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Identification of Superior Recombinant Lines for Yield and Leaf Rust Resistance in the Cross between DWR 162 X NIL PBW 343 in Bread Wheat (*Triticum aestivum* L.)

G. Anil Kumar[1*], R. R. Hanchinal[2], Shrinivas Desai[3] and Suma Biradar[4]

[1]Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad, India
[2]Chairperson, Protection of Plant Varieties and Farmers Rights Authority,
Ministry of Agriculture, Govt. of India, New Delhi, India
[3]Department of Genetics and Plant Breeding, Professor, University of Agricultural Sciences, Dharwad, India
[4]Department of Genetics and Plant Breeding, Junior Breeder, Wheat, MARS, University of Agricultural Sciences, Dharwad, India
*Corresponding Author E-mail: anil.gsid@gmail.com

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ABSTRACT

F_3 and F_4 lines of the cross between DWR 162 X NIL PBW 343 were evaluated for yield and leaf rust resistance. The genetic variability studies in F_3 generation indicate high mean and wider range for all the traits under evaluation. High PCV (phenotypic coefficient of variation) and moderate GCV (genotypic coefficient of variation) were recorded for the traits number of tillers per plant and grain yield per plant. In F_4 generation high PCV (phenotypic coefficient of variation) and moderate GCV (genotypic coefficient of variation) were recorded for the traits, number of tillers per plant and grain yield per plant.). For leaf rust infection, high PCV and GCV, moderate heritability and high GAM was recorded.

Key words: *Triticum aestivum L., Leaf rust resistance, Recombinant lines, PCV and GCV.*

INTRODUCTION

Wheat (*Triticum aestivum* L. em Thell), a cereal grass of the family *Graminae* (*Poaceae*) and genus *Triticum*, is the world's largest cereal crop. It is the most widely cultivated and important food crop in the world. It provides about a fifth of the calories consumed by man and acts as a staple food in many developing as well as developed countries. The world population is expected to reach 9 billion in 2050. With the growing world population, world wheat demand is estimated to increase by 60 percent in 2050. It is thus evident that wheat production rapidly needs to be increased [1].Rusts are the most devastating fungal diseases posing a threat towards realizing the potential yield in wheat, worldwide. Wheat is attacked by three rust species: leaf rust (*Puccinia triticina* Eriks.), stem rust (*P. graminis* f. sp. *graminis* Eriks. & E. Henn) and stripe (yellow) rust (*P. striiformis* Westend.). Leaf rust caused by *Puccinia triticina* is the most common and widely distributed of the three wheat rusts.

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MATERIAL AND METHODS

The F_3 and F_4 populations of the cross DWR 162 X NIL PBW 343 were evaluated for eight quantitative traits, yield and leaf rust resistance in a replicated randomized complete block design.

Observations were recorded on Morphological characters. The data obtained was subjected to statistical analysis to find out mean, range, genotypic and phenotypic coefficient of variation, heritability (h^2), genetic advance (GA) and genetic advance over mean (GAM).

Field screening for leaf rust

On the appearance of symptoms, rust severity (percentage) and response of the plants to disease were assessed using a modified Cobb's scale [8]. The final disease severity data for the leaf rust was converted into Coefficient of Infection (CI) by multiplying severity with a constant value for field response given in Table 1 [10, 11].

Screening F_4 lines for leaf rust resistance in rust screening nursery

F_3 and F_4 lines were screened for leaf rust resistance with leaf rust pathotype 77-5, and mixture of races. Plants that were observed to show resistance phenotypically were subjected to molecular marker analysis for confirmation for the presence of *Lr24* and *Lr28* leaf rust resistant genes.

Molecular confirmation for the presence of leaf rust resistance genes *Lr24* and *Lr28* in F_3 and F_4 populations

Superior plants for productivity in F_3 and F_4 generation selected based on Morphological characters, were subjected to foreground selection using molecular markers SCS719 linked to *Lr24* and SCS421 and Xwmc313 linked to *Lr28* genes.

PCR analysis

DNA was isolated from young leaves by a modified CTAB method [5]. PCR reactions were performed in a total volume of 25 µl, containing 1× PCR buffer, 200µM of each dNTP, 20 ng of each primer, 1 U of Taq DNA polymerase (Banglore Genei Pvt. Ltd., India) and 100 ng of genomic DNA in a PTC-200 thermal cycler (MJ Research). Primers were synthesized from Sigma Aldrich Pvt. Ltd., Bangalore, India. The sequence of primers and the PCR conditions used for amplification presented in. Amplified PCR products were resolved in 2% agarose gel, stained with ethidium bromide.

Genes tagged	Molecular markers	Primer sequence 5'---3'	Amplification product size(bp)	References
Lr24	SCAR:SCS719	F: TCG TCC AGA TCA GAA TGT G R: CTC GTC GAT TAG CAG TGA G	719	[9]
Lr24	SCAR:SCS1302	F: CGC AGG TTC CAA TAC TTT TC R: CGC AGG TTC TAC CTA ATG CAA	607	[6]
Lr28	SCAR:SCS421	F: ACA AGG TAA GTC TCC AAC CA R: AGT CGA CCG AGA TTT TAA CC	570	[4]

RESULTS AND DISCUSSION

Days to 50 per cent flowering

Moderate PCV (11.33) and low GCV (8.16) values coupled with moderate heritability (52%) and moderate genetic advance over mean (12.11%) in F_3 generation and Moderate PCV (10.65) low GCV(7.64) coulped with moderate heritability (51%) and genetic advance over mean (11.29%) in F_4 generation were recorded for days to 50 per cent flowering.

Plant height (cm)

Moderate PCV (12.01) and moderate GCV (10.22) values coupled with high heritability (72%) and moderate genetic advance over mean (17.92%) in F_3 population and moderate PCV (10.23) and low GCV (8.05) values coupled with high heritability (62%) and moderate genetic advance over mean (13.05%) in F_4 population of cross DWR 162 X NIL PBW 343 were recorded for plant height.

Number of productive tillers per plant

High PCV (29.56) and low GCV (4.07) values coupled with low heritability (2%) and low genetic advance over mean (1.15%) in F_3 population and high PCV (23.06) and low GCV (5.62) values coupled with low heritability (6%) and low genetic advance over mean (2.82%) in F_4 population were observed for number of productive tillers per plant.

Spike length (cm)

Moderate PCV (11.58) and low GCV (8.14) values coupled with moderate heritability (49%) and moderate genetic advance over mean (11.79%) in F_3 population and moderate PCV (11.02) and low GCV (9.12) values coupled with low heritability (68%) and moderate genetic advance over mean (15.55%) in F_4 population were observed for spike length.

Spikelets per spike

Low PCV (7.86) and low GCV (3.9) values coupled with low heritability (25%) and low genetic advance over mean (3.99%) in F_3 population and high PCV (8.11) and low GCV (5.32) values coupled with moderate heritability (43%) and low genetic advance over mean (7.19%) in F_4 population were observed for number of spikelets per spike.

Grain yield per plant (g)

Mean grain yield per plant was 20.5 g and 19.5 g with a range of 3 to 56.5 g and 8 to 46 g in F_3 and F_4 populations respectively. High PCV (21.61) and moderate GCV (13.61) values coupled with moderate heritability (39.66%) and moderate genetic advance over mean (17.66%) in F_3 population and high PCV (26.66) and moderate GCV (16.02) values coupled with moderate heritability (36.11%) and low genetic advance over mean (19.83%) in F_4 population of the cross DWR 162 X NIL PBW 343 were observed for grain yield per plant.

Thousand grain weight (g)

Moderate PCV (12.86) and low GCV (6.34) values coupled with low heritability (24.31%) and low genetic advance over mean (6.44%) in F_3 population and moderate PCV (13.22) and low GCV (8.11) values coupled with moderate heritability (37.63%) and moderate genetic advance over mean (10.25%) in F_4 population were observed for thousand grain weight.

Resistance to leaf rust (Natural condition)

High PCV (47.92) and GCV (31.57) values coupled with moderate heritability (43.4%) and high genetic advance over mean of (42.84%) in F_3 population and high PCV (42.46) and GCV (27.63) values coupled with moderate heritability (42.34%) and high genetic advance over mean of (37.04%) in F_4 population of cross DWR 162 X NIL PBW 343 were recorded for leaf rust incidence under natural condition.

Protein content (%)

Low PCV (7.26) and GCV (5.92) values coupled with high heritability (66.49%) and low genetic advance over mean (9.94%) were observed in F_3 generation. In F_4 generation low PCV (8.12) and GCV (6.59) values coupled with high heritability (65.87%) and moderate genetic advance over mean (11.02%) were recorded.

Observed and expected number of plants with leaf rust resistance genes in F_3 and F_4 generation

Based on the joint segregation of SCAR markers linked to *Lr24* and *Lr28* in F_3 generation, out of 60 plants screened using linked markers, 24 plants carried both *Lr24* and *Lr28* as against an expectation of 33.75, 10 plants showed only *Lr24* as against an expectation of 11.25, 12 plants showed presence of only *Lr28* as against the expectation of 11.25, and 4 plants as against an expectation of 3.75, had neither of the genes in F_3 generation with a non-significant $\div^2$ value of 3.02 (table $\div^2$ value 7.82) as revealed from the results of $\div^2$ test, indicating the goodness of fit (Table 4 and Plate 8 to 10).

In F_4 generation out of 70 plants screened using linked markers, 28 plants carried both *Lr24* and *Lr28* as against an expectation of 39.375, 16 plants showed only *Lr24* as against an expectation of 13.125, 14 plants showed presence of only *Lr28* as against the expectation of 13.125, and 6 plants as against an expectation of 4.375, had neither of the genes in F_4 generation with a non-significant $\div^2$ value of 4.58 (table $\div^2$ value 7.82) as revealed from the results of $\div^2$ test, indicating the goodness of fit (Table 5).

Evaluation of the F_4 lines with *Lr24* and *Lr28* in the background of DWR 162

F_4 lines were evaluated for their agronomic characters for judging the superiority of the lines. The results of evaluation of eight F_4 lines are presented in Table 6. Line F_4 -25 (26.80 g) followed by line F_4 -28 (25.90 g) recorded highest grain yield per plant among the eight lines. Line F_4 -17 recorded low grain yield per plant (14.70 g) as compared to rest of the lines. Remaining lines were on par with each other for grain yield. Line F_4 -25 showed highest (43.10 g) thousand grain weight followed by line F_4 -17(38.63 g). For rest of the characters all the nine lines were found to be on par with each other.

Table 1: Leaf rust reaction, code for field response and response value

Reaction	Code	Field response	Response value
No disease	0	No visible infection	0.0
Resistant	R	Necrotic areas with or without minute uredia	0.2
Moderately resistant	MR	Small uredia present surrounded by necrotic area	0.4
Moderately resistant, Moderately susceptible	MRMS	Small uredia present surrounded by necrotic areas as well as medium uredia with no necrosis but possible some distinct chlorosis.	0.6
Moderately susceptible	MS	Medium uredia with no necrosis but possible some distinct chlorosis	0.8
Moderately susceptible susceptible	MSS	Medium uredia with no necrosis but possible some distinct chlorosis as well as large uredia with little or chlorosis present	0.9
Susceptible	S	Large uredia and little or no chlorosis present	1.0

Table 2: Genetic variability studies for yield and rust resistance in F_3 generation of the cross DWR 162 X NIL PBW 343

Characters	Mean	Min	Max	PCV	GCV	h2	GA	GAM%
Days to 50 per cent flowering	75	56	97	11.33	8.16	51.87	9.08	12.11
Plant height (cm)	70	47.5	91.2	12.01	10.22	72.41	12.54	17.92
Tillers per plant	15	2	27	29.56	14.20	23.08	2.11	14.05
Spike length (cm)	11	6.6	13.5	11.58	8.14	49.41	1.30	11.79
Number of spikelets per spike	22	15	25	7.86	3.9	24.62	0.88	3.99
Grain yield per plant (g)	20.5	3	56.5	21.61	13.61	39.66	3.62	17.66
Thousand grain weight (g)	36.2	26	45.6	12.86	6.34	24.31	2.33	6.44
Protein (%)	13.72	11	14	7.26	5.92	66.49	1.36	9.94
Average coefficient of infection of leaf rust	11.45	0	42	47.92	31.57	43.4	4.91	42.84

Table 3: Genetic variability studies for yield and rust resistance in F_4 generation of the cross DWR 162 X NIL PBW 343

Characters	Mean	Min	Max	PCV	GCV	h2	GA	GAM%
Days to 50 per cent flowering	71	60	89	10.65	7.64	51.46	8.02	11.29
Plant height (cm)	72	44	86	10.23	8.05	61.92	9.40	13.05
Tillers per plant	12	6	24	23.06	15.11	42.93	2.45	20.40
Spike length (cm)	12	6	15	11.02	9.12	68.49	1.87	15.55
Number of spikelets per spike	19	13	21	8.11	5.32	43.03	1.37	7.19
Grain yield per plant (g)	19.5	8	46	26.66	16.02	36.11	3.87	19.83
Thousand grain weight (g)	34	21	48	13.22	8.11	37.63	3.48	10.25
Protein (%)	14	12	15	8.12	6.59	65.87	1.54	11.02
Average coefficient of infection of leaf rust	10.34	0	38	42.46	27.63	42.34	3.83	37.04

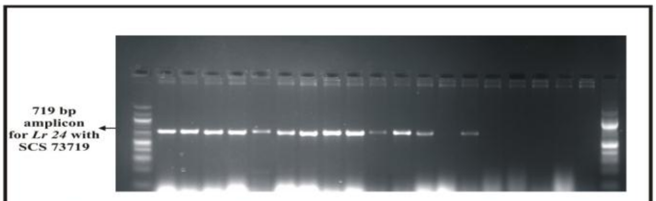

Plate 8: Molecular confirmation for the presence of *Lr24* in F_3 and F_4 generations using SCAR marker SCS 73719

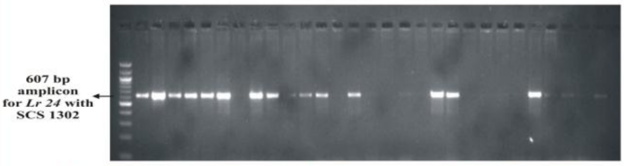

Plate 9: Molecular confirmation for the presence of *Lr24* in F_3 and F_4 generations using SCAR marker SCS 1302

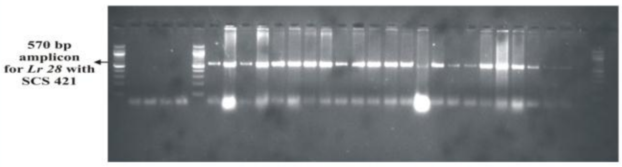

Plate 10: Molecular confirmation for the presence of *Lr28* in F_3 and F_4 generations using SCAR marker SCS 421

Table 4: Observed and expected number of plants with *Lr24*, *Lr28* and *Lr24* and *Lr28* in F_3 generation of DWR 162 X PBW 343 based on joint segregation of linked SCAR markers SCS1302 (*Lr24)* and SCS421(*Lr28*)

Sl. No.	Genotypic class	Number of plants		Cal χ^2	Tab χ^2
		Observed	Expected		
1	A_ B_(9)	24	33.75	3.02	7.82
2	A_ bb (3)	10	11.25		
3	aaB_(3)	12	11.25		
4	aabb (1)	4	3.75		

A= *Lr24*, B= *Lr28*

Table 5: Observed and expected number of plants with *Lr24*, *Lr28* and *Lr24* and *Lr28* in F_4 generation of DWR 162 X PBW 343 based on joint segregation of linked SCAR markers SCS1302 (*Lr24)* and SCS421(*Lr28*)

S No	Genotypic Class	Number of plants		Cal χ^2	Tab χ^2
		Observed	Expected		
1	A_ B_(9)	28	39.375	4.58	7.82
2	A_ bb (3)	16	13.125		
3	aaB_(3)	14	13.125		
4	aabb (1)	6	4.375		
Total		64	70		

Table 6: Evaluation of selected F_4 lines of the cross of DWR 162 X NIL PBW 343 with leaf rust resistance genes *Lr24* and *Lr28*.

F_4 Line No.	Days to fifty percent flowering	Plant height (cm)	Number of tillers per plant	Spike length (cm)	Number of spikelets per spike	Grain yield per plant (g)	Thousand grain weight (g)	Protein content (%)	ACI for leaf rust
F_4-17	76.00	75.50	10.00	11.10	18.00	14.70	38.63	11.20	4.00
F_4-21	71.00	77.00	13.00	9.50	19.00	16.00	36.45	12.56	2.00
F_4-25	81.00	92.00	16.00	11.40	22.00	26.80	43.10	13.90	0.00
F_4-27	72.00	80.00	9.00	10.30	19.00	18.30	33.90	10.60	2.00
F_4-28	67.00	74.00	14.00	10.70	20.00	25.90	36.70	11.53	3.00
F_4-29	87.00	83.00	12.00	10.50	19.00	20.10	37.83	10.58	4.00
F_4-39	70.00	88.00	9.00	9.20	17.00	15.00	34.03	12.67	4.00
F_4-42	74.00	84.00	15.00	10.30	20.00	16.80	37.30	13.17	2.00
DWR 162	68.00	93.00	16.00	11.42	18.00	21.22	37.76	11.65	54.00
NIL PBW 343 (*Lr24* + *Lr28*)	83.80	85.40	11.70	10.40	17.00	18.30	41.10	12.15	5.33
CD at 5%	10.52	8.09	6.81	1.58	2.42	12.26	8.08	1.30	6.17
S.Em ±	4.43	3.57	3.12	0.56	0.85	5.72	2.96	0.46	3.14

CONCLUSION

The genetic variability studies in F_3 generation (Table 2) indicate high mean and wider range for all the traits under evaluation. High PCV (phenotypic coefficient of variation) and moderate GCV (genotypic coefficient of variation) were recorded for the traits number of tillers per plant and grain yield per plant and moderate PCV and moderate GCV for the trait, plant height. Moderate PCV and low GCV for the traits, days to 50 per cent flowering, spike length and thousand grain weight was observed. Low PCV and GCV were observed for number of spikelets per spike and percent protein content. Very narrow difference between the values of GCV and PCV indicated that the environmental effect was small for the expression of these characters. In F_4 generation (Table 3) high PCV (phenotypic coefficient of variation) and moderate GCV (genotypic coefficient of variation) were recorded for the traits, number of tillers per plant and grain yield per plant. All the other characters showed moderate levels of PCV and low GCV. This is in accordance with the findings of Subhashchandra[12]. Reports of high and moderate levels of PCV and GCV in wheat for different quantitative traits was reported by Abinasa[2] in early segregating generations.

High heritability for the trait plant height was observed in both F_3 and F_4 generations. In F_4 generation, the trait, spike length also exhibited high heritability. All the other traits showed moderate to low heritability in both F_3 and F_4 generations. In F_3 generation moderate to low estimates of genetic advance (as percentage of mean) was observed for all the traits. In F_4 generation high GAM with moderate heritability was observed for the trait tillers per plant. This suggests that this trait is governed by additive gene action and selection could be effective in early segregating generations for this trait. All the other traits showed moderate to low GAM. Moderate heritability with low genetic advance was also observed by [3] and [7]. For leaf rust infection, high PCV and GCV, moderate heritability and high GAM was recorded, suggesting that there is high variability for this trait in both F_3 and F_4 populations of the cross DWR 162 X NIL PBW 343.

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