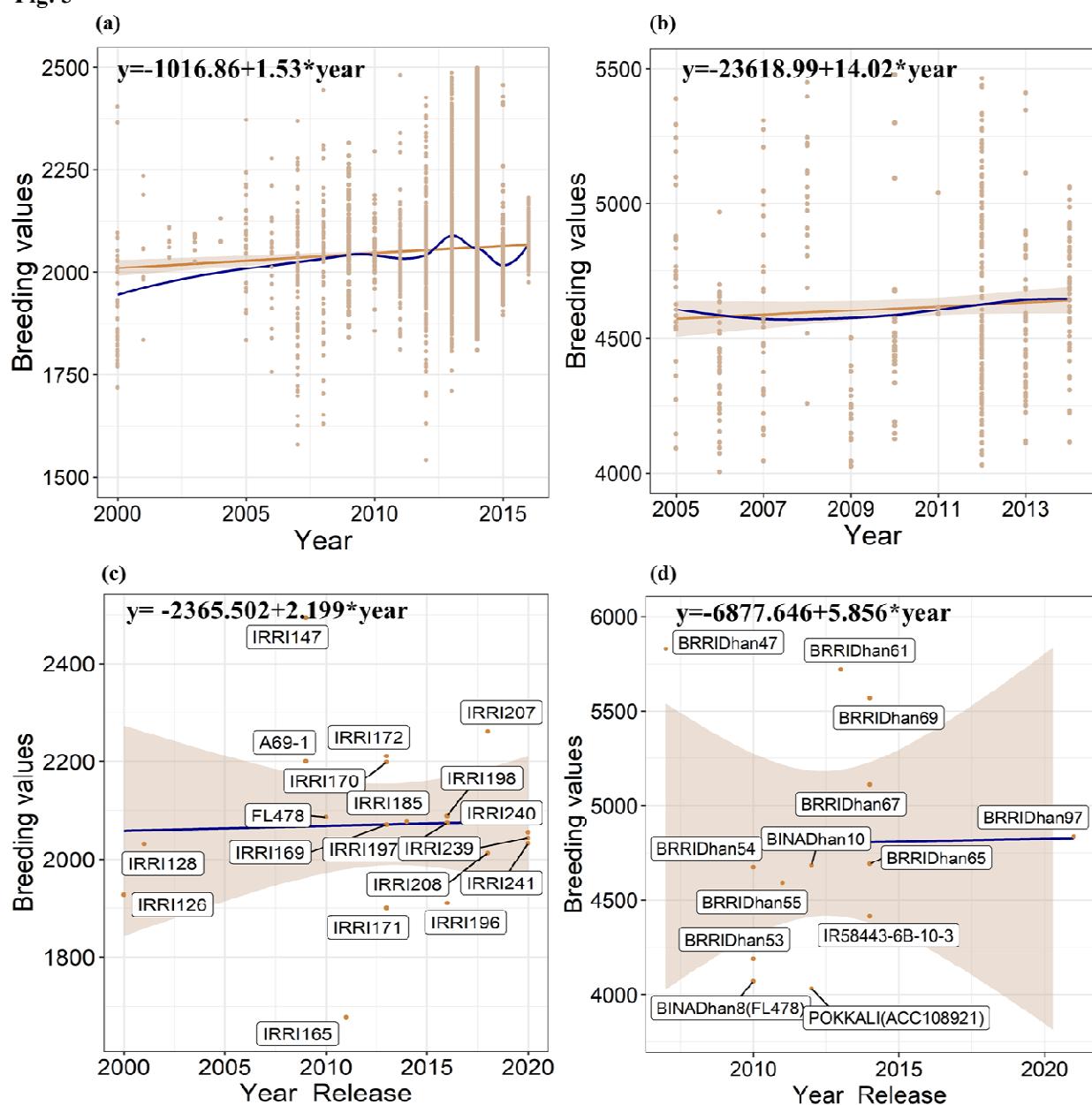
Fig. 2



457

458 (a) Connectivity of all the unique genotypes in the Philippines dataset, (b) Bangladesh dataset across years were
 459 plotted in form of heatmap. The numbers in each box represent common genotypes between each of the year
 460 combinations. Since the checks and promising varieties were repeatedly bred and tested in the successive years for
 461 evaluating their performance, the datasets represent apt connectivity across years. (c) The barplot depicts the
 462 classification of the genotypes tested across years of origin in the trials organized at IRRI, Philippines from 2000-
 463 2016. The differential coloration in each bar represents varying breeding strategies employed in the respective years.
 464 As the figure depicts single and backcrosses were initiated in the initial years which were added by double three-
 465 way complex crosses in the later years in the breeding program. (d) The boxplot depicts the range of each of the
 466 breeding strategies in terms of breeding values. However, the ranges slightly differ in each of the cases, the
 467 backcrosses possessed a wider range followed by single and three way crosses. The numbers of each of the cross
 468 combinations formulated across years have also been depicted in the brackets besides each cross type. The
 469 maximum were single cross combinations followed by backcrosses.
 470

Fig. 3

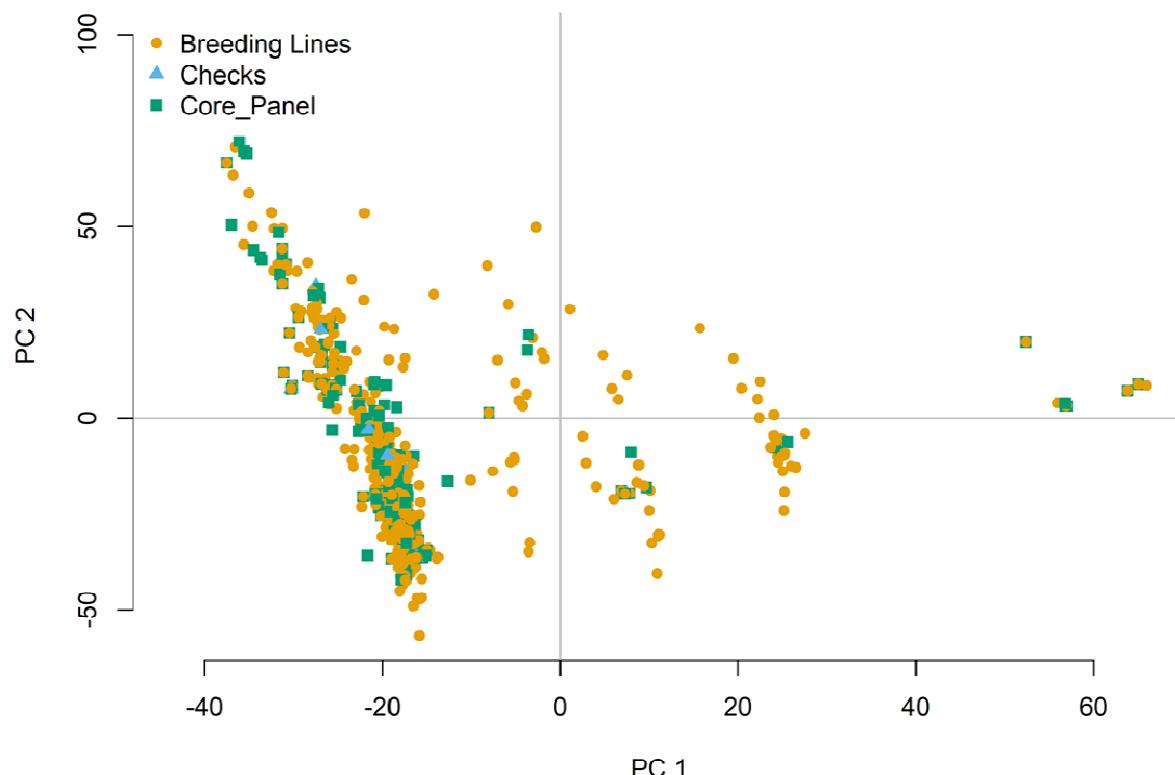


471

472 (a) Trends in genetic gain from IRRI's salinity breeding program comprising 12 years of breeding trials, 2008 to
473 2019. (b) Trends in genetic gain from salinity breeding program in Bangladesh comprising 10 years of breeding
474 trials, 2005 to 2014. In both (a) and (b), The x-axis depicts the year of origin, and the y-axis portrays the breeding
475 values or BLUPs of the genotypes. The dots represent the breeding values or BLUPs respective to each year. The
476 slope in Peru represents the genetic trends using linear regression, and the slope in dark blue portrays the genetic
477 trend using a non-parametric approach using loess regression. (c) The trends in genetic gain for the released varieties
478 across years bred for salinity tolerance at IRRI. (d) The trends in genetic gains for the released varieties across years
479 bred in Bangladesh. The gains were likewise estimated by regressing the grain yield breeding values on the year of
480 origin.

481

Fig 4.



482

483 PCA-Biplot depicting the complete set of unique breeding lines obtained from the historical breeding trials in IRRI-
484 HQ. The elite core panel breeding lines selected based on the superior breeding values have been depicted by violet
485 color and checks by blue color. The biplot was obtained and plotted using the first two principal components using
486 the pedigree matrix. The elite core panel lines overlap the complete set of breeding lines and represent the diversity
487 of whole collection as a useful future breeding resource.

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631

632 **Acknowledgments**

633 We thank the Bill and Melinda Gates Foundation for funding and supporting this study. We
634 would also like to acknowledge our respect and thankfulness to Dr. Hans Bhardwaj (Platform
635 Leader, Rice Breeding Innovations, IRRI) for his support and encouragement. The authors also
636 thank IRRI rainfed breeding program breeders and technical staff, who have conducted these
637 trials since 2003 and generated the data used in this study. We would like to thank all IRRI
638 members who have contributed to maintaining the B4R database to gather and preserve valuable
639 breeding trial information.

640 **Funding**

641 The AGGRi Alliance project funded the study, “Accelerated Genetic Gains in Rice Alliance”
642 funded by the Bill and Melinda Gates Foundation through the grant ID OPP1194925- INV
643 008226

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663 **Contributions**

664 WH, SB, AK designed the concept and study. AK and WH wrote the manuscript. WH and AK
665 performed the data analysis. GG, RKS, AR bred the salinity breeding materials and helped
666 gather the trial data. JR, AG, TSC, MC, and MA helped compile data sets. GG, RKS, AR, HK,
667 RI, and VKS revise the manuscript and gave suggestions and incorporations to finalize the
668 manuscript. All authors read and approved the manuscript.

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672 Laguna 4031, Philippines.

673 **Ethics declarations: NA**

674 **Conflict of interest**

675 The authors declare that the research review was conducted without any commercial or
676 economic associations that could be construed as a potential conflict of interest.

677 **Additional information**

678 The phenotypic data from the historical trials and scripts for analysis are available at the GitHub
679 repository and can be accessed through the following links:

680 https://github.com/whussain2/Genetic_Trend_Rice_Drought

681 <https://github.com/whussain2/Analysis-pipeline>

682 **Publisher's notes**

683 **Supplementary Information**

684 **Rice Salinity Breeding at IRRI: Genetic Gains Achieved and Future Breeding Resource**

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698 **Supplementary Figures:**

Supplementary Fig. 1

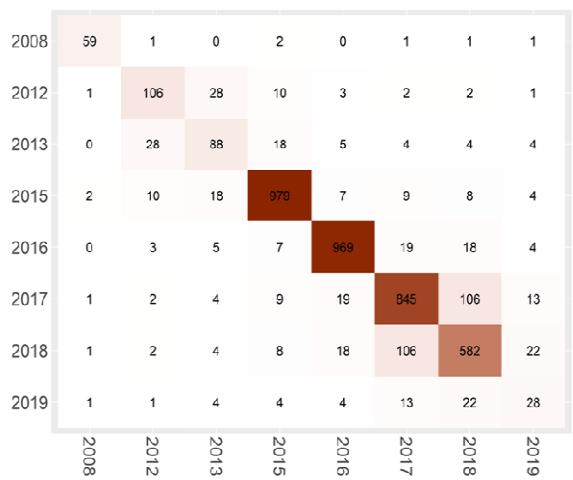
(a)



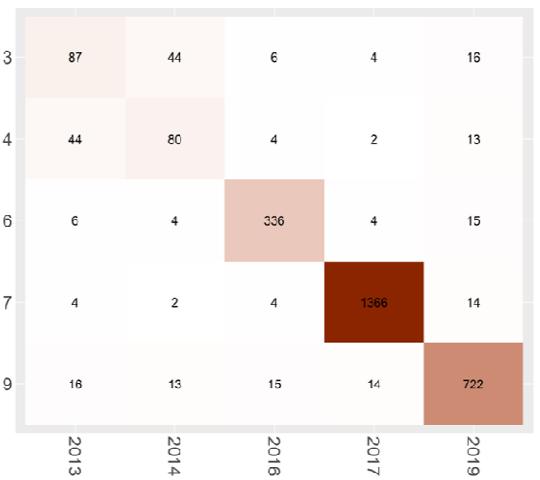
(b)



(c)



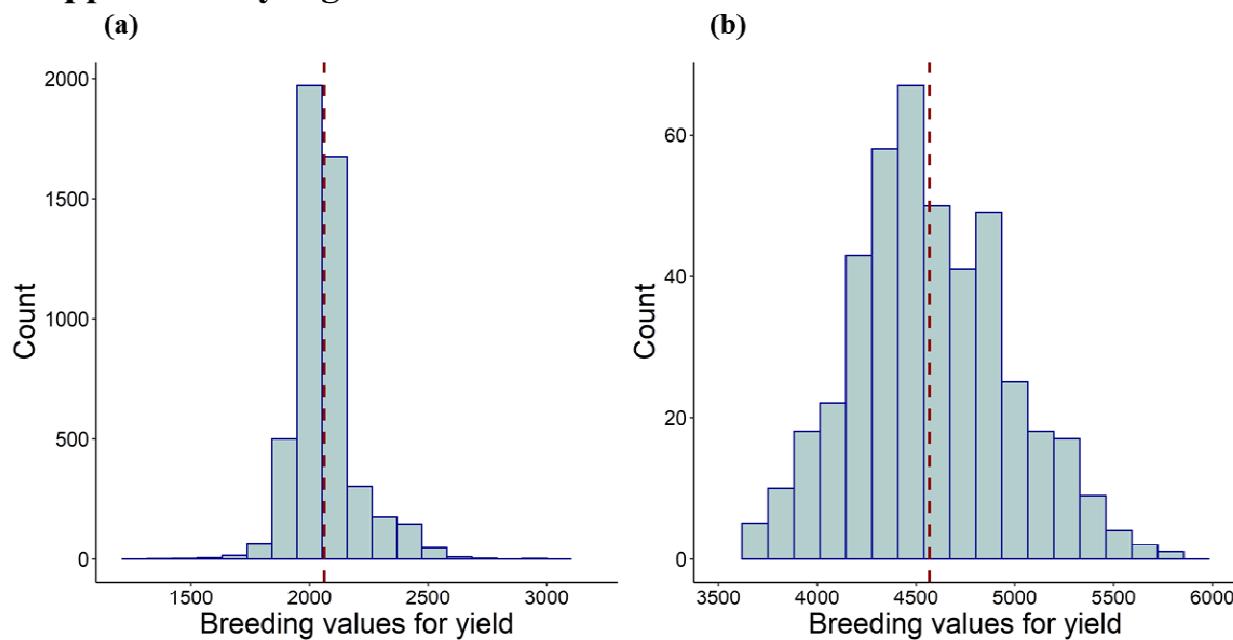
(d)



699

700 The figures depicted in brown depict the connectivity of the unique genotypes from Bangladesh evaluated during (a)
 701 Aman season, (b) Boro season; The heat maps plotted in blue depict the connectivity of the unique genotypes from
 702 the Philippines dataset tested during the (c) wet season, (d) dry season. The numbers in each box represent common
 703 genotypes between each year combination. Both datasets represent apt connectivity across years since the checks
 704 and promising varieties were repeatedly bred and tested in successive years to evaluate their performance.

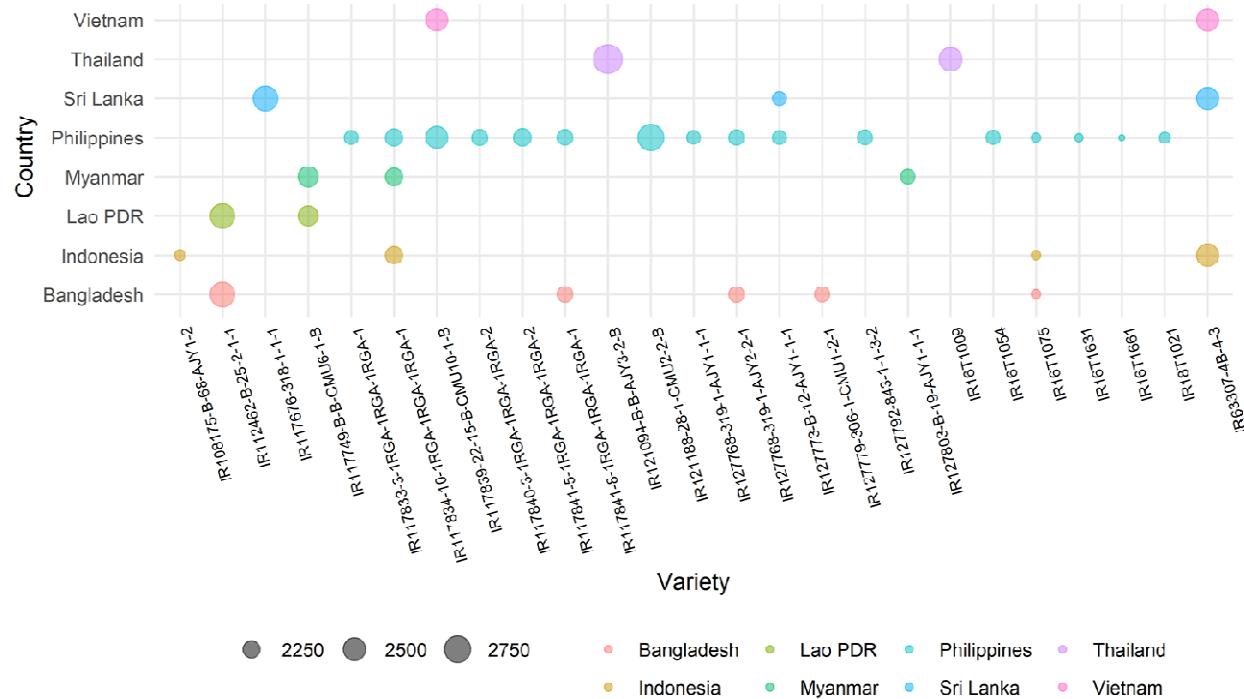
Supplementary Fig. 2



705

706 The distribution of the breeding values for grain yield (kg/ha) of the genotypes screened under reproductive stage
 707 salinity stress at **(a)** Philippines and **(b)** Bangladesh has been demonstrated. In each of the figures the red margin
 708 portrays the mean of the breeding values in each of the cases.

Supplementary Fig. 3

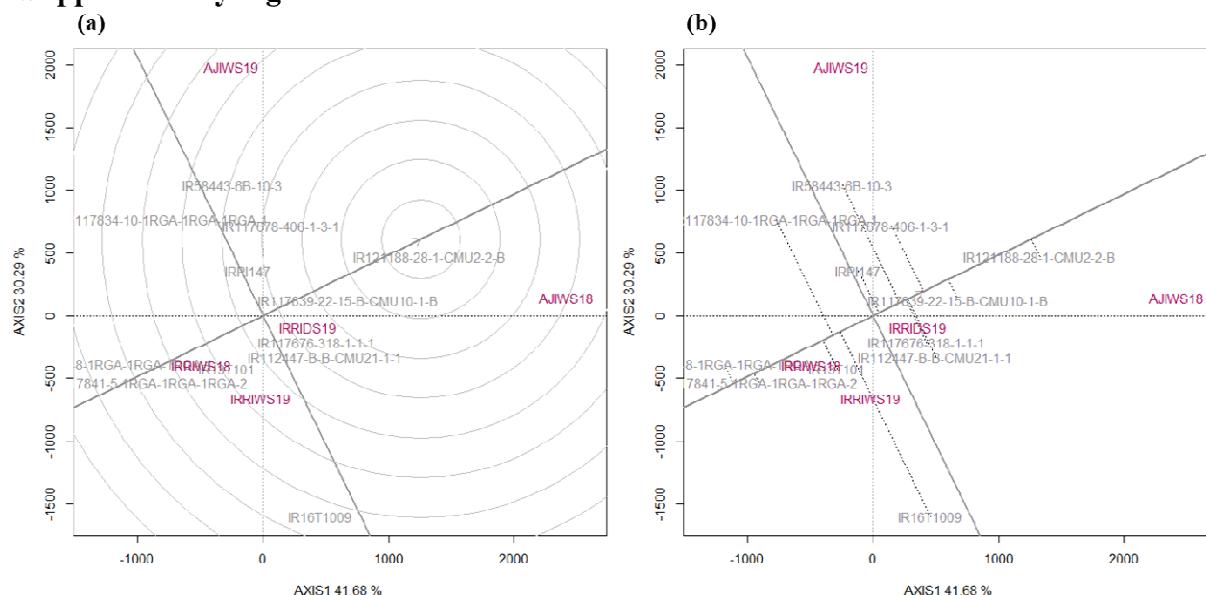


709

710 Salt tolerant historical breeding lines bred across 12 years from 2008-2019 at IRRI, Philippines and were selected
 711 for nominations across 8 countries for the year 2021-22. The breeding values for each of the countries have been

712 depicted by different colors. The more the circumference of the circle, higher the breeding values. The highest
713 breeding value was for the genotype IR121094-B-B-AJY3-2-B, nominated for Thailand depicted in violet color,
714 followed by IR121188-28-1-CMU2-2-B, nominated for the Philippines depicted in cyan color.

Supplementary Fig. 4



715

716 The goodness of fit of the biplot, explaining a total of 71.97% of test centric data (G+GE) has been represented in
717 the figures. The percentages of GGE explained by the top two PC axes were estimated for ranking genotypes based
718 on their relative performance and ranking genotypes relative to the ideal genotype. **(a)** Ranking of genotypes with
719 reference to the “ideal genotype”, as shown by the arrow. The genotypes are raked in descending order starting from
720 the arrow of ideal genotype. **(b)** Comparative depiction of genotypes based on their mean performance and stability
721 portraying the average-environment coordination view of GGE biplot.

722