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Efficiency of honeycomb selection design in early segregating generation in ADT45 × Nona Bokra cross under salt stress

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Salt stress is a major abiotic stress that limits rice productivity worldwide including India. As modern rice varieties are salt sensitive, infusing salt tolerance through breeding is a viable farmer-friendly approach. Breeding salt tolerant rice varieties has been slow due to complexity of the trait and high Genotype × Environment interaction in the salt affected field. Selection practiced in such a situation using conventional selection design (CSD), in the presence of competition, would be misleading. On the otherhand, selection is effective in the absence of competition in honeycomb selection and counteracts the disturbing effects of competition on effectiveness of selection. In the present study, we tested the efficiency of honeycomb selection design (HSD) in early generation of a rice cross genetically in improving the yield and practice selection. All the characters studied in both the design showed non normal distribution except for panicle length in CSD. All the characters studied in both the design had lower coefficient of variation, high mean and high standard deviation in the HSD compared to CSD. Large number of genes with duplicate epistasis governs days to flowering whereas panicle length and single plant yield are governed by few number of genes with complimentary epistasis. Twenty nine F_2 plants each in CSD and HSD were selected based on mean and plant index (SPY), respectively. Plants selected in HSD recorded higher percentage of increase over base population compared to CSD and found HSD to be superior to CSD because of enhanced phenotypic expression in the former by eliminating confounding effects of negative correlation between yielding and competitive ability.

Keywords: Abiotic stress, *Oryza sativa*, Paddy, Rice

Among varied abiotic stresses that limit crop productivity, salt stress is regarded important next to drought. It influences 20% of irrigated land (approx. 45 million ha) and 2% (approx. 32 million ha) of dryland worldwide, that accounts to one-third of total rice growing areas[1]. Rice (*Oryza sativa* L.), one of the most important staple crops in many countries where it forms main source of food, is considered salt sensitive[1]. Rice crop responds differently to salt stress during its growth period as evident from its tolerance at germination and active tillering stages and sensitive at early seedling and reproductive stages[2]. Between these two sensitive stages, reproductive stage tolerance is as critical as it finally decides the grain yield under salt stress[3].

Plant breeders normally consider densely grown field plot as the unit of phenotyping and evaluation for plant breeding purposes, but this fails to improve the efficiency of selection and the corresponding genetic gain[4]. This can be overcome by honeycomb selection design (HSD) which accomplishes three distinct functions *viz.*, effective sampling for environmental diversity, effective selection among and within genetic entries and simultaneous selection [5,6]. In light of the above facts, the present investigation was undertaken with the objective of testing the efficiency of HSD in studying the genetics of yield and practising selection for yield under salt stress condition.

Materials and Methods

Plant material

The experimental material consisted of F_2 segregating population generated from a cross between ADT 45 and Nona Bokra. ADT 45, a popular short duration rice variety of Tamil Nadu with agronomic traits such as heavy tillering, a short stature and early maturing, but susceptible to salinity, was used as the female parent. Nona Bokra, an *indica* land race of West Bengal, is highly salt tolerant and extensively used in breeding for salt tolerance similar

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to that of Pokkali, was used as the male parent. CSR10, a short duration salt-tolerant rice variety was used as check in this experiment. True F_1 seeds were identified based on molecular marker analysis (data not shown) and 35 true F_1 plants were raised. Out of 35 plants, F_2 seeds from one single F_1 plant was used as experimental material in the study. A total of 1200 F_2 plants were raised in 40 rows with 30 plants each at a spacing of 20 × 15 cm in conventional selection design (CSD) along with parents. In case of HSD, 1500 plants (1285 F_2 plants and 215 CSR10 plants) were raised in 50 rows with 30 plants each at a spacing of 90 cm between rows and 100 cm between plants. (Fig. 1). In un-replicated honeycomb trials, plants are allocated in the field randomly so that the plants were included within a moving ring of a certain size. In the unreplicated-1 (UNR-1) honeycomb design, the single check CSR10 (salt tolerant early check) occupied 14.3% of the positions accounting for 215 plants occurring adjacent to every plant. Evaluation of each plant was done by comparing the yield of each plant, positioned in the center of the ring, with the yield of its neighboring plants within the ring in which a plant was selected only if it out yields the other plants within the ring[5] and the size of the circle determines the intensity of selection. The choice of the size of the moving ring depends on the genetic structure, size of the population and degrees of soil homogeneity.

Fig. 1 — Layout of Honeycomb Selection Design (HSD)

Phenotyping under saline field condition

The trial plots were irrigated with sodic bore-well water to impose the salt stress and to characterize the stress nine piezometers were placed in the field and the root zone water samples collected at fortnightly interval for measuring EC (Electrical conductivity measured in dSm-1) and pH. The EC of the root zone water (Table 1) ranged from 1.20 dSm^{-1} to 2.75 dSm^{-1} whereas pH (Table 2) ranged between 8.19 and 9.45. Highest spatial range was observed at 76 days after sowing with 1.46-2.75 dSm^{-1} for EC and at 15 days before sowing with 8.28-9.37 for pH. While highest temporal range was observed at piezometer spot No. 5 with 1.80-2.75 dSm^{-1} for EC and with 8.31-9.41 for pH. Great spatial and temporal heterogeneity were observed in the nine spots and over fortnight interval showing that the field exhibited tremendous soil

Table 1 — Characterization of stress (A) EC; and (B) pH of root zone water at peizometer spots

Peizometer spots	5 DBS	15 DAS	36 DAS	60 DAS	76 DAS	86 DAS	95 DAS	102 DAS	114 DAS	Temporal range
(A) EC of root zone water at peizometer spots										
1	1.78	1.75	1.95	1.80	2.19	2.26	2.30	2.24	2.15	1.75-2.30
2	1.28	1.21	1.36	1.47	1.46	1.48	1.42	1.32	1.28	1.21-1.48
3	1.20	2.08	2.10	2.10	1.95	1.84	1.93	1.51	1.60	1.20-2.10
4	1.60	1.98	1.89	1.93	1.86	1.92	1.59	1.53	1.75	1.53-1.98
5	1.99	1.91	1.80	1.89	2.75	2.63	2.47	2.30	2.19	1.80-2.75
6	1.77	1.75	1.62	1.74	1.84	1.94	2.06	2.18	1.81	1.62-2.18
7	1.94	1.85	1.74	1.76	1.94	2.31	1.84	1.88	1.95	1.74-2.31
8	1.98	1.89	1.75	1.80	2.22	2.45	2.65	2.44	2.12	1.75-2.65
9	1.80	1.78	1.72	1.42	1.68	1.60	1.68	1.44	1.53	1.42-1.80
Spatial range	1.20-1.99	1.21-2.08	1.36-2.10	1.42-2.10	1.46-2.75	1.48-2.63	1.42-2.65	1.32-2.44	1.28-2.19	
(B) pH of root zone water at peizometer spots										
1	8.59	8.55	8.51	8.52	8.38	8.40	8.88	8.80	8.70	8.38-8.88
2	8.81	8.76	8.80	8.90	8.83	9.03	9.14	9.02	8.92	8.76-9.14
3	9.37	9.34	9.45	9.35	8.54	8.74	9.18	9.43	8.92	8.54-9.45
4	8.88	8.84	8.92	9.06	8.25	8.63	8.98	9.11	9.29	8.25-9.29
5	8.41	8.31	8.36	8.50	9.10	8.54	8.90	9.21	9.41	8.31-9.41
6	8.63	8.54	8.65	8.72	8.30	8.76	9.04	8.84	9.01	8.30-9.04
7	8.71	8.67	8.73	8.80	8.45	8.83	8.93	8.82	9.05	8.45-9.05
8	8.66	8.58	8.69	8.80	8.28	8.95	9.14	9.01	8.80	8.28-9.14
9	8.28	8.19	8.31	8.45	8.36	8.70	8.94	8.82	8.68	8.19-8.94
Spatial range	8.28-9.37	8.19-9.34	8.31-9.45	8.45-9.35	8.25-9.10	8.40-9.03	8.88-9.14	8.80-9.43	8.68-9.41	

[DBS, Days before sowing; and DAS, Days after sowing]

Table 2 — Test of normality, skewness, kurtosis of phenotypic characters in F_2 population of ADT45 × Nona Bokra under conventional selection design (HSD) and honeycomb selection design (HSD)

Particulars	Conventional selection design (CSD)					Honeycomb selection design (HSD)				
	DAF (days)	PHT (cm)	PTN (No.)	PNL(cm)	SPY (g)	DAF (days)	PHT (cm)	PTN (No.)	PNL(cm)	SPY (g)
W value	0.91	0.96	0.99	0.99	0.90	0.95	0.99	0.99	0.97	0.90
Probability	<0.0000	<0.0000	<0.0243	<0.2612	<0.0000	<0.0000	<0.0000	<0.0000	<0.0000	<0.0000
Skewness	–0.40**	0.39**	0.22**	–0.02	1.04**	0.73**	0.01	0.36**	0.56**	1.44**
T-value	–3.92	3.82	2.16	–0.20	10.20	10.74	0.15	5.29	8.24	21.18
Kurtosis	–1.20**	–0.70**	0.04	0.11	0.34	0.98**	–0.11	0.04	5.94**	3.16**
T-value	–5.91	–3.45	0.20	0.54	1.67	7.15	–0.80	0.29	43.36	23.07

[*,**Significance at 5% and 1%, respectively]

heterogeneity. This imposed the need for effective sampling of soil heterogeneity so that plants can be selected from low and high salinity spots. The weather was moderate sunny with intermittent rainfall during the crop period with minimum temperature of 24-27°C and maximum temperature of 31°C-37°C. A mean relative humidity of 72-92% and 47-74% was observed during morning and evening, respectively. A total rainfall of 1604 mm with 61 rainy days was recorded during June-December 2019. Normal agronomic package of practices was adopted in the trial crops.

The phenotypic observation such as days to flowering (DAF), plant height (PHT), number of productive tillers (PTL), panicle length (PNL) and single plant yield (SPY) (adjusted to 14% moisture) were recorded from all the plants on single plant basis in HSD and in randomly chosen 240 plants among 1200 plants in CSD.

Statistical analysis

The test for normality and statistical parameters like range, mean, variance, standard deviation, coefficient of variation, skewness, kurtosis were estimated using statistical package STAR version 2.0.1 software. For the plants raised in HSD, JMP Add-In (Version 13.2.1)[5], a computer program was used for construction of un-replicated honeycomb design and for the analysis of data to select the best entries and the best plants per entry[5]. The column SPY was assigned to the moving ring response and moving ring of 18 plants (MR = 18) was selected. In the un-replicated honeycomb designs, selection of the best plants was performed based on the Plant Index ($PI = (x/\bar{x}_r)^2$) which measures the plant yield devoid of the confounding effects of soil heterogeneity, where x is the yield of each plant and $\bar{x}_r$ is the mean yield of the surrounding plants within the specified moving ring[5]. Based on PI (SPY), best F_2 plants were selected. Whereas in conventional selection design, plants exceeding mean ± 2 SE for single plant yield and other desirable characters were selected.

Results

Test of normality of phenotypic characters in CSD and HSD

Only panicle length length in conventional selection design showed normal distribution (Table 2) while remaining characters such as days to flowering, plant height, number of productive tillers, single plant yield in both CSD and HSD and panicle length in HSD showed non normal distribution. Only days to flowering in CSD showed significant negative skewness (Table 2), whereas the remaining characters such plant height (0.39), number of productive tillers (0.22) and single plant yield (1.04) in CSD and days to flowering (0.73), number of productive tillers (0.36), panicle length (0.56) and single plant yield (1.44) in HSD were significantly and positively skewed. Panicle length (5.94) and single plant yield (3.16) showed significant leptokurtic distribution with kurtosis value more than 3. While days to flowering (-1.20) and plant height (-0.70) in CSD and days to flowering (0.98) in HSD showed significant platykurtic distribution (Table 2). Days to flowering in CSD was negatively skewed with platykurtic distribution while plant height in CSD and days to flowering in HSD were positively skewed with platykurtic distribution. Panicle length and single plant yield in HSD were positively skewed with leptokurtic distribution.

Descriptive statistics of phenotypic characters in CSD and HSD

Days to flowering ranged from 61 days to 94 days (Table 3 and Fig. 2) with a CV of 16.53 %, mean of 79.62 days and S.D of 9.98 days in CSD. In HSD, the CV of 15.32 %, mean of 83.66 days and S.D of 12.83 days were observed. It ranged from 61 days to 126 days. Similar trend was followed for all characters studied *i.e.*, plant height recorded high CV of 25.67% in CSD compared to 22.74% in HSD which is lower. Plant height ranged from 55 cm to 180 cm with low mean and SD of 105.25 cm and 27.02 cm compared to HSD that ranged from 48 cm to 242 cm with mean

Table 3 — Descriptive statistics of phenotypic characters in F_2 population of ADT45 × Nona Bokra under conventional selection design (CSD) and honeycomb selection design (HSD)

Particulars	Conventional selection design (CSD)					Honeycomb selection design (HSD)				
	DAF (days)	PHT (cm)	PTN (No.)	PNL(cm)	SPY (g)	DAF (days)	PHT (cm)	PTN (No.)	PNL(cm)	SPY (g)
Range	61.00-94.00	55.00-180.00	2.00-34.00	15.5-29.00	2.08-136.70	61.00-126.00	48.00-242.00	5.00-79.00	10.50-35.20	1.02-317.90
Mean	79.62	105.25	16.03	22.32	40.81	83.66	118.98	36.17	25.82	62.45
Variance	99.67	730.20	34.50	6.72	890.20	164.30	732.60	155.30	10.16	1692.30
Std. Dev	9.98	27.02	5.87	2.59	29.83	12.83	27.06	12.46	3.19	41.13
CV	16.53	25.67	36.60	11.61	75.11	15.32	22.74	34.45	14.60	65.87
T-value	61.00-94.00	55.00-180.00	2.00-34.00	15.5.-29.00	2.08-136.70	61.00-126.00	48.00-242.00	5.00-79.00	10.50-35.20	1.02-317.90

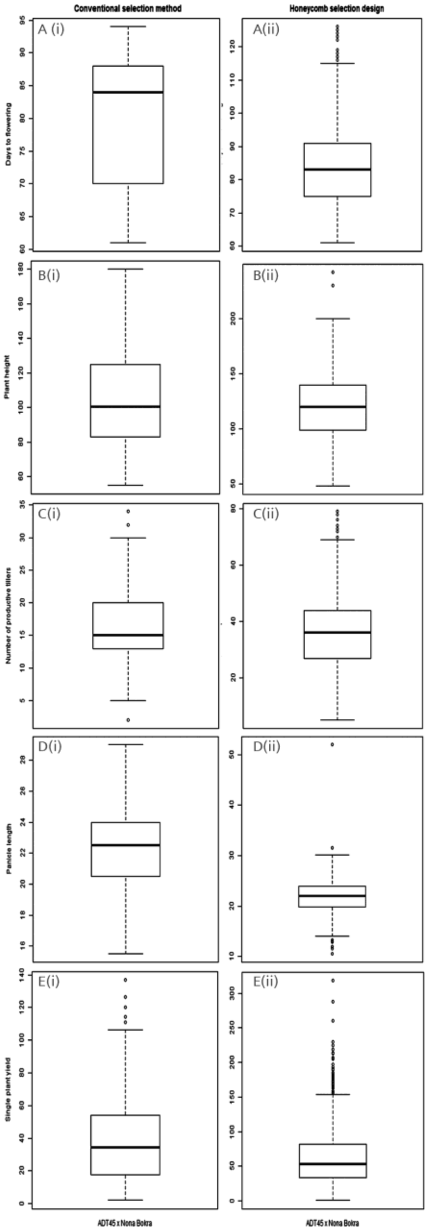

Fig. 2 — Distribution of mean, standard deviation and coefficient of variation in (i) conventional selection; and (ii) honeycomb selection design. (A) Days to flowering; (B) Plant height; (C) No. of productive tillers; (D) Panicle length; and (E) Single plant yield

and SD of 118.98 cm and 27.06 cm, respectively. While number of productive tillers varied from 2 to 34 with CV of 36.60%, mean of 16.03 and SD of 5.87 in CSD and in HSD it varied from 5 to 79 with CV of 34.45, mean of 36.17 and SD of 12.46. For panicle length, it ranged from 15.50 cm to 29 cm with CV of 11.61%, mean of 22.32 cm and SD of 2.59 cm in CSD. In HSD, it ranged from 10.50 cm to 35.20 cm with a CV of 14.60%, mean of 25.82 cm and SD of 3.19 cm. Single plant yield ranged from 2.08 g to 136.70 g with CV of 75.11, mean of 40.81 g and SD of 29.83 g in CSD and in HSD, it ranged from 1.02 g to 317.90 g with CV of 65.87%, mean of 62.45 g and SD of 41.13 g.

Early generation selection of best F_2 plants in CSD and HSD

The success of a plant breeding programmes rely on our ability to identify in the F_2 plants carrying genes for high and stable crop yield since they are carriers of fixed favorable genes and heritable superiority. In CSD, 29 plants accounting for 12.08% selection intensity were selected based on desirable traits such as high single plant yield, early/medium maturing and semi dwarf plant type (Table 4). In these plants single plant yield ranged from 44.73 g (entry 109) to 100.90 g (entry 139), plant height from 70 cm (entry 130) to 102.00 cm (entry 133) and days to flowering ranged from 63 days (entry 58) to 92 days (entry134). The selected plants accounted for 12.08% selection intensity.

In HSD, based on PI (SPY), 100 plants were ranked best. Out of the 100 best plants, 29 plants (Table 4) accounting for high selection intensity of 2.26% were selected based on other desirable characters such early/medium duration, semi dwarf characters apart from high single plant yield. In the selected plants single plant yield ranged from 110.20 g (entry 263) to 235.20 g (entry 636), plant height from 73 cm (entry 742) to 143 cm (entry 473) and days to flowering from 64 days (entry 405) to 110 (entry 724).

Table 4 — Selected plants of CSD and HCD

Entry	PHT	DAF	PTL	PNL	SPY	Entry	DAF	PHT	PTL	PNL	SPY
130	70.00	84.00	20.00	24.00	61.52	74	77.00	128.00	41.00	21.10	165.64
172	70.00	90.00	20.00	25.50	74.65	8	64.00	120.00	48.00	26.90	184.80
121	75.00	76.00	17.00	24.00	54.96	1053	93.00	84.00	56.00	28.30	146.16
1	75.00	84.00	25.00	20.50	64.20	1189	95.00	96.00	51.00	28.70	189.72
178	80.00	81.00	25.00	25.50	65.45	91	83.00	123.00	27.00	24.80	137.97
91	82.00	89.00	26.00	24.00	54.89	559	84.00	90.00	79.00	22.20	177.75
115	83.00	86.00	25.00	21.00	54.66	63	81.00	118.00	53.00	23.50	181.26
102	83.00	86.00	20.00	24.50	59.37	1220	91.00	130.00	59.00	22.10	169.92
202	83.00	86.00	20.00	24.50	59.37	473	92.00	143.00	52.00	27.00	218.40
211	83.00	86.00	20.00	24.50	59.37	1074	89.00	122.00	49.00	25.30	136.71
50	85.00	72.00	19.00	20.00	53.44	563	82.00	130.00	42.00	24.50	162.96
134	85.00	92.00	21.00	23.50	90.70	1036	88.00	75.00	68.00	22.70	213.93
79	88.00	82.00	26.00	22.50	63.64	608	78.00	132.00	50.00	24.20	230.00
46	90.00	70.00	15.00	23.60	48.90	636	83.00	90.00	48.00	19.00	235.20
103	90.00	85.00	15.00	20.50	49.71	1192	98.00	125.00	60.00	15.40	134.40
203	90.00	85.00	15.00	20.50	49.71	173	82.00	75.00	39.00	21.80	127.14
212	90.00	85.00	15.00	20.50	49.71	700	98.00	110.00	68.00	21.00	212.84
109	92.00	70.00	18.00	22.30	44.73	1114	88.00	94.00	38.00	23.00	115.52
209	92.00	70.00	18.00	22.30	44.73	546	89.00	132.00	58.00	23.50	142.10
218	92.00	70.00	18.00	22.30	44.73	293	78.00	105.00	56.00	17.00	137.20
28	93.00	78.00	19.00	22.00	59.78	1009	79.00	94.00	63.00	18.50	185.85
123	95.00	86.00	30.00	22.50	69.88	724	110.00	139.00	64.00	24.30	181.76
128	96.00	86.00	21.00	22.50	56.22	887	78.00	118.00	42.00	26.60	140.70
116	100.00	90.00	17.00	22.50	45.56	983	89.00	95.00	48.00	19.00	181.44
139	100.00	67.00	21.00	20.50	100.90	739	79.00	110.00	36.00	16.00	128.52
158	101.00	91.00	29.00	24.50	78.71	686	80.00	120.00	72.00	19.40	168.48
189	101.00	91.00	29.00	24.50	78.71	742	75.00	73.00	31.00	17.10	152.83
133	102.00	86.00	20.00	20.00	56.86	263	103.00	100.00	29.00	23.50	110.20
58	102.00	63.00	21.00	22.50	71.73	405	64.00	120.00	59.00	21.30	155.76

Table 5 — Percentage increase of selected plants in Conventional selection design (CSD) and Honeycomb selection design (HSD)

Particulars	Mean of base F2 population (g)	Mean of 29 selected plants (g)	Percentage increase over mean of base population (%)
CSD	40.81	60.92	49.28
HSD	62.45	166.38	166.42

Mean of the selected plants were 60.92 g and 166.38 g for single plant yield in CSD and HSD, respectively. For single plant yield, percentage increase of F_2 mean was worked out for the selected plants of CSD and HSD. It was found that selected plants in HSD had higher percentage increase of 166.42% over F_2 base population compared to selected plants in CSD which had a percentage increase of 49.28% over F_2 base population raised in CSD (Table 5).

Discussion

In HSD, systematic entry allocation, instead of random entry allocation in case of RCBD and lattice design, and multiple replicates not only ensures effective sampling of spatial heterogeneity but also counteracts its detrimental effects on selection efficiency. Additionally, geneticists and breeders need not worry with the pattern and orientation of soil heterogeneity to decide on the layout of the field plan, the shape, size and orientation of the plots and grouping of plots into blocks[7]. As a result, single plant evaluation under *nil*-competition is effective because (i) it reduces masking effects of negative correlation between yielding and competitive ability; (ii) it maximizes the range of genotypic expression; and (iii) it improves the validity of the mean by reducing the CV of single plant yield. Therefore, honeycomb selection design (HSD) is considered an ideal phenotyping platform to study genetics of any quantitative traits specifically yield and its components and to breed varieties for higher and stable yield in a shorter period.

The normality test revealed that the F_2 population did not show normal distribution for all the characters studied in both the designs except for panicle length in CSD. This may be due to loss of few F_2 plants on account of incompatibility between two parents (ADT45 × Nona Bokra), meiotic distortion and may also be due to phenomenon of linkage drag and linkage disequilibrium[8]. Positive skewness, observed for plant height, number of productive tillers and single plant yield in CSD (with competition), is due to transposition of low yielding plants from the left to the right tail of the curve reflecting the magnitude of the negative correlation between yielding and competing ability. Positive skewness observed, for days to flowering, number of productive tillers, panicle length and single plant yield in HSD (without competition), is due to the elimination of low yielding plants from the left tail by efficient selection reflecting magnitude of selection efficiency as stated by Kyriakou & Fasoula[9] and Pasini and Bos[10]. However, significant positive skewness of the individual plant yield distribution of the F_2 obtained by growing the population at a very wide plant spacing contrasts with the usually expected symmetrical yield distribution for F_2 generation[10].

All the characters in both CSD and HSD showed platykurtic distribution except for panicle length and single plant yield in HSD which showed leptokurtic distribution with kurtosis value more than 3. Negatively skewed platykurtic distribution was observed for days to flowering in CSD revealing that this trait is controlled by large number of genes displaying duplicate (additive × additive) epistasis. Hence, a mild selection is required for a rapid genetic gain. Positively skewed platykurtic distribution was observed for plant height in CSD and days to flowering in HSD suggesting that large number of genes displaying complementary epistasis controls these traits and hence intense selection is required for a rapid genetic gain. Further, positively skewed leptokurtic distribution observed for panicle length and single plant yield in HSD suggests that few numbers of genes with complementary epistasis control the expression of the trait and hence intense selection is required for a rapid genetic gain. Similar study supporting the above results of skewness and kurtosis had been reported in segregating population (F_2 and F_3) of rice[10,11], RIL population of rice[8].

The main drawback of the conventional selection design is that plants in F_2 -F_4/F_5 (early generations) are heterogeneous. Therefore, they are quite unstable in their response to environmental interaction which makes early selection difficult because the individual is the unit of selection and the effects of variation in salinity across a field plot can result in some loss of segregates with genes for salt tolerance. Hence, selection was delayed until F_6-F_8 generation to reduce the environmental effects[12]. Importantly, plant selections are done at dense stands conditions that "mimic" the farming conditions which is questionable whether segregating generations can stimulate farming conditions[13]. Indeed, performance of plants in dense stand and nil competition are not correlated when heterogeneous populations (in the presence of strong competitor genotypes) are evaluated due to inverse association between yielding and competitive ability[13]. Therefore, selection of heterogenous F_2-F_4 lines at commercial planting densities under salt stressed field with wider spatial variability, appears senseless. Under this scenario, in self-pollinating, segregating populations, the frequency of individuals with all favourable alleles is reduced with generations. In addition, genotype × environment interaction hinders selection and genetic gain for the character like grain yield.

To increase breeding efficiency the most appropriate unit of plant phenotyping corresponds to the individual plant grown unhindered in the absence of competitive interactions. In doing so, phenotypic expression and corresponding phenotypic variance are maximized, but the CV (coefficient of variation) of single-plant yields is minimized. As a result the spatial heterogeneity is effectively controlled[4] Honeycomb field designs accomplishes the above criteria (i) by assessing the yield potential of single plants after reducing the confounding effects of competition and soil heterogeneity and (ii) by taking advantage of soil heterogeneity to select for stability of performance. Under these conditions, the phenotypic range of trait expression is maximized and the true genetic potential can be measured[5]. Although the mean is a measure of the yielding ability of a genotype, stable performance over locations and years is essential and the phenotypic standard deviation should also be used to assess stability of performance.

In this study, all the characters had lower coefficient of variation, high mean and standard deviation in the honeycomb selection design compared to conventional selection design (Fig. 2). From the results, it was found that the characters influenced by environment such as number of productive tillers, single plant yield showed drastic difference between the two designs, confirming earlier reports stating mean, phenotypic standard deviation, coefficient of variation of single plant yield are affected differently in absence *vs*. presence of competition[10]. The competition environment reduced the mean and standard deviation drastically and increased the CV of single plant yield[10]. The increased CV under competition is due to higher reduction rate of mean compared to phenotypic standard deviation[13]. Thus, mean is a much more sensitive parameter for measuring how much competition reduce the range of phenotypic expression and differentiation. The higher the load of deleterious genes, the larger the CV and the more positively skewed the yield distribution. In contrast, lower the load of deleterious genes, the smaller the CV and the more negatively skewed the yield distribution. Single-plant yield and stability are reduced under high load of deleterious genes and increased under low load of deleterious genes[13]. Stand uniformity is measured by CV of single-plant yields and it has been shown to constitute a very reliable measure of crop yield potentials[6].

Results of the present study showed that the selected plants of HSD had higher percentage of increase over the base population compared to CSD showing the efficiency of HSD for enhancing phenotypic expression. This is further supported by results of studies in wheat[14] showing that selection for individual plant yield is more effective at wider spacing where interplant competition is likely to be less important or absent. The above selected plants in HSD is expected to have high response to selection compared to CSD as evidenced in mungbean, rice and cotton[4] where HSD outperformed other selection methods like panicle row selection, conventional pedigree selection, single seed descent method and bulk method. In addition early generation selection is effective in HSD[5] while ineffective in conventional pedigree selection[5] on account of superiority of selecting plants in F_2 and subsequent segregating generation in HSD as a result of evaluation of individual plant performance for yield under low density and application of moving circle selection. Hence, HSD samples effectively for environmental diversity than random allocation of progeny line and thus minimizes the environmental variance[5]. Thus, honeycomb selection designs are advanced experimental designs with innovative properties that enable effective sampling of soil heterogeneity ensuring that all plants are allocated under comparable growing conditions[14].

Conclusion

In the present study, honeycomb selection design (HSD) was found to be superior to conventional selection design (CSD) because of enhanced phenotypic expression in the former due to elimination of confounding effects of negative correlation between yielding and competitive ability. Thus, it is concluded that HSD can be effectively used for studying genetics of yield components and in early generation selection as result of enhanced phenotypic expression at wider spacing.

Conflict of interest

Authors declare that no competing interests.

References

1 Chakraborty K, Chattaopadhyay K, Nayak L, Ray S, Yeasmin L, Jena P, Gupta S, Mohanty SK, Swain P & Sarkar RK, Ionic selectivity and coordinated transport of Na+ and K+ in flag leaves render differential salt tolerance in rice at the reproductive stage. *Planta*, 250 (2019) 1637.

2 Ganie SA, Molla KA, Henry RJ, Bhat KV & Mondal TK, Advances in understanding salt tolerance in rice. *Theor Appl Genet*, 132 (2019) 851.

3 Singh RK, Kota S & Flowers TJ, Salt tolerance in rice: seedling and reproductive stage QTL mapping come of age. *Theor Appl Genet*, 134 (2021) 3495.

4 Fasoula VA, Prognostic breeding: a new paradigm for crop improvement. *Plant Breed Rev*, 37 (2013) 347.

5 Fasoula V, Thompson K & Mauromoustakos A, The prognostic breeding application JMP Add-In Program. *Agronomy*, 9 (2019) 25.

6 Fisher RA, Breeding wheat for increased potential yield: Contrasting ideas from Donald and Fasoulas and the case for early generation selection under nil competition. *Field Crop Res*, 252 (2020) 107782.

7 Tokatlidis I, Sampling the spatial heterogeneity of the honeycomb model in maize and wheat breeding trials: Analysis of secondary data compared to popular classical designs. *Exp Agric*, 52 (2015) 290.

8 Mohannath TNG, Vaishali G, Biradar M,& Hittalmani S, Response of a new IR50/Moroberekan recombinant inbred population of rice (*Oryza sativa* L.) from an i*ndica* × *japonica* cross for growth and yield traits under aerobic conditions. *Euphytica*, 152 (2006) 161.

9 Omirou M, Ioannides MI & Fasoula DA, Optimizing resource allocation in a cowpea (*Vigna unguiculata* L. Walp.) landrace through whole-plant field phenotyping and non-stop selection to sustain increased genetic gain across a decade. *Front.Plant Sci.* 10 (2019) 949.

10 Fischer RA & Rebetzke GJ, Indirect selection for potential yield in early generation, spatial planting of wheat and other small grain cereals. *Crop Pasture Res*, 69 (2018) 439.

11 Savitha, P& Kumari RU, Studies on skewness, kurtosis and parent progeny regression for yield and its related traits in segregating generations of rice. *Oryza*, 52 (2015) 86.

12 Abhilash R, Thirumurugan T, Sassikumar D & Chitra S, Genetic studies in F_2 for biochemical traits in rice (*Oryza sativa* L.) *Electronic J Plant Breed*, 9 (2018) 1067.

13 Tokatlidis IS, Crop adaptation to density to optimize grain yield: breeding implications. *Euphytica*, 213 (2017) 92.

14 Fisher RA, Ramos OHM, Monasterio IO & Sayre KD, Yield response to plant density, row spacing and raised beds in low latitude spring wheat with ample soil resources: An update. *Field Crop Res*, 232 (2019) 95.