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Genetic Diversity Studies in Rice for Bacterial Leaf Blight Resistance

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ABSTRACT
Bacterial blight (BLB), caused by Xanthomonas oryzae PV. oryzae (Xoo) is one of the most destructive diseases active in the major rice growing countries of Asia. In field level screening, the genotypes PY5 and Kadaikannan showed immune against rice BLB. Under artificial condition, IR 11C 114, Adukan and Kadaikannan shows resistant to bacterial leaf blight. The trait single plant yield showed positive significant correlation with plant height (0.21), number of productive tillers (0.19) and thousand grain weight (0.37).

Keywords: *Bacterial leaf blight, Darwin, PDI, association analysis and genetic divergence*

1.INTRODUCTION

The genus of rice *Oryza* constitutes more than 20 species, among them only two species are cultivable. The Asian rice *Oryza sativa*, is cultivated in Asia and Japan while the African rice *Oryza glaberrima* is cultivated in West Africa (Watanabe, 1979). The cultivated rice (*Oryza sativa* L.) (2n=24) is a monocotyledon angiosperm belongs to the family Poaceae and is widely cultivated in tropical and subtropical regions (Ezuka and Kaku, 2000). More than 2.7 billion people in the world consume rice as staple food. Besides providing employment for more than one billion directly or in allied and supported activities (Das et al., 2014). In India, rice crop is cultivated in an area of 433.88 lakh hectares with a production and productivity of 104.32 million tonnes and 2404 kg/ha respectively (Annual Report 2016-17, Dept. of Agriculture, Cooperation and Farmers Welfare). In Tamil Nadu, rice crop occupy an area of 21 lakh hectares with a production of 93 million tonnes (Policy Note 2015-16, Minister for Housing, Urban Development and Agriculture, Govt. of Tamil Nadu). To sustain self-sufficiency and to meet food grain requirement of future, India has to produce 135–140 million tones of rice by 2030. Rice gets affected by more than 70 diseases by the infection of bacteria, fungi, and viruses. Among them bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv *oryzae* (*Xoo*) is the important disease around the rice cultivated areas (Khan, 1996). Disease incidence occurs at all growth stages of rice crop, causing drastic yield losses ranging between 20 and 30 per cent. The disease severity can cause a yield loss up to 80 per cent and is influenced by various crop stage, environmental conditions (28 to 34°C) and degree of susceptibility of the genotypes (Ou, 1985; Shin et al., 1992; Mew et al., 1993; Noh et al., 2007).

2. MATERIALS AND METHODS

2.1. Plant materials: Mature seeds of One hundred and fourteen rice rice germplasm were used.

2.2 Screening for Bacterial Leaf Blight Resistance

2.2.1. Field level screening:

Each germplasm is evaluated for the BLB resistance by calculating percentage disease index and by giving scales to the respective PDI reading. For one germplasm, twenty plants are taken for evaluating the BLB resistance. The Percentage Disease Index (PDI) and scales for evaluating the BLB resistance was analyzed based on the method suggested by Nagendran et al., (2013).

$$\text{Percentage Disease Index (PDI)} = \frac{\text{Sum of all Numerical Ratings}}{\text{Total No of leaves graded}} \times \frac{100}{\text{Maximum Grade obtained}}$$

Scoring system used to evaluate breeding lines for BLB resistance in the field (IRRI, 2006 & Rafi et al., 2013)

Scale	Disease Leaf Area (%)	Description
0	0	Immune
1	1-10	Resistant
3	11-25	Moderate resistant
5	26-50	Moderate susceptible
7	51-75	Susceptible
9	76-100	Highly susceptible

2.2.2. Glass house screening:

Under greenhouse condition all the germplasm were tested against *Xoo*, the causal agent of BLB in rice crop. Seed of rice germplasm were sown in pots containing fertile soil. Rice plants were grown under glasshouse conditions. For the inoculation 10 ml of sterile distilled water poured into culture plates of most aggressive strain and maintained the inoculum concentration @109 CFU/ml. Plants with fully fresh and expanded leaves were inoculated by leaf cutting method before panicle initiation (Kauffman et al., 1973). The sterile scissors was dipped in the inoculum and clipped the 3 leaves per plant approximately 2-3 cm from tip of each leaf. BLB lesions on clipped leaves were observed 15 days after inoculation.

Scoring system used to evaluate breeding lines for BLB resistance in the glasshouse (IRRI, 2006)

S.No	Lesion length (cm)	Description
1.	0-5	Resistant
2.	5-10	Moderately resistant
3.	10-15	Moderately susceptible
4.	>15	Susceptible

2.3. Characterization for Morphological traits:

Twenty five days old seedlings of the selected genotypes are transplanted in the experimental plots of AC & RI, KKM at a spacing of 20 cm × 20 cm (within and between rows) in two replications with standard agronomic practices. Data was recorded on six plants from each genotype for morphological traits like plant height, number of productive tillers per plant, panicle length, single plant yield, days to fifty per cent flowering, number grains per panicle, thousand grain weight, grain length and grain breadth.

2.4. Association analysis

The genotypic correlation between yield and its component traits and between the grain quality traits were worked out as per the methods suggested by Johnson et al., (1955).

Genotypic correlation coefficient $r_g(xy) = \frac{COV_g(xy)}{\sqrt{(\sigma^2_g x)(\sigma^2_g y)}}$

Where,

$r_g(xy)$ = genotypic correlation coefficients,

$COV_g(xy)$ = genotypic covariance between the traits 'x' and 'y',

$\sigma^2_g x$ = genotypic variance of the trait 'x',

$\sigma^2_g y$ = genotypic variance of the trait 'y',

x = dependent variable x and

y = independent variable y.

The significance of genotypic correlation coefficient was tested by referring to the standard table given by Snedecor et al., (1961).

2.5. Genetic divergence

The genetic divergence among one hundred genotypes was estimated by Mahalanobis (1949) D^2 statistics for 9 quantitative characters.

The calculation of D^2 values involved following steps.

Calculation of D^2values

For each combination, the D^2 are calculated as follows

$$D^2 = \sum (Y_i^1 - Y_j^2)$$

The D^2 values were calculated using the software Window Stat. The computed values were tested for significance. The average inters and intra cluster distance tables were obtained from the software output.

2.5.1. Group constellation and cluster diagram

The grouping of the genotypes into cluster was done in Window Stat by using Tocher's method (Rao *et al.*, 2002). The cluster diagram was drawn using the D^2 tables.

3. RESULTS AND DISCUSSION

3.1. Screening of BLB resistance under natural and artificial conditions

The morphological screening of one hundred and fourteen rice varieties against the bacterial leaf blight pathogen under field condition was carried out. Here, there was no bacterial inoculum applied as well as there was no artificial favorable condition given to

promote the pathogen growth. In order to test the host pathogen interaction at natural environment, the field screening of rice germplasm were carried out. Among the resistant genotypes PY 5 and Kadaikannan found to be immune with the lowest PDI value of 0.00 and 0.00 per cent respectively. The genotypes, Jai Shree Ram and Kurukot registered highest PDI value of 95.00 and 78.57 per cent respectively. The result for this screening of rice genotypes based on field screening were presented in the table 1.

Ramalingam et al., 2017 screened twenty five rice genotypes for bacterial leaf blight resistance and revealed that the improved rice lines in the genetic backgrounds of Samba Mahsuri, ASD 16, ADT 43 and IR 24 exhibited the higher level of resistance to most of the pathotypes studied, whereas the rice lines derived from ADT 47 background exhibited more susceptibility.

Rafi et al., (2013) evaluated the BLB resistance in the rice field for calculating the disease severity and to find out the severity of BLB disease under absence of BLB resistance. The genotype, PY 5 and Kadaikannan which was immune in field screening, was found to be moderately resistant under the artificial glasshouse condition, but other naturally screened resistant germplasm IR 11C 114 were resistant under artificial screening. The field screened resistant germplasm IR 12L 138, ACK 12001 and Veethiruppu were moderate resistant in artificial screening. This variation in resistance can be due to escape mechanism adopted by germplasm in the rice field or due to the precise procedure of clipping method, which can facilitate easy entry of BLB pathogen.

In the glasshouse screening, among the resistant genotypes IR 11C 114, Adukan and Kadaikannan recorded mean lesion length 3.5, 5.2 and 5.3 cm respectively while the susceptible genotypes Srilanka, IR 64, Navarai, IR 72, IRRI 163, TP 10008, Kottara Samba, Varapukudaichan, Abhya and Kullakar exhibited mean lesion length of 22.8 cm, 22.5 cm, 21.9 cm, 21.6 cm, 21.3 cm, 21.3 cm, 21.2 cm, 20.8 cm, 19.4 cm and 19.1 cm respectively. The result for this screening of rice genotypes based on glasshouse screening were presented in the table 2.

In a similar experiment Mubassir et al., (2016) screened ten IRRI advanced rice lines and seventeen rice varieties against the bacterial leaf blight pathogen and adjusted IRBB5, IRBB21, IRBB60 and IRBB65 as resistant to BLB.

3.2.1. Variability on quantitative traits in rice germplasm

In general, estimation of PCV was higher than their corresponding GCV however good correspondence was observed between GCV and PCV for all biometric traits. Higher magnitude of phenotypic and genotypic coefficients of variation were observed for the traits single plant yield (31.67 and 31.46), number of filled grains per panicle (31.67 and 31.46), number of productive tillers (26.20 and 25.10) and 1000 grain weight (20.58 and 20.43). The results for coefficient of variation were presented in table 3 and figure 1.

The findings are in accordance with the works of Vivek *et al.,* (2005) and Ramanjaneyulu *et al.,* (2014). In the present study, estimates of genetic parameters revealed that the phenotypic co-efficient was slightly higher than the genotypic co-efficient of variability for all the characters studied, which indicated that they all interacted with the environment. However, the narrow difference between GCV and PCV, gave evidence that the variability existing among the genotypes was mainly due to their genetic makeup. For morphological characters similar observations were made by Anbanandan *et al.,* (2009). The high magnitude of PCV and GCV indicates less influence of environment on these traits. Selection based on these traits would be effective. In present study, single plant yield and number of filled grains per panicle recorded higher PCV and GCV. This result is in conformity with the findings of Augustina *et al.,* (2013) and Vanisree *et al.,* (2013).

Higher heritability was observed for the traits studied and it ranged from 76.60 per cent (grain breadth) to 99.40 per cent (days to 50 per cent flowering). The heritability of number of grains per panicle, thousand grain weight, plant height, single plant yield, number of productive tillers and panicle length recorded were 98.70 per cent, 98.50 per cent, 98.50 per cent, 98 per cent, 91.80 and 83.40 per cent respectively. Heritability along with genetic gain is a more useful criterion in predicting the resultant effect for selecting the best individual (Vanisree *et al.,* 2013). Higher genetic advance as per cent of mean was observed for single plant yield (68.20%) followed by number of grains per panicle (64.40%), number of productive tillers (49.54%), thousand grain weight (41.77%), plant height (39.03%), days to fifty per cent flowering

(24.42%) and grain breadth (23.80%). Moderate genetic advance as per cent of mean was observed for grain length (18.83%) and panicle length (17.51%).

The results for coefficient of variation, heritability and genetic advance as per cent of mean were presented in table 3 and figure 2.

High heritability and genetic advance as per cent of mean was observed for the traits. All the traits selected for the study has moderate to high variations with almost high genetic advance and heritability. Similar results of high genetic advance along with high heritability were obtained by Augustina *et al.*, 2013 and Vanisree *et al.*, 2013 for plant height, number of productive tillers per plant and number of filled grains per panicle. Besides Chanbeni *et al.*, 2012 reported for plant height and number of filled grains per panicle. The success of any crop breeding programme depend on the choice of parents based on their mean performance.

3.3. Trait association in rice germplasm

The association analysis revealed the positive or negative relationship of different traits to the yield. The genotypic correlations between traits indicated the direction and magnitude of correlated responses to selection, the relative efficiency of indirect selection and permit calculation of optimal multiple trait selection indices (Falconer and Mackay, 1967). It was observed that there was positive significant correlation between single plant yield and number of tillers per panicle besides positively significant correlation reported between single plant yield and thousand grain weight. Lang et al., (2014) reported a negative correlation of days to fifty per cent flowering with rice single plant yield. Early flowering indicated short life cycle and early maturing varieties are advantageous in areas with short rainfall duration because they grow faster during the vegetative phase and are thus more competitive with weeds utilizing less water (Khush, 1995).

Plant height was significant and positively correlated with the panicle length (0.29), and days to fifty per cent flowering (0.56), thousand grain weight (0.41), grain breadth (0.23) and single plant yield (0.21). Similarly, the number of productive tillers was significant and positively correlated with the single plant yield (0.19). The negative significant correlation was noticed among days to 50 per cent flowering and grains per panicle (-0.20). Number of productive tillers was significant and negatively correlated with the traits days to fifty per cent flowering. Number of panicle length showed negatively significant correlation with the trait number of grain per panicle (-0.20). The number of productive tillers and thousand grain weight were positive and significantly correlated with the yield. The relationship between the traits observed on all the germplasm lines was given in the table 4 and figure 3. This present study of correlation was in the agreement with the findings of Bagheri et al., (2011), Sabesan et al., (2010) and Khan et al., (2009).

3.4. Variability studies among rice germplasm using morphological studies

In the cluster diagram formed by the Tocher's method, eighteen major clusters were formed (Table 5). Large number of germplasm were present in cluster I with 38 individuals, followed by 30 germplasm in cluster II, 20 germplasm in cluster IV 4 germplasm in XI cluster and remaining clusters were all contain single genotype. The pattern of group constellation proved the existence of significant amount of variability. Similar findings also reported by Ramanjaneyulu *et al.*, (2014). The intra and inter cluster average distance among eighteen clusters were variable and depicted in the table 6. The highest intra cluster distance was recorded for cluster XI (29.02) followed by cluster V (27.28) and lowest intra cluster average distance was recorded by cluster I (20.17) and V (24.77). Genotypes belonging to clusters separated by the higher genetic distance may be used in hybridization programme to obtain a wide spectrum of variation among the segregants. Hybridization programme involving genetically diverse parents belonging to different distant clusters would provide the opportunity for bringing together gene constellations of diverse nature (Ramanjaneyulu *et al.*, (2014).

Cluster XI and XVII showed maximum inter cluster distance of 67.45 followed by cluster X and XVII (63.39). The lowest inter cluster distance was noticed between XIII and XIV (17.78), followed by the clusters VIII and XV (18.82). To realize much variability and high heterotic effect, Beevi and Venkatesan (2015) recommended that parents should be selected from two clusters having wider inter cluster distance. Thus, the divergence of the one hundred and fourteen genotypes used in the study may be due to involvement of different ancestral pedigree or uncommon parentage.

4. Conclusion

Rice germplasm screened under field condition, one per cent were immune towards bacterial blight, nine per cent genotypes were resistant, thirty two per cent of them were found to be moderately resistant, thirty five per cent of rice genotypes were moderately susceptible, eighteen per cent were susceptible to bacterial blight and one per cent rice germplam are highly susceptible to rice bacterial blight (BLB). Under glasshouse conditions, 0.87% were resistance to BLB, 20% of them were found to be moderately resistant, 45% of rice germplasm were moderately susceptible and 33% of susceptible towards bacterial blight. Genotypic correlation studies for morphological characters revealed that Single plant yield showed positive and significant correlation with plant height (0.219), number of productive tillers (0.191) and thousand grain weight (0.370). On the basis of Mahalanobis *D*2 statistics, the one hundred and fourteen genotypes were grouped into eighteen clusters.

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Table1. Score for BLB resistance under Field Screening

S.No	Germplasm	Percentage Disease Index (PDI)	Scale	Description
1.	Kadaikannan	0	0	Immune
2.	PY5	0	0	Immune
3.	Veethiruppu	10	1	Resistant
4.	Jai Shree Ram	95	9	Highly Susceptible
5.	Kurukot	78.57	9	Highly Susceptible

Table2. Scores obtained in artificial screening of rice germplasm

S.NO	Germplasm	Mean Lesion length (cm)	Description
1.	IR 11C 114	3.5	Resistant
2.	Adukan	5.2	Moderately Resistant
3.	Kadaikannan	5.3	Moderately Resistant
4.	Navarai	21.9	Susceptible
5.	IR 64	22.5	Susceptible
6.	Srilanka	22.8	Susceptible

Table3. Variance components, genetic advance (GA) and heritability observed for the quantitative traits

COMPONENTS	PH	NPT	PL	SPY	DFF	NGP	TGW	GL	GB
G.V.	609.40	17.62	4.61	222.46	96.71	2140.10	10.59	0.63	0.15
P.V.	618.58	19.20	5.53	226.92	97.25	2168.32	10.76	0.71	0.19
GCV	19.09	25.10	9.31	33.43	11.88	31.46	20.43	9.70	13.20
PCV	19.23	26.20	10.19	33.77	11.92	31.67	20.58	10.29	15.08
Heritability (%)	98.50	91.80	83.40	98.00	99.40	98.70	98.50	88.80	76.60
G.A. (% m)	39.03	49.54	17.51	68.20	24.42	64.40	41.77	18.83	23.80

PH - Plant height, NPT - No of productive tillers, PL - Panicle length, SPY - Single plant yield, DFF - Days to 50% flowering, NGP - Number of filled grains per panicle, TGW - 1000 grain weight, GL - Grain Length, GB - Grain breadth, GCV - Genotypic Coefficient of Variance, PCV - Phenotypic Coefficient of Variance and G.A. (% m) - Genetic Advance Per cent of mean.

Table4. Genotypic correlation among various biometric traits in rice genotypes

	PH	NPT	PL	DF	NGP	TGW	GL	GB	SPY
PH	1	-0.20*	0.29*	0.56*	-0.12	0.41*	-0.07	0.23*	0.21*
NPT		1	-0.06	-0.20*	-0.02	0.15	-0.19*	-0.18*	0.19*
PL			1	0.33*	-0.20*	0.15	-0.03	0.14	0.16
DF				1	-0.20*	0.32*	-0.10	0.07	0.15
NGP					1	-0.14	-0.17	-0.07	-0.06
TGW						1	-0.07	-0.02	0.37*
GL							1	0.07	0.07
GB								1	0.00
SPY									1

PH - Plant height, NPT - No of productive tillers, DFF - Days to 50% flowering, NGP - Number of filled grains per panicle, TGW - 1000 grain weight, GL - Grain Length, GB - Grain breadth, PL - Panicle length and SPY - Single plant yield.

Table5. Composition of *D2* cluster for rice genotypes

Cluster	Number of genotypes	Name of the genotypes
1.	38	TN 1, Co 50, IR 12L 104, IRRI 104, Co 39, IR DL 25 CA, ADT 37, IR 12L 115, IR BL 5M, BB 8, ASD 18,CR 1009, IR 12L 107, Uma, ASD 16, AD BIO 09518, IR 12L 342, Co 43, IR 72, TPS 4, ACK 12001, ADT 43, IR 12L 214, BPT 5204, PY 5, ACK 14004, LFR 293,IR 64, ADT 42, IR 11L 433, IR 50, IRRI C 134, IR 12L 138, ADT 48, White Sannam, Adukan, IR 11C 114 and IRRI 163.
2.	30	Kadaikannan, Kalakeri, Kitchadi samba, Karsamba, Veethiruppu, Kalakeri, Annada, Chinnapunjan, Jaya, Kuliyadichan, Thondi, Surakuruvai, Maranellu, Navarai, Anjali, Purple Puttu, Virendra, Sahbhagi Dhan, Rajalakshmi, Athira, Kurukot, Gowri, Kaivara Samba, Gowni, Swarna, Bharathi, Kayamma, White Ponni, Meikuruvai and Abhya.
3.	1	SR22B
4.	20	ACK 13005, ADT 39, Kullakar, Srilanka, Kalyani, Kavya, Jai Shree Ram, Kalyani, Sadabahar, ADT 46, IR 11C 465, CR Dhan 70, JGL 1798, IR 11T 193, Co 49, HH-Z17-Y16-Y3-Y2, JGL 348, Kuruvai Kalanjium, TP10008 and Namcheonbyeo.
5.	9	IR 12L 110, MDU 5, Co 45, ADT 41, PY 2, Co 51, Kalinga 3, Pusa Basmati and TP 10106.
6.	1	IR 10A 240
7.	1	Kottara Samba
8.	1	IR 20
9.	1	Vanaprabha

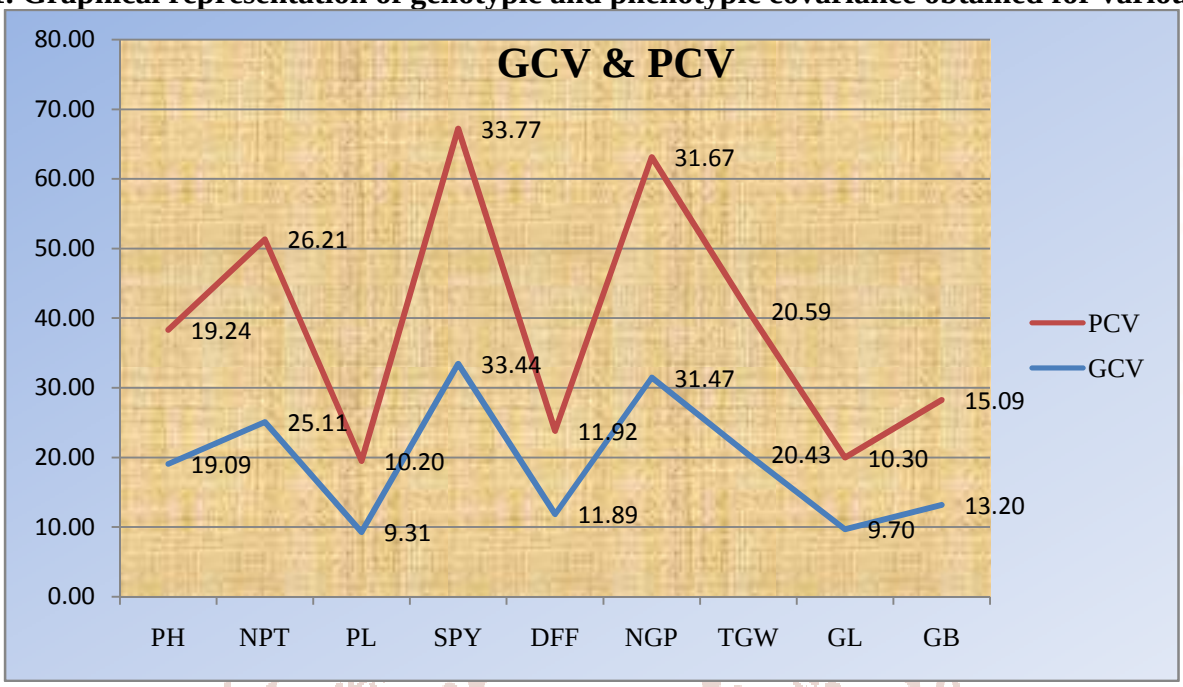
Cluster	Number of genotypes	Name of the genotypes
10.	1	Thooyamalli
11.	4	Mapillai Samba, Varapukudaichan, Mattai and Dhalaheera.
12.	1	Poonkar
13.	1	Kerala Gandhakasala
14.	1	Kattanur
15.	1	Seeraga Samba
16.	1	JGL 3855
17.	1	IR-BL-TAR-PI (Co)
18.	1	Krishna Hemavathi

Table6. Average inter and intra cluster D^2 values for rice genotypes

Cluster	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.
1.	20.19	37.28	22.32	25.85	32.42	24.99	36.48	24.85	32.85	44.28	48.44	37.32	38.58	39.88	36.15	30.26	30.19	46.16
2.		20.35	33.91	33	42.07	45.31	26.52	34.53	43.1	26.4	31.22	28.51	25.73	30.02	33.06	44.47	52.81	34.25
3.			0	19.49	28.92	33.33	33.78	31.12	29.05	33.21	40.95	39.88	41.48	43.59	40.38	25.19	41.98	46.99
4.				24.77	31.39	34.71	32.99	31.11	30.73	35.3	40.22	38.47	38.8	41.67	38.81	29.4	42.21	44.81
5.					27.28	44.58	45.17	35.8	34.61	46.42	45.89	52.46	45.95	50.62	41.9	34.56	45.23	43.04
6.						0	39.66	29.23	36.15	53.48	56.69	33	46.85	45.82	44.85	39.09	28.84	58.43
7.							0	33.55	40.18	26.36	32.99	28.77	35.64	31.5	38.15	38.57	50.56	47.65
8.								0	45.65	47.97	48.57	34.55	32.75	28.53	18.82	41.52	33.11	34.96
9.									0	40.66	42.6	46.11	50.92	57.44	55.63	25.91	46.32	59.19
10.										0	25.47	38.42	39.99	43.86	48.69	42.2	63.39	49.04
11.											29.02	43.11	44.08	49.09	49.06	49.34	67.45	46.21
12.												0	31.56	31.55	39.18	51.13	49.15	47.42
13.													0	17.78	23.3	46.76	44.88	26.62
14.														0	20.58	49.9	44.35	33.15
15.															0	49.32	42.6	21.6
16.																0	38.19	57.43
17.																	0	56.25
18.																		0

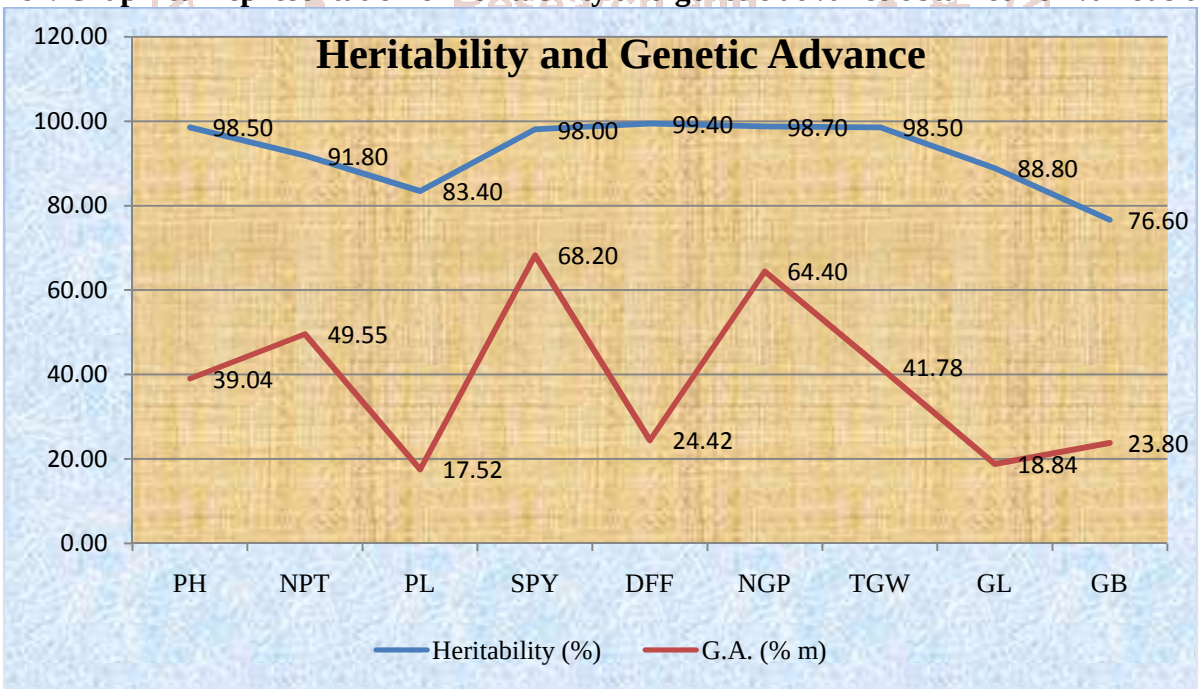
Intra cluster – Diagonal values, Inter cluster – Off diagonal values

Figure1. Graphical representation of genotypic and phenotypic covariance obtained for various traits



1. PH - Plant height
2. NPT - No of productive tillers
3. PL - Panicle length
4. SPY - Single plant yield
5. DFF - Days to 50% flowering
6. NGP - Number of filled grains per panicle
7. TGW - 1000 grain weight
8. GL - Grain Length
9. GB - Grain breadth

Figure2. Graphical representation of heritability and genetic advance obtained for various traits



1. PH - Plant height
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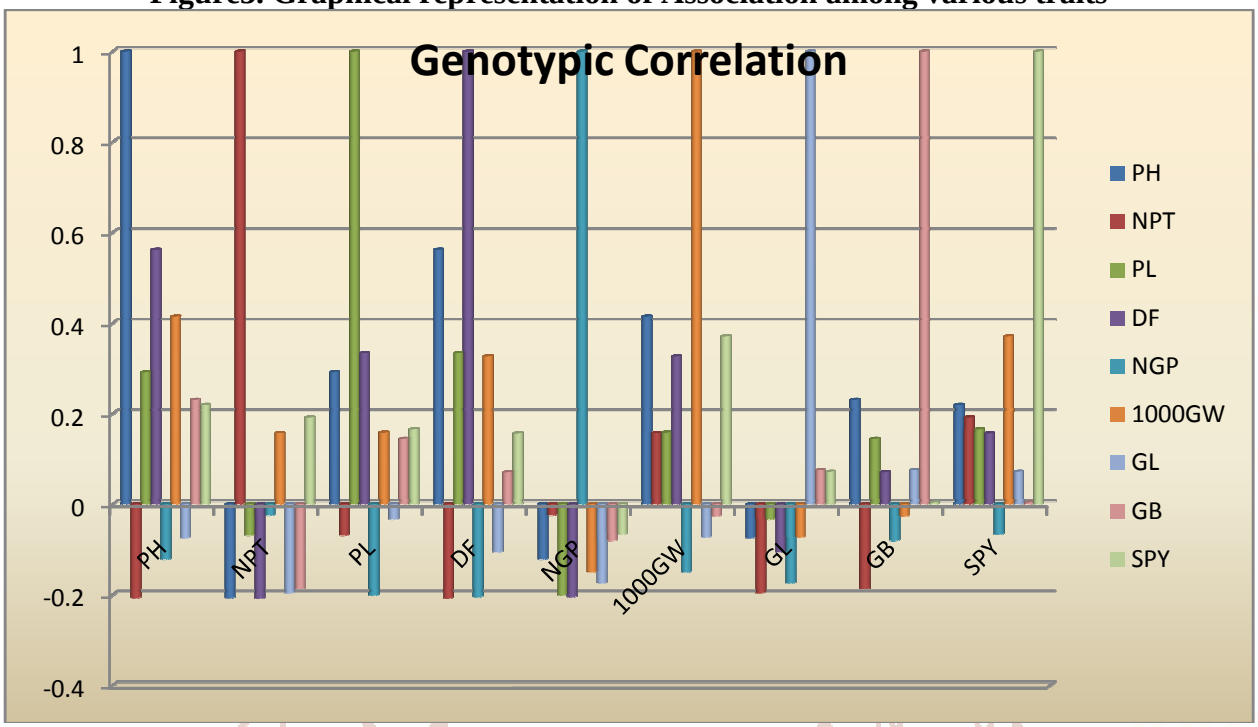
4. SPY - Single plant yield
5. DFF - Days to 50% flowering
6. NGP - Number of filled grains per panicle
7. TGW - 1000 grain weight
8. GL - Grain Length
9. GB - Grain breadth

Figure3. Graphical representation of Association among various traits



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