

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.710.164>

Molecular and Phenotypic Characterization for Leaf Rust Resistance in Bread Wheat (*Triticum aestivum* L.)

Arati Yadawad*, R.R. Hanchinal, H.L. Nadaf, S.A. Desai,
Suma Biradar and V. Rudranaik

Department of Genetics and Plant Breeding, University of Agricultural Sciences,
Dharwad-58000, Karnataka, India

*Corresponding author

ABSTRACT

Keywords

Marker assisted selection, Leaf rust, Resistance genes, *Triticum aestivum*

Article Info

Accepted:
12 September 2018
Available Online:
10 October 2018

Present study aimed at molecular and phenotypic characterization of F₃ population of a cross between leaf rust susceptible but well adapted bread wheat variety DWR 162 and resistant variety PBW 343 carrying highly effective alien genes *Lr24* and *Lr28* to identify recombinant lines with high productivity and leaf rust resistance. Fifty nine F₃ lines with seven checks were evaluated for yield and yield contributing traits in randomized block design (RBD) with two replications. These lines were also screened against most virulent pathotypes of leaf rust 77-5 (121R63-1) in rust screening nursery. SCAR markers, namely SCS1302₆₀₇ and SCS421₅₇₀ linked to *Lr24* and *Lr28* genes facilitated identification of individual lines possessing the targeted genes. To identify the lines that mostly resemble 'DWR 162' variety, DUS (Distinctiveness Uniformity Stability) characterization was done. Nine lines were identified in F₃ generation, possessing high yield potential and leaf rust resistance genes, *Lr24* and *Lr28* with the desirable features of DWR 162. Availability of a combination of two major rust resistance genes would facilitate the breeding program for developing new leaf rust resistant and high yielding cultivars.

Introduction

Bread wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide (second after maize), providing staple food for 35 % of the world population. Leaf rust caused by the fungus *Puccinia triticina* Eriks. is one of the most common foliar diseases of wheat worldwide (Oelke and Kolmer, 2004; Mebrate *et al.*, 2008). Severe leaf rust epidemics can cause yield losses up to 40% (Knott, 1989). Use of resistant wheat cultivars is the most economic and environmentally

safe way to reduce losses caused by leaf rust. Till date 71 genes for resistance to leaf rust have been cataloged in wheat (Singh *et al.*, 2012). Most *Lr* genes confer race-specific seedling resistance and are vulnerable to defeat by new virulent races. Greater durability of resistance could be achieved through combinations of race-specific genes (Kolmer *et al.*, 2008a, 2008b).

MAS has allowed concurrent introgression of more than one targeted gene into otherwise elite crop varieties, and thus has been found to

improve significantly the efficiency and effectiveness of conventional breeding (Gupta *et al.*, 2010).

Available molecular markers, tightly linked to desired *Lr* genes help in the selection of individuals with introduced genes, within segregating populations. In wheat, gene pyramiding using MAS has been achieved for resistance against leaf rust (Cox *et al.*, 1994; Gupta *et al.*, 2005; Singh *et al.*, 2004; Nocente *et al.*, 2007; Revati *et al.*, 2010 and Chhuneja *et al.*, 2011).

Present study aimed at molecular and phenotypic characterization of F₃ population from a cross between the leaf rust resistant parent PBW 343 carrying *Lr24* and *Lr28* and a susceptible parent DWR 162 to identify recombinant lines resembling DWR 162 with high productivity and leaf rust resistant genes *Lr24* and *Lr28*.

Materials and Methods

The bread wheat genotype DWR 162, which is susceptible to leaf rust evolved by pedigree method from a cross between Kavakaz/Buho//Kalyanasona/Bobwhite, was selected as female parent. 'PBW 343' a ruling variety of North Western Plains Zone with two effective leaf rust resistance genes, *Lr24* and *Lr28* was used as a male parent.

The desired crosses were made in the polyhouse and in fields of Dr. Sanjay Rajaram Wheat Laboratory, All India Coordinated Wheat Improvement Project (AICWIP), Main Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad and alternate generations were grown in off season at Indian Agricultural Research Institute, Regional Research Station, Wellington, Tamil Nadu. The recommended package of practices was followed for raising the crop.

F₁ seeds of the cross DWR 162 X PBW 343 were sown in spaced planting and individual plants of F₁'s of the cross DWR 162 X PBW 343 were confirmed for the presence of *Lr24* and *Lr28* genes using SCAR markers linked to leaf rust resistance genes *Lr24* and *Lr28*. Individual plants negative for donor gene under transfer were removed. Individual F₁ plants with *Lr24* and *Lr28* were selfed to get F₂ population and 121 individual F₂ plants were grown in the field in space planting. These plants were confirmed for the presence of *Lr24* and *Lr28* and the F₂ plants which carry both *Lr24* and *Lr28* were selfed to get F₃ population for evaluation and selection of superior recombinant lines.

A total of 59 F₃ lines along with two parents, DWR 162, PBW 343 and five checks *viz.*, UAS 304, HI 977, GW 322, UAS 304 and HS 240 were sown in two replications. Observations were recorded on DUS characters and also Morpho-physiological characters on five randomly selected plants in each progeny row line of two replications.

Disease screening

One of the replication of the F₃ segregating progeny was sown at rust screening nursery for screening of leaf rust resistance at the seedling stage with leaf rust pathotype 77-5, virulent on recurrent parent 'DWR 162'. On the appearance of symptoms, rust severity (percentage) and response of the plants to disease were assessed using a modified Cobb's scale (Peterson *et al.*, 1948).

The final disease severity data for the leaf rust was converted into an Average Coefficient of Infection (ACI) by multiplying severity with a constant value for field response (Stubbs *et al.*, 1986 and Roelf *et al.*, 1992). Leaf rust screening along with molecular marker analysis was used to follow the genes during these selfing generations.

DNA isolation, PCR conditions and electrophoresis

Based on DUS characters and Morpho-physiological characters recorded for 59 lines of F₃ in two replications, only superior lines with high productivity and DWR 162 type characters were selected and 10 plants per line were selected randomly and confirmed for the presence of *Lr24* and *Lr28* genes.

DNA isolation of parental genotypes and backcross progenies was carried out from one-month-old plants using a CTAB method (Dellaporta *et al.*, 1983). The PCR amplification was carried out in a reaction mixture of 20 µl containing 200M dNTPs (MBI; Fermentas, Lithuania, USA), 0.75 U Taq DNA polymerase (MBI; Fermentas, Lithuania, USA), 5 pmole of each primer, 20–30 ng template DNA and 10 X PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM MgCl₂). PCR cycle consisted of an initial denaturation for 5 min at 94°C, followed by 40 cycles each with 1 min at 94°C, 1 min at annealing temperature (which differs for different primers), with a final extension of 7 min at 72°C. The details of primers sequence and the PCR conditions used for amplification are presented in Table 1. Amplified PCR products were resolved in 2% agarose gel and stained with ethidium bromide.

Results and Discussion

Although major resistance genes have many disadvantages (Ayliffe *et al.*, 2008), they are still widely used in wheat resistance breeding. In recent years developments in molecular marker techniques and marker identification have facilitated the spread of MAS. This is particularly true in the field of breeding wheat for leaf rust resistance, where PCR-based markers are already available for almost half of the 60 or more designated resistance genes and alleles. Furthermore, all the effective resistance genes designated so far can be

traced in segregating progeny populations by means of MAS.

The present work was aimed to identify superior lines for yield and leaf rust resistance with two major leaf rust resistance genes *Lr24* and *Lr28* in F₃ population of DWR 162 X PBW 343. In F₁ to F₃ self-generations, selection for the leaf rust resistance genes was performed with SSR and SCAR markers for identifying plants carrying both *Lr24* and *Lr28*, followed by visual selection for better phenotype among the plants carrying both the genes. The homozygous lines were identified and evaluated for yield parameters.

Validation of linked molecular markers

The presence of expected desired /genes in the parent PBW343 used for transfer of leaf rust resistance was verified by studying DNA polymorphism or lack of it for the linked markers with DWR 162. These markers were consistent with amplification of specific marker fragment size of 320bp with SSR marker Xwmc313, 570bp fragment with SCAR marker S421570 for *Lr28* and 719bp fragment with the SCAR marker SCS719, 607bp fragment with the SCAR marker SCS1302 for *Lr24* in PBW 343. This confirmed the presence of both resistance genes in PBW 343 and their absence in DWR 162. This proved the efficiency of SCAR markers SCS719 and SCS421 in marker assisted selection. These markers viz., SCS719 and SCS421 have been effectively utilized for MAS by Revati *et al.*, (2010) and Chhuneja *et al.*, (2005). SCAR marker SCS1302₆₀₇ was employed and proved effective for validating the presence of *Lr24* in HS424 (Pal *et al.*, 2015). SCAR markers were used for screening of *Lr24*, *Lr 25*, and *Lr 37* genes (Robert *et al.*, 1999), whereas, STS and RAPD markers were used to validate the presence of *Lr 9*, *Lr 35*, and *Lr 29*, respectively (Vida *et al.*, 2009) (Table 2 and Fig. 3).

Fig.1 Screening of F₃ lines for the presence of leaf rust resistance genes

Fig.1a Lr28 with SCAR marker SCS 421

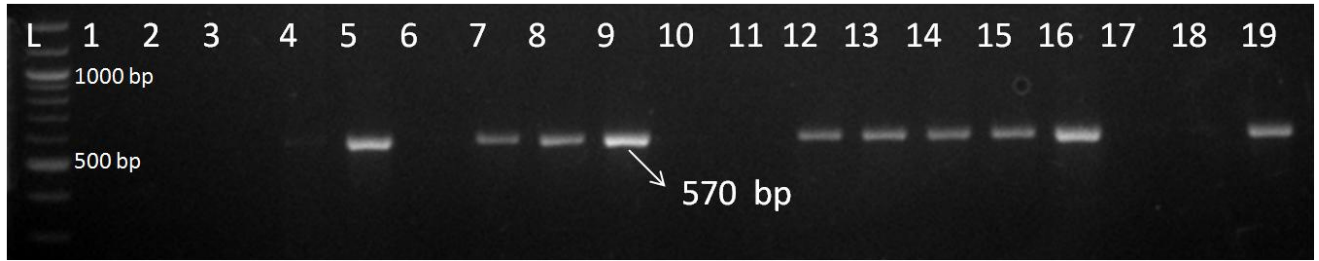


Fig.1b Lr24 with SCAR marker SCS 719

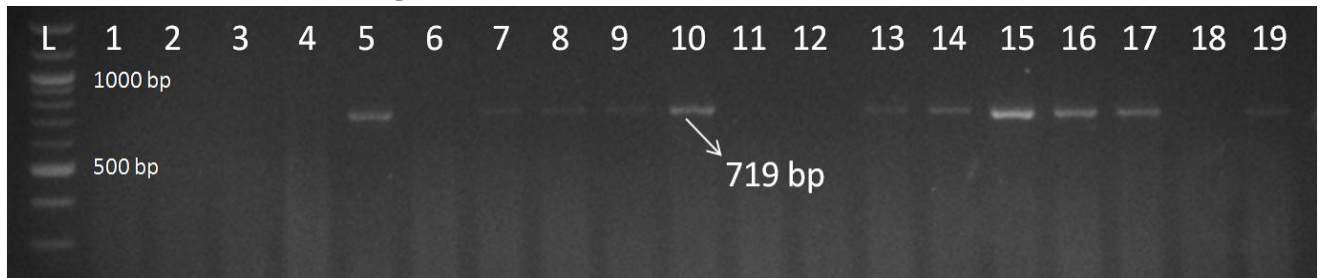


Fig.1c Lr24 with SCAR marker SCS 1302

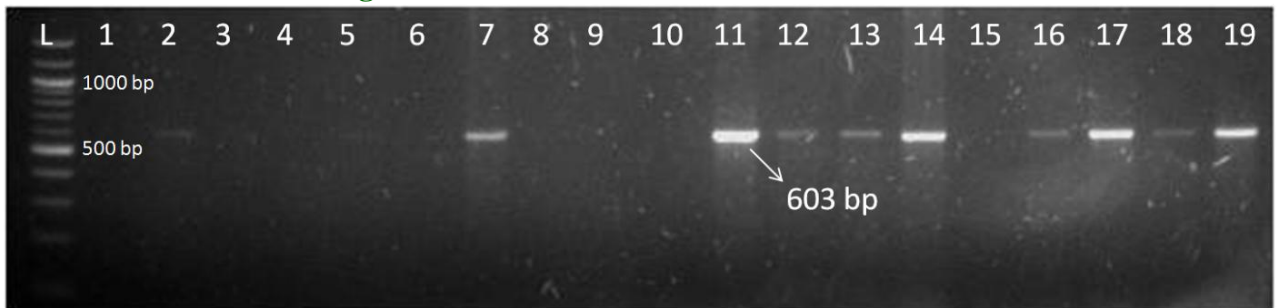


Table.1 Molecular markers for foreground selection of rust resistance Genes used in the study

Genes tagged	Molecular markers	Primer sequence 5'---3'	Amplification product size. bp)	References
<i>Lr24</i>	SCAR:SCS719	F: TCG TCC AGA TCA GAA TGT G R: CTC GTC GAT TAG CAG TGA G	719	Prabhu <i>et al.</i> , (2004).
<i>Lr24</i>	SCAR:SCS1302	F: CGC AGG TTC CAA TAC TTT TC R: CGC AGG TTC TAC CTA ATG CAA	607	Gupta <i>et al.</i> , (2006).
<i>Lr28</i>	SCAR:SCS421	F: ACA AGG TAA GTC TCC AAC CA R: AGT CGA CCG AGA TTT TAA CC	570	Cherukuri <i>et al.</i> , (2005).
<i>Lr28</i>	SSR:Xwmc313	F: GCA GTC TAA TTA TCT GCT GGC G R: GGG TCC TTG TCT ACT ATG TCT	320	Bipinraj <i>et al.</i> , (2011)

Table.2 Superior F₃ lines with rust resistance and *Lr24* and *Lr28* genes in the cross of DWR 162 X PBW 343

F ₃ 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)	Yield per meter row (g)	Thousand grain weight (g)	Protein content (%)	ACI for leaf rust
17	67.00	79.00	11.00	9.60	19.00	14.70	73.40	38.63	13.60	8.00
21	75.00	78.00	14.00	9.50	20.00	16.00	80.00	36.45	13.90	2.00
25	70.00	81.00	16.00	10.40	22.00	17.80	89.20	43.10	12.90	0.00
27	71.00	80.00	9.00	10.30	20.00	18.30	91.30	33.90	13.60	2.00
28	66.00	77.00	11.00	9.70	20.00	17.10	85.60	36.70	12.60	12.00
29	77.00	83.00	13.00	10.30	19.00	20.10	100.40	37.83	12.80	8.00
39	71.00	78.00	10.00	9.50	19.00	16.50	82.40	34.03	13.50	4.00
42	76.00	74.00	15.00	10.30	20.00	16.80	83.80	37.30	12.70	2.00
59	74.00	73.00	13.00	9.60	20.00	25.90	129.40	35.88	12.90	4.00
DWR 162	66.00	86.00	20.00	10.60	19.00	23.42	96.50	38.67	14.65	65.00
Improved PBW 343 (<i>Lr24</i> + <i>Lr28</i>)	84.90	66.40	11.70	9.40	18.00	12.30	56.50	31.10	12.15	5.33
CD at 5%	12.52	10.09	8.81	1.58	2.42	16.16	80.84	8.38	1.30	8.87
S.Em±	4.43	3.57	3.12	0.56	0.85	5.72	28.62	2.96	0.46	3.14

Agronomic evaluation

A total of 66 lines involving 59 F₃ families along with two parents and five checks for yield, quality and rust resistance were evaluated for yield and its component traits. The analysis of variance for all the F₃ lines revealed that both the parents and segregating lines differed significantly ($P \leq 0.01$) for all the traits under study, showing the existence of abundant genetic variability among the genotypes. Similar findings of high variability in segregating F₃ families of wheat have been reported (Yadawad *et al.*, 2015a).

DUS characterization for 26 DUS characters was done identification of individual lines which have recovered most of the characters of DWR 162. Out of fifty nine lines, fifteen F₃ lines in both replications recorded similar characters of DWR 162 based on DUS indicating high resemblance with DWR 162. Selection of BC1F₂ plants for the recovery of genetic background of recurrent parent DWR162 based on twenty Six DUS characters has been reported (Yadawad *et al.*, 2015b).

Screening in leaf rust screening nursery

Fifty nine F₃ segregating progeny rows of rust screening nursery were screened for leaf rust resistance with leaf rust pathotype 77-5, and mixture of races. Out of fifty nine lines, twenty two lines were found to be homozygous resistant, since all the plants within the lines were completely resistant, whereas twenty nine lines found to be segregating with both resistant and susceptible plants within the family. Only eight lines were found to be homozygous susceptible. These twenty two lines which were found to be homozygous resistant were need to be subjected to molecular marker analysis for confirmation of the presence of *Lr24* and *Lr28* leaf rust resistant genes.

Yadawad *et al.*, (2015b) reported selection of nine homozygous resistant lines from screening of 59 F₃ progeny rows for leaf rust incidence in rust screening nursery.

Molecular marker analysis

Molecular marker analysis was performed on selected twenty two lines which were found to be resistant to identify the homozygous lines carrying both the leaf rust resistant genes.

Out of these, 13 lines showed segregation for *Lr24* or *Lr28* genes indicating that these F₂ plants were heterozygous for these two genes. Nine lines found to give uniform amplification with all the markers indicating that these lines are homozygous for both the genes.

The alien segment carrying *Lr24* does not impose any deleterious effect on yield as several cultivars carrying *Lr24* have been released for cultivation in India has been reported by Singh *et al.*, (2007). Similar report of evaluation of Forty one families of F₃ generation for three markers, *Lr 24*, *Lr 28*, and *Lr 9* (Charpe *et al.*, 2012) revealed segregation for eight families, several families were either non-segregating for one or more markers and only one family showed no segregation for all the three markers SCS5550, SCS73719 and SCS421570 indicating the homozygosity for all the three genes.

These nine lines superior for agronomic traits with two major leaf rust resistance genes *Lr24* or *Lr28* in the desirable background of DWR 162 were advanced to F₄. These lines can be used as genetic resource in future wheat breeding programs for improving both yield and leaf rust resistance. Further Multi-year / multilocation trials are planned for these lines to evaluate their potential for release for commercial cultivation.

References

- Ayliffe, M., Singh, R. and Lagudah, E. 2008. Durable resistance to wheat stem rust. *Current Opinion in Plant Biology*, 11: 187-192.
- Bipinraj, A., Honrao, B., Prashar, M., Bhardwa, S., Rao, S. and Tamhankar, S. 2011. Validation and identification of molecular markers linked to the leaf rust resistance gene Lr28 in wheat. *J. Applied Genetics*, 52: 171-175.
- Charpe, A., Koul, S., Gupta, S.K., Singh, A., Pallavi, J.K. and Prabhu, K.V. 2012. Marker assisted gene pyramiding of leaf rust resistance genes *Lr 9*, *Lr 24* and *Lr 28* in a bread wheat cultivar HD 2329. *J. Wheat Research*, 4: 20-28.
- Chhuneja, P., Vikal, Y., Kaur, S., Singh, R., Juneja, S., Bains, N.S., Berry, O., Sharma, A., Gupta, S. K., Charpe, A., Prabhu, K.V. and Dhaliwal, H.S. 2011. Marker-assisted pyramiding of leaf rust resistance genes Lr24 and Lr28 in wheat. *Triticum aestivum*. *Indian J. Agricultural Sciences*, 81(3): 214-218.
- Cox, T. S., Raupp, W. J., Gill, B. S. 1994. Leaf rust-resistance genes, *Lr41*, *Lr42* and *Lr43* transferred from *Triticum tauschii* to common wheat. *Crop Science*, 34: 339-343.
- Dellaporta, S.L., Wood, J. and Hicks, J.B. 1983. A plant DNA mini-preparation: version II. *Plant Molecular Biology Reporter*. 1: 19-21.
- Gupta, P.K., Langridge, P., Mir, R.R. 2010. Marker-assisted wheat breeding: present status and future possibilities. *Molecular Breeding*. 26: 145-161.
- Gupta, S., Charpe, A., Koul, S., Prabhu, K.V., Haq, Q.M. 2005. Development and validation of molecular markers linked to an *Aegilops umbellulata*-derived leaf rust resistance gene, Lr9, for marker-assisted selection in bread wheat. *Genome*, 48: 823-830.
- Knott, D.R. 1989. The wheat rusts-breeding for resistance. Monographs on theoretical and applied genetics. Vol 12. Springer, Berlin Heidelberg New York.
- Kolmer, J.A., Chen, X. and Jin, Y. 2008a. Diseases which challenge global wheat production the wheat rusts. In: B.F. Carver, editor, *Wheat: Science and trade*. Wiley-Blackwell, Ames, IA.
- Kolmer, J.A., Long, D.L. and Hughes, M.E. 2008b. Physiological specialization of *Puccinia triticina* on wheat in the United States in 2006. *Plant Disease*, 92: 1241-1246.
- Mebrate, S.A., Oerke, E.C., Dehne, H.W. and Pillen, K. 2008. Mapping of the leaf rust resistance gene Lr38 on wheat chromosome arm 6DL using SSR markers. *Euphytica*, 162: 457-466.
- Nocente, F., Gazza, L., Pasquini, M. 2007. Evaluation of leaf rust resistance genes Lr1, Lr9, Lr24, Lr47 and their introgression into common wheat cultivars by marker-assisted selection. *Euphytica*, 155: 329-336
- Oelke, L.M. and Kolmer, J.A. 2004. Characterization of leaf rust resistance in hard red spring wheat cultivars. *Plant Disease*, 88: 1127-1133.
- Pal, D., Bhardwaj, S.C., Sharma, D., Kumari, S.M., Patial, V. and Pratima, S. 2015. Assessment of genetic diversity and validating rust resistance gene sources using molecular markers in wheat (*Triticum aestivum* L). *Sabrao Journal of Breeding and Genetics*, 47: 89-98.
- Peterson, R.F., Campbell, A.B. and Hannah, A.E. 1948. A diagram scale for estimating rust severity on leaves and stems of cereals. *Canadian J. Research. Section C*, 26: 496-500.
- Prabhu, K.V., Gupta, S.K., Charpe, A., Koul, S., Cherukuri, D.P. and Dhaliwal, H.S. 2003. Molecular markers detect redundancy and miss-identity in genetic stocks with alien leaf rust resistance

- genes *Lr32* and *Lr28* in bread wheat. *J. Plant Biochemistry and Biotechnology*, 12: 123-129
- Revathi, P.S., Tomar, M., Vinod, S. and Singh, N.K. 2010. Marker assisted gene pyramiding of leaf rust resistance genes *Lr24*, *Lr28* along with stripe rust resistance gene *Yr15* in wheat. *Triticum aestivum* L. *Indian J. Genetics and plant breeding*, 70(40): 349-354.
- Robert, O., Abelard, C. and Dedryve, F. 1999. Identification of molecular markers for the detection of the yellow rust resistance gene *Yr17* in wheat. *Molecular Breeding*, 5: 167-175.
- Roelfs, A.P., Singh, R.P. and Saari, E.E. 1992. Rust Diseases of Wheat: Concepts and Methods of Disease Management, Mexico. CIMMYT.
- Singh, D., Mohler, V., and Park, R.F. 2012. Discovery, characterization and mapping of wheat leaf rust resistance gene *Lr71*. *Euphytica*. 190: 131-136
- Singh, R., Datta, D., Priyamvada Singh, S., Tiwari, R. 2004. Marker- assisted selection for leaf rust resistance genes *Lr19* and *Lr24* in wheat (*Triticum aestivum* L.). *J. Appl. Genet.*, 45: 399-403.
- Stakman, E.C., Stewart, D.M. and Loegering, W.Q. 1962. Identification of physiological races of *Puccinia graminis* var. *tritici*. Agricultural Research Service E617. USDA, Washington DC.
- Stubbs, R.W., Prescott, J.M., Saari, E.E. and Dubin, H.J. 1986. Cereal Disease Methodology Manual, CIMMYT: Mexico.
- Vida, G., Gal, M., Uhrin, A., Veisz, O., Syed, N.H. and Flavell, A J. 2009. Molecular markers for the identification of resistance genes and marker-assisted selection in breeding wheat for leaf rust resistance. *Euphytica*, 170: 67-76.
- Yadawad Arati, Hanchinal, R. R., Nadaf, H. L., Desai, S. A., Suma Biradar and Rudra Naik, V. 2015a. Genetic variability and heritability estimates for yield attributes and leaf rust resistance in F₃ population of wheat (*Triticum aestivum* L). *The Bioscan*. 10(2): 935-938.
- Yadawad Arati., Hanchinal, R.R., Nadaf, H.L., Desai, S., Suma Biradar and Rudranaik, V. 2015b. Improvement of leaf rust resistance in bread wheat variety DWR162. *Triticum aestivum* L. through marker assisted selection. *Indian J. Genetics and Plant Breeding*, 75(2): 256-259.

How to cite this article:

Arati Yadawad, R.R. Hanchinal, H.L. Nadaf, S.A. Desai, Suma Biradar and Rudranaik, V. 2018. Molecular and Phenotypic Characterization for Leaf Rust Resistance in Bread Wheat (*Triticum aestivum* L.). *Int.J.Curr.Microbiol.App.Sci*. 7(10): 1469-1476.
doi: <https://doi.org/10.20546/ijcmas.2018.710.164>