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RESEARCH ARTICLE

Efficiency of honeycomb selection design and validation of molecular markers in early generation selection in rice

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Abstract

Breeding for salt tolerance is complex and tedious due to its genetics and environmental interaction. Honeycomb Selection Design (HSD), a paradigm for conventional breeding, provides an opportunity to breed density-independent cultivars under nil competition. The present study was attempted to study the effectiveness of HSD in selecting salt-tolerant genotypes in F_2 and F_3 populations of ADT (R) 45 × Nona Bokra rice cross raised in unreplicated honeycomb design (UN-1) in F_2 generation and replicated honeycomb design (R-37) in F_3 generation respectively at wider spacing of 100 cm x 90 cm. The F_2 plants were selected based on plant index and F_3 plants were selected based on plant and stability index. Plant index-based selection in F_2 population identified 35 best performing genotypes accounting for 4.13 % selection intensity and they were forwarded to raise F_3 population. In F_3 population, 28 plants were selected based on plant index and stability index accounting for 3.63 % selection intensity. Such low selection intensity in HSD leads to rapid fixation of additive alleles that are indispensable for advancing through selection. In F_2, 35 selected plants showed an increase of 213.86 % over the total mean of F_2. Yield of F_3 progenies significantly improved over the respective selected F_2 plants showing positive genetic gain. In addition, the best performing 28 plants selected in F_3 population were from 15 and 12 progeny lines in normal and saline condition among 35 individual entries selected in F_2 population. Further, best 100 F_2 and F_3 plants were subjected to genotyping using nine markers linked to salt tolerance. The correlation between salt tolerance markers and observed morphological traits was weak in F_2 which may be due to a lack of coverage of all the regions of salt tolerance quantitative trait loci (QTLs) and may also be due to unidentified regions of the chromosome conferring tolerance to salinity stress. Hence, selection for yield in the early generations (F_2 and F_3) of ADT (R) 45 × Nona Bokra rice cross using the HSD improves the efficiency of breeding procedure by reducing the number of genotypes to be tested in subsequent, expensive yield trials, thereby increasing genetic gain per unit cost.

Keywords: early generation selection; genetic gain; selection intensity

Introduction

Salinity causes significant yield reduction and it is considered as a predominant abiotic stress (1). Regardless of the cause, high salt concentration in the root zone severely affects normal plant growth and development, resulting in reduced crop productivity or crop failure. About 2.34 million hectare area of rice growing area in India is salt affected leading to production loss of 17.85 % with monetary loss of 15.77 % (2). Conventional breeding for salt tolerance in rice entails various breeding (selection) methods like backcrossing, pedigree selection, bulk and modified bulk method, recombination breeding and anther culture. Improving the salt tolerance of crops would not only lead to the effective use of saline-alkali land but also support the sustainable agriculture and alleviate the world food crisis (3).

Selection efficiency in conventional breeding is influenced by density and interplant competition, soil heterogeneity, genotype-by-environment (GxE) interaction, heterozygosity and lack of exploitation of the adaptive variation released constantly by the genome in response to environmental stimuli (4-7). In addition, in the pace of progress, breeding salt-tolerant rice is slow owing to varied factors such as complexity of the traits, need of efficient selection criteria, absence of reliable and repeatable screening methodology and variation of tolerance with ontogeny (8). Further, direct selection in field sites for quantitative traits such as salt tolerance is difficult because of uncontrollable environmental factors adversely affecting the precision and repeatability of trials (9). This high variability in soil salinity and environmental interactions makes it questionable whether breeding should be conducted for

tolerance or for high yield (10).

The main pitfall of this conventional methodology is that plants in F_2-F_4/F_5 (early generations) are heterogeneous and therefore 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 generations to reduce the environmental effects (10). Importantly, plant selections are done at dense stand conditions that "mimic" the farming conditions which is questionable whether segregating generations can stimulate farming conditions (11). 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 (11). Therefore, the 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 without selection. In addition, genotype × environment interaction hinders selection and genetic gain for the character like grain yield.

The success of a breeding program depends on our ability to identify F_2 plants that carry genes associated with high and stable crop yields, as these plants possess fixed favourable genes and heritable advantages. Consequently, the likelihood of selecting plants with increased gene fixation (extreme individuals) that drive progress through selection is higher in the F_2 generation than in any other. The probability of retaining a superior line at early generation decreases with increased non-additive effects and low heritabilities. Thus, early generation selection should be used for populations or traits with little non-additive effect, coupling linkage and high heritability (12).

To enhance efficiency in plant breeding, the ideal unit for plant phenotyping in selection is an individual plant grown without competition. This ensures maximized phenotypic expression and variance, minimizes the coefficient of variation (CV) for single-plant yield (SPY) and effectively manages spatial heterogeneity (5, 7, 13). Under these conditions, the phenotypic range of trait expression is maximized and the true genetic potential can be measured (6). Selecting the appropriate unit for plant phenotyping in the field is essential to maximize the efficiency of selection in plant breeding programs and achieve measurable genetic gains.

The honeycomb breeding method emerged after a systematic search for the barriers that reduce the efficiency of early generation selection for yield (5, 6). The confounding effects of soil heterogeneity (environmental variation) and genotypic variation of individual plants (in F_2 and F_3 generation) on SPY are effectively accounted for in honeycomb field designs by positioning each plant at the center of a moving replicate. This design enables efficient selection for yield stability through the strategic exploitation of soil heterogeneity, achieved by arranging plants of each sibling line in a triangular grid pattern. HSD samples environmental diversity effectively than random allocation of progeny line and thus minimizing environmental variance (14).

Despite huge success in identifying QTLs in rice for salt tolerance, yield and grain quality attributes, the success of marker assisted selection (MAS) for these traits has been limited due to lack of consistency (9) and therefore, validation of these markers in alternative breeding populations are necessary.

With the view of the above facts, the present study was conducted in early generation (F_2 and F_3) of ADT (R) 45 x Nona Bokra rice cross using HSD, to prevent the loss of fixed alleles that are nearing homozygosity.

Materials and Methods

Plant material

ADT (R) 45, a popular short duration rice variety, is susceptible to saline conditions but has the ideal traits of heavy tillering and short stature, chosen as the female parent of the cross. Nona Bokra, a land race of West Bengal, which is highly salt tolerant and extensively used in breeding for salt tolerance like Pokkali, possesses undesirable traits such as tallness (prone to lodging), poor yielding, red kernel, bold grain type and photo sensitiveness. But it is reported to have good tillering capacity under salt stress. Hence chosen as a male parent of the cross. Initially, a cross between ADT (R) 45 × Nona Bokra was made to create true F_1 seeds, which acted as base material for creating the actual experimental material (F_2 and F_3) in which the study was carried out. The trial was carried out in the farm of Pandit Jawaharlal Nehru College of Agriculture and Research Institute, Karaikal, during the Kharif seasons of 2020 and 2021.

The experiment started with the F_2 material, which was raised under saline conditions in an unreplicated honeycomb selection design (HSD; UN-1) consisting of 847 F_2 plants and 141 CSR 10 plants, which was used as a tolerant check. The best performing 35 plants, selected in the F_2 generation were advanced to raise the F_3 generation under both saline and normal conditions in a replicated honeycomb design (R-37). Saline trial consists of 770 F_3 plants (35 selected plants were replicated 22 times each, creating 770 F_3 plants) along with two checks, CSR 10 and TRY 2 which were replicated 22 times each. As in saline trial, the normal trial also consists of the same number of F_3 plants (770) along with checks CSR 10 and ADT (R) 45, which were also replicated 22 times each. In HSD, plants were raised at a wider spacing of 100 cm x 90 cm. All the recommended agronomic package of practices were adopted during the entire crop growth.

Methods

The methodology adopted was phenotyping of all plants planted in HSD and selection of best performing genotypes having high yielding, early to medium maturing and semi dwarf nature under saline condition in F_2 generation and forwarding it to F_3 generation and again selecting the best performing genotypes under saline and normal conditions. And to compare the selected genotypes under both conditions to identify the best lines that perform better under both conditions. In addition to field phenotyping, genotyping was also done in both F_2 and F_3 generations to confirm the presence of salt tolerant genes in plants selected through HSD.

Observations recorded

Days to flowering, plant height, number of productive tillers and SPY were recorded in single plant basis for all the plants raised in HSD.

Stress level

In saline trial of both F_2 and F_3 generations, the field was irrigated with saline borewell water to impose the salt stress to identify the salt tolerant plants. Four piezometers were placed in the field and the

root zone water sample was collected for measuring the EC and pH periodically to characterize the stress level in both F_2 and F_3 generation. In F_2 generation, the recorded EC ranged from 0.74 dSm^{-1} to 1.92 dSm^{-1}, pH ranged from 7.1 to 9.15. In F_3 generation, the recorded EC value range was from 1.20 dS m^{-1} to 1.81 dS m^{-1} and pH range from 7.88 to 8.48.

HSD analysis

HSD selection and analysis were performed using the Prognostic Breeding Application JMP Add-In Program. In the unreplicated honeycomb selection design, selection of best performing plants was based on the Plant Index (PI) value calculated using SPY data. The PI compares and adjusts the yield of each plant relative to the mean yield of 18 plants surrounding it within the moving-ring design (15).

$$PI\ (SPY) = (x/\bar{x}_r)^2 \qquad \text{Eqn. 1}$$

where x is the yield of the individual plant and $\bar{x}_r$ is the mean yield of the surrounding plants within the specified moving ring. This index measures the yield performance of each plant independently of soil heterogeneity, thereby facilitating the selection of superior genotypes in the unreplicated honeycomb selection design (16-18). Replicated honeycomb selection design was analysed based on PI, Stability Index (SI) and the Plant Prognostic Equation (PPE), which is the product of the PI and SI. The formation of moving replicates or rings ensures that plants of each line are positioned at the vertices of a moving triangular grid. This arrangement facilitates effective sampling of soil heterogeneity and enables the estimation of the SI,

$$SI = (\bar{x}_g/s)^2 \qquad \text{Eqn. 2}$$

where $\bar{x}_g$ and S represent the mean plant yield and standard deviation, respectively, for the plants of a given line within the moving grid (16-18). The product of the PI and SI gives the PPE which measures the crop yield potential of each plant and is used for the selection of best plants (16-18).

$$PPE = (x/\bar{x}_r)^2\ (\bar{x}_g/s)^2 \qquad \text{Eqn. 3}$$

where x is the yield of the individual plant, $\bar{x}_r$ is the mean yield of the surrounding plants within the specified moving ring, $\bar{x}_g$ and s represent the mean plant yield of each line and its progenies in that generation and the standard deviation respectively.

The PI, SI and PPE are dimensionless indices. Their application facilitates rapid progress during the early stages of plant selection and enhances annual genetic gain by effectively increasing selection pressure to retain only the best performing plants (17, 18).

Parental polymorphism survey

A total of 55 Simple Sequence Repeat (SSR) primer pairs were surveyed for identifying parental polymorphism between the two parents (ADT (R) 45 and Nona Bokra). Among 55 SSR primers, nine primers were exhibiting polymorphism and those primers were used to amplify the DNA of top 100 selected plants in both F_2 and F_3 generation under saline condition. These nine primers were reported to be linked to various salt tolerance QTLs in seedling and reproductive stages in different mapping populations having one parent as Nona Bokra. Supplementary Table S1 provides the sequence details of the nine polymorphic SSR primer used to amplify the DNA.

Molecular marker analysis

Correlation analysis was performed to tag and confirm potential SSR markers linked to the traits such as number of tillers and SPY in both F_2 and F_3 generation using MS excel. Salt tolerance markers identified in the background of Nona Bokra were used for analysis. Top 100 best performing plants from F_2 generation selected based on PI and top 100 best performing plants from F_3 generation selected based on PI, SI and PPE using JMP Add-In program were subjected to molecular analysis. The molecular analysis was carried using nine SSR primers linked to salt tolerance.

Results and Discussion

The isolation environment allows the application of high selection intensities, optimizes heritability and broadens the phenotypic differentiation, thus fully satisfying the equation for response to selection.

Early generation selection in HSD

Only 841 plants survived out of 847 in F_2 generation. Out of 814 F_3 plants, only 804 and 790 plants survived under saline and normal condition respectively. The missing plants posed no issue, as they did not influence the yield of neighbouring plants due to the ultrawide spacing and they did not affect the selection efficiency because of the presence of large number of plants within the moving ring. The number of plants fixed to be present in each complete moving ring was 18, but only 23 plants out of 35 selected plants from the F_2 generation under saline condition had complete moving ring of 18 plants. Similarly, out of 28 selected plants under saline condition from F_3 generation, only 12 plants had a complete moving ring of 18 plants and under normal condition, only 5 plants had a complete moving ring of 18 plants out of 28 selected plants. The lack of plants in a complete moving ring was due to the missing plants because of death of susceptible plants. Some were due to an incomplete moving ring because of border plants. Selection of lines for yield and stability should be performed as early as the F_2 and F_3 generations, as the expected frequency of high-yielding genotypes declines with each successive generation of selfing without selection (19).

The 35 plants in F_2 were selected based on other desirable characters such as early-medium maturing, semi dwarf and short bold/medium slender white rice (Table 1), accounting for 4.13 % selection intensity. While the 28 plants selected in F_3 for saline and normal condition (Table 2, 3), accounted for 3.63 % selection intensity. Such an ultra-high selection pressure leads to rapid fixation of additive alleles that are indispensable for advance through selection (18). Higher selection pressure in the absence of competition environment and the application of the moving round grid contributed towards the higher efficiency of honeycomb design (20). This is critical because there is a substantial decrease in the expected frequency of high yielding genotypes with each generation of selfing without selection (6).

The total mean (SPY) of F_2 (988 plants) recorded was 20.71 g. The mean of 35 selected plants (65.00g) showed a percentage increase of 213.86 % over total mean population. The total mean (SPY) of F_3 (814 plants) was found to be 29.02 g. The F_3 population showed an increase of 40.12 % over the total mean SPY of F_2 plants. The percentage increase in SPY of the 28 selected F_3 plants under saline and normal condition over check varieties was calculated and presented in Table 4 and 5 respectively. Under saline condition, the highest percentage increase over CSR 10 and TRY 2 was found in S776-1 with an increase of 172.62 % over CSR 10 and 132.86 % over TRY 2, followed by S749-1 with an increase of 118.08 % over CSR 10

Table 1. List of 35 selected best performing individual plants in F_2 population

Sl. No.	Selected plants	DAF	PHT	PTL	SPY	MR plants (SPY) at MR = 18	MR mean (SPY) at MR = 18	PI (SPY) at MR = 18	Grain type	Colour	Replication in F_3
1	8	114.00	95.00	30.00	61.44	11	25.25	5.92	Short bold	White	8-(1-22)
2	46	114.00	64.00	57.00	76.84	15	21.10	13.27	Short bold	White	46-(1-22)
3	91	95.00	100.00	30.00	38.69	18	15.67	6.10	Basmati type	Red	91-(1-22)
4	97	109.00	100.00	25.00	31.40	10	10.10	9.67	Medium slender	White	97-(1-22)
5	104	81.00	105.00	27.00	67.25	17	20.86	10.40	Medium slender	Red	104-(1-22)
6	153	119.00	89.00	27.00	48.35	18	22.96	4.43	Medium slender	White	153-(1-22)
7	181	114.00	111.00	27.00	60.27	18	18.00	11.21	Long slender	Red	181-(1-22)
8	204	112.00	105.00	46.00	53.18	18	21.19	6.30	Short bold	White	204-(1-22)
9	222	110.00	89.00	54.00	89.10	18	19.75	20.36	Long bold	Red	222-(1-22)
10	240	113.00	102.00	51.00	60.79	18	17.96	11.45	Short bold	Red	240-(1-22)
11	242	117.00	85.00	59.00	70.63	18	19.51	13.11	Basmati type	Red	
12	251	109.00	102.00	31.00	77.98	18	20.58	14.35	Long bold	White	251-(1-22)
13	259	112.00	100.00	59.00	92.26	15	20.66	19.95	Medium slender	Red	259-(1-22)
14	272	114.00	95.00	41.00	78.43	18	17.24	20.69	Medium slender	Red	272-(1-22)
15	290	121.00	82.00	41.00	103.6	17	21.89	22.42	Medium slender	Red	290-(1-22)
16	309	114.00	100.00	30.00	56.28	18	19.93	7.97	Medium slender	White	309-(1-22)
17	377	104.00	112.00	50.00	65.00	18	19.94	10.63	Short bold	White	377-(1-22)
18	386	112.00	88.00	44.00	32.04	18	14.59	4.82	Medium slender	White	386-(1-22)
19	390	115.00	128.00	51.00	78.19	15	21.54	13.18	Long bold	White	390-(1-22)
20	403	112.00	127.00	25.00	49.13	17	19.15	6.58	Long bold	White	403-(1-22)
	TRY 2										**TRY 2-(1-22)**
21	416	99.00	116.00	59.00	49.47	18	26.64	3.45	Short bold	White	416-(1-22)
22	428	86.00	100.00	61.00	56.94	18	16.46	11.96	Short bold	Red	428-(1-22)
23	531	119.00	105.00	38.00	79.96	18	20.61	15.05	Long bold	Red	531-(1-22)
24	634	99.00	127.00	51.00	68.14	18	30.86	4.88	Short bold	white	634-(1-22)
25	675	97.00	116.00	19.00	43.76	18	20.74	4.45	Long bold	White	675-(1-22)
26	683	98.00	69.00	76.00	60.33	12	27.00	4.99	Short slender	White	683-(1-22)
27	703	103.00	124.00	39.00	37.22	18	13.17	7.99	Short bold	White	703-(1-22)
28	721	109.00	125.00	24.00	53.86	18	22.30	5.83	Long slender	White	721-(1-22)
29	724	88.00	147.00	34.00	79.83	18	15.52	26.46	Medium slender	Red	724-(1-22)
30	739	101.00	100.00	32.00	41.27	18	15.38	7.20	Medium slender	White	739-(1-22)
31	749	113.00	100.00	66.00	109.9	12	21.41	26.34	Short bold	Red	749-(1-22)
32	751	114.00	94.00	40.00	78.05	18	27.39	8.12	Short bold	White	751-(1-22)
33	776	113.00	92.00	52.00	70.52	18	21.08	11.19	Long bold	White	776-(1-22)
34	811	116.00	115.00	56.00	116.30	15	20.59	31.91	Long bold	Red	811-(1-22)
35	825	113.00	87.00	49.00	38.83	11	14.60	7.07	Medium slender	White	825-(1-22)
	CSR 10										**CSR 10-(1-22)**

DAF: Days after flowering; PHT: Plant height; PTL: Productive tillers; SPY: Single plant yield; MR: Moving replicate; PI (SPY): Plant index of single plant yield.

Table 2. Selection of superior F_3 individual plants based on PI, SI, PPE of SPY and desirable traits under saline condition

Sl. no.	Selected plants	DAF	PHT	PTL	SPY	MR plants (SPY)	MR mean (SPY)	PI (SPY)	SI (SPY)	PPE (SPY)	LB ratio	Grain type	Colour
1	S8-1	107.00	140.00	62.00	110.81	10	29.97	13.67	4.43	60.57	2.9	Medium slender	White
2	S776-1	113.00	114.00	60.00	94.19	18	32.79	8.25	6.68	55.12	3.2	Long slender	White
3	S776-2	82.00	105.00	58.00	71.05	15	25.27	7.91	6.68	52.82	3.0	Long slender	White
4	S776-3	99.00	108.00	37.00	72.49	17	26.95	7.23	6.68	48.33	3.1	Long slender	White
5	S724-1	82.00	103.00	44.00	64.37	11	23.91	7.25	6.02	43.64	3.1	Long slender	Red
6	S153-1	114.00	162.00	47.00	88.09	17	36.52	5.82	7.23	42.08	2.9	Medium slender	White
7	S181-1	108.00	159.00	34.00	61.76	15	26.33	5.5	7.60	41.83	3.0	Long slender	Red
8	S776-4	105.00	111.00	48.00	73.6	15	29.93	6.05	6.68	40.41	3.1	Long slender	White
9	S776-5	83.00	99.00	27.00	60.53	18	25.79	5.51	6.68	36.80	3.1	Long slender	White
10	S749-1	106.00	106.00	46.00	70.68	15	25.26	7.83	4.44	34.77	3.8	Extra-long slender	Red
11	S222-1	114.00	137.00	61.00	109.73	16	29.99	13.39	2.50	33.40	2.9	Medium slender	Red
12	S181-2	98.00	146.00	31.00	54.63	15	26.58	4.22	7.60	32.11	3.0	Long slender	Red
13	S181-3	108.00	148.00	33.00	54.10	12	27.58	3.85	7.60	29.25	3.0	Long slender	Red
14	S259-1	102.00	118.00	38.00	62.23	17	31.06	4.01	6.57	26.39	3.0	Long slender	Red
15	S776-6	97.00	109.00	38.00	50.09	15	25.54	3.85	6.68	25.69	2.5	Long bold	White
16	S825-1	100.00	112.00	61.00	48.32	18	23.97	4.06	6.30	25.59	3.0	Short slender	White
17	S259-2	98.00	137.00	27.00	61.76	17	31.95	3.74	6.57	24.57	2.5	Medium slender	Red
18	S259-3	101.00	141.00	31.00	47.98	18	24.91	3.71	6.57	24.39	2.5	Medium slender	Red
19	S259-4	108.00	131.00	31.00	59.97	18	31.36	3.66	6.57	24.05	2.5	Medium slender	Red
20	S724-2	108.00	109.00	31.00	45.63	18	23.12	3.89	6.02	23.45	2.9	Medium slender	Red
21	S91-1	101.00	102.00	41.00	50.12	18	22.43	5.00	4.52	22.56	3.0	Long slender	Red
22	S721-1	91.00	119.00	42.00	50.58	18	23.29	4.72	4.78	22.52	3.0	Long slender	White
23	S8-2	100.00	128.00	72.00	65.13	16	29.04	5.03	4.43	22.29	2.4	Long bold	White
24	S153-2	109.00	148.00	27.00	37.04	18	21.11	3.08	7.23	22.26	2.8	Medium slender	White
25	S153-3	115.00	134.00	22.00	41.93	18	24.21	3.00	7.23	21.69	2.8	Medium slender	White
26	S825-2	109.00	106.00	32.00	51.36	15	29.37	3.06	6.30	19.25	2.9	Medium slender	White
27	S309-1	108.00	100.00	69.00	50.10	18	30.95	2.62	6.75	17.69	2.7	Medium slender	White
28	S222-2	113.00	152.00	45.00	68.62	18	26.67	6.62	2.50	16.52	2.7	Long bold	Red

SI(SPY): Stability index of single plant yield; LB ratio: Length and breadth ratio; PPE (SPY): Plant prognostic equation of single plant yield.

Table 3. Selection of superior F_3 individual plants based on PI, SI, PPE of SPY and desirable traits under normal condition

Sl. no.	Selected plants	DAF	PHT	PTL	SPY	MR plants (SPY)	MR mean (SPY)	PI (SPY)	SI (SPY)	PPE (SPY)	LB ratio	Grain type	Colour
1	N153-1	89.00	150.00	49.00	200.31	17	42.01	22.73	7.10	161.41	3.01	Long slender	White
2	N749-1	85.00	130.00	88.00	202.78	17	48.27	17.65	4.73	83.47	3.30	Long slender	Red
3	N204-1	83.00	140.00	45.00	181.70	17	48.04	14.31	5.71	81.68	2.93	Medium slender	White
4	N272-1	85.00	150.00	77.00	293.80	18	49.90	34.67	2.29	79.38	2.93	Medium slender	Red
5	N153-2	86.00	129.00	59.00	174.01	11	54.66	10.13	7.10	71.94	2.40	Long bold	White
6	N97-1	83.00	117.00	41.00	83.36	15	36.26	5.29	11.08	58.57	2.46	Short bold	White
7	N46-1	83.00	73.00	30.00	88.40	16	40.71	4.72	11.02	51.97	2.93	Medium slender	White
8	N721-1	90.00	100.00	29.00	131.04	18	43.26	9.17	5.01	45.97	3.38	Long slender	White
9	N749-2	83.00	114.00	49.00	152.38	17	52.82	8.32	4.73	39.36	2.88	Medium slender	Red
10	N403-1	78.00	121.00	53.00	85.83	18	37.35	5.28	7.35	38.81	2.31	Short bold	White
11	N776-1	85.00	90.00	36.00	85.54	17	40.98	4.36	8.51	37.09	2.95	Medium slender	White
12	N749-3	86.00	121.00	59.00	136.06	11	50.22	7.34	4.73	34.72	2.84	Medium slender	Red
13	N251-1	83.00	98.00	45.00	118.24	17	63.78	3.44	7.44	25.57	2.35	Long bold	White
14	N739-1	85.00	88.00	51.00	121.11	16	42.00	8.31	3.05	25.36	3.07	Long slender	White
15	N739-2	85.00	88.00	53.00	155.56	14	57.06	7.43	3.05	22.67	1.88	Short bold	White
16	N259-1	81.00	58.00	21.00	87.92	15	49.33	3.18	6.96	22.11	3.07	Long slender	Red
17	N251-2	83.00	113.00	52.00	112.28	16	68.18	2.71	7.44	20.18	3.03	Long slender	White
18	N403-2	81.00	112.00	40.00	94.12	10	57.87	2.64	7.35	19.44	3.01	Long slender	White
19	N377-1	85.00	98.00	33.00	75.52	17	36.70	4.23	4.55	19.27	3.05	Short slender	White
20	N251-3	86.00	60.00	27.00	61.35	15	38.42	2.55	7.44	18.97	3.00	Medium slender	White
21	N683-1	81.00	120.00	83.00	146.24	18	66.18	4.88	3.78	18.46	3.00	Medium slender	White
22	N251-4	86.00	82.00	44.00	99.06	14	63.37	2.44	7.44	18.18	3.42	Basmati type	White
23	N222-1	81.00	78.00	42.00	94.30	17	50.72	3.46	5.06	17.49	2.98	Medium slender	Red
24	N776-2	90.00	82.00	35.00	91.55	15	64.91	1.99	8.51	16.93	3.09	Long slender	White
25	N222-2	83.00	72.00	20.00	94.46	14	53.16	3.16	5.06	15.98	1.92	Short bold	Red
26	N222-3	86.00	61.00	51.00	72.71	18	42.26	2.96	5.06	14.98	2.98	Medium slender	Red
27	N683-2	79.00	120.00	49.00	115.87	15	58.24	3.96	3.78	14.96	2.96	Medium slender	White
28	N222-4	85.00	58.00	56.00	73.46	17	44.19	2.76	5.06	13.98	1.93	Short bold	Red

Table 4. Percentage increase of SPY in 28 F_3 selected plants over CSR 10 and TRY 2 salt tolerant checks under saline condition

Sl. no.	Selected progeny lines in F_3	SPY (g) of Selected progeny lines in F_2	Selected plants in F_3	SPY (g) of selected plants in F_3	SPY (g) of CSR 10 nearer to selected plants	SPY (g) of TRY 2 nearer to selected plants	% increase over CSR 10	% increase over TRY 2
1	8	61.44	S8-1	110.81	59.17	45.51	87.27	143.48
2	8	61.44	S8-2	65.13	48.84	40.45	33.35	61.01
3	91	38.69	S91-1	50.12	34.55	34.23	45.07	46.42
4	153	48.35	S153-1	88.09	48.84	40.45	80.36	117.78
5	153	48.35	S153-2	37.04	32.00	22.40	15.75	65.36
6	153	48.35	S153-3	41.93	21.54	22.83	94.66	83.66
7	181	60.27	S181-1	61.76	37.44	35.80	64.96	72.51
8	181	60.27	S181-2	54.63	34.79	37.32	57.03	46.38
9	181	60.27	S181-3	54.10	31.28	35.61	72.95	51.92
10	222	89.1	S222-1	109.73	50.29	62.28	118.19	76.19
11	222	89.1	S222-2	68.62	31.79	37.47	115.85	83.13
12	259	92.26	S259-1	62.23	48.84	40.45	27.42	53.84
13	259	92.26	S259-2	61.76	36.98	34.68	67.01	78.09
14	259	92.26	S259-3	47.98	34.79	34.56	37.91	38.83
15	259	92.26	S259-4	59.97	38.03	31.97	57.69	87.58
16	309	56.28	S309-1	50.10	34.55	35.61	45.01	40.69
17	721	53.86	S721-1	50.58	38.03	37.72	33.00	34.09
18	724	79.83	S724-1	64.37	31.42	31.97	104.87	101.35
19	724	79.83	S724-2	45.63	33.39	31.97	36.66	42.73
20	749	109.85	S749-1	70.68	32.41	31.79	118.08	122.33
21	776	70.52	S776-1	94.19	34.55	40.45	172.62	132.86
22	776	70.52	S776-2	71.05	32.22	35.00	120.52	103.00
23	776	70.52	S776-3	72.49	34.79	34.68	108.36	109.03
24	776	70.52	S776-4	73.60	31.28	45.51	135.29	61.72
25	776	70.52	S776-5	60.53	38.03	37.32	59.16	62.19
26	776	70.52	S776-6	50.09	36.98	34.05	35.45	47.11
27	825	38.83	S825-1	48.32	34.55	45.51	39.86	6.17
28	825	38.83	S825-2	51.36	32.22	35.00	59.40	46.74

Table 5. Percentage increase of SPY in 28 F_3 selected plants over ADT (R) 45 and TRY 2 checks under normal condition

Sl. no.	Selected plants in F_3	SPY (g) of selected plants	SPY (g) of ADT (R) 45 nearer to selected plants	SPY (g) of TRY 2 nearer to selected plants	% increase over ADT (R) 45	% increase over TRY 2
1	N153-1	200.31	90.80	76.62	120.61	161.43
2	N749-1	202.78	75.36	76.62	169.08	164.66
3	N204-1	181.70	66.77	69.34	172.13	162.04
4	N272-1	293.80	67.59	69.34	334.68	323.71
5	N153-2	174.01	59.19	72.05	193.99	141.51
6	N97-1	83.36	54.36	59.66	53.35	39.73
7	N46-1	88.40	50.33	77.42	75.64	14.18
8	N721-1	131.04	61.36	90.25	113.56	45.20
9	N749-2	152.38	71.58	77.42	112.88	96.82
10	N403-1	85.83	59.19	50.54	45.01	69.83
11	N776-1	85.54	50.33	56.14	69.96	52.37
12	N749-3	136.06	57.94	59.66	134.83	128.06
13	N251-1	118.24	67.59	99.40	74.94	18.95
14	N739-1	121.11	75.25	73.98	60.94	63.71
15	N739-2	155.56	63.22	99.40	146.06	56.50
16	N259-1	87.92	50.33	77.42	74.69	13.56
17	N251-2	112.28	90.80	76.62	23.66	46.54
18	N403-2	94.12	61.36	69.83	53.39	34.78
19	N377-1	75.52	71.58	70.87	5.50	6.56
20	N251-3	61.35	46.93	56.14	30.73	9.28
21	N683-1	146.24	59.19	72.05	147.07	102.97
22	N251-4	99.06	67.59	69.34	46.56	42.86
23	N222-1	94.30	44.00	70.55	114.32	33.66
24	N776-2	91.55	71.58	77.42	27.90	18.25
25	N222-2	94.46	61.36	69.83	53.94	35.27
26	N222-3	72.71	57.55	50.08	26.34	45.19
27	N683-2	115.87	71.58	73.98	61.87	56.62
28	N222-4	73.46	65.39	50.08	12.34	46.69

and 122.33 % over TRY 2. Similar results of high percentage increase of F_2 (SPY) over check CSR 10 were obtained for the F_2 generation. Under normal condition, the highest percentage increase was found to be present in the line N272-1 when compared with ADT (R) 45 and TRY 2. It had an increase of 334.68 % over ADT (R) 45 and an increase of 323.71 % over TRY 2. Followed by the line N153-2 with an increase of 193.99 % over ADT (R) 45 and 141.51 % over TRY 2. The efficacy of HSD in boosting phenotypic expression was demonstrated by the larger percentage rise in chosen HSD plants above the base population and check varieties (21).

Hence the plants selected based on PI (SPY) will reflect positive response to selection with increased genetic gain and efficiency.

Efficiency of HSD in selection stable high yielding plants

The 35 selected plants showed a percentage increase of 213.86 % over total mean population in F_2. The best performing 28 plants selected in F_3 population under saline condition were from 12 progeny lines and plants within those progeny lines (Fig. 1) among 35 individual entries selected in F_2 population. The 12 progeny lines were S8, S91, S153, S181, S222, S259, S309, S721, S724, S749, S776 and S825 as in F_2 population. Similarly, the best performing 28 plants selected in F_3 population under normal conditions were from 15 progeny lines and plants within those progeny lines (Fig. 2) among 35 individual entries selected in F_2 population. The 15 progeny lines were N46, N97, N153, N204, N222, N251, N259, N272, N377, N403, N683, N721, N739, N749 and N776 as in F_2 population. As HSD control effectively the confounding effects of competition, heterozygosity and soil heterogeneity, the number of selected plants

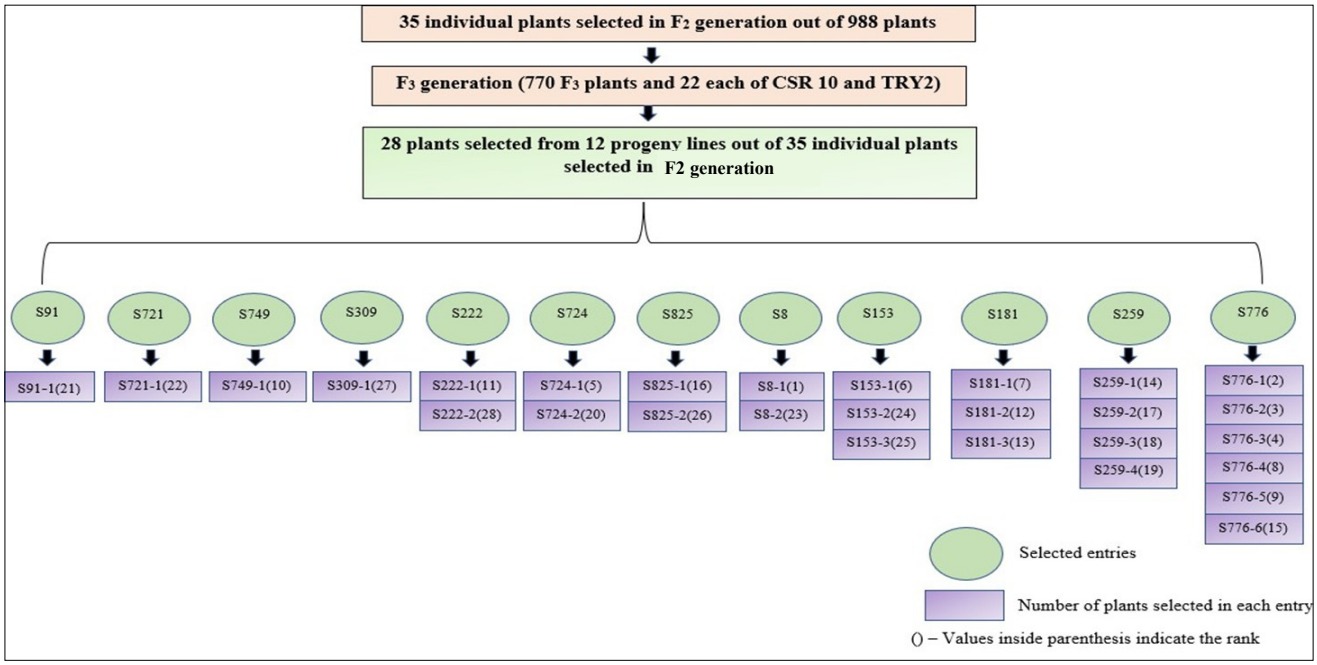

Fig. 1. Flowchart of 28 selected plants in F_3 generation under saline condition.

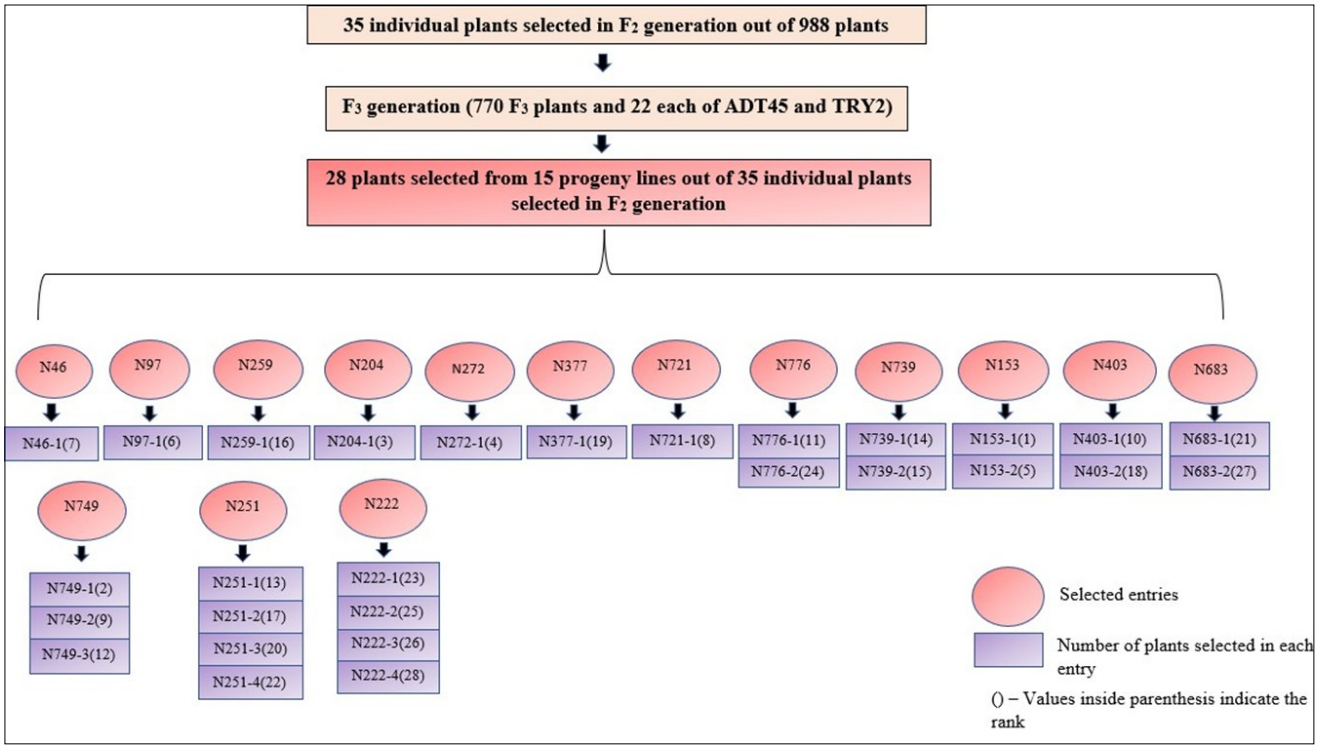

Fig. 2. Flowchart of 28 selected plants in F_3 generation under normal condition.

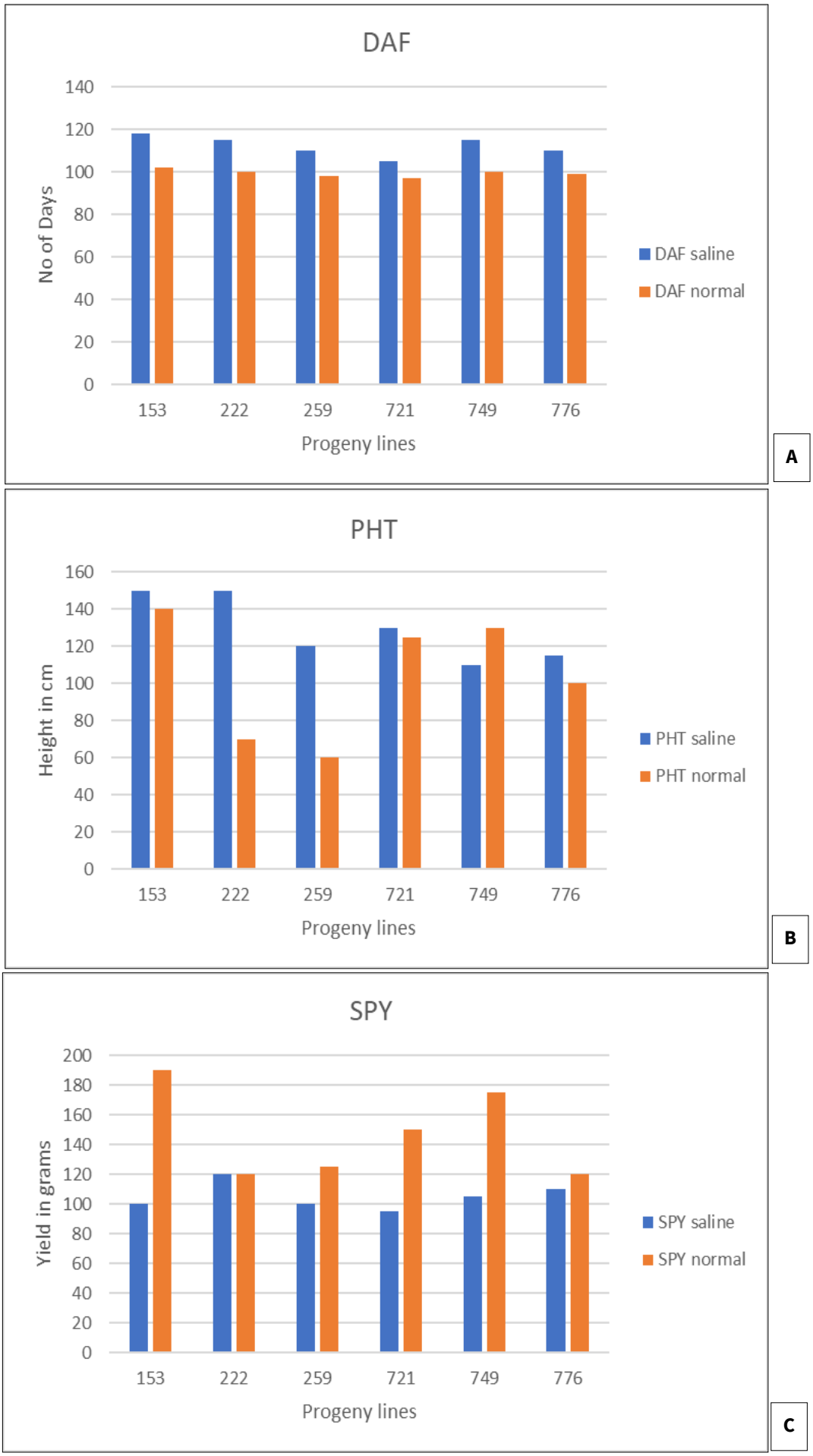

Fig. 3. Graphical representation of six F_3 selected progeny lines performing better under both saline and normal conditions, based on traits (a) days to flowering (DAF); (b) Plant height (PHT); (c) Single plant yield (SPY).

in this selection method can be reduced significantly, where the response to selection attains maximum values (18). Progeny lines and plants selected within those progeny lines like 153, 222, 259, 721, 749 and 776 were selected under both saline and normal conditions. Based on the recorded observations for days to flowering, plant height and single plant yield characters, the progeny line S776 (Fig. 3) was found to be early to medium maturing, semi-dwarf, high yielding along with desirable grain types like medium and long slender with white pericarp colour under both conditions. The experiment is mainly focused on identifying the genotypes that are suitable for cultivating under saline conditions. However, these six progeny lines were identified to be performing better under both conditions. Hence, density-neutral cultivars developed from these progeny lines in the future would give better results under both conditions i.e., there won't be any yield penalty if a cultivar developed from these lines is either planted under saline or normal conditions.

Similar reports showing the efficiency of the honeycomb method in promoting genetic gains for grain yield were observed for rice (22-23), durum wheat (24), spring wheat (25), winter rye (26), spring rye (27), oat (28), mung bean (29), faba bean (30), cotton (31-20) and maize (32 -33).

The results of this study are consistent with previous findings, where selecting top plants using the PPE under wide plant spacing (1.25 m) led to a significant increase in the prolificacy of the superior selected plants (33) and prolificacy can be increased effectively only under wide plant spacings (5). Thus, selection for individual plant yield is more effective at wider spacings where interplant competition is absent resulting in a significant response (24).

Study of marker association

Marker assisted selection (MAS) allows early selection for traits of interest, multiple cycles of selection in a year and pyramiding of tolerance components from different genetic resources. MAS for abiotic stress (except submergence tolerance) was not as effective as biotic stress. The identified marker in different backgrounds sometimes produces inconsistent results in MAS because identified QTLs in greenhouses behave differently in field conditions. The inconsistency and variability of QTLs in different genetic backgrounds and environments have limited their applications in breeding programs (9).

Therefore, it is essential to validate the identified markers within the target genetic background and environmental conditions before employing them in further MAS programs. In the F_2 generation, spikelet sterility showed a significant but weak negative correlation with total salt tolerance marker score, seedling stage salt tolerance marker score and reproductive stage salt tolerance marker score (Table 6). Similarly, the number of productive tillers exhibited a significantly weak negative correlation only with the seedling stage salt tolerance marker score. Whereas in F_3generation, none of the combinations has recorded significant correlation (Table 6). The failure to detect QTL related to salt tolerance might be due to absence of the SALTOL segment in chromosome one, in the recombinants or that the SALTOL marker being validated was weakly associated to salt tolerant genes due to the quantitative nature of the gene. In addition, it may be due to lack of coverage of all the regions of salt tolerance and yield related QTLs. Hence, addition of some more QTLs conferring salt tolerance especially at reproductive stage would improve the correlation for the characters.

Conclusion

In HSD, plants are planted at wider spacing, which helps in minimising the confounding effect of uneven soil conditions, ensuring that differences in plant performance are due to genetics and not due to soil variation, which is crucial when breeding for salt tolerance. Therefore, selecting for yield in the early generations using HSD enhances the efficiency of the breeding process by reducing the number of genotypes that need to be evaluated in later, costly yield trials, thereby increasing the genetic gain per unit cost.

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Authors' contributions

TS conceptualized the study, supervised the research process and assisted with final editing and suggestions for the manuscript. VAF designed the experiment and analyzed the data. RA^1 and YC contributed equally for carrying out the lab and field research work, manuscript preparation. KJ, VV and JC provided guidance on experimentation and correcting the manuscript. RIP, RA^2, SAMK and NM contributed to editing and participated in the manuscript's preparation. All authors have read and approved the final version of the manuscript [RA^1 stands for Rajam A and RA^2 for Rajeshwari A].

Compliance with ethical standards

Conflict of interest: The authors do not have any conflict of interest to declare.

Ethical issues: None

Table 6. Correlation of productive tillers, spikelet sterility with salt tolerance marker score in F_2 and F_3 generation

Traits	Total salt tolerance marker score		Seedling stage salt tolerance marker score		Reproductive stage salt tolerance marker score	
	F_2	F_3	F_2	F_3	F_2	F_3
Productive tillers	-0.151	0.048	-0.281**	0.157	0.172	0.085
Spikelet sterility	-0.350**	0.192	-0.272**	0.184	-0.252*	0.143

***** Significance at 5 % (0.197)

*Significance at 1 % (0.256)

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