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Breeding for high yielding rice (*Oryza sativa* L.) varieties and hybrids adapted to aerobic (non-flooded, irrigated) conditions — I. Preliminary evaluation of a large number of improved germplasm lines

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Abstract

During wet season 2003, over 2600 improved germplasm lines, including 600 drought tolerant introgression lines, 800 indica/tropical japonica derivatives and 800 hybrid rice (*Oryza sativa* L.) breeding materials were screened in a puddled field (clay soil) under non-flooded, irrigated (aerobic) conditions with Rasi and Vandana as check varieties. The checks, known for their water stress tolerance were planted at regular intervals (after every ten entries) to serve as controls. In both the checks, within as well as between the strips, there was no variation for days to heading, whereas a significant variation was found for grain yield character. Apart from visual selection, three different methods utilizing appropriate statistics were employed for identifying promising genotypes. Among the three methods studied, the bias due to overestimation of strip mean of the check variety (method 1) and identification of false positives by nearest-neighbor analysis (method 2) were eliminated by adopting standardized grid selection procedure (method 3). Further, it was found to be very consistent and effective and therefore a suitable method for employing in large breeding populations and/over wide range of environmental variations in the experimental field.

Key words: Aerobic rice, moisture stress, days to heading, grain yield, nearest-neighbor analysis, standardized grid selection

Introduction

The abundant water environment in which rice grows best, differentiates it from all other important crops. But, water is becoming increasingly scarce. Per capita availability of water resource declined by 40-60% in many Asian countries between 1955 to 1990 [1] and more than 50% of the population in developing countries is exposed to polluted water resources. For various reasons such as diminishing rainfall, depletion of ground water resources and increasing demand from other sectors, the availability of water for agriculture will diminish both in quantity and quality in the years to come [2]. Yet, more rice needs to be produced with less and less water to feed the ever increasing population. Of all the crops grown under irrigation, more than 50-60% of the irrigation water is used for rice, the staple food for nearly half the world's population. Since more than 90% of the world's rice is produced and consumed in Asia, water scarcity will be a threat to food security in this region. Future rice production will therefore depend primarily on developing and adopting strategies that will use water more efficiently in irrigation schemes. While significant advances have been made in irrigation management, the genetic research to alter the basic water requirement of rice has not been encouraging. In Brazil and Latin America, traditional uplands were converted into intensive, market integrated cultivation systems with sprinkler irrigation facility suitable for high input management. This system of rice cultivation with controlled irrigation facility became popularly known as 'aerobic rice'. Traditionally, aerobic rice is grown in rainfed uplands with low or no inputs. With declining water availability for agricultural use, aerobic rice cultivation is expected to expand into the irrigated, intensive and high productivity cropping systems [3]. However, there are variations in the use of term 'aerobic rice' [4] under puddled soil condition which is invariably followed to minimize irrigation water loss. The genetic improvement of rice for aerobic (non-flooded) environments has not been understood well and major efforts on this front are lacking. Keeping these in view, we conducted preliminary studies involving more than 2600 improved germplasm lines to examine yield variation and to identify genotypes suitable for cultivation under limited water use.

Materials and methods

During wet season 2003, over 2600 improved germplasm lines including 600 drought tolerant introgression lines, 800 *indica/japonica* derivatives and 800 hybrid rice breeding materials etc. as detailed in Table 1 were evaluated in field under limited water conditions. The seeds were sown on 30th June, 2003 and seedlings transplanted in a puddled field on 4th August, 2003.

Table 1. Categories of genetic material screened under aerobic (non-flooded, irrigated) conditions

Category	Number
Drought tolerant introgression lines	600
Indica / tropical *japonica* derivatives	800
Hybrid rice breeding lines	800
Released varieties	225
Released hybrids and their parents	50
DH lines and some donor germplasm	175
Total	2650

Each entry was represented by a single row of 3.5 m length, a spacing of 20 cm between rows and 15 cm between plants was followed. After every ten entries, drought tolerant check varieties Rasi and Vandana were planted. Thus, over 2600 entries were planted in 6 strips of 100 m length. The field lay out depicting arrangement of screening material and check varieties is presented in Fig. 1.

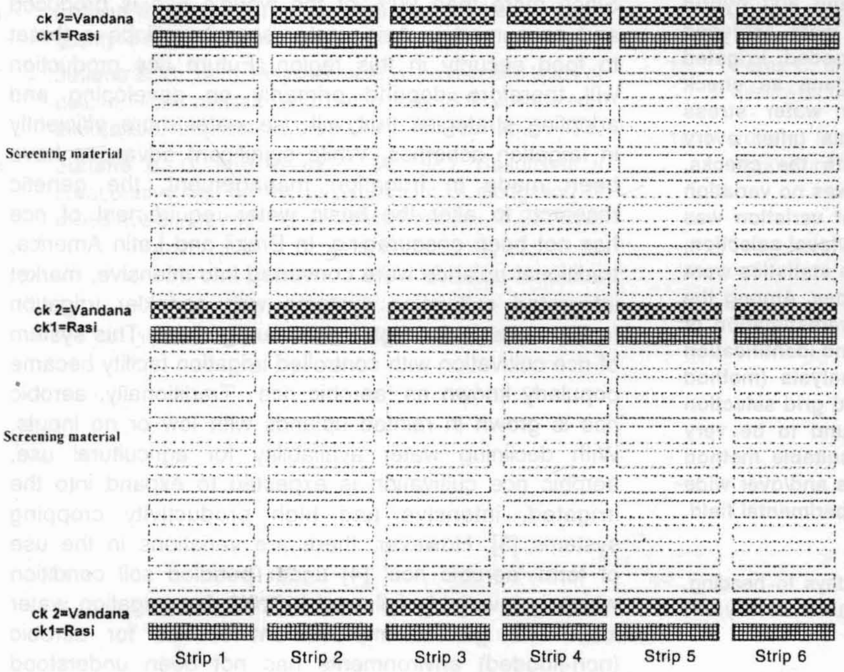


Fig. 1. Field layout

After planting, the experimental plot was maintained as rainfed. Water accumulated due to few rains was completely drained on 23rd August, 2003 and the field was allowed to dry for about 20 days. On September 12th a flush irrigation was given followed by top dressing with urea. Subsequently, the crop was maintained without any irrigation till maturity. The amount of rain received during the crop growth i.e., from planting to maturity is shown in Fig. 2. Each entry was evaluated visually for plant height, tillering ability and heading. At maturity, five plants from each entry including the checks were chosen at random for recording grain yield.

Identification of desirable genotypes: In order to identify desirable genotypes, three different methods in addition to visual selection were employed. In the first method, the entries with higher grain yield than the overall mean of the check variety in each strip were identified. That is, each strip would have different mean value for the check variety depending on the extent of stress. In the second method, Nearest-Neighbor analysis as detailed in MSTATC was used. Neighbor analysis is an iterative process which estimates the true yield of several varieties that are randomly arranged in a long narrow field. In this method, each plot/entry will be compared with its neighbors on either side and update yield estimate of that plot/entry. It is possible to iterate this process as many times as desired. The third method was analogous to Gardner's [5] stratification technique, wherein, each strip is divided into several grids each containing ten test entries and two check varieties on both sides. The grain yield of test entries was standardized against the mean yield of check variety in each grid and expressed as percentage of check variety's performance. It is presented as $\hat{Y}_{ij} = (X_{ij} - \bar{X}_j) / \bar{X}_j \times 100$, where, Y_{ij} is the estimated yield of ith entry in jth grid as percentage over the check variety, X_{ij} is the observed yield of the ith entry in jth grid and $\bar{X}_j$ is the mean yield of check variety in the jth grid. The three methods were compared for their efficiency in identifying desirable genotypes.

Results and discussion

Table 2 shows mean days to heading of check varieties Rasi and Vandana in six strips. On the overall basis, the variety Vandana took 69 days for heading, while Rasi required 81 days, showing a difference of 10-12 days between the check varieties. The variation in heading of checks between strips was insignificant with CV ranging from 0.0 to 2.7%. These results amply indicate that days to heading is less varying within a given condition unlike other yield traits. This is probably associated with high heritability of this trait. Under normal irrigated conditions, differences between replications for heading are less common, while the present investigation demonstrate that even under varying stress levels, heading of a given check was not affected. A number of studies indicate that heading is a simply inherited trait and is less influenced by environmental variations within the field. Two sets of major genes reported to affect heading differentially. The first set of genes (two or three) affect Basic

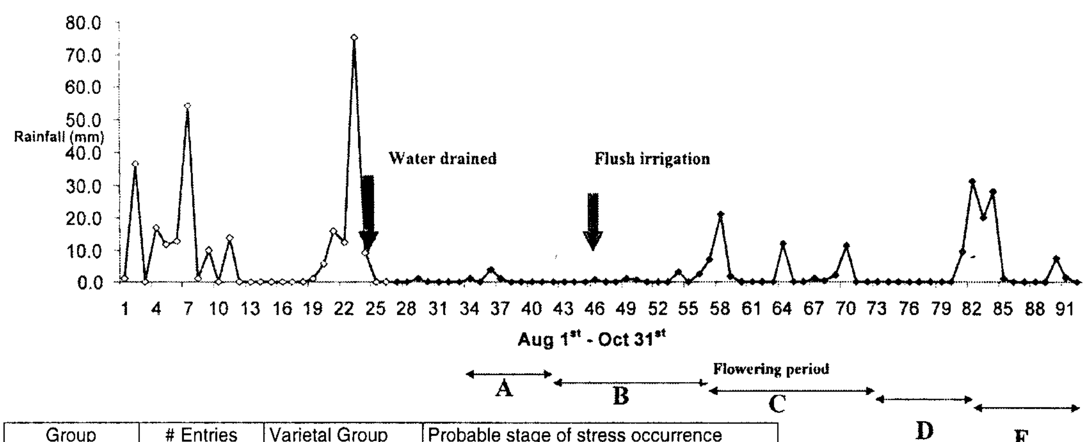
Table 2. Variation in Days to heading of check varieties, Rasi and Vandana under aerobic condition

Rasi							Vandana					
S1	S2	S3	S4	S5	S6		S1	S2	S3	S4	S5	S6
80	79	84	84	84	82		71	69	69	67	69	67
82	79	82	84	84	82		69	69	69	69	69	67
80	79	79	82	84	79		71	69	67	69	69	67
80	79	79	84	84	79		69	69	69	67	69	67
80	79	79	84	84	79		71	69	69	69	69	69
82	79	79	84	84	79		69	69	69	69	69	69
79	79	79	84	84	79		69	69	69	69	69	69
79	79	79	84	84	79		69	69	69	69	69	69
80	79	79	84	84	79		69	69	69	69	69	69
79	79	84	84	84	79		69	69	69	69	69	69
80	79	84	84	84	79		69	69	67	69	69	69
80	79	84	82	84	82		69	69	69	69	69	69
80	79	84	84	84	82		69	69	67	69	69	69
79	79	84	84	84	82		69	69	69	69	69	69
80	82	84	84	84	82		69	69	69	69	69	69
80	82	84	84	84	79		70	69	69	69	69	69
80	79	84	84	84	80		69	69	69	69	69	69
80	79	84	84	84	80		69	69	69	69	69	69
80	79	84	84	84	84		69	69	69	69	69	69
79	79	84	79	84	82		69	69	69	69	69	69
80	79	82	79	84	84		69	69	69	69	69	69
79	79	84	79	84	84		69	69	69	69	69	69
79	79	84	79	84	82		69	69	69	69	69	67
79	79	84	79	84	84		69	69	69	69	69	69
79	79	84	79	84	84		69	69	69	69	69	67
79	79	84	84	84	84		69	69	69	69	69	
							69	69	67	69	69	
79.8	**79.2**	**82.5**	**82.7**	**84.0**	**81.2**	**Mean**	**69.3**	**69.0**	**68.7**	**68.9**	**69.0**	**68.5**
1.02	1.03	2.7	2.6	0	2.5	CV(%)	0.95	0	1.05	0.77	0	1.27

S = Strip

Vegetative Growth (BVG) [6] of which one major gene, Ef-1 is known to control BVG is located on chromosome 10 [7]. The second set of genes, which include Se-1 (Lm), Se-3, Se-4, Se-5, Se-6, Se-7, E1, E2 and E3 [8-13] are reported to affect Photoperiod Sensitivity (PS).

The grain yield of check varieties Rasi and Vandana in different strips along with plots/entries associated with decreasing yield trends are presented in Table 3. The average strip yield of Vandana varied from 31.5 g to 68.4 g with CV's ranging from 26.8 to 49.4% while that of Rasi ranged from 54.1 g to 87.6 g with CV's ranging from 15.7 to 29.1% indicating larger variations in the yields of Vandana within and between the strips compared to Rasi. However, unlike heading, the variation in grain yield of check varieties was in general high. It has long been observed that yield being a polygenic trait, has low heritability, particularly under stress situations compared to normal irrigated conditions. This is probably why direct selection for yield under moisture stress has not been given much importance as it is understood to have poor heritability which affects selection efficiency. Further, difficulty in imposing uniform stress on a large diverse breeding population at a defined physiological stage is challenging. Thus, such selection programs were not only expensive, but also slow in attaining progress [14-16]. On the contrary, Blum [17] obtained relatively higher heritability estimates for grain yield under stressed (0.79) compared to control (0.62) conditions. In two different years in a doubled haploid population, Lafitte and Courtois [18] reported heritability estimates for grain yield under control and stress to be 0.75, 0.49, 0.20 and 0.20 respectively. Contrary to the findings of Babu *et al.*, [19], Lanceras *et al.*, [20] found that heritability



Group	# Entries	Varietal Group	Probable stage of stress occurrence
A	6	Vandana	Mild-post flowering
B	77	Rasi	Panicle development
C	316	IR 64	Tillering and panicle development
D	172	Pusa Basmati	Vegetative
E	25	Amulya	Vegetative

Fig. 2. Distribution of rainfall, flowering period and stage of occurence of stress

Table 3. Grain yield (g) of check varieties, Rasi and Vandana in different strips along with plots showing declining yield trends (PDYT)

Strip-1					Strip-2					Strip-3				
Vandana		Rasi			Vandana		Rasi			Vandana		Rasi		
Plot No	GY (g)	Plot No	GY (g)	PDYT	Plot No	GY (g)	Plot No	GY (g)	PDYT	Plot No	GY (g)	Plot No	GY (g)	PDYT
1	91.5	13	82.4	15-23	1	79.6	13	53.2	2-4					
12	46.9	25	111.4	29-36	12	38.3	25	31.8	13-15	1	99.9	13	56.1	8-19
24	41.0	37	96.2	40-51	24	24.7	36	68.0	24-27	24	60.7	25	63.7	27-29
36	32.2	49	79.8	92-96	35	24.6	47	68.8	61-63	36	27.5	37	77.9	56-62
48	28.1	61	63.4	112-116	46	24.5	58	58.7	66-70	60	33.9	49	77.3	75-81
60	39.1	73	99.2	126-141	57	51.7	69	48.6	74-80	72	40.4	61	82.3	95-105
72	29.6	84	87.0	166-172	68	23.6	81	95.6	91-93	84	22.5	96	72.6	142-146
95	29.0	96	65.4	190-193	80	20.7	91	49.2	147-179	95	48.6	109	40.4	156-174
108	49.8	109	95.5	201-213	90	36.6	103	43.8	183-187	120	31.1	121	79.0	179-186
119	70.6	120	81.6	221-228	102	28.0	114	38.4	203-210	132	45.1	143	73.8	214-218
131	65.3	142	94.1	299-302	147	35.0	136	44.8	216-221	154	50.2	155	65.7	234-241
153	60.0	154	101.3	306-308	203	33.0	148	61.0	228-238	166	38.7	177	52.2	253-257
164	58.4	175	71.6	312-324	245	30.0	170	47.3	245-251	188	27.3	199	46.6	268-274
186	60.9	197	97.0	328-333	278	23.4	214	61.0	257-266	232	26.9	221	41.0	295-297
208	48.4	219	84.0	339-344	321	29.0	256	74.7	269-330	265	25.0	243	44.5	309-312
229	36.0	240	87.4	389-392	342	21.0	289	42.0	334-401	308	23.1	264	55.7	322-326
250	46.2	262	76.8	411-413	363	23.0	310	48.8	419-424	352	20.6	276	37.0	331-334
261	56.4	273	101.0		383	24.0	352	48.8		410	24.3	319	36.3	343-349
283	66.4	294	86.0		401	28.5	373	41.0		431	30.0	386	48.5	365-368
304	50.3	315	79.7				393	48.8		450	51.6			381-385
326	49.6	337	73.4				405	61.7						439-442
348	32.3	358	75.2											
369	40.5	378	103.1											
389	28.8	398	83.8											
409	37.9	417	64.6											
426	48.0	434	64.8											
448	53.2													
Mean	**48.01**		**84.83**		**Mean**	**31.54**		**54.10**		**Mean**	**38.28**		**58.37**	
CV(%)	31.5		15.7		CV(%)	43.9		26.7		CV(%)	49.4		27.3	

Table 3 contd...

Strip-4					Strip-5					Strip-6				
Vandana		Rasi			Vandana		Rasi			Vandana		Rasi		
Plot No	GY (g)	Plot No	GY (g)	PDYT	Plot No	GY (g)	Plot No	GY (g)	PDYT	Plot No	GY (g)	Plot No	GY (g)	PDYT
1	97.0	25	63.4	3-22	1	110.4	13	121.6	10-12					
12	29.0	61	67.3	29-35	12	35.6	25	81.5	26-29	1	102.5	9	58.0	5-8
24	47.2	97	111.0	42-47	36	37.4	37	77.6	35-37	8	27.5	21	40.1	19-23
36	53.8	109	73.0	65-70	47	53.5	48	61.4	41-46	49	50.4	31	69.9	30-50
48	50.1	121	78.1	75-81	58	33.5	59	37.2	48-66	70	73.2	50	35.0	63-65
72	43.8	132	85.5	85-87	82	29.1	71	100.3	73-91	80	69.0	71	91.2	80-81
84	48.1	143	60.3	93-96	93	74.1	94	107.8	96-103	136	65.8	115	94.9	87-89
96	39.1	155	64.0	111-115	149	72.3	105	88.2	107-113	190	62.7	126	77.3	103-110
154	34.8	177	47.5	147-150	159	51.8	117	50.6	117-137	211	75.2	158	60.6	124-131
209	27.0	220	39.0	156-163	168	39.7	139	119.9	140-145	232	60.6	179	109.6	138-160
253	20.4	242	46.5	168-174	182	52.4	150	106.5	151-171	244	85.1	200	94.1	167-173
265	23.0	264	54.0	180-185	193	55.2	174	103.7	197-233	266	57.0	222	87.0	239-240
287	21.2	276	33.0	202-207	226	49.0	187	98.1	242-254	276	55.1	243	83.6	249-253
309	36.0	298	36.0	210-219	245	36.2	195	118.3	270-274	286	47.2	311	80.4	268-284
331	29.0	342	35.6	225-227	264	53.4	208	107.8	278-302	351	70.7	362	65.5	292-294
387	27.3	364	40.6	255-261	270	64.2	217	102.3	310-313	385	69.1	374	116.5	307-318
427	25.6	409	47.7	291-297	279	61.2	233	50.7		417	85.0	407	71.8	362-365
448	70.7	434	58.1	333-336	313	63.3	240	97.6		424	100.6	434	100.2	392-401
				365-369			249	70.6		449	74.5			
				376-380			257	85.7						
				388-394			288	72.7						
							298	66.7						
Mean	**40.17**		**55.96**		**Mean**	**54.02**		**87.58**		**Mean**	**68.40**		**78.57**	
CV(%)	48.5		29.1		CV(%)	35.8		27.5		CV(%)	26.8		28.5	

GY = Grian yield; PDYT = Plots showing declining yield trends for grain yield under stress was very low (0.39). However, Atlin *et al.*, [21] suggested that selection for grain yield in a carefully designed and well managed stress environments is more rewarding rather than making selection under highly variable conditions.

The nearest-neighbor analysis generates a map file indicating the plots which are associated with declining yield trends (PDYT). Such plots/entries showing declining yield trends are presented in Table 3.

A careful examination of these and the yield of nearby check varieties reveal that the trend in check yields closely correspond to the PDYT in each strip which suggests that heterogenity in soil moisture and nutrient availability are very well reflected in the yield values of check varieties. In other words, whenever the yield of test entries were low they were always reflected in the performance of check varieties. This kind of information is very useful for adjusting the yields of test entries in a given strip with large variations for effective comparison among the test entries.

The distribution of rainfall during the crop growth and at the time of flowering of 596 entries which were selected on visual basis is presented in Fig. 2. Based on the heading date and the distribution of rainfall during the season, five groups were made as detailed in the figure. The group-A included six entries, where Vandana a rainfed upland variety flowered earliest. The entries in this group are expected to have experienced mild stress during post flowering period. The group-B consisted of 77 entries with Rasi, another variety known to adapt to water stress as a representative. The group-C was the largest having 316 entries with varieties Annada and IR 64 as the representatives which started flowering 55 days after planting. *Indica* / tropical *japonica* derivatives and drought tolerant introgression lines predominated this group. The group-D was the second largest with 172 entries consisting predominantly *indica* / tropical *japonica* derivatives. These belonged to maturity group of Pusa Basmati 1. The group-E was the late maturity group with 25 entries. It should be noted that under this kind of screening, most entries experience a mild stress throughout crop growth period.

Given that the yield under stress is a low heritable trait and the estimates vary largely between experiments, it remains to be asked as to how to select for desirable/promising entries or genotypes under stress environments. In order to find effective method for selection of desirable genotypes under large scale testing of breeding material/populations, we examined three different methods. The results on number of genotypes selected against Rasi and their average yield in each of the methods is presented in Table 4.

Table 4. Comparison of different methods of identifying desirable genotypes

Particulars\ methods	Overall strip mean of check variety (1)	Nearest-neighbor analysis (2)	Standardised grid selection (3)
No. of selected entries	220	306	360
Mean yield (g /5pl) of selected entries	78.3	66.4	68.4

Of the three methods, highest number of genotypes were selected in the 3rd (Standardized grid selection) method followed by nearest-neighbor analysis (2nd method). Highest mean yield of 78 g of selected entries was observed in the 1st method followed by the 3rd method (68.4 g). In the 1st method, the mean yield of check variety was overestimated because of higher yields recorded along the ends of strip and alleyways provided for irrigation. Because of this overestimation, some of the entries located at relatively more stressed areas within a strip recorded lower yields. Although they were really potential entries for the water stress conditions, yet they got unselected. Thus, less number of entries were selected by this method resulting in higher mean value.

In the nearest neighbor analysis a good number of entries (306) were selected. However, this method was observed to be slightly biased. As described in methodology, the yield of every plot/entry is readjusted relative to its neighbors. Thus, some genotypes that do not have good *per se* performance also get overestimated due to its neighbors leading to selection of certain false positive entries. On the other hand, the 3rd method, eliminates such bias, wherein the yields of each of the test entries are expressed in terms of mean of check variety in the respective grid.

Gridding of the field or Gardner's stratification technique is very important and reduces the large environmental variations between plots. There have been numerous examples [22] to show that the efficiency of selection is greatly enhanced by reduced phenotypic variation and lowered selection differential which in turn resulted greater selection response. A method similar to the one presented in this paper was adopted by Bos [23] for adjusting the grid mean in order to select the variable number of individuals among grids which further improved selection efficiency.

Among the three methods considered in the present study to identify promising genotypes, the Standardized Grid Selection method was found to be consistent. Entries identified by this method were also identified in other methods, but not *vice-versa*. Though visual selection also helps in identifying most of the promising entries, the number of entries not identified by other methods remained very high. Further, when heritability of yield under stress is observed to be low, the number of unsuccessful selections on visual basis would be very high.

Based on the foregoing results it is concluded that heading is a highly heritable trait even under stress conditions as indicated by low variation unlike grain yield which exhibited significant variation between plots even within the strip. The standardized grid selection method was found to be very effective while dealing with large breeding populations and/environmental variations.

In essence, this attempt to segment, examine and group together homogenous plots or promising genotypes in a large experimental area under varying stress conditions has not only been successful, but it also helped to identify suitable method for evaluation of a large number of genotypes.

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