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Multivariate analysis of wild rice MAGIC population under sodic soil condition

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Abstract

A study was conducted with 98 wild rice MAGIC lines under sodic soil conditions to estimate potential variation among rice genotypes. Observations on 12 morphometric traits were subjected to multivariate analyses viz., PCA and cluster analysis to assess genetic diversity. A correlogram was generated to depict the association among the traits. Results of ANOVA suggested the existence of significant variability among the lines. PCA showed that PC1 and PC2 represented 54 per cent of variation. PC1 accounted for the highest variance (38 %) for six characters followed by PC2 which accounted for (15 %) for four characters. Flag leaf area, plant height, panicle length, flag leaf length, flag leaf breadth and panicle breadth were identified as vital traits contributing to variability. Based on hierarchical cluster analysis, the genotypes were grouped into eight clusters. Correlation analysis suggested that the traits viz., number of productive tillers, number of grains per panicle, panicle length and 100 grain weight had a significant correlation with grain yield. Based on *per se* performance and correlation, the genotypes WRM 6, WRM 10, WRM 22, WRM 29 and WRM 105 were identified as superior and could be exploited as genetic stocks for rice improvement under sodic soil conditions.

Key words: Rice, MAGIC, principal components, cluster analysis, correlation

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's cardinal staple food crops contributing to the food security of the majority of the global population and accounts for about 50–80 per cent of daily calorie intake (International Rice Genome Sequencing Project, 2005; Food and Agriculture Organization, 2019). The application of new possibilities in rice breeding is an expected prospect to release competitive genotypes in comparison with traditional ones (Pauk *et al.*, 2009). The Multi-Parent Advanced Generation Inter-Cross (MAGIC) strategy has been devised to integrate a multitude of alleles and to enable high resolution mapping and a high rate of recombination (Cavanaugh *et al.*, 2008). MAGIC populations are large sets of recombinant inbred lines (RILs) that are a genetic mosaic of multiple founder parents. They represent an important pre-breeding resource, not only for the final lines obtained but also for the possibility of developing

new combinations. Wild rice germplasm is considered a valuable genetic resource for improving rice cultivars.

It is assessed that approximately 950 million hectares of arable land globally, involving 250 million hectares of irrigated land, is affected by salinity (Yamaguchi and Blumwald, 2005; Shahbaz and Ashraf, 2013). It is one of the most devastating abiotic stresses in rice, and the salt-affected soils account for about 20 per cent of the total paddy cultivating area (Zhou *et al.*, 2016). A high level of sodium in the soil causes osmotic stress on cell water relations and increases the toxicity of sodium in the cytosol leading to damage to cell metabolism (Gupta *et al.*, 2020). Salt tolerant plants maintain a low cytosolic Na⁺ / K⁺ ratio through some strategies viz., extrusion of excess Na⁺ through roots and compartmentalization in different parts, which in turn decreases Na⁺ concentration

in the cytosol. This osmotic adjustment aids in overcoming adverse salt effects, thus producing a considerable yield (Chakraborty *et al.*, 2018). The easiest and eco-friendly way to address the problem is through the development of salt tolerant varieties (Quijano-Guerta and Kirk, 2002). Adequate knowledge of genetic variation among different genotypes is a preliminary step in the breeding program for the selection and production of new varieties (Kumbhar *et al.*, 2015; Ahmed *et al.*, 2016). Multivariate analysis is used to measure the degree of difference between the genotypes. Principle Component Analysis (PCA) and cluster analysis are used to assess variation as multivariate methods (Maji and Shaibu, 2012; Tiwari *et al.*, 2020). PCA is used to study diversity and to determine the influence of several characteristics on diversity. Cluster analysis is used when we need to classify genotypes according to genetic or agronomic traits into different groups (Shabir *et al.*, 2013). Considering these facts, a study was carried out to assess genetic components of variability and genetic diversity among rice MAGIC lines under sodic soil conditions.

MATERIALS AND METHODS

The F_{5,6} generation material of wild rice magic lines developed in IRRRI by crossing eight different *Oryza* species viz., (*O. sativa*, *O. nivara*, *O. rufipogon*,

O. meridionalis, *O. glaberimma*, *O. barthi*, *O. glumaepatula*, and *O. longistaminata*) obtained from the Department of Rice, Tamil Nadu Agricultural University, Coimbatore was used in the present study (Table 1). The experimental materials were raised in Anbil Dharmalingam Agricultural College and Research Institute, Trichy under sodic soil condition (EC -1.5 dSm⁻¹, pH – 8.75 and ESP 42.4) during November 2020, in randomized block design with three replications by adopting a spacing of 20 × 20 cm. Observations on 12 morphological traits viz., days to 50% flowering, plant height (cm), flag leaf length (cm), flag leaf breadth (cm), the number of productive tillers, panicle length (cm), panicle breadth (cm), the number of grains per panicle, 100 grain weight (g), flag leaf area (cm²), grain yield per plant (g) and spad meter reading, were recorded in five random plants in each replication. The mean for all the characters was subjected to Analysis of Variance (ANOVA) based on the model proposed by Panse and Sukhatme (1969). The principal component analysis was carried out as suggested by Rao (1952). Statistical analysis was performed using different software such as TNAU STAT to analyse ANOVA, STAR 3.0 for cluster diagram and R Studio to calculate PCA and correlogram.

RESULTS AND DISCUSSION

The ANOVA revealed that a highly significant difference

Table 1. List of genetic materials used in the study

S.No.	Genotypes name	S.No.	Genotypes name	S.No.	Genotypes name	S.No.	Genotypes name
1	<i>O. sativa</i>	26	WRM 26	51	WRM 75	76	WRM 116
2	NSCRE222	27	WRM 27	52	WRM 77	77	WRM 117
3	<i>O. glaberimma</i>	28	WRM 29	53	WRM 78	78	WRM 119
4	<i>O. sativa ssp. P₂</i>	29	WRM 30	54	WRM 79	79	WRM 120
5	WRM 1	30	WRM 51	55	WRM 80	80	WRM 121
6	WRM 2	31	WRM 52	56	WRM 82	81	WRM 122
7	WRM 3	32	WRM 53	57	WRM 85	82	WRM 124
8	WRM 6	33	WRM 54	58	WRM 88	83	WRM 125
9	WRM 7	34	WRM 55	59	WRM 89	84	WRM 126
10	WRM 8	35	WRM 56	60	WRM 90	85	WRM 127
11	WRM 10	36	WRM 57	61	WRM 91	86	WRM 129
12	WRM 11	37	WRM 60	62	WRM 92	87	WRM 130
13	WRM 12	38	WRM 61	63	WRM 95	88	WRM 131
14	WRM 13	39	WRM 62	64	WRM 97	89	WRM 132
15	WRM 14	40	WRM 63	65	WRM 98	90	WRM 133
16	WRM 15	41	WRM 64	66	WRM 105	91	WRM 134
17	WRM 16	42	WRM 65	67	WRM 106	92	WRM 135
18	WRM 17	43	WRM 66	68	WRM 107	93	WRM 136
19	WRM 18	44	WRM 67	69	WRM 108	94	WRM 137
20	WRM 19	45	WRM 68	70	WRM 109	95	WRM 138
21	WRM 20	46	WRM 69	71	WRM 111	96	WRM 139
22	WRM 21	47	WRM 70	72	WRM 112	97	WRM 140
23	WRM 22	48	WRM 72	73	WRM 113	98	WRM 158
24	WRM 23	49	WRM 73	74	WRM 114		
25	WRM 25	50	WRM 74	75	WRM 115		

existed for all the 12 characters studied (**Table 2**). Wide variability was observed among the genotypes for all the morphological traits as evinced by a wide range of the traits. Among the traits, the number of grains per panicle exhibited a wide variation ranging from 73.33 g (WRM 25) to 324.67g (WRM 10) with a mean of 128.58 g. WRM 29 recorded the longest plant height of 161.33 cm and the shortest was observed in WRM 80(71.67 cm) with a mean value of 119.23 cm.

Flag leaf length showed larger variation ranged from 74.67 cm (WRM 17) to 18 cm (WRM 80) with an average of 41.11cm. Flag leaf breadth was broad in WRM 105 (2.30 cm) and narrow in WRM 25 (0.80 cm) with a mean of 1.31cm. Maximum numbers of productive tillers were produced in WRM 85 (22) and minimum in WRM 30 (2.33) with an average of 11.99. Panicle length was longer in WRM 1 (35.83 cm) and shorter in WRM 80 (14.33 cm) with a mean value of 26.07cm. The line WRM 21 produced the longest panicle breadth of 1.93 cm and WRM 80 produced the shortest panicle breadth of 0.77cm with a mean of 1.21cm.

The highest 100 grain weight was recorded in WRM 6 (3.71 g) and the lowest in WRM 60 (1.87 g) with an average grain weight of 2.93 g. A larger flag leaf area was recorded in WRM 21 (66.63 m²) and the shorter of 11.53 m² was recorded in WRM 80 with a mean of 2.93 m². The average day for 50 per cent flowering was 104.8 days and the genotype WRM 27 was observed to be early flowering (81.67 days) and WRM 22 recorded late flowering (131 days). As regards the maximum grain yield was recorded by WRM 10 (69.0 g).

Principal component analysis (PCA) is a statistical technique to reduce a large set of data into smaller

components, without losing the essential information, by taking into consideration the interrelationship among the variables. In the present study, PCA analysis pooled all the 12 traits into four major principal components each with eigenvalues greater than one, which accounted for 74 per cent of total variation (**Fig. 1**). PC1 axis alone accounted for 38 per cent of variation and the major contributing traits towards variation were flag leaf area, plant height, panicle height and flag leaf length. PC2 axis accounted for 15 per cent of the total variation, which was explained by the traits number of productive tillers and grain yield per plant (**Table 3**). The first two PC axes together explained more than 50 per cent of the variation, which indicated a high degree of association between the traits under consideration (Subramanian *et al.*, 2019). Similarly, Tuhina-khatun *et al.* (2015), based on a study of 22 morphological traits in 43 upland rice genotypes, reported that 74 per cent of the total variability was contributed by the first four principal components.

Individual PCA plots revealed the distribution of genotypes based on both PC1 and PC2 scores and the colour variations assigned depended on the quality of representation of each genotype in PC axes (**Fig.2**). The genotypes (WRM 112, 127, 79, 80, 25 and 10) had the highest quality representation of the PC axes and among them, the genotypes (WRM 10, 25 and 80) were the most diverse genotypes as they had higher interaction with PC1 axis. The MAGIC population was classified into seven groups (**Fig. 3**) and the genotypes belonging to the same grouping are similar performing lines. The biplot (PC1 vs PC2) showed the interaction among the characters and also their interaction with each genotype (**Fig 4**). The vector length of each trait reflects its contribution towards the total divergence, the higher the length more the contribution. In the present study, the trait flag leaf

Table 2. Summary statistic and variance of ANOVA for 12 quantitative traits

S.No.	Character	Variance from ANOVA	Mean	Maximum	Minimum	SE	CD(p=05)	CD(p=01)
1.	Days to 50% flowering	707.18**	104.81	131.00 (WRM 22)	81.67 (WRM 27)	1.64	4.55	5.99
2.	Plant height (cm)	785.82**	119.23	161.33 (WRM 29)	71.67 (WRM80)	4.48	12.43	16.36
3.	Flag leaf length (cm)	298.10**	41.11	74.67 (WRM 17)	18.00 (WRM80)	4.88	13.52	17.79
4.	Flag leaf breadth(cm)	0.15**	1.31	2.30 (WRM 105)	0.80 (WRM 25)	1.16	0.44	0.57
5.	Number of productive tillers	57.39**	11.99	22.00 (WRM 85)	2.33 (WRM 30)	1.12	3.09	4.07
6.	Panicle length(cm)	35.92**	26.07	35.83 (WRM 1)	14.33 (WRM 80)	1.68	4.65	6.12
7.	Panicle breadth(cm)	0.15**	1.22	1.93 (WRM 21)	0.77 (WRM80)	0.13	0.37	0.49
8.	Number of grains per panicle(g)	4125.4**	128.58	324.67(WRM 10)	73.33 (WRM 25)	8.37	23.20	30.55
9.	100 grain weight(g)	0.22**	2.93	3.71 (WRM 6)	1.87 (WRM 60)	0.06	0.18	0.24
10.	Flag leaf area (m ²)	418.29**	34.93	66.63 (WRM 22)	11.53 (WRM55)	6.22	17.25	22.70
11.	Grain yield per plant (g)	247.21**	16.92	69.00 (WRM 10)	3.33 (WRM80)	1.73	4.80	6.31
12.	SPAD index	41.61**	36.46	48.13 (WRM 10)	27.53 (WRM109)	2.35	6.50	8.56

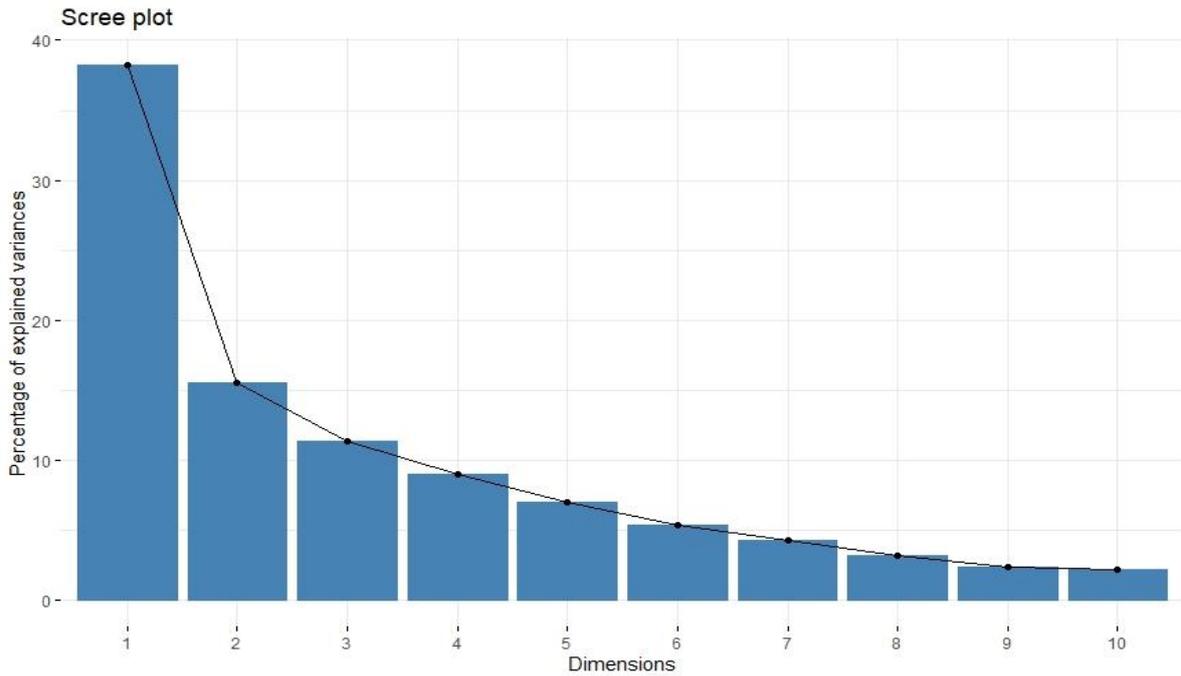


Fig. 1. Scree plot for twelve principal component axes

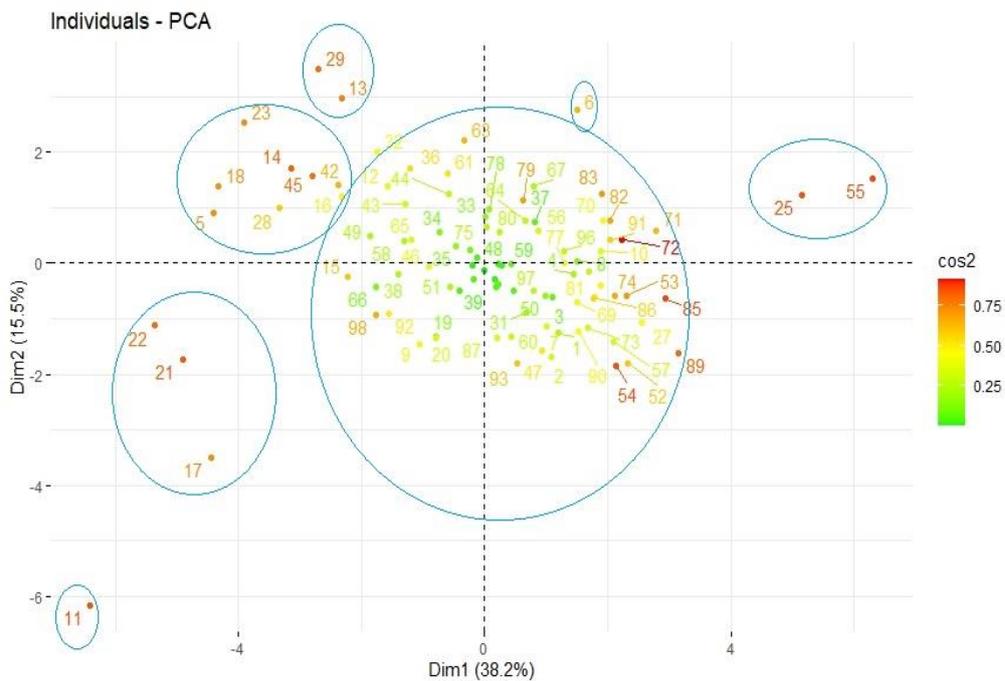


Fig. 2. Individual PCA between PC1 and PC2

NB : The corresponding WRM lines for the serial numbers mentioned in the figure are furnished in Table 1

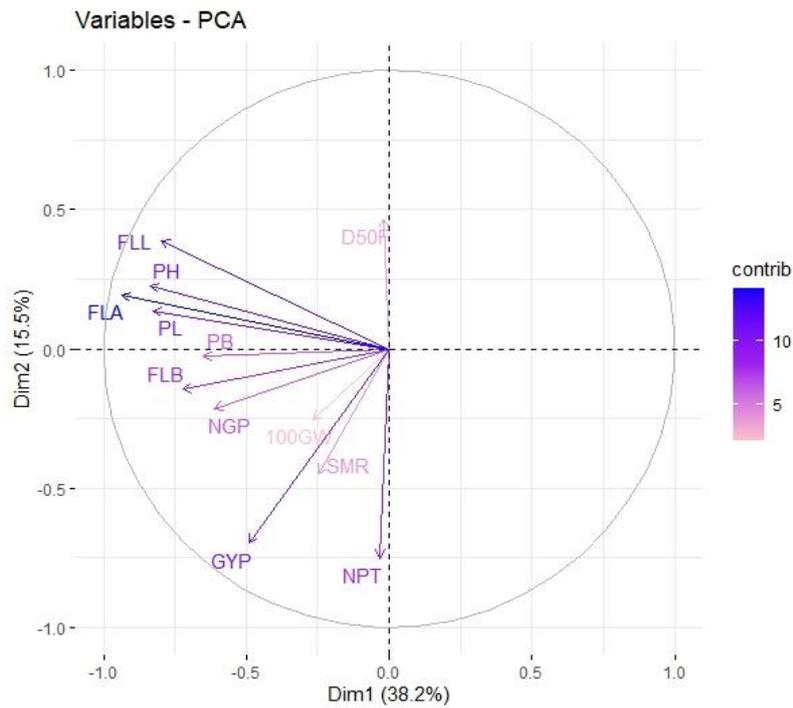


Fig. 3. Variable PCA between PC1 and PC2

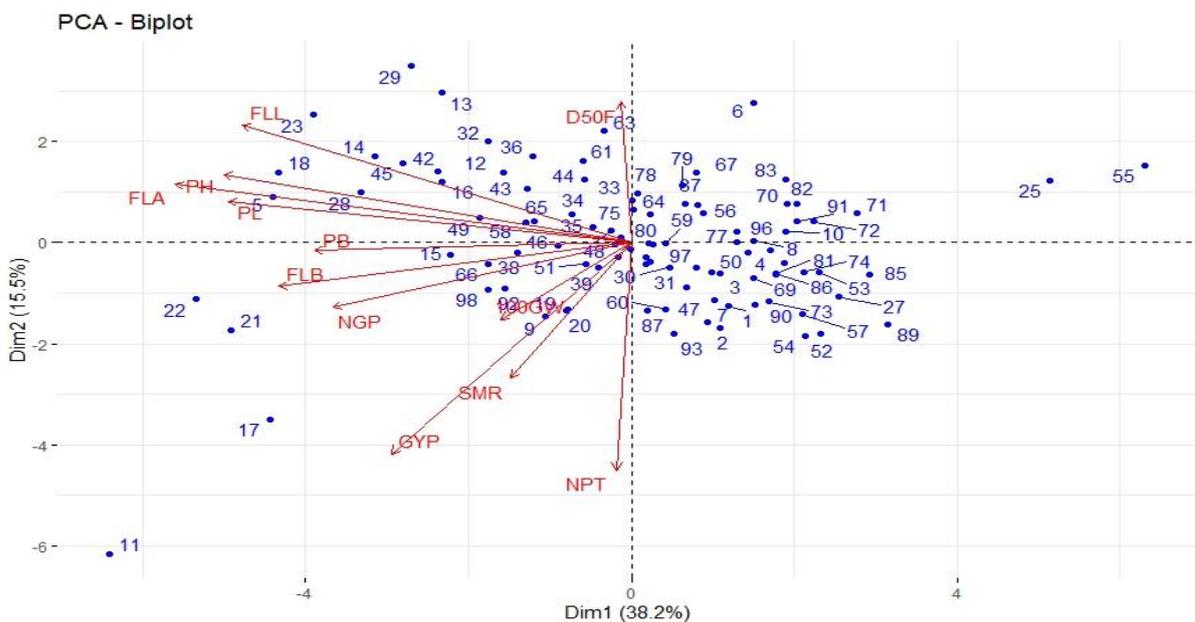


Fig. 4. Biplot between PC1 and PC2

NB: The corresponding WRM lines for the serial numbers mentioned in the figure are furnished in Table 1

Table 3. Eigenvectors, standard deviation, the proportion of variation, Eigenvalues and cumulative proportion of twelve quantitative traits

Statistics	Eigenvectors											
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
Standard deviation	2.14	1.36	1.16	1.04	0.91	0.80	0.71	0.62	0.53	0.51	0.41	0.1223
Proportion of variance	0.38	0.15	0.11	0.09	0.06	0.05	0.04	0.03	0.02	0.02	0.01	0.00
Cumulative proportion	0.38	0.53	0.65	0.74	0.81	0.86	0.90	0.93	0.96	0.98	0.99	1.00
Eigen values	4.58	1.85	1.35	1.08	0.83	0.64	0.50	0.38	0.28	0.26	0.17	0.01

area had the maximum length indicating predominance in contribution towards the total diversity followed by flag leaf length, plant height, panicle length and grain yield per plant. The angle between the trait vectors is an indication of the direction of association between the traits. An acute angle ($<90^\circ$) between vectors indicates a positive correlation, whereas an obtuse angle ($>90^\circ$) indicates a negative correlation and a right angle (90°) indicates no correlation. Out of the 12 traits, ten traits showed a positive correlation with grain yield per plant except for the trait days to 50% flowering, which had a negative correlation.

The genotypes that are projecting towards a trait vector would be the best performing for that particular trait. All the genotypes that are present alongside the trait vectors of the same quadrant tend to perform better for those traits in general. The genotypes (WRM 10, 16, 20 and 21) along with other genotypes present in the particular quadrant were better performing for a number of productive tillers, grain yield per plant, 100 grain weight, the number of grains per panicle, flag leaf breadth, panicle breadth and SPAD index. However, the genotypes (WRM 10, 16, 20 and 21) were the best performing lines for the traits grain yield per plant, 100 grain weight, the number of grains per panicle and flag leaf breadth since these were directly projecting the corresponding trait vectors. Similarly, the genotypes (WRM 1, 17, 22, 29, 30) and other genotypes present in the quadrant were the ones performing better for panicle length, flag leaf area, plant height, flag leaf length and days to 50% flowering. Those genotypes, which are present in the opposite direction to these vectors, viz., (WRM 80 and 25), were poor performing for all the traits under consideration.

To study the diversity among the genotypes, cluster analysis was carried out using Ward's method (**Fig. 5**). The 98 MAGIC lines were grouped into eight clusters of which cluster V was the largest grouping with 28 lines followed by cluster VI with 20 lines. The MAGIC lines WRM 25 and WRM 80 were grouped in cluster 2. The clustering pattern formed by Ward's method was not entirely identical with one formed by PCA. The genotype (WRM 10) formed a solitary cluster in PCA, while in the case of Wards method, it was grouped in the seventh cluster along with genotypes (WRM 16), (WRM 20) and (WRM 21). The genetic diversity among the MAGIC lines could be attributed to the meiotic events and genetic

recombination during their development phase. The highest genetic distance was between cluster I and cluster VIII hence, the genotypes from these clusters can be used as parents in the hybridization programme for sodicity tolerance.

A correlogram is a pictorial representation of the strength of correlation among the component traits. In the present study, grain yield per plant had a significant and positive correlation with the number of productive tillers per plant (0.52), the number of grains per panicle (0.44), flag leaf breadth (0.41), 100 grain weight (0.34), panicle length (0.31), flag leaf area (0.3), spad meter reading (0.28) and plant height (0.21) (**Fig. 6**). A strong positive correlation of traits namely, the number of productive tillers, the number of grains per panicle, panicle length and 100 grain weight with yield is quite imperative. But the significant correlation of flag leaf length, flag leaf breadth and flag leaf area clearly indicated their role in the accumulation of photosynthates for the developing grains. Selection based on these traits could result in a proportionate increase in grain yield. The results are in agreement with Guru *et al.* (2016), Prasannakumari *et al.* (2020), Rashid *et al.* (2014) and Sathishkumar *et al.* (2020) for plant height and the number of productive tillers per plant, Guru *et al.* (2016) for the number of grains per panicle, Worede *et al.* (2014) for panicle length, Kumar *et al.* (2018) for 100 grain weight, Rahman *et al.* (2013) for spad meter reading, Bhattachara *et al.* (2019) for flag leaf area.

Flag leaf area was observed to record a highly significant positive correlation with flag leaf length (0.87), plant height (0.79), panicle length (0.72), flag leaf breadth (0.71), panicle breadth (0.57) and the number of grains per panicle (0.47). Hence, these characters can be simultaneously improved to improve yield potential by increasing photosynthetic efficiency. These results are in agreement with Bing *et al.* (2006) for flag leaf length and flag leaf breadth, Rashid *et al.* (2014) for panicle length and the number of grains per panicle. The number of grains per panicle exhibited a positive and significant relationship with panicle length (0.41), flag leaf length (0.38), plant height (0.37), flag leaf breadth (0.36), panicle breadth (0.35) and spad meter reading (0.33). Similar findings have been reported in previous studies by Yadav and Kashyap, (2020) for panicle length and Rashid *et al.* (2014) for plant height. A number of productive tillers had a negative and significant correlation with flag leaf

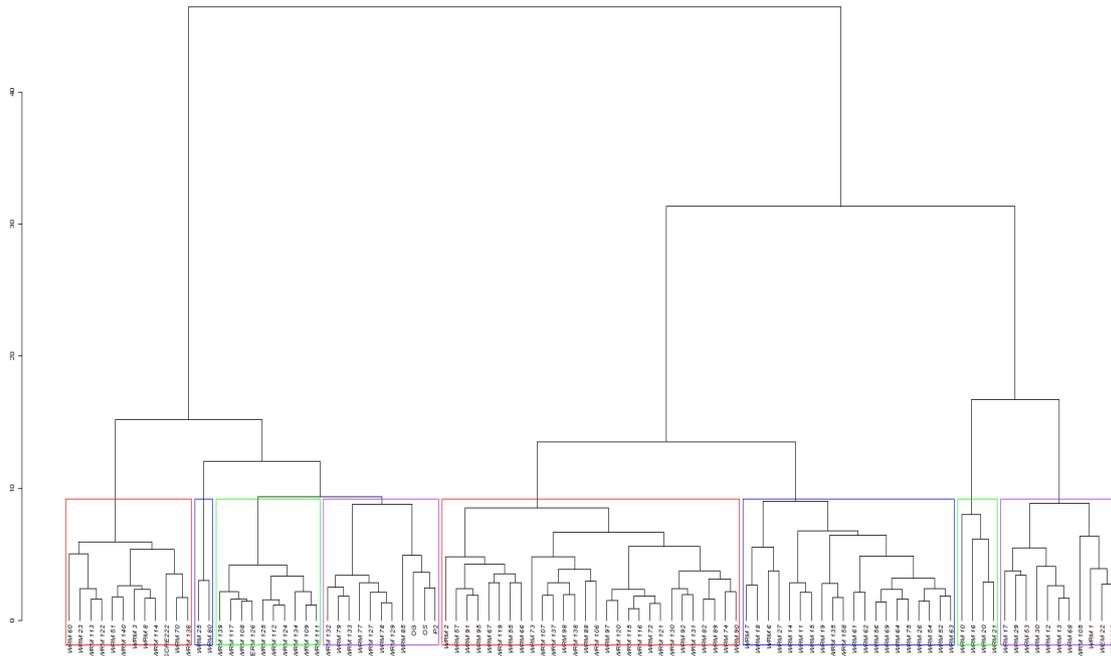


Fig. 5. Cluster analysis of ninety eight rice MAGIC lines using ward's method

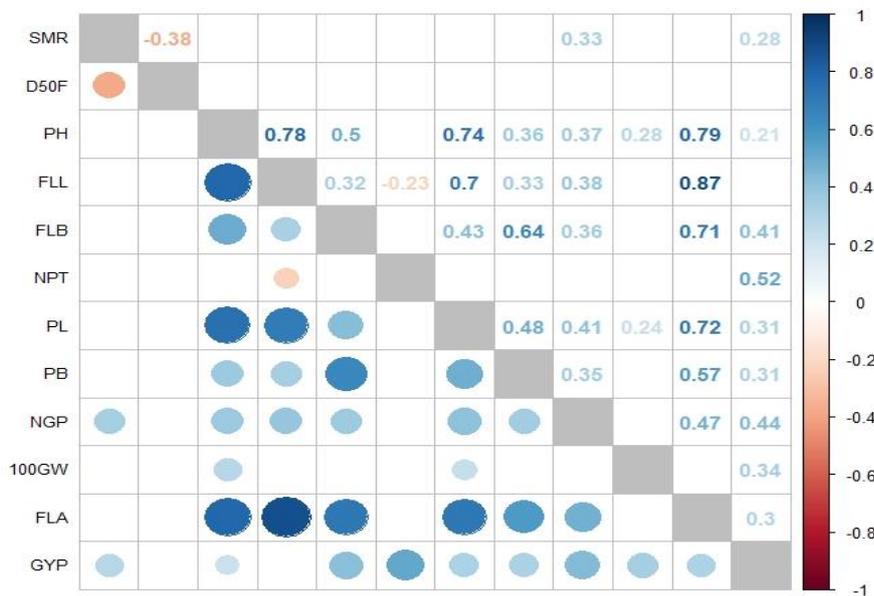


Fig. 6. Correlogram indicating the correlation in yield and attributing traits

SMR- spad meter reading, D50F- Days to 50% flowering, PH- Pant height, FLL- Flag leaf length, FLB- Flag leaf breadth, NPT- Number of productive tillers, PL – Panicle length, PB- Panicle breadth, NGP- Number of grains per panicle, 100GW- 100 grain weight, FLA – Flag leaf area, GYP- Grain yield per plant

Circles- corresponding to relative correlation(r) values, Numerical- genotypic correlation; Circle- phenotypic correlation; Dark colour- high correlation values; Light colour- low correlation values; Red colour- negative correlation, Blue colour- positive correlation

length (-0.23). In the figure, the strength of positive association between traits is indicated as solid circles of different sizes in different grades of blue colour, while the negative association is indicated as solid circles in different intensities of red colour. The empty squares in the figure showed non-significant correlation between the traits (**Fig.6**).

In the present study, it was observed that the rice MAGIC lines exhibited considerable variability for most of the quantitative traits and hence they could be exploited as genetic stocks for crop improvement. Based on performance *per se* for grain yield and component traits with better correlation with yield, the genotypes (WRM 6, 10, 22, 29 and 105) were identified as better performers under sodic soil conditions. PCA analysis identified flag leaf area, plant height, panicle length, flag leaf length, flag leaf breadth and panicle breadth as vital traits contributing to variability. Analysis of genetic diversity based on hierarchical clustering also suggested the existence of genetic diversity among the lines. Hybridization of lines from distant clusters could result in superior segregants with sodicity tolerance.

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