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Genetic Architecture and Association Studies for Grain Yield and its attributing Traits in Recombinant Inbred Lines for Sodicity Tolerance in Rice (*Oryza sativa* L.)

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ABSTRACT

The present study was undertaken at NSP Unit 6 Agricultural Research Farm and Main Experimental Station of Acharya Narendra Dev University of Agriculture and Technology, Uttar Pradesh, India, from July to November 2020, in which genetic variability and heritability of 250 recombinant inbred lines (F_7) and their parents PUSA 44 and CSR 43 were evaluated and correlation studies were performed for grain yield and yield contributing traits. The experiment was conducted in two replications at two different and naturally occurring sodic conditions following α-lattice design. Analysis of variance revealed significant variations among the lines depicting inherent variability between lines. For attributes, viz., plant height, effective tillers plant^{-1}, spikelet fertility percentage, test weight and grain yield plant^{-1}, high phenotypic and genotypic coefficient of variation estimates were observed for both the locations indicating good scope for selection based on these traits. However, the attributes viz., test weight and effective tillers plant^{-1} values depicted the pertinent role of the environment and thus, require careful assessment in selection programs concerning these traits. For days to 50% flowering, filled grains panicle^{-1}, spikelet fertility percentage, and grain yield plant^{-1}, moderate heritability was discovered with a high genetic advance as a percentage of mean, indicating that there is less influence of environment over these traits. Correlation studies revealed a significant and positive association with these yield attributing traits. Mutual association between component traits exhibit variable responses at both locations, validating genotype and environment interactions among traits with respect to yield.

KEYWORDS: Correlation, GCV, heritability, PCV, rice, RILs, sodicity

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1. INTRODUCTION

Rice (*Oryza sativa* L.) is the primary food crop consumed by 1/3rd of the World's population. It accounts for 35–75% of the calorie intake of more than 3.5 billion humans (Krishnamurthy et al., 2016; Snehi et al., 2022). In 2020, global milled rice production was 504.74 mt, with average productivity of 4.60 t ha^{-1} across 164.19 million hectares (mha) (Anonymous, 2021). In 2020, India produced 102.36 mt of rice in an area of 40.10 mha. in India, with a productivity of 2.5 t ha^{-1} (Anonymous, 2021). A grain yield gain of 1.0–1.2% $year^{-1}$ in rice is necessary beyond 2020 to feed the expanding world population (Anonymous, 2013).

Globally ~953 mha area has Salt-Affected Soil (SAS), i.e., 6.2% of the world area, 1/5th of the world arable land and 1/3rd of irrigated agricultural area (Anonymous, 2014; Machado and Serralheiro, 2017). Of these, 52% are sodic soil. About 3.77 mha area of soil in India is affected by sodicity stress (Arora et al., 2016; Kumar and Sharma, 2020). Sodic soil is distributed in the Indo-Gangetic plains (1.78 mha), the arid and semi-arid regions of western and central India, and Peninsular India (Kumar and Sharma, 2020). Uttar Pradesh (U.P.) shares 20.3% SAS across the country consisting of 35.6% sodic soil and 1.3% saline soil. In specific ways, soil sodicity generated by $NaHCO_3$ and Na_2CO_3 is more problematic than soil salinization induced by neutral salts like NaCl and Na_2SO_4. (Zhang et al., 2012). Sodic soil has a pH range of 8.5 to 10.5 (> saline soil), a high Exchangeable Sodium Percentage (ESP>15), a high Sodium Absorption Ratio (SAR>13) and an Electrical Conductivity (EC) of less than 4 dSm^{-1}. Excess sodium on the exchange complex and a high concentration of carbonate/bicarbonate anions turns the soil black.

Sodicity is one of the pressing concerns of all the abiotic problems that rice comes across throughout its growing stage, restricting its output and productivity. About 30% of the world's rice growing land is affected by salinity (Wang et al., 2012; Hopmans et al., 2021). Rice is considered an especially salt–susceptible cereal, and the crop response to sodicity stress varies with the growth stage (Munns and Tester, 2008; Liang et al., 2015; Sheoran et al., 2021). High pH and poor soil structure cause phosphorus, zinc, iron insufficiency, and boron toxicity. When plants are harmed by sodicity, they collect less critical nutrients from the soil. (Upadhyay et al., 2020). Reduced germination rate, stunted plant growth, limited root development, low tillering, spikelet sterility and number, low test weight and yield, uneven field growth, poor root growth, leaf rolling, low harvest index, leaf browning, delayed flowering, reduced seed set due to lower pollen viability and mortality are some of the pertinent morphological signs a plant shows when grown in sodic soil. Extensive field trials also showed yield losses ranging from 36 to 69% in rice cultivated on sodium salt-affected soils compared to normal ones (Qadir et al., 2014). Therefore, understanding the genetic architecture of phenotypic traits governing tolerance under sodic conditions is paramount.

Given the scenario mentioned above, Recombinant Inbred Lines (RILs) were developed from PUSA 44 (Susceptible) and CSR 43 (Tolerant) and grown under natural sodicity to evaluate their genetic variability, heritability and correlation for yield and its attributing traits to select the same for plant breeding programme.

2. MATERIALS AND METHODS

2.1. Crossing and development of RILs

The genetic materials consisted of 250 RILs generated from PUSA 44 as female and CSR 43 as male parent (Table 1). The cross was made between them in *kharif* (July, 2016), at the Agricultural Research Farm of the Institute of Agricultural Sciences, Banaras Hindu University (BHU), Varanasi and F_{1s} were obtained. The F_{1s} were validated through molecular markers, and a single F_1 plant was grown at ICAR-National Rice Research Institute (NRRI) in *rabi* (January, 2016). The main season crop was grown at BHU, Varanasi, while off-season at ICAR-NRRI, Cuttack using Single Seed Descent (SSD) method up to F_7 generation. Finally, 250 RILs were developed in October, 2019 (Figure 1).

Table 1: Profile of parents used in the crossing program

	PUSA 44 (Parent I)	CSR 43 (Parent II)
Parentage	IARI-5901-2 × IR-8	KDML105×IR4630-22-2-5-1-3×IR20925-33-3-1-28
Year and place of release	1994 from IARI, New Delhi	2011 from CSSRI, Karnal
Plant height	Semi dwarf (95–110 cm)	Semi dwarf (95 cm)
Maturity duration	140 days	110 days
Grain type	Long and slender	Long and slender
Response against sodicity stress	Susceptible (Krishnamurthy et al., 2016)	Tolerant (pH 10) (Singh et al., 2013)
Average score for sodicity tolerance	0	3

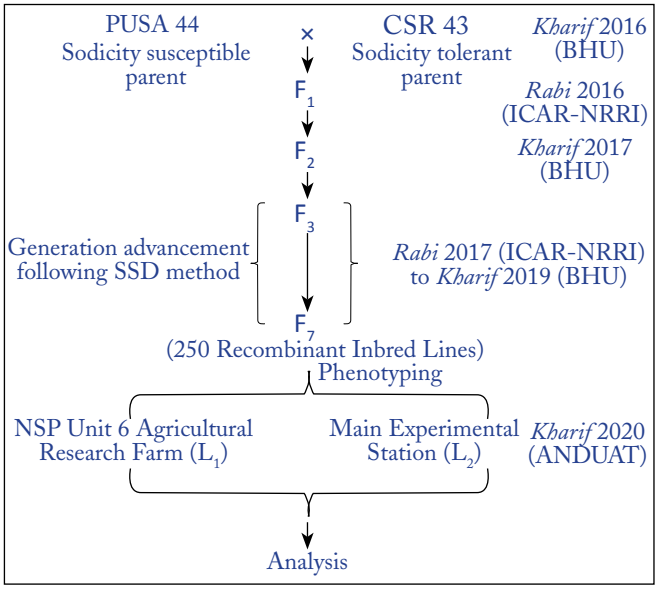

Figure 1: Genealogical representation for the development of recombinant inbred lines

2.2. *Experimental site and evaluation of RILs*

The 250 RILs were evaluated in July–November 2020 at NSP Unit 6, Agricultural Research Farm (L_1) and Main Experimental Station (L_2) of Acharya Narendra Dev University of Agriculture and Technology (ANDUAT), Kumarganj, Ayodhya. In the Gangetic alluvium of Eastern Uttar Pradesh, Ayodhya is located in the semi-arid zone with a latitude of 26°47' North and a longitude of 81°12' East, at the height of 113 m above mean sea level, with monsoon supplying 80% of total precipitation. The soil samples of both the locations of ANDUAT were collected at pre-transplanting and post-harvesting. Afterwards, the samples were mixed thoroughly in equal proportion and were subjected to pH, Electrical Conductivity (EC), Exchangeable Sodium Percentage (ESP), and Sodium Absorption Rate (SAR) analysis. Post Analysis, the two locations (L_1 and L_2) were found to have varying sodicity conditions. L_1 was found to have a pre-transplanting and post-harvest pH of 9.6 and 9.2, respectively, while the EC (at 25°C), ESP and SAR were 0.65 dSm^{-1},75.57 and 19.42 $mmoleL^{-1}$. The pre-transplanting and post-harvesting pH analysis of L_2 was 9.0 and 8.7, respectively, whereas the EC (at 25°C), ESP and SAR were 2.24 dSm^{-1}, 52.38 and 14.28 $mmoleL^{-1}$. Accordingly, the soil at L_1 and L_2 were classified as moderately and slightly sodic, respectively.

Sowing of F_7 seeds nursery was done. Later, after 21 days, F_7 seedlings were transplanted at two locations (L_1 and L_2) of ANDUAT following the α-lattice design of the experiment with two replications of 20×20 cm^2 spacing. The data used for analysis were an average of five plants $line^{-1}$ from each replication for the traits viz., Days to 50% flowering, Days to maturity, Plants height (cm), Filled grains $panicle^{-1}$, Unfilled grains $panicle^{-1}$, Spikelet fertility percentage, Test weight (g) and Grain yield $plant^{-1}$ (g). Standard agronomic practices were followed as per recommendation.

2.3. *Statistical analysis*

Analysis of variance (ANOVA) was performed over the mean phenotypic data collected in the study using the 'R' programming language environment for α-lattice design to know the mean sum of square values of all the traits. Using 'R' programming language Bartlett's test was performed to determine the homogeneity of variances. The GCV and PCV were calculated according to Burton and Devane's 1953 technique and categorized as recommended by Sivasubramanian and Menon, 1973. Broad sense heritability (h^2_{BS}) and Genetic Advance (GA) were calculated using Allard's formula, 1960, while heritability estimates were classified using the approach given by Johnson et al.,1955. The Correlation analysis was carried out using the R package and classified according to the method provided by Searle, 1965.

3. RESULTS AND DISCUSSION

3.1. *Analysis of variance*

No pooling of ANOVA was done since the variances between the two data groups were not homogeneous. Analysis of variances was highly significant ($p<0.01$) for all traits (Tables 2 and 3) sown at two locations and screened under a natural sodium environment except for days to 50 % flowering, Plant height at L_1, while days to flowering,

Table 2: ANOVA for 11 yield attributing traits in recombinant inbred lines (RILs) sown at L_1

Sources	d.f.	DF	DM	PH (cm)	ET	PL (cm)	FGPP	UFGPP	SF%	TW (g)	SPAD	GYPP (g)
Treatments	251	2561.3	3729.3**	967.69	57.430**	142.55**	2562.7**	938.58**	1170.49**	107.79**	420.56**	124.72**
Replications	1	1679.4	1292.2	1445.32	133.783	681.19	4838.6	1921.58	2209.31	307.25	889.32	140.15
Blocks$	17	2387.9	3646.7	746.72	56.387	74.73	1789.3	608.83	759.74/	88.58	253.87	132.90
Error	234	1980.3	1859.7	699.02	28.366	110.83	1256.3	315.49	392.78	58.76	194.84	73.44

**: $p< 0.01$; $: ignoring treatments

Table 3: ANOVA for 11 yield attributing traits in recombinant inbred lines (RILs) sown at L_2

Sources	d.f.	DF	DM	PH (cm)	ET	PL (cm)	FGPP	UFGPP	SF%	TW (g)	SPAD	GYPP (g)
Treatments	251	2230.3	4348.4	900.4**	88.43**	153.4**	507.19**	1142.8**	624.91**	125.79**	368.2	101.21**
Replications	1	1197.4	1738.3	1856	316.3	336.23	798.6	2259.7	266.09	364.48	836.2	176.8
Blocks$	17	1940.1	4049.1	773.4	61.64	98	409.79	793.3	420.1	104.32	510.2	84.67
Error	234	1794.6	3567	462.3	49.61	77.74	200.81	819.8	275.55	63.89	279.7	45.01

**: $p< 0.01$; $: ignoring treatments; d.f.: degree of freedom; DF: Days to 50% flowering; DM: Days to maturity; PH: Plant height (cm) ; ET: Effective tillers plant^{-1}; PL: Panicle length (cm); FGPP: Filled grain panicle^{-1}; UFGPP: Unfilled grain panicle^{-1}; SF%: Spikelet fertility percentage; TW: Test weight; SPAD: Soil plant analysis development (Chlorophyll content); GYPP: Grain yield plant^{-1}

days to maturity, panicle length and SPAD were found to be insignificant at L_2. The analysis underlines the significant level of variability present among the advanced lines. These can be selected for improvement and genetic analysis studies.

3.2. Genetic Variability

Variability parameter estimates were shown in Tables 4 and 5. Based on PCV and GCV, a comparison was made between the relative magnitudes of the phenotypic and genotypic variations of the characteristics. The PCV was comparatively higher than GCV for traits for both locations. At L_1, panicle length (10.386) showed the highest difference between PCV and GCV, followed by grain yield plant^{-1} (7.272). At the same time, the lowest difference was recorded for days to maturity (1.399). Similarly, at L_2, the highest difference existed for effective tillers plant^{-1} (11.084), while filled grains panicle^{-1} (1.647) recorded the lowest difference. PCV was found to be highest for effective tillers plant^{-1} (38.331), followed by panicle length (25.633) and grain yield plant^{-1} (25.357). Moderate PCV was reported to be of test weight (19.981), followed by SPAD (13.374), filled grains panicle^{-1} (12.065) and spikelet fertility percentage (11.947). The lowest PCV was found for days to maturity (5.842) and unfilled grain panicle^{-1} (7.325). The highest GCV at L_1 was reported to be of effective tillers plant^{-1} (29.243). Panicle length (15.247), test weight (14.718), grain yield plant^{-1} (18.085), and SPAD (10.408) fell in the range of moderate GCV, while the lowest GCV was exhibited by days to maturity (4.443) and days to 50% flowering (5.973). The highest PCV displayed at L_2 was by effective tillers plant^{-1} (40.783). Moderate PCV was shown by panicle length (20.471), grain yield plant^{-1} (19.173), and test weight (17.069). In contrast, days to maturity (7.929) and filled grains panicle^{-1} (8.718) displayed the lowest PCV.

For some characteristics, however, apparent variation was linked to genetic architecture and the environment; thus, selection can be deceiving in these cases. Although the traits under consideration are quantitative and follow genotype×environment interaction, the observation

Table 4: Estimates of genetic variability parameters for 11 yield attributing traits for L_1

Sources	DF	DM	PH (cm)	ET	PL (cm)	FGPP	UFGPP	SF%	TW (g)	SPAD	GYPP (g)
Mean	69.12	124.45	42.79	6.68	13.09	54.79	68.31	45.58	15.12	31.32	12.44
σ^2g	290.5	934.8	134.33	14.532	15.86	653.2	311.545	291.355	24.515	112.86	25.641
σ^2p	2270.8	2794.5	833.35	42.898	126.69	1909.5	627.035	879.135	83.275	307.7	99.086
PCV (%)	9.988	5.842	12.55	38.331	25.633	12.065	7.325	11.947	19.981	13.374	25.357
GCV (%)	5.973	4.443	7.95	29.243	15.247	9.227	6.150	9.064	14.718	10.408	18.085
h^2_{BS}	12.793	33.451	16.12	33.876	12.519	34.208	49.685	33.141	29.439	36.679	25.878
GA (1%)	16.277	47.215	12.42	5.924	3.762	39.911	33.219	26.237	7.173	17.179	6.878
GAM (1%)	23.550	37.936	29.03	88.729	28.744	72.847	48.627	57.562	47.442	54.857	55.275

σ^2g: Genotypic variance; σ^2p: Phenotypic variance; PCV (%): Phenotypic coefficient of variation expressed in percentage; GCV (%): Genotypic coefficient of variation expressed in percentage; h^2_{BS}: Broad sense heritability in percent; GA (1%): Genetic advance in percent at selection intensity (k)=1%, GAM (1%) : Genetic advance as percent of mean expressed in percent at selection intensity (k)=1%

Table 5: Estimates of genetic variability parameters for 11 yield attributing traits for L_2

Sources	DF	DM	PH (cm)	ET	PL (cm)	FGPP	UFGPP	SF%	TW (g)	SPAD	GYPP (g)
Mean	74.90	128.03	43.63	7.07	16.02	49.76	61.90	42.04	18.28	31.54	15.25
σ^2g	317.8	390.7	219.05	19.41	37.83	153.19	161.5	174.68	30.95	44.25	28.1
σ^2p	2612.4	3957.7	681.35	69.02	115.57	354	981.3	450.23	94.84	323.95	73.11
PCV (%)	9.545	6.195	11.710	40.783	20.471	8.718	9.041	10.956	17.069	13.452	19.173
GCV (%)	5.637	3.473	8.818	29.699	15.484	7.071	5.758	8.647	12.901	8.178	15.097
h^2_{BS}	12.167	9.872	32.149	28.122	32.733	43.274	16.458	38.798	32.634	13.660	38.435
GA (1%)	16.604	16.582	22.406	6.238	9.396	21.739	13.765	21.980	8.485	6.564	8.775
GAM (1%)	22.167	12.952	51.357	88.264	58.660	43.690	22.235	52.280	46.413	20.813	57.535

σ^2g: Genotypic variance; σ^2p: Phenotypic variance; PCV (%): Phenotypic coefficient of variation expressed in percentage; GCV (%): Genotypic coefficient of variation expressed in percentage; h^2_{BS}: Broad sense heritability in percent; GA (1%): Genetic advance in percent at selection intensity (k)=1%, GAM: Genetic advance as percent of mean expressed in percent at selection intensity (k)=1%

supported good scope for selecting attributes with higher differences between PCV and GCV. The traits showing maximum PCV should be carefully considered for selection despite environmental influence. These variability results follow Bisen et al.,2019; Gupta et al., 2020.

3.3. Heritability and genetic advance

The degree of phenotypic variation induced by gene activity is measured by heritability (h^2). According to Johnson and colleagues, 1995, heritability alone does not offer a substantial quantity of genetic improvement resulting from genotype selection. Concurrently using genetic advance as a percent of the mean (GAM) was recommended to supplement this. GAM is the increase in the selected family's mean over the baseline population. Heritability and expected GAM indicate the mode of action in expressing traits, which help choose an appropriate breeding methodology. Broad sense heritability (h^2_{BS}), according to Lush, 1949, is the ratio of genotypic to phenotypic variation expressed in percent. The traits under evaluation ranged from low to high heritability, with the majority expressing moderate values. Genetic advance as percent of mean was categorized into the levels such as low, moderate and high genetic advance described by Falconer, 1989. Moderate heritability coupled with high GAM were exhibited by filled grains panicle^{-1} (34.208; 72.847), effective tillers plant^{-1} (33.876; 88.729) and spikelet fertility percentage (33.141; 57.562) at L_1 suggested preponderance of both additive and non-additive gene interactions. Hence simple selection may be used to take advantage of these component traits. L_2 data exhibited moderate h^2_{BS} with high GAM for panicle length (32.783; 58.660), grain yield plant^{-1} (38.435; 57.535), spikelet fertility percentage (38.798; 52.280) and

Table 6: Phenotypic correlation coefficient among 11 yield attributing traits at L_1

Sources	DF	DM	PH (cm)	ET	PL (cm)	FGPP	UFGPP	SF%	TW (g)	SPAD	GYPP (g)
DF	1										
DM	0.896**	1									
PH	-0.443**	0.567**	1								
ETs	0.059	0.596**	0.206**	1							
PL	0.106	0.166	-0.389**	0.121	1						
FGPP	0.505**	0.443**	-0.409**	0.303**	0.683**	1					
UFGPP	0.664**	0.593**	0.107	-0.496**	0.625**	-0.526**	1				
SF%	0.249**	-0.576**	0.420**	0.737**	0.753**	0.826**	-0.685**	1			
TW	0.233**	0.197**	-0.563**	0.809**	0.862**	0.756**	-0.431**	0.473**	1		
SPAD	0.024	-0.893**	-0.079**	0.096	-0.107	0.507**	-0.154	0.084	0.644**	1	
GYPP	0.595**	0.533**	-0.672**	0.795**	0.38**	0.711**	-0.391**	0.848**	0.847**	0.145	1

**p< 0.01

Table 7: Phenotypic correlation coefficient among 11 yield attributing traits at L_2

Sources	DF	DM	PH (cm)	ET	PL (cm)	FGPP	UFGPP	SF%	TW (g)	SPAD	GYPP (g)
DF	1										
DM	0.934**	1									
PH	-0.370**	0.882**	1								
ETs	0.677**	0.121	-0.459**	1							
PL	0.017	0.165**	-0.128	-0.135**	1						
FGPP	0.613**	0.577**	-0.505**	0.795**	0.81**	1					
UFGPP	0.072	0.601**	0.597**	-0.583	0.752**	-0.539**	1				
SF%	0.194**	-0.450**	0.570**	0.735**	0.169**	0.813**	-0.259**	1			
TW	0.412**	0.105	-0.352**	0.82**	0.575**	0.787**	-0.554**	0.852**	1		
SPAD	-0.879**	-0.884**	-0.51**	0.115	0.103	0.626**	0.114	0.1	0.792**	1	
GYPP	0.688**	0.185**	-0.764**	0.830**	0.867**	0.918**	-0.556**	0.834**	0.847**	0.351**	1

**$p < 0.01$

plant height (32.149;51.357). Similarly, test weight (32.634; 46.413) and filled grains panicle^{-1} (43.274; 43.690) showed moderate heritability with moderate GAM (Tables 4 and 5)

3.4. Correlation studies among grain yield and its contributing traits

The correlation coefficient in Tables 6 and 7 show how two variables are related. Yield is the ultimate trait of concern for plant breeders. It is visualized that yield is a complex trait governed by several components characters. The relationship between component characters might be owing to pleiotropic gene activity, linkage, or, more often, both. (Gupta et al., 2020). Grain yield plant^{-1} showed a strong, non-negative and significant relationship with plant height (0.672), effective tillers plant^{-1} (0.795) and filled grains panicle^{-1} (0.711), spikelet fertility percentage (0.848), test weight (0.847). Days to 50% flowering (0.595) and days to maturity (0.533) showed a positively significant and moderately strong correlation with grain yield plant^{-1}. Traits viz., panicle length, unfilled grains panicle^{-1}, showed a weak correlation with grain yield plant^{-1} at L_1.

At L_2, most of the traits had a significant, strong and positive correlation with grain yield plant^{-1} viz., days to 50% flowering (0.688), effective tillers plant^{-1} (0.830), panicle length (0.867) and filled grain panicle^{-1} (0.918). In contrast, the days to maturity showed a positively significant but weak association with grain yield plant^{-1}. Plant height showed a significant negative correlation with grain yield plant^{-1} for both locations.

3.5. Mutual correlations among the contributing component traits

At L_1, days to maturity hold a significantly positive and very strong correlation with days to 50% flowering (0.896). At the same time, it shows a moderately strong but significant positive association (0.567) with plant height. In contrast, day to maturity has a moderately significant but negative correlation with spikelet fertility percentage (-0.576). Another trait, viz., plant height, displayed a moderately strong but negative correlation with days to 50% flowering (-0.443) and a very weak correlation with effective tillers plant^{-1} (0.206). It revealed a moderately strong significant and negative correlation with filled grain panicle^{-1} (-0.409) and test weight (-0.563). Panicle length and spikelet fertility percentage showed a strong, positively significant mutual correlation (0.753). Test weight showed a very strong and significant relation with filled grains panicle^{-1} (0.756) and SPAD (0.644). Spikelet fertility percentage shared a moderately weak and significantly positive association with test grains weight.

At L_2, plant height displayed a negatively significant and moderately weak correlation with days to 50% flowering (-0.370). A non-significant, non-positive relationship was exhibited for panicle length and plant height (-0.128) and among effective tillers plant^{-1} and panicle length (-0.135). Test weight showed a moderately weak but significant negative correlation with plant height (-0.352), and SPAD revealed a highly significant and positive correlation with test grains weight (0.792). Effective tillers plant^{-1} were found to be non significantly correlated with days to 50% maturity (0.121). Filled grain panicle^{-1} was negative but highly significant with plant height (-0.505).

4. CONCLUSION

RILs under investigation showed a broad range of variability. Spikelet fertility percentage, test weight, panicle length and filled grains plant^{-1} can be used as

selection indices for improvement. They had a strong, positive and significant correlation with Grain yield Plant^{-1}. Unfilled grain panicle^{-1} showed a positive association with plant height. The data revealed early maturing lines should be selected as these showed a positive and significant relationship with effective tillers plant^{-1} and test weight. The study indicated a need for careful selection for Plant height since it is negatively correlated with unfiled grains per panicle.

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