

Diversity analysis of indica rice (*Oryza sativa* L.) genotypes against low and high temperature stress

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Abstract: Morphological characterization of 470 rice (*Oryza sativa* L.) accessions including five checks collected from different regions country were assessed using 19 quantitative characters traits. Accessions were evaluated in a field experiment in an augmented experimental design. The aim of the research was to study variations and to select lines that could be used as potential parents in future breeding programs. A principal components plot and distance between genotypes in different cluster groups were used to group the accessions. The rice genotypes grouped into divergent cluster 12 and 18 are expected to give promising and desirable recombinants in the segregating generations. Also, traits contributing maximum to genetic divergence viz. Seed vigor followed by Fertile Grains/ Panicle, Fertile Grains/ Panicle and panicle length may be utilized in selecting genetically diverse parents.

Key words: genetic divergence, cold tolerance, rice, inter cluster distance

I. Introduction:

Rice has become a commodity of strategic significance and the fastest growing food source in India, any reduction in the rice production will have large scale implications on food security. The recent studies have predicted a reduction of 4 % in rice production due to climate change. It is estimated that for every 1^oC rise in temperature there is a reduction of 0.5 tons in rice yields (1). To meet the food demand of the growing population and to achieve food security in the country, the present production levels need to be increased by 2 million tonnes every year, which is possible through heterosis breeding and other innovative breeding approaches. To increase the present levels of heterosis for yield, there is a need to identify and utilize genetically divergent parents for inter and intra specific crosses in rice (2). A systematic evaluation and characterization of germplasm lines not only help in identification of superior and genetically divergent germplasm lines but also provide information on the utility of the genetic resources (3). Characterization of accessions provides the information on morphological and agronomic aspects of the material that is essential for the gene bank management (4). Therefore, the present investigation was undertaken to study the genetic variability for yield and its component characters in various rice germplasm lines. In the present study several germplasm accessions were evaluated for their cold tolerance during seedling stage and heat tolerance during reproductive phase.

II. Materials And Methods:

A set of 470 rice germplasm accessions collected from different parts of country were tested for their cold tolerance in a phytotron. Five checks AK Dhan, N-22, Prasanna, RP-Bio and Vikas were used. The seeds of the germplasm lines were allowed to germinate at 35^oC in a growth chamber for 3 days. The germinated seeds were surface-sterilized with 1% sodium hypochlorite solution and washed with distilled water. 30 uniformly germinated seeds from each accession were sown in germination trays and cultured in growth cabinet where the temperature was maintained at 25^oC with 70% relative humidity. At one-leaf stage the seedlings were subjected to cold stress at 6^oC during night time and 8^oC during day with a relative humidity of 70% for 7 days after which the temperature was adjusted gradually back to 25^oC. The observations on the growth of seedlings were recorded from the 5th day of cold treatment. The survived lines at 20th day were transferred to field to evaluate their agronomic performance and tolerance to heat during reproductive phase. A field experiment was conducted by using augmented design during Rabi, 2012 at DRR farm, ICRISAT Campus Patancheru, Hyderabad. India. Situated at 17.53^oN latitude, 78.27^o E longitude and altitude of 545m above mean sea level. The augmented experimental design introduced (5) was used for the experiment. An Augmented Experimental Design (AED) is usually useful for testing a large number of genotypes in early generations when valid statistical analyses are needed particularly when seed supplies are too limited to permit replication. The basic concept of augmented design construction is to establish a standard replicated design using checks for which sufficient seeds are available. Each replicate forms a complete block, incomplete block, or cell, depending on the standard design. Additional unassigned plots are created within

each replicate and un-replicated entries for which there are insufficient seeds. Entries are then assigned to these plots in the form of an incomplete block design. Seedlings were transplanted at 21 day old with a spacing of 20X20 cm. The field experiment was carried out in summer with maximum temperatures crossing 40°C. One seedling was transplanted per hill and the inter-plot spacing was 40 cm. A plot size of 1.2x5 m with 6 rows was used for each accession in the field. Recommended cultural practices are followed. Morphological data were collected for nineteen quantitative characters at appropriate growth stage of rice plant following the descriptor for Rice *Oryza sativa* L. (6).

III. Results And Discussion:

The analysis of variance revealed significant differences among the 470 genotypes for all the nineteen characters studied (Table 1). The results indicated high variances for most of the characters, which may favour the selection and its further utilization in recombination-breeding programmes in the mere future. The quantum of genetic divergence was also assessed by cluster analysis using Mahalanobis's Euclidean squared distances which grouped the entire material into more precised clusters and estimates the average distance between them. The Euclidean squared distance grouped the material into 8 clusters.

Table. 1. Analysis of variance for different traits in rice germplasm

	Block (ignoring Treatments)	Treatment (eliminating Blocks)	Checks	Checks+Var vs. Var.	ERROR
d.F	4	474	4	470	16
Tillers/Plant	966.46 ***	23.59 ***	6.7	23.73***	4.9
Effective Tillers/ Plant	106.65 ***	14.46	3.34	14.56	8.91
Plant Height cm	2512.62***	219.40 *	1351.70 ***	209.77 *	99.85
Leaf Length cm	551.92 ***	139.06 ***	405.50***	136.79 ***	19.93
Leaf Width cm	0.35***	0.04	0.11*	0.04	0.03
Panicle Length cm	131.77***	12.01***	64.97***	11.56***	1.8
Sterility %	2882.25***	381.75*	5394.24***	339.09	169.07
Yield/ Plant	189.71**	32.67	564.93***	28.14	33.36
100-seed Wt	0.36**	0.18**	0.26**	0.18**	0.05
Seed Density	775.70***	141.36	357.46*	139.52	76.36
Grains/ Panicle	7676.18*	1215.72	2603.06	1203.91	1767.13
Fertile Grains/ Panicle	6675.63	1528.31	15504.14**	1409.36	2608.07
Pollen Viability	423.21 ***	71.98*	491.04***	68.41*	29.34
Days to 50% Flowering	123.06**	14.93	15.26	14.92	25.49
Germination %	1000.55***	908.5***	12.26	916.13***	15.97
Coleoptiles Length (cm)	12.57	31.72**	25.6	31.78**	10.74
Radical Length (cm)	17.36	22.66	15.97	22.72	22.71
Seed Growth (cm)	51.79	98.61	67.35	98.88	58.82
Seed vigor	399585.16	1053480.1	618118	1057185	586843

The 470 rice genotypes were grouped into 22 clusters (Table.2). Among the clusters, cluster III was the largest comprising of 46 genotypes followed by cluster VII (37), cluster XIII (34), cluster V (33), cluster 10,14 (30) and cluster 25 having twenty-four genotypes. Cluster 22,20,18 are having twenty genotypes. Cluster IV is comprising of four genotypes only. It is interesting to observe that most of the genotypes of one cluster were adapted to only one region. The clustering pattern reflects the closeness between the clusters and the geographical adaptation of the genotypes (7).

Table .2. Rice genotypes included in different clusters

Group	n	Cluster Members --->
1	13	38 48 64 86 121 162 222 229 277 325 435 441 473
2	14.	14 29 30 42 47 51 125 130 152 169 185 233 373 390
3	46	1 3 7 9 11 17 28 31 34 37 46 60 62 63 66 72 73 75 87 103 107 144 216 226 237 239 272 285 297 309 321 332 353 363 365 366 372 377 381 385 395 420 423 429 434 464
4	4	67 356 380 467
5	33	4 43 49 65 71 76 94 105 111 112 114 150 172 183 187 221 223 234 236 247 262 276 284 308 328 348 361 374 375 431 433 471 472
6	17	93 101 104 120 126 128 129 131 136 157 161 167 168 174 177 178 182
7	37	10 25 44 45 50 80 83 85 92 98 110 146 147 159 199 203 241 248 282 291 295 301 302 311 324 342 345 350 355 362 369 396 402 418 422 437 469
8	12	99 106 115 127 132 142 149 164 175 181 184 186
9	19	113 151 154 155 158 166 176 179 180 188 189 194 198 202 209 210 211 232 274
10	30	96 97 109 117 133 170 204 206 207 208 217 225 227 259 261 269 279 378 379 383 406 407 408 412 414 442 449 450 451 459
11	6	196 245 250 397 409 410
12	13	100 108 124 134 135 140 163 200 212 230 231 252 463
13	34	8 12 16 23 32 35 70 119 122 123 137 139 156 165 201 215 228 235 238 243 256 273 288 337 346 354 364 370 376 389 393 424 439 458
14	30	219 224 253 254 257 263 268 271 280 287 294 296 298 306 319 327 333 343 349 388 391 394 400 401 426 445 446 452 455 461
15	18	5 6 24 54 88 192 193 246 255 303 310 313 316 318 326 382 428 468
16	23	267 281 289 290 299 320 322 330 331 335 336 340 341 344 352 367 368 392 405 411 413 440 447
17	17	13 18 21 40 118 190 278 286 300 304 305 312 317 334 338 351 357
18	20	143 148 160 173 205 220 242 244 249 270 339 387 398 403 415 427 438 443 453 454
19	23	68 69 77 78 81 102 145 195 197 214 251 258 260 265 275 358 386 399 417 419 444 466 470
20	20	2 33 52 56 58 61 84 138 264 283 307 314 323 347 360 371 425 430 432 457
21	24	15 22 27 41 53 55 57 59 74 90 91 116 141 153 171 240 266 404 416 421 436 448 456 465
22	20	19 20 26 36 39 79 82 89 95 191 213 218 292 293 315 329 359 384 460 462

The average intra and inter cluster D2 values are presented in Table 3. Most of the intra clusters were closely related and cluster D2 values ranged from 45.4(cluster 22) to 10.6(cluster 9). The intra cluster distance analysis revealed that the maximum divergence was observed in cluster 22(45.4). It is reported that genotypes within the cluster with high degree of divergence would produce more desirable breeding materials for achieving maximum genetic advance. The other clusters showing high intra cluster values were cluster 3,17,10,12,7 and cluster 9 is having least intra cluster value(8).

Inter cluster D2 values ranged from 183(cluster 12) to 19.9(cluster 18). From the inter cluster D2 values of the twenty two clusters, it can be observed that the highest divergence was between cluster 12and 22while, lowest divergence was noticed between cluster 20and 18 (19.9). Highly divergent genotypes would produce a broad spectrum of variability in the subsequent generation enabling further selection and improvement, which would facilitate successful breeding of rice. Thus hybrids developed from the distant cross may produce high magnitude of heterosis or desirable transgressive segregants, which would facilitate successful breeding of rice (9).

The number of times that each yield component appeared in first rank and its respective percent contribution towards genetic divergence is presented in Table 4..The results showed that the contribution of Seed vigor towards genetic divergence was highest (96.47%) by taking 107693 times ranking first, followed by Fertile Grains/ Panicle (1.24%) by 1389 times, Grains/ Panicle (1.23%) by 1374 times, Germination % (0.61%) by 680 times. Seed density (0.13%) by 142 times, Sterility % (0.12%) by 138 times. Plant Height, Effective Tillers/ Plant, Leaf Width, Panicle Length, Yield/ Plant, 100-seed Wt, Days to 50% Flowering, Coleoptiles Length, Radical Length and Seed Growth did not contribute towards genetic divergence among the genotypes under investigation (10).

Table .3. Average intra and inter cluster distance values of rice genotypes

	1Cluster	2Cluster	3Cluster	4Cluster	5Cluster	6Cluster	7Cluster	8Cluster	9Cluster	10Cluster	11Cluster	12Cluster	13Cluster	14Cluster	15Cluster	16Cluster	17Cluster	18Cluster	19Cluster	20Cluster	21Cluster	22Cluster
1 Cluster	17.6	25.3	55.5	71.8	28.7	24.5	26.3	27.2	38.3	42.0	34.4	34.5	51.7	26.4	38.5	36.5	27.5	30.5	41.7	29.8	45.4	112.6
2 Cluster		18.9	60.6	65.0	36.9	25.8	32.3	37.6	44.4	40.3	33.7	61.2	46.4	27.3	41.6	43.3	30.9	41.2	42.1	33.6	46.3	106.3
3 Cluster			40.1	69.1	67.3	68.8	73.9	73.2	84.8	87.7	80.0	104.4	96.5	63.4	67.0	74.2	54.8	86.4	99.3	76.8	49.0	66.5
4 Cluster				24.2	51.2	60.3	63.4	78.0	83.0	90.1	65.5	80.8	97.0	92.3	109.4	133.9	78.8	111.7	126.2	100.2	115.9	154.0
5 Cluster					15.8	20.8	27.3	24.7	32.0	48.4	32.2	35.5	42.8	47.5	56.5	67.4	34.9	44.7	61.6	41.3	78.1	146.6
6 Cluster						16.0	25.2	25.1	32.1	38.8	29.6	37.4	35.4	35.8	45.1	32.4	29.4	38.0	46.6	31.1	63.7	130.7
7 Cluster							20.4	32.5	48.6	58.6	42.8	62.0	58.1	45.8	64.9	65.8	46.8	44.9	57.0	45.5	74.1	146.5
8 Cluster								17.3	21.4	37.4	27.8	40.5	37.4	33.4	37.0	46.1	27.2	25.1	36.8	23.9	61.1	145.4
9 Cluster									10.6	25.1	22.7	31.9	33.3	33.9	28.4	47.2	26.1	25.6	30.9	22.8	66.6	156.0
10 Cluster										24.6	28.5	42.8	44.9	30.1	27.6	40.9	29.5	33.3	32.8	27.1	58.3	139.7
11 Cluster											17.9	36.0	37.9	32.5	39.0	51.6	29.1	34.0	38.9	28.5	65.8	150.7
12 Cluster												20.7	45.3	61.1	58.7	76.9	43.4	56.0	70.5	47.1	102.6	183.2
13 Cluster													26.5	50.5	47.3	62.2	38.0	48.5	49.2	36.5	77.0	149.0
14 Cluster														16.9	21.9	25.3	25.0	25.5	26.1	21.2	52.4	104.9
15 Cluster																13.1	21.3	21.5	27.5	26.4	20.9	99.0
16 Cluster																	16.5	29.3	29.3	31.4	26.6	99.3
17 Cluster																		15.5	30.5	36.7	21.8	103.7
18 Cluster																			15.8	22.6	19.9	142.4
19 Cluster																				17.3	22.3	142.6
20 Cluster																					14.9	125.7
21 Cluster																						38.9
22 Cluster																						45.4

Table.4. Relative contribution of different traits to genetic diversity in rice

Source	Times Ranked	Contribution %
1 Tillers/Plant	7	0.01
2 Effective Tillers/ Plant	0	0.00
3 Plant Height cm	137	0.12
4 Leaf Length cm	58	0.05
5 Leaf Width cm	0	0.00
6 Panicle Length cm	0	0.00
7 Sterility %	138	0.12
8 Yield/ Plant	0	0.00
9 100-seed Wt	0	0.00
10 Seed Density	142	0.13
11 Grains/ Panicle	1374	1.23
12 Fertile Grains/ Panicle	1389	1.24
13 Pollen Viability	10	0.01
14 Days to 50% Flowering	0	0.00
15 Germination %	680	0.61
16 Coleoptiles Length (cm)	0	0.00
17 Radical Length (cm)	0	0.00
18 Seed Growth (cm)	0	0.00
19 Seed vigor	107693	96.47

IV. Conclusion:

In the present investigation, it is suggested that hybridisation programme within the divergent cluster 12 and 18 are expected to give promising and desirable recombinants in the segregating generations. Also, traits contributing maximum to genetic divergence viz. Seed vigor followed by Fertile Grains/ Panicle, Fertile Grains/ Panicle and panicle length may be utilized in selecting genetically diverse parents.

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