



RESEARCH ARTICLE

Development of near isogenic lines (NILs) for leaf rust resistance utilizing advanced generation segregating lines of RIL population in wheat (*Triticum aestivum* L.)

Ramesh Bhurta, Subhash Bijarania, Naman Raj, Anupama Singh, Ajay K. Chandra, Priyanka Agarwal, Hariom Shukla, Raghunandan K., Niharika Mallick, Vinod and Shailendra K. Jha*

Abstract

Near-isogenic lines (NILs) are useful genetic resources for basic genetic studies and further understanding of associated molecular mechanisms. The NILs can be developed through standard methods like backcross breeding or advanced generation segregating lines. The present study aimed the development of NILs for leaf rust resistance from the advanced generation segregating lines with residual heterozygosity. Advanced generations segregating lines/heterogeneous inbred families (HIFs) segregating for contrasting infection types (IT; 1 and 3) for leaf rust were identified from the recombinant inbred lines (RILs) between cross of *T. timopheevii* derived introgression line Selection G12, a resistant parent and a susceptible parent Agra local. The molecular analysis using polymorphic SSR markers between parents indicated a high level of similarity with 97.07 and 96.49% resemblance among the contrasting NIL pairs from HIF1 and HIF5, respectively. These NILs may serve as valuable resources for conducting fine mapping and expression analysis of leaf rust resistance in wheat and, therefore, will help to identify candidate gene(s) for leaf rust resistance in Selection G12.

Keywords: Near isogenic lines, heterogeneous inbred families, recombinant inbred lines, leaf rust

Introduction

Bread wheat (*Triticum aestivum* L., AABBDD, $2n = 6x = 42$) is a globally cultivated crop vital in providing calories for human beings. It constitutes approximately 20% of humans' total calories and feeds over 35% of the global population (Brenchley et al. 2012). With the world's population continuously growing, there is a persistent need to enhance crop production. To achieve this, crop breeders frequently harness genetic diversity to enhance and protect the crop yield and quality (Zhou et al. 2021). Protecting crops from major losses due to biotic stress like rust requires genetic modification for resistance genes. The rapid utilization of new resistance genes needs an understanding of the associated genetic and molecular mechanisms. The near isogenic lines (NILs) are valuable for comprehending genetic and molecular analysis. They are referred to as strains with almost identical genetic compositions, except for a few loci (Muehlbauer et al. 1988; Young et al. 1988). Specifically, the NILs are developed to minimize the influence of varied genetic backgrounds, thereby eliminating potential interactions between the target gene and the diverse background genes (Zhu et al. 2015). Despite originating from diverse progeny resulting from specific crosses, NILs

are ideally brought to a state of homozygosity and the least difference from each other. This ensures that their genetic composition becomes stable and almost similar. Once the lines reach this homozygous state, they become immortal because their genetic makeup remains constant and can be perpetually utilized in numerous replications for various experiments.

Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

***Corresponding Author:** Shailendra K. Jha, Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India, E-Mail: jhashail78@gmail.com

How to cite this article: Bhurta R., Bijarania S., Raj N., Singh A., Chandra A.K., Agarwal P., Shukla H., Raghunandan K., Mallick N., Vinod and Jha S.K. 2023. Development of near isogenic lines (NILs) for leaf rust resistance utilizing advanced generation segregating lines of RIL population in wheat (*Triticum aestivum* L.). Indian J. Genet. Plant Breed., **83**(4): 482-489.

Source of support: CSIR and DST Project No: ECR/2017/000700.

Conflict of interest: None.

Received: Aug. 2023 **Revised:** Oct. 2023 **Accepted:** Nov. 2023

NILs are valuable resources in various genetic studies and breeding applications. Numerous NILs have been created with resistance against various diseases in the context of crop plants. These NILs have found extensive use in activities such as genetic mapping of resistance genes (Liet al. 2019; Parra et al. 2021; Han et al. 2022), investigating gene interactions (Jain et al. 2019), cloning of *R* genes (Ouibrahim et al. 2014), exploring differential expression (Dhariwal et al. 2011; Gidhi et al. 2023), and analysing gene regulation (Chandra et al. 2020; Bhurta et al. 2022; Hurali et al. 2022). Further, characterization of QTLs directly in breeding programs poses a challenge, primarily due to the broad genomic intervals associated with most identified QTLs (Mia et al. 2019). A potential solution to this issue is the development of NILs. Creating NILs makes it possible to convert quantitative traits into Mendelian factors, thus facilitating the identification of candidate genes and closely linked markers (Liu et al. 2009).

Development of NILs can be accomplished using common methods, viz., backcrossing and marker-assisted selection, depending on the available resources (Yuan et al. 2017). In its simplest form, introgression lines involve the incorporation of a single target locus from a donor parent into a recipient parent with an otherwise identical genetic background. Most of the NILs have recently been generated through a marker-assisted backcross breeding approach (Singh et al. 2017; Mallick et al. 2022a; Mallick et al. 2022b). This approach greatly aids NIL development in a defined genetic background, which is primarily popular cultivars and hence replaces otherwise popular but susceptible varieties. However, through the incorporation of a fast generation cycling system (FGCS) (Zheng et al. 2013), along with the adoption of the heterogeneous inbred family (HIF) method as well as repeated DNA marker-assisted selection (MAS) described by Tuinstra et al. (1997), the duration for NIL development can be significantly reduced. The HIF method proves valuable in creating various sets of NILs with diverse genetic backgrounds stemming from a single cross (Wang et al. 2019). With this approach, it is possible to achieve approximately six generations per year (Yan et al. 2017). This expedited process offers a promising solution to the traditionally lengthy and labor-intensive nature of NIL development. Therefore, the present study targeted the development of NILs for *LrSelG12* utilizing phenotypic as well as integrating markers in a segregating line of RIL population/HIF for leaf rust resistance from resistant stock Selection G12 with Agra local as susceptible parent. The *LrSelG12* was reported to be a broad-spectrum leaf rust resistance gene (Singh et al. 2017) identified in *T. timopheevii*-derived introgression line Selection G12.

Materials and methods

Plant materials

The plant materials used in this study included the bread wheat genotype Agra local as the susceptible parent and *T. timopheevii* IL Selection G12 as the resistant parent for leaf rust. Agra local is one of the most susceptible genotypes for leaf rust at the seedling stage and has always been a choice in various genetic and mapping studies (Rani et al. 2020; Nymagoud et al. 2022). The IL Selection G12 was developed at ICAR-IARI through introgression of the *Triticum timopheevii* segment in bread wheat background (Guha et al. 1996) and was further utilized for the identification of leaf rust resistance gene *LrSelG12* (Singh et al. 2017).

Identification of segregating RIL families for leaf rust resistance

The leaf rust resistant introgression line, Selection G12, was crossed with the susceptible genotype Agra local to develop a large RIL population with a target to fine map the leaf rust resistance gene *LrSelG12*. The true F_1 plants were selfed to generate F_2 seed, and a large F_2 population was screened with leaf rust pathotype 77-5. The F_2 population was further selfed to generate the $F_{2:3}$ lines. The $F_{2:3}$ population was screened by taking 12 to 15 plants from each line. In addition, two seeds from each $F_{2:3}$ family were sown in the field as a hill with a row-to-row distance of 25 cm and a hill-to-hill distance of 25 cm. From the $F_{2:3}$ generation onwards until the $F_{6:7}$ generation, two seeds from each family were planted in the field. After 25 days of sowing, when the seedlings were established, one seedling was uprooted, and only a single plant was retained for each line. In each generation, a single spike from each plant was harvested, and two seeds from each plant were sown in the field to advance to the next generation. This process was repeated to develop the $F_{6:7}$ generation. The RIL population in the $F_{6:7}$ generation was phenotyped for leaf rust by taking 12 to 15 seedlings from each line. The lines with residual heterozygosity at the *LrSelG12* locus have segregated for leaf rust resistance. The 20 plants from each segregating family were grown and evaluated for phenotypic similarity among the plants of the same family. The five families segregating for leaf rust resistance and having the most homogeneous plants were selected and designated as HIF1 to HIF5. The single plants from each family were selfed and harvested separately. The twenty seedlings from each plant of each HIF family were phenotyped for leaf rust. The homozygous resistant and homozygous susceptible plants from each family were identified as NIL-R1 and NIL-S1 from HIF-1 to NIL-R5 and NIL-S5 from HIF-5, respectively. Among the five NIL pairs, only two NIL pairs named NIL-R1 and NIL-S1 from HIF-1 and NIL-R5 and NIL-S5 from HIF5 were finally selected for molecular analysis to confirm their similarity at genomic

level through polymorphic SSR markers. The plan for NIL development from RIL families with residual heterozygosity is depicted in Fig. 1.

Phenotyping for leaf rust

The parents, F₁, F₂, F_{2:3}, F_{6:7} RILs and HIF for NILs were screened against *Puccinia triticina* pathotype, 77-5 at the seedling stage. The initial uredospore inoculum for these rust pathotypes was obtained from ICAR-Indian Institute of Wheat and Barley Research (IIWBR), Regional Station in Flowerdale, Shimla. The inoculum was reproduced on the susceptible wheat cultivar Agra local at ICAR-IARI, New Delhi. Further, to carry out the leaf rust screening, the test materials were planted in aluminum trays with dimensions of 4 × 10 × 3 inches and kept in a glasshouse. About seven to ten days old seedlings were inoculated by spraying the uredospore inoculum of rust onto them using a hand sprayer. The inoculation mixture consisted of uredospores suspended in water and a drop of Tween 20. Following inoculation, the trays were placed in glass chambers with high humidity for 48 hours before being transferred to glasshouse benches

(Rani et al. 2020). The infection types were recorded 10 to 12 DPI (days post inoculation), following the disease scoring scale by Stakman et al. (1962).

DNA isolation, PCR amplification and marker analysis

To extract DNA, leaf samples were taken from 3 to 4 week-old plants of Selection G12, Agra Local, NIL-R1, NIL-S1, NIL-R5, and NIL-S5. The CTAB technique, described by Murray and Thompson in 1980, was used to extract and maintain DNA at a concentration of 25 ng/μL. A total of 142 SSR markers showing polymorphic between the parents, spanning wheat's A, B, and D genomes, were used to analyze the resemblance between resistant and susceptible NILs. PCR amplification was carried out in a 10 μL reaction volume, consisting of 2 μL of genomic DNA, 3 μL of 2× Taq PCR Master Mix, and 2 μL of nuclease-free water. The cycling program was: Initial denaturation: 95°C for 5 minutes; 35 cycles of denaturation (95°C for 30–60 seconds), annealing (50–61°C, 30–60 seconds), extension (72°C for 30–60 seconds) followed by final extension at 72°C for 10 minutes. The PCR products were then separated on a 3.5% agarose gel and visualized using a UV transilluminator gel documentation system.

Determination of similarity between NILs

A collection of 650 SSRs (Simple Sequence Repeats) spread throughout the wheat genome was utilized to identify genetic markers that differ between the resistant and susceptible parents. The specific sequences of these markers were obtained from the GrainGenes database (<https://wheat.pw.usda.gov/GG3/>). These SSR markers were employed to survey genetic polymorphisms between the parents. The similarity between the contrasting NIL pairs was calculated using the following formula: Similarity between contrasting NILs (%) = (Number of monomorphic markers between contrasting NILs / Total number of polymorphic markers between parents) × 100, i.e., Similarity (%) = [Monomorphic A + Monomorphic B + Monomorphic H / (A + B + H)] × 100. Here, A represents the amplicon of markers indicating the resistant parent allele, "B" stands for the susceptible parent allele, and "H" denotes the heterozygous allelic locus. The similarity estimation was carried out in the best two NIL pairs, i.e., NIL-R1, NIL-S1, NIL-R5 and NIL-S5.

Results

Development of NILs from the segregating RIL families with residual heterozygosity

The resistant parent Selection G12 was highly resistant, while the susceptible parent Agra Local was found to have a contrasting susceptible reaction. The F₁ plants had susceptible reactions, although the reaction type was slightly lower than that of the susceptible parent. The screening of F₂ plants revealed segregation of 232 resistant

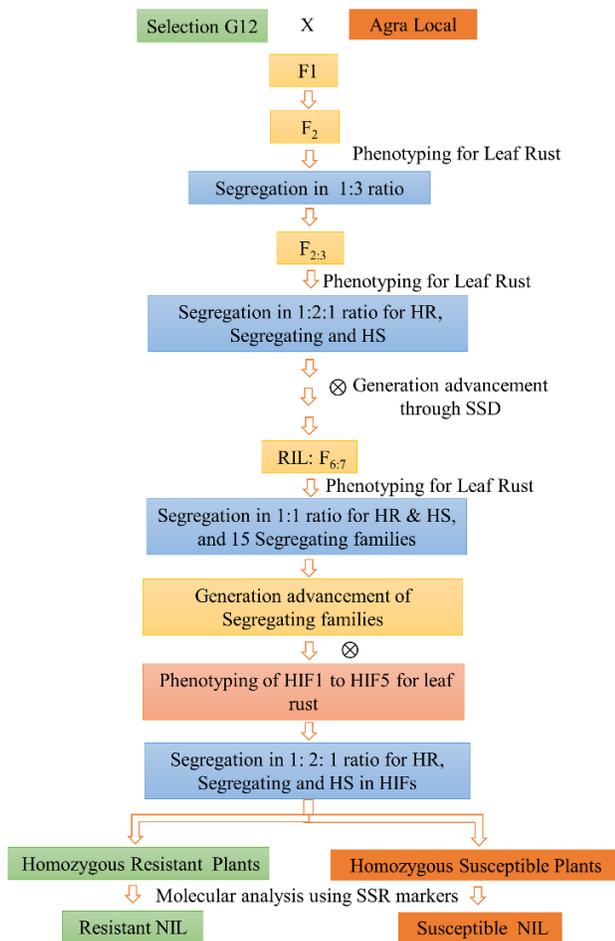


Fig. 1. Schematic illustration of the development of NILs with contrasting leaf rust resistance from advanced generations segregating RIL families from the cross of Selection G12/Agra Local

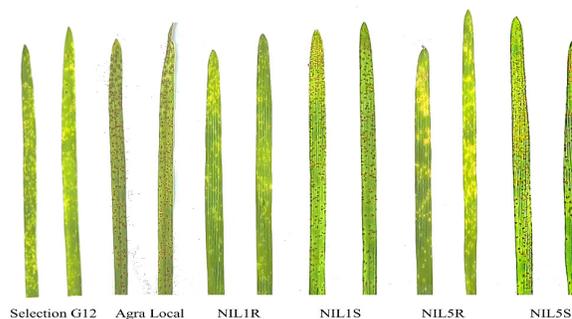
Table 1. Segregation ratio (Res.: Resistant and Suscp.: Susceptible in F_2 , HR: Homozygous Resistant, Seg.: Segregating, and HS: Homozygous Susceptible in $F_{2:3}$ and onwards) across the different generations for leaf rust resistance in the population of Selection G12/Agra Local

Generation	Number of plants				Expected ratio	χ^2 value	p-value
	Res./HR	Seg.	Suscp./HS	Total			
Sel. G12	10	0	0	10	-	-	-
Agra Local	0	0	10	10	-	-	-
F_1	0	0	15	15	-	-	-
F_2	232	-	678	910	1:3	0.12	0.730
$F_{2:3}$	236	442	222	910	1:2:1	0.82	0.663
$F_{6:7}$	446	15	439	900	1:1	0.30	0.581
$F_{7:8}$ HIF-1	6	9	5	20	1:2:1	0.30	0.861
$F_{7:8}$ HIF-2	4	10	6	20	1:2:1	0.40	0.819
$F_{7:8}$ HIF-3	4	11	5	20	1:2:1	0.30	0.861
$F_{7:8}$ HIF-4	4	12	4	20	1:2:1	0.80	0.670
$F_{7:8}$ HIF-5	5	11	4	20	1:2:1	0.30	0.860

and 678 susceptible individuals, displaying a ratio of 1 resistant to 3 susceptible (χ^2 value - 0.12, p -value - 0.730). The F_2 generation was further advanced to create the $F_{2:3}$ generation. In the $F_{2:3}$ generation, 236 families were homozygous for resistance, 442 were segregating, and 222 were homozygous susceptible. These segregating families adhered to a Mendelian ratio of 1:2:1 (χ^2 value - 0.82, p -value - 0.663). Then, $F_{2:3}$ families were precisely advanced until the $F_{6:7}$ generation using the single seed descent method for RIL development. During this process, a single spike from each family from the $F_{2:3}$ generation and onward was selected separately from each plant. $F_{6:7}$ was segregated in a ratio of 1 resistant :1 susceptible (χ^2 value - 0.30, p -value - 0.581). In the $F_{6:7}$ generation, 15 residual segregating families were identified. Five families with maximum visual homogeneity were selected from these segregating families and designated as HIF-1 to HIF-5. The selfed seeds from 20 plants from each of these five HIFs were subjected to phenotypic evaluation for disease reaction. All HIFs displayed a segregation ratio of 1 resistant: 2 segregating: 1 susceptible, HIF-1 (χ^2 value - 0.30, p -value - 0.861), HIF-2 (χ^2 value - 0.40, p -value - 0.819), HIF-3 (χ^2 value - 0.30, p -value - 0.861). HIF-4 (χ^2 value - 0.80, p -value - 0.670) and HIF-5 (χ^2 value - 0.30, p -value - 0.860). The details of each generation with their segregation pattern and disease response are provided in [Table 1](#). In the following generation, homozygous resistant and homozygous susceptible individuals showing phenotypic similarities but contrasting leaf rust resistance were selected and designated as NILs.

Phenotyping of NILs for leaf rust

Five HIFs from the advanced generation RIL population were identified in the Selection G12 and Agra local cross. A set of NIL-R(IT; 1) and NIL-S (IT 33+) were selected from each HIF. Such HIF-derived plants were evaluated for leaf

**Fig. 2.** Disease reaction of parents and NILs with leaf rust pathotype 77-5

rust resistance at seedling stage three common pathotypes 77-5 ([Fig. 2](#)), 77-9 and 106. All the selected NILs showed a high level of resistance (ITs ;1- to ;1) against all the three pathotypes used, and their corresponding susceptible NILs showed ITs 3 to 33+ ([Table 2](#)). This showed that disease reactions were consistent on all the NILs against different pathotypes.

The resemblance between NILs using polymorphic SSR markers

The NILs obtained from the heterogeneous inbred family (HIF) were genotyped using polymorphic SSR markers. Of the approximately 650 SSR markers used for the parental polymorphism survey, 142 SSRs revealed a polymorphism between the susceptible parent Agra Local and the resistant parent Selection G12. The identified polymorphic markers were used for genotyping the (NIL-R1, NIL-S1, NIL-R5, and NIL-S5) along with the parents ([Fig. 3](#)).

These polymorphic markers span all 21 linkage groups of the wheat genome, with the number of markers per linkage group varied, ranging from 6 (1D, 4D and 6B) to 12 (2A) based on consensus maps ([Somers et al. 2004](#); [Song et al.](#)

Table 2. Infection types of selected NILs against leaf rust pathotypes

	Pathotype 77-5		Pathotype 77-9		Pathotype 106	
	NIL-R	NIL-S	NIL-R	NIL-S	NIL-R	NIL-S
HIF1	;1-	33+	;1	33+	;1	33+
HIF2	;1	33+	;1	33+	;1	33+
HIF3	;1	33+	;1	33+	;1	33+
HIF4	;1	33+	;1	33+	;1	33+
HIF5	;1-	33+	;1	33+	;1	33+

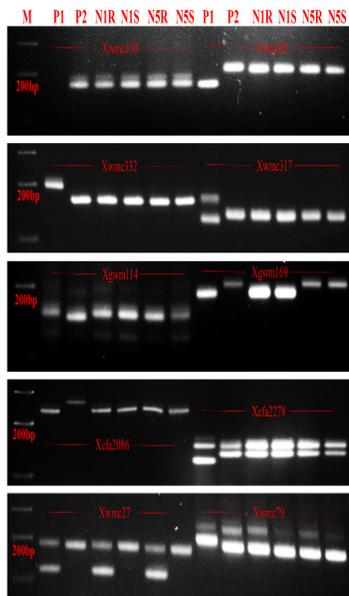


Fig. 3. A representation gel picture showing polymorphic SSR markers between Selection G12 (P1), Agra Local (P2) and NIL pairs from HIF1 and HIF5

2005). The extent of similarity, as indicated by the number of similar amplicon patterns of polymorphic markers between parents, was 97.07% between the NIL-1R and NIL-1S and 96.49% between the NIL-5R and NIL-5S. Further construction of graphical genotyping shows segments of both the parents in HIF-derived NILs (Fig. 4). The graphical depiction demonstrated that the susceptible parent genome was present in the higher part of most of the chromosomes in both the NILs (Fig. 4).

Discussion

HIFs are created through selfing and selection breeding (Jain

1 and Allard, 1960). These HIFs represent advanced inbred lines and typically segregate only for a small region within the genome. Utilizing molecular markers makes it faster and easier to identify HIFs that exhibit segregation for the specific marker(s) within the genomic region of interest. HIF-derived NILs have been employed in various studies to validate quantitative trait loci (QTLs) (Loudet et al. 2005; Diaz et al. 2006; Bai et al. 2010; Liu 1 et al. 2011; Maet et al. 2012; Tuyen et al. 2013). Additionally, these NILs have been instrumental in narrowing down the genomic region containing the QTLs (Yamanaka et al. 2005; Liu et al. 2009; Liu et al. 2011).

The research described in this study involves the development of NILs from HIFs of Selection G12 and Agra Local that exhibit segregation for rust resistance. These NILs can be subsequently employed to identify and validate the loci/gene(s) associated with rust resistance in *T. timopheevii* derived IL Selection G12. The advanced generations resulting from the cross, Selection G12/Agra local, which involved parents contrasting for leaf rust resistance, included a small number of families that showed segregation for leaf rust resistance. A total of 5 such segregating/HIFs were selected from the F_{6,7} generation. From these segregating families, plants contrasting for rust resistance were selected. Seedling evaluation of NILs indicated that these differed significantly for rust resistance and were phenotypically similar for other traits. Plants arising from the same HIF and displaying segregation solely for rust resistance while not showing variation in other traits were carefully chosen as NILs. Hu et al. (1997) developed the closely related inbred lines resistant and susceptible to powdery mildew from a segregating F₅ family and identified RAPD markers linked to the *PM 1* gene for resistance to powdery mildew in wheat. These NILs serve as valuable genetic resources for additional genetic investigations (Yeri et al. 2014).

Phenotypic analysis for leaf rust of these NILs showed that the difference between the NIL pairs was consistent across the different pathotypes. Since NIL pairs have different alleles only at the long arm of chromosome 5B and the short arm of chromosome 1B and 4A region, differences in phenotype can be attributed to the genes in these segments. These can be further validated through fine mapping of rust resistance in these NILs.

The availability of DNA markers enables the determination of genomic regions that exhibit dissimilarities or similarities (isogenic) among the NILs. In the non-availability of such

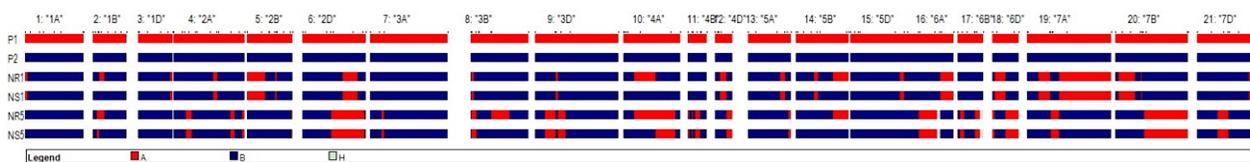


Fig. 4. Graphical representation showing the parental segment distribution in HIF-derived NILs

markers, phenotypic selection can serve the purpose. This study selected NILs based on phenotypic differences for leaf rust resistance. Recovering a specific genomic region in HIF-derived NILs is only possible by assessing the overall genomic heterozygosity using markers (Sherif et al. 2023). A set of NILs was derived from five HIFs of Selection G12 and Agra local. The background genome similarity among these NILs was examined following a parental survey using several SSR markers covering the wheat genome. Genotyping analysis was performed with 142 polymorphic markers between both parents. The results indicated a notably high loci similarity among the NILs within each set. For instance, the NILs (NIL-1R and NIL-1S) derived from HIF-1 exhibited the highest genome similarity of 97.07%. Similarly, the NIL-5R and NIL-5S derived from HIF-5 displayed a genome similarity of 96.49%. As a result, the plants selected from each HIF could be considered genuine NILs because they exhibited phenotypic differences solely for rust resistance. Furthermore, based on graphical genotyping data, these NILs possessed contrasting alleles only at a few SSR loci while maintaining identical alleles at most other loci.

Previously, the development of HIF-derived NIL has been reported in various plant species, such as rice (Kobayashi et al. 2006), sorghum (Tuinstra et al. 1997, 1998), wheat (Hu et al. 1997), barley (Nduulu et al. 2002), Arabidopsis (Bikard et al. 2009), soybean (Ikeda et al. 2009) and in peanut for rust resistance (Yeri et al. 2014). Additionally, studies on NILs with differing resistance to leaf rust have been conducted using backcross breeding methods in wheat (Mallick et al. 2022a; Mallick et al. 2022b). However, this current study represents the development of NILs from HIFs/RILs in wheat for leaf rust resistance.

NILs obtained from HIFs offer a distinct advantage compared to backcross-derived NILs in understanding the expression of specific loci under various genomic contexts. The NILs derived from HIFs represent a diverse set of recombination events, which allows for a comprehensive examination of the impact of different genetic backgrounds on the expression of the locus in question (Tuinstra et al. 1997). This characteristic also facilitates the study of epistatic interactions and the degree of penetrance for the targeted genomic region. Furthermore, this unique feature of HIF-derived NILs allows for selecting an optimal genetic background that maximizes the expression of the genetic loci. In contrast to backcross-derived NILs, those derived from HIFs do not exhibit a strict similarity or resemblance to the recurrent parent, which offers increased genetic diversity and flexibility (Haley et al. 1994).

The broad spectrum of allele representation is a clear indication of the diverse recombination events occurring within the HIFs, which is also clearly visible in the present study (Fig. 4). Consequently, the NILs identified in this study, exhibiting diverse recombination events can serve

as valuable tools for investigating the expression of rust resistance loci. These NILs can also identify candidate genes associated with leaf rust resistance in Selection G12. They will also aid in dissecting specific *timopheevii* genomic regions associated with the leaf rust resistance in selection G12.

Authors' contribution

Conceptualization of research (SKJ, V); Designing of the experiments (SKJ, V, NM, RK); Contribution of experimental materials (SKJ,V); Execution of field/lab experiments and data collection (RB,SB, NR, AS, HS); Analysis of data and interpretation (RB, AKC, PA, RK, SKJ); Preparation of the manuscript (RB, SKJ, NM, V, PA).

Acknowledgments

The first author is grateful to CSIR for the financial assistance from JRF and SRF during their study at PG School, ICAR-IARI, New Delhi. The authors also thank the ICAR-IIWBR, Research, Regional Station, Flowerdale, Shimla, for providing pure inoculum of *P. triticina* pathotypes. The corresponding author express his gratitude to DST for funding the project "Fine mapping of broad-spectrum new leaf rust resistance gene in *Triticum timopheevii* derivative 'Selection G12'"(File No: ECR/2017/000700).

References

- Bai X., Luo L., Yan W., Kovi M. R., Zhan W. and Xing Y. 2010. Genetic dissection of rice grain shape using a recombinant inbred line population derived from two contrasting parents and fine mapping a pleiotropic quantitative trait locus *qGL7*. *BMC Genet.*, **11**: 1–11.
- Bhurta R., Hurali D. T., Tyagi S., Sathee L., Adavi B., Singh D., Mallick M., Chinnusamy V., Vinod and Jha S. K. 2022. Genome-wide identification and expression analysis of the *thioredoxin (trx)* gene family reveals its role in leaf rust resistance in wheat (*Triticum aestivum* L.). *Front. Genet.*, **13**: 563.
- Bikard D., Patel D., Le Mett e C., Giorgi V., Camilleri C., Bennett M. J. and Loudet O. 2009. Divergent evolution of duplicate genes leads to genetic incompatibilities within *A. thaliana*. *Science*, **323**(5914): 623–626.
- Brenchley R., Spannagl M., Pfeifer M., Barker G. L. A., D'Amore R., Allen A. M., McKenzie N., Kramer M., Kerhornou A., Bolser D. and Kay S. 2012. Analysis of the bread wheat genome using whole-genome shotgun sequencing. *Nature*, **491**(7426): 705–710.
- Chandra S., Satapathy L., Basu S., Jha S. K., Kumar M. and Mukhopadhyay K. 2020. Characterization of the leaf rust responsive ARF genes in wheat (*Triticum aestivum* L.). *Plant Cell Rep.*, **39**: 1639-1654.
- Dhariwal R., Vyas S., Bhaganagare G. R., Jha S. K., Khurana J. P., Tyagi A. K., Parbhu K.V., Balyan H. S. and Gupta P. K. 2011. Analysis of differentially expressed genes in leaf rust infected bread wheat involving seedling resistance gene *Lr28*. *Funct. Plant Biol.*, **38**(6): 479-492.
- Diaz C., Saliba-Colombani V., Loudet O., Belluomo P., Moreau L., Daniel-Vedele F., Morot Gaudry J. and Masclaux-Daubresse C. 2006. Leaf yellowing and anthocyanin accumulation are two genetically independent strategies in response to

- nitrogen limitation in *Arabidopsis thaliana*. *Plant Cell Physiol.*, **47**(1): 74–83.
- Gao S., Jiang Y., Zhou H., Liu Y., Li H., Liu C. and Zheng Z. 2023. Fine mapping of a Fusarium crown rot resistant locus on chromosome arm 6HL in barley by exploiting near isogenic lines, transcriptome profiling, and a large near isogenic line-derived population. *Theor. Appl. Genet.*, **136**(6): 1–12.
- Guha G., Bhowal J. G. and Dasgupta S. K. 1996. New sources of resistance to stem, leaf and stripe rusts from crosses of *Triticum aestivum* L. and *T. timopheevii* Zhuk. *Annu. Wheat Newslett.*, **42**: 113.
- Gidhi A., Mohapatra A., Fatima M., Jha S.K., Kumar M. and Mukhopadhyay K. 2023. Insights of auxin signaling F-box genes in wheat. *Protoplasma*, **260**: 723–739.
- Haley S. D., Afanador L. K., Miklas P. N., Stavely J. R. and Kelly J. D. 1994. Heterogeneous inbred populations are useful as sources of near-isogenic lines for RAPD marker localization. *Theor. Appl. Genet.*, **88**(3–4): 337–342.
- Han J., Dong S., Liu X., Shi Y., Beckles D.M., Gu X., Miao H. and Zhang S. 2022. Fine mapping a quantitative trait locus underlying seedling resistance to gummy stem blight using a residual heterozygous lines-derived strategy in cucumber. *Front. Plant Sci.*, **13**: 968811.
- Holbrook C. C., Timper P., Dong W., Kvien C. K. and Culbreath A. K. 2008. Development of near-isogenic peanut lines with and without resistance to the peanut root-knot nematode. *Crop Sci.*, **48**(1): 194–198.
- Hu X. Y., Ohm H. W. and Dweikat I. 1997. Identification of RAPD markers linked to the gene *PM 1* for resistance to powdery mildew in wheat. *Theor. Appl. Genet.*, **94**: 832–840.
- Hurali D. T., Bhurta R., Tyagi S., Sathee L., Sandeep A. B., Singh D., Mallick N., Vinod and Jha S. K. 2022. Analysis of NIA and GSNOR family genes and nitric oxide homeostasis in response to wheat-leaf rust interaction. *Sci Rep.*, **12**(1): 803.
- Ikeda T., Ohnishi S., Senda M., Miyoshi T., Ishimoto M., Kitamura K. and Funatsuki H. 2009. A novel major quantitative trait locus controlling seed development at low temperature in soybean (*Glycine max*). *Theor. Appl. Genet.*, **118**: 1477–1488.
- Jain P., Dubey H., Singh P.K., Solanke A.U., Singh A.K. and Sharma T.R. 2019. Deciphering signalling network in broad spectrum Near Isogenic Lines of rice resistant to *Magnaporthe oryzae*. *Sci. Rep.*, **9**(1): 16939.
- Jain S. K. and Allard R. W. 1960. Population studies in predominantly self-pollinated species, I. Evidence for heterozygote advantage in a closed population of barley. *Proc. Natl. Acad. Sci.*, **46**(10): 1371–1377.
- Kobayashi S., Araki E., Osaki M., Khush G. S. and Fukuta Y. 2006. Localization, validation and characterization of plant-type QTLs on chromosomes 4 and 6 in rice (*Oryza sativa* L.). *Field Crops Res.*, **96**(1): 106–112.
- Li Z., Xue Y., Zhou H., Li Y., Usman B., Jiao X., Wang X., Liu F., Qin B., Li R. and Qiu Y. 2019. High-resolution mapping and breeding application of a novel brown planthopper resistance gene derived from wild rice (*Oryza rufipogon* Griff). *Rice*, **12**(1): 1–13.
- Liu T., Mao D., Zhang S., Xu C. and Xing Y. 2009. Fine mapping SPP1, a QTL controlling the number of spikelets per panicle, to a BAC clone in rice (*Oryza sativa*). *Theor. Appl. Genet.*, **118**: 1509–1517.
- Liu Y., Zhu X. Y., Zhang S., Bernardo M., Edwards J., Galbraith D. W., Leach J., Zhang G., Liu B. and Leung H. 2011. Dissecting quantitative resistance against blast disease using heterogeneous inbred family lines in rice. *Theor. Appl. Genet.*, **122**: 341–353.
- Loudet O., Gaudon V., Trubuil A. and Daniel-Vedele F. 2005. Quantitative trait loci controlling root growth and architecture in *Arabidopsis thaliana* confirmed by heterogeneous inbred family. *Theor. Appl. Genet.*, **110**: 742–753.
- Ma J., Yan G. J. and Liu C. J. 2012. Development of near-isogenic lines for a major QTL on 3BL conferring Fusarium crown rot resistance in hexaploid wheat. *Euphytica*, **183**: 147–152.
- Mallick N., Jha S. K., Agarwal P., Kumar S., Mall A., Choudhary M. K., Chandra A.K., Bansal S., Saharan M.S., Sharma J.B. and Vinod. 2022. Marker-assisted transfer of leaf and stripe rust resistance from *Triticum turgidum* var. *durum* cv. Trinakria to wheat variety HD2932. *Front. Genet.*, **13**: 941287.
- Mallick N., Jha S. K., Agarwal P., Mall A., Kumar S., Choudhary M. K., Bansal S., Saharan M. S. and Sharma J. B. 2022. Marker-assisted improvement of bread wheat variety HD2967 for leaf and stripe rust resistance. *Plants*, **11**(9): 1152.
- Mia M. S., Liu H., Wang X. and Yan G. 2019. Multiple near-isogenic lines targeting a QTL hotspot of drought tolerance showed contrasting performance under post-anthesis water stress. *Front. Plant Sci.*, **10**: 271.
- Muehlbauer G. J., Specht J. E., Thomas-Compton M. A., Staswick P. E. and Bernard R. L. 1988. Near-isogenic lines—a potential resource in the integration of conventional and molecular marker linkage maps. *Crop Sci.*, **28**(5): 729–735.
- Murray M. G. and Thompson W. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.*, **8**(19): 4321–4326.
- Nduulu L. M., Mesfin A., Muehlbauer G. J. and Smith K. P. 2002. Effect of Chevron alleles at two Fusarium head blight resistance QTL determined using near-isogenic lines. In 2002 National Fusarium Head Blight Forum Proceedings, U.S. Wheat & Barley Scab Initiative. East Lansing: MI State Univ. Press. Elanger, KY, p 35.
- Nymagoud S., Tyagi S., Chandra A. K., Agarwal P., Niranjana M., Mallick N., Raghunandan K., Jha S. K. and Tomar S. M. S. 2022. Development and characterisation of leaf rust resistant *Triticum timopheevii*-derived introgression lines in hexaploid wheat. *Indian J. Genet. Plant Breed.*, **82**(04).
- Ouibrahim L., Mazier M., Estevan J., Pagny G., Decroocq V., Desbiez C., Moretti A., Gallois J.L. and Caranta C. 2014. Cloning of the *Arabidopsis rwm1* gene for resistance to Watermelon mosaic virus points to a new function for natural virus resistance genes. *Plant J.*, **79**(5): 705–716.
- Parra L., Nortman K., Sah A., Truco M. J., Ochoa O. and Michelmore R. 2021. Identification and mapping of new genes for resistance to downy mildew in lettuce. *Theor. Appl. Genet.*, **134**: 519–528.
- Rani K., Raghu B. R., Jha S. K., Agarwal P., Mallick N., Niranjana M., Sharma J.B., Singh A.K., Sharma N.K., Rajkumar S. and Vinod. 2020. A novel leaf rust resistance gene introgressed from *Aegilops markgrafii* maps on chromosome arm 2AS of wheat. *Theor. Appl. Genet.*, **133**: 2685–2694.
- Sherif A., Zhang B., Wu B., Hu Y., Li S., Zhou X., Elbadri A.M., Elshenawy M., El-Badawy M.E.M., Hassan I.O. and Xing Y. 2023. A pseudo-near isogenic F₂ population strategy for rapid QTL cloning. *Mol. Breed.*, **43**(8): 61.
- Singh A. K., Sharma J. B., Vinod, Singh P. K., Singh A. and Mallick

- N. 2017. Genetics and mapping of a new leaf rust resistance gene in *Triticum aestivum* L. × *Triticum timopheevii* Zhuk. derivative 'Selection G12'. *J. Genet.*, **96**: 291-297.
- Singh M., Mallick N., Chand S., Kumari P., Sharma J. B., Sivasamy M., Jayaprakash P., Prabhu K.V., Jha S.K. and Vinod. 2017. Marker-assisted pyramiding of *Thinopyrum*-derived leaf rust resistance genes *Lr19* and *Lr24* in bread wheat variety HD2733. *J. Genet.*, **96**: 951-957.
- Somers D. J., Isaac P. and Edwards K. 2004. A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.*, **109**: 1105–1114.
- Song Q. J., Shi J. R., Singh S., Fickus E. W., Costa J. M., Lewis J., Gill B. S., Ward R. and Cregan P. B. 2005. Development and mapping of microsatellite (SSR) markers in wheat. *Theor. Appl. Genet.*, **110**: 550–560.
- Stakman E. C., Stewart D. M. and Loegering W. Q. 1962. Identification of physiologic races of *Puccinia graminis* var. *tritici*. USDA Washington.
- Tuinstra M. R., Ejeta G. and Goldsbrough P. 1998. Evaluation of near-isogenic sorghum lines contrasting for QTL markers associated with drought tolerance. *Crop Sci.*, **38**(3): 835–842.
- Tuinstra M. R., Ejeta G. and Goldsbrough P. B. 1997. Heterogeneous inbred family (HIF) analysis: a method for developing near-isogenic lines that differ at quantitative trait loci. *Theor. Appl. Genet.*, **95**: 1005–1011.
- Tuyen D. D., Zhang H. M. and Xu D. H. 2013. Validation and high-resolution mapping of a major quantitative trait locus for alkaline salt tolerance in soybean using residual heterozygous line. *Mol. Breed.*, **31**: 79–86.
- Wang X., Liu H., Liu G., Mia M. S., Siddique K. H. and Yan G. 2019. Phenotypic and genotypic characterization of near-isogenic lines targeting a major 4BL QTL responsible for pre-harvest sprouting in wheat. *BMC Plant Biol.*, **19**(1): 1-10.
- Yamanaka N., Watanabe S., Toda K., Hayashi M., Fuchigami H., Takahashi R. and Harada K. 2005. Fine mapping of the *FT1* locus for soybean flowering time using a residual heterozygous line derived from a recombinant inbred line. *Theor. Appl. Genet.*, **110**: 634–639.
- Yan G., Liu H., Wang H., Lu Z., Wang Y., Mullan D., Hamblin J. and Liu C. 2017. Accelerated generation of selfed pure line plants for gene identification and crop breeding. *Front. Plant Sci.*, **8**: 1786.
- Yeri S. B., Shirasawa K., Pandey M. K., Gowda M. V. C., Sujay V., Shriswathi M., Nadaf H. L., Motagi B. N., Lingaraju S. and Bhat A.R.S. 2014. Development of NIL s from heterogeneous inbred families for validating the rust resistance QTL in peanut (*Arachis hypogaea* L.). *Plant Breed.*, **133**(1): 80–85.
- Young N. D., Zamir D., Ganai M. W. and Tanksley S. D. 1988. Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the *tm-2a* gene in tomato. *Genetics*, **120**(2): 579–585.
- Yuan G., Wan Y., Li X., He B., Zhang Y., Xu B., Wang S., Xie W., Zhou X. and Wu Q. 2017. Development of near-isogenic lines in a parthenogenetically reproduced thrips species, *Frankliniella occidentalis*. *Front. Physiol.*, **8**: 130.
- Zheng Z., Wang H. B., Chen G. D., Yan G. J. and Liu C. J. 2013. A procedure allowing up to eight generations of wheat and nine generations of barley per annum. *Euphytica*, **191**: 311–316.
- Zhou Y., Bai S., Li H., Sun G., Zhang D., Ma F., Zhao X., Nie F., Li J. and Chen L. 2021. Introgressing the *Aegilops tauschii* genome into wheat as a basis for cereal improvement. *Nat. Plants*, **7**(6): 774–786.
- Zhu X., Lei Y., Yang Y., Baxter S. W., Li J., Wu Q., Wang S., Xie W., Guo Z. and Fu W. 2015. Construction and characterisation of near-isogenic *Plutella xylostella* (Lepidoptera: Plutellidae) strains resistant to Cry1Ac toxin. *Pest Manag. Sci.*, **71**(2): 225–233.