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Wheat Line “Selection 212” A Source of Broad Spectrum Resistance to Leaf Rust Pathotypes

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ABSTRACT

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A leaf rust resistant stock ‘Selection212’ was assessed for resistance against array of leaf rust pathotypes and identified to be a source of resistance to all these pathotypes. The resistant stock Selection212 including two susceptible checks Agra Local and Kharchia local were evaluated against 16 pathotypes of leaf rust, *Puccinia triticina* (*Pt*) pathogen. The Selection 212 showed high degree of broad spectrum resistance at seedling stage to diverse pathotypes of leaf rust pathogen in India and effective at adult plant stage against two most prevalent pathotypes 77-5 of leaf rust and pathotype 40A of stem rust. Selection 212 was found resistant to leaf rust pathotypes, 12-3, 12-4, 77, 77A, 77A-1, 77-3, 77-5, 77-6, 77-7, 77-8, 77-10, 104-1, 104-4, 106, 107-1 and 108. Some of the pathotypes like 77-7, 77-8 and 77-10 are known to have virulence against important resistance genes like *Lr9*, *Lr19* and *Lr28*. Therefore, Selection 212 seems to be a source of broad spectrum resistance for leaf rust and can be used for further investigation by molecular studies, so that resistance can be exploited for marker assisted wheat breeding.

Introduction

Wheat is a major staple food crop being consumed by 30 percent population of the world (Eversole *et al.*, 2014) and is grown in all the continents. Globally wheat is grown on 219.70 million hectares’ area with production of 755.2 mt (Source: USDA, 2017), while in India cultivated on an area of 30.60 million hectares with production of 98.61 mt, with highest ever productivity of 3.22 t/ha

according to 3rd advance estimate (2017-18) of government of India. In view of growing population, we need higher food grain yield. The yield can be increased through enhancing productivity or reducing the losses caused by biotic and abiotic stresses. Among several biotic stress causing damage to wheat crop, three rust diseases i.e. leaf, stem and stripe rust, are the most prevalent and reported from all wheat growing countries (Roelfs *et al.*, 1992). The rusts are known to reduce wheat

production worldwide and causing significant losses to the yield. Among three wheat rusts, leaf rust (*Puccinia triticina*. f. sp. *tritici* Eriks) is most common in all wheat growing regions (Roelfs *et al.*, 1995). Leaf rust could result in major losses due to higher number of infection cycles during crop growth in congenial environment. They have potential to cause yield losses up to 50 percent, if infection starts at early growth stage (Huerta-Espino *et al.*, 2011). There are few leaf rust resistance genes which have been commercially exploited so far in Indian cultivar are *Lr1*, *Lr3*, *Lr9*, *Lr10*, *Lr13*, *Lr14a*, *Lr17*, *Lr19*, *Lr23*, *Lr24*, *Lr26*, *Lr28* and *Lr34* (Bhardwaj *et al.*, 2010) but only *Lr24* is all plant stage resistance gene for which virulence is not known in India. However, virulence already has been reported for *Lr24* in some countries (Singh, 1991). The frequent emergence of new pathotypes in wheat rust necessitates search of new broad spectrum resistance sources to be utilized in breeding program.

Efforts have been made for the search of novel and effective sources of resistance by geneticists and plant breeders throughout the world, in this regard till date 79 leaf rust resistance (*Lr*) genes were identified (McIntosh *et al.*, 2017; Qureshi *et al.*, 2018). The present study is a step towards identifying another novel broad spectrum resistance gene by screening genetic stock "Selection212" with different pathotypes of leaf rusts pathogen at seedling stage.

Materials and Methods

Plant material

The wheat line Selection212 ($2n = 42$), a homoeologous recombinant of wheat (*T. aestivum*) and rye (*Secale cereale*), was developed at the Division of Genetics, IARI, New Delhi (Singh, 1991). Susceptible cultivars used in current study were Agra

Local and Kharchia Local, a highly susceptible genotypes against Indian pathotypes of all three rust at seedling as well as adult stage (Mehta, 1929), hence were used as control as well as for multiplication of rust inoculum.

Pathogen

The single spore culture inoculum of 16 pathotypes of *Puccinia triticina*. f. sp. *tritici* Eriks (*Pt*) procured from IIWBR, Regional Station, Flowerdale, Shimla during 2016-17 and 2017-18 were used for present study. In addition, a stem rust, *Puccinia graminis* f.sp. *tritici* (*Pgt*) pathotype 40A was used for screening at adult plant stage.

Multiplication of rust inoculum

Urediniospore of pathotypes was multiplied in glass house on Agra local (Joshi *et al.*, 1988). Susceptible host, Agra Local was sown in 4" plastic pots filled with thoroughly mixed garden soil. Seeds were sown and irrigated for proper germination. After 10 days, seedlings were ready for inoculation. Rust inoculum was mixed with non-toxic talcum powder and applied on the surface of leaves. After inoculation water was sprayed on seedlings so as to deposit small dew droplets on the leaves. The inoculated seedlings were transferred to humidity chamber for next 48 hours. After incubation of 48 hours, seedlings were taken out and transferred to the glasshouse benches for sporulation. The sporulation appeared after 12-13 days of inoculation under optimum conditions. Urediniospore inoculum was collected every alternate day by gently tapping and used for multipathotype testing.

Multipathotype test

Selection212 along with susceptible genotype Agra Local and Kharchia Local were sown in 4" pot for evaluation with different pathotypes

of leaf rust. 10 days old seedlings were inoculated by freshly multiplied urediniospores *Pt* and *Pgt* pathotypes separately as water suspension using a drop of Tween 20 (Polysorbate 20) as surfactant. The inoculated seedlings were kept in humidity chamber for 48 hours and then shifted to glass house benches. The infection types were recorded after 12 days of inoculation based on 0-4 scale of standard classification given by Stakman *et al.*, (1962). The infection type 0, 1 and 2 were classified as resistant reaction while infection types 3 and 4 were grouped as susceptible.

Results and Discussion

The resistant stock Selection212 as well as susceptible cultivars Agra Local and Kharchia Local were screened with 16*Pt* pathotypes including 10 pathotypes not tested earlier and infection types were recorded on these lines are presented in Table 1. 'Selection212' expressed resistant infection types against all 16 pathotypes tested with, ranging from '0;' to 'X'. In contrast, Agra Local and Kharchia Local showed susceptible reaction with infection type ranging from '3' to '3⁺' against all these pathotypes used in the study (Figure 1). Results suggested that leaf rust resistance gene in 'Selection212' showed hypersensitive reaction at seedling stage to different pathotypes of leaf rust indicating its effectiveness and broad-spectrum of resistance.

Selection212 and Agra Local were tested at adult plant stage by inoculating the flag leaf and penultimate leaf with *Pt* pathotype 77-5 and *Pgt* pathotypes 40A. Selection212 expressed high degree of hypersensitive resistant response in contrast to highly susceptible response by check Agra Local with both the pathotypes (Figure 2). The effectiveness of Selection212 at adult plant stage suggests its effectiveness at all plant stage.

The result of multipathotype test indicates that Selection212 is source of broad spectrum resistance to leaf rust pathotypes. Sharma and Singh in 2000 assessed Selection212 with 25 leaf rust and 20 stem rust pathotypes and found Selection212 to be resistant to all pathotypes. In present study, Selection212 was found to be resistant to additional 10*Pt* pathotypes.

The leaf rust resistance genes *Lr1*, *Lr3*, *Lr9*, *Lr10*, *Lr13*, *Lr14a*, *Lr17*, *Lr19*, *Lr23*, *Lr24*, *Lr26*, *Lr28* and *Lr34* are exploited so far in Indian cultivar (Bhardwaj *et al.*, 2010). The avirulence/virulence pattern of these pathotypes according to Prasad *et al.*, (2017) suggested that all these leaf rust resistance genes except *Lr24* were susceptible to pathotypes 12-4, 77A, 77A-1, 77-6, 77-7, 77-8, 77-10, 104-1 and 104-4 in other hand Selection212 were shown resistant reaction indicating its differential response against leaf rust pathotypes and could be a new source of resistance to leaf rust pathotypes.

The leaf rust resistance gene in Selection212 was located cytogenetically on chromosome 2B and named as *LrSel212* (Sharma and Singh, 2001), out of 16 *Pt* pathotypes in present study 10 pathotypes were not tested earlier and found avirulent against resistance genes like *Lr 4*, *5*, *6*, *7*, *8*, *24*, *25*, *32*, *39*, *41*, *42*, *45*, *47* (Prasad *et al.*, 2017) with only *Lr25* (McIntosh,1988) and *Lr45* (McIntosh *et al.*, 1995) is derived from *Secale cereale*. But none of the resistance genes identified from avirulence pattern is located on chromosome 2B while leaf rust resistance gene in Selection212 was reported earlier to be located on 2B chromosome cytologically (Table 2).

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earlier and found avirulent against resistance genes like *Lr 4, 5, 6, 7, 8, 24, 25, 32, 39, 41, 42, 45, 47* (Prasad *et al.*, 2017) with only *Lr25* (McIntosh, 1988) and *Lr45* (McIntosh *et al.*, 1995) is derived from *Secale cereale*. But none of the resistance genes identified from avirulence pattern is located on chromosome 2B while leaf rust resistance gene in Selection212 was reported earlier to be located on 2B chromosome cytologically.

The number of leaf rust resistance genes already designated on chromosome 2B are *Lr13, Lr16, Lr23, Lr35* and *Lr73* (Dyck *et al.*, 1966; Dyck and Samborski, 1968; McIntosh and Dyck, 1975; Kerber and Dyck, 1990; Park *et al.*, 2014). Avirulence/virulence pattern (Prasad *et al.*, 2017) for these resistance genes with 16 pathotypes used in study suggested that among the leaf rust resistance genes *Lr13* and *Lr16* have shown susceptibility to total 15 pathotypes used in study while Selection212 has showed resistance to these pathotypes in the present multipathotype testing. Hence presence of *Lr13* and *Lr16* in Selection212 can be ruled out.

Similarly, *Lr23* has made ineffective by 6 *Pt* pathotypes (77-5, 77-6, 77-7, 77-10, 104-1 and 104-4) to which Selection212 has shown resistant response thereby ruling out the presence of *Lr23* in Selection212. The leaf rust resistance genes *Lr13, Lr16, Lr23* were found to be susceptible against pathotype 77-5 whereas Selection212 was resistant (Sharma and Singh, 2000). The resistance genes *Lr35* and *Lr73* are adult plant resistance (APR) genes and effective only at adult stage of the plant growth and seedlings of plants with these genes shows susceptibility but Selection212 has seedling resistance (which is effective throughout the plant life) to all the pathotypes tested with earlier and in present study again ruling out the presence of these genes in Selection212. The above-mentioned logics of absence of *Lr13, Lr16, Lr23, Lr35* and *Lr73* in Selection212 suggest a strong

case for identification and mapping of a new leaf rust resistance gene named *LrSel212*. Pathotypes 77-5 apart from being most prevalent is also the most virulent that makes ineffective most of the *T. aestivum* originated seedling resistance genes for leaf rust like *Lr 1, 2a, 2b, 2c, 3, 10, 11, 13, 14b, 15, 16, 17, 20, 27+31, 30, 33, 49* (Prasad *et al.*, 2017) indicating that the leaf rust resistance in Selection212 effective against pathotype 77-5 is different from the rust resistance genes susceptible to this pathotype.

The spectrum of infection types produced by Selection212 neither compares with any of the known resistance gene(s) present in differential lines used in the Indian differential sets nor can be explained by combination of more than one *T. aestivum* derived genes based on avirulence/virulence analysis of 16 pathotypes used in the study indicating that the leaf rust resistance in Selection212 could be a new source of resistance.

Leaf rust pathotypes 77-5 and 77-6 made ineffective important resistance genes like *Lr13, Lr16, Lr23* and *Lr35*. However Selection212 has been tested with pathotype 77-5 and 77-6 in current study exhibits resistance reaction against these pathotype suggesting its effectiveness to combat with new virulent pathotypes of leaf rust.

Therefore, to cope with dynamic nature of rust pathogen we need to broaden the genetic base of present cultivar by investigating new source of resistance. As Selection212 showing broad spectrum resistance to total 35 pathotypes of leaf rust including 10 new pathotypes in present studies and 25 pathotypes in previous study (Sharma and Singh, 2000), therefore Selection212 could be effective source of broad spectrum resistance to leaf rust pathotypes. At the outset resistance in Selection212 was considered to be different from earlier report in case of leaf rust resistance.

Table.1 Infection types on Selection212, Agra Local and Kharchia Local against 16 pathotypes of leaf rust (*P. triticina*) at seedling stage of plant growth at mean temperature range 20-280 C
 0 (Immune); (Very resistant), 1(Resistant), 2(Moderately resistant), X (Mesosthetic), 3 (Moderately susceptible), ‘=’:Uredia much smaller than typical and at the low limit for the infection type, ‘-’: Uredia smaller than normal, ‘N’ indicates more than usual degree of necrosis

S. No.	Pathotypes	Selection212	Agra Local	Kharchia Local
1	12-3	X ⁻	33 ⁺	3 ⁺
2	12-4	;1 ⁻	3 ⁺	33 ⁺
3	77	X ⁻	3 ⁺	3 ⁺
4	77A	;	3	33 ⁺
5	77A-1	;1	33 ⁺	3 ⁺
6	77-3	;1	3 ⁺	3 ⁺
7	77-5	;1 ⁺	3 ⁺	3 ⁺
8	77-6	X ⁻	3 ⁺	3 ⁺
9	77-7	X ⁻	X	33 ⁺
10	77-8	;1 ⁻	3	3 ⁺
11	77-10	;1	33 ⁺	33 ⁺
12	104-1	;1 ^N	3 ⁺	3 ⁺
13	104-4	;1	3 ⁺	33 ⁺
14	106	0;	3 ⁺	3
15	107-1	;1 ⁻	3	3
16	108	;1 ⁻	3	3

Table.2 Avirulence pattern of different resistance genes with their location, source and reaction types

Lr gene	Location	Source	IT	References
Lr4-8	abandoned			
Lr 24	3DL	<i>Th.ponticum</i>	0; to 1	McIntosh <i>et al.</i> , 1976
Lr 25	4BS	<i>Secale cereale</i>	0;, 1, 12	McIntosh, 1988
Lr 32	3D	<i>T. tauschii</i>	1 ⁺ 2 ⁻	Kerber, 1987
Lr 39	2DS	<i>T. tauschii</i>	12 ⁻	Raupp <i>et al.</i> , 2001
Lr 41	1D	<i>T. tauschii</i>	;	Cox <i>et al.</i> , 1994
Lr 42	1D	<i>T. tauschii</i>	; to ;1	Cox <i>et al.</i> , 1994
Lr 45	2AS	<i>Secale cereale</i>	;12	McIntosh <i>et al.</i> , 1995
Lr 47	7AS	<i>Ae. speltoides</i>	;	Dubcovsky <i>et al.</i> , 1998
LrSel212	2B	<i>Secale cereale</i>	X ⁻	Sharma and Singh, 2001

Fig.1

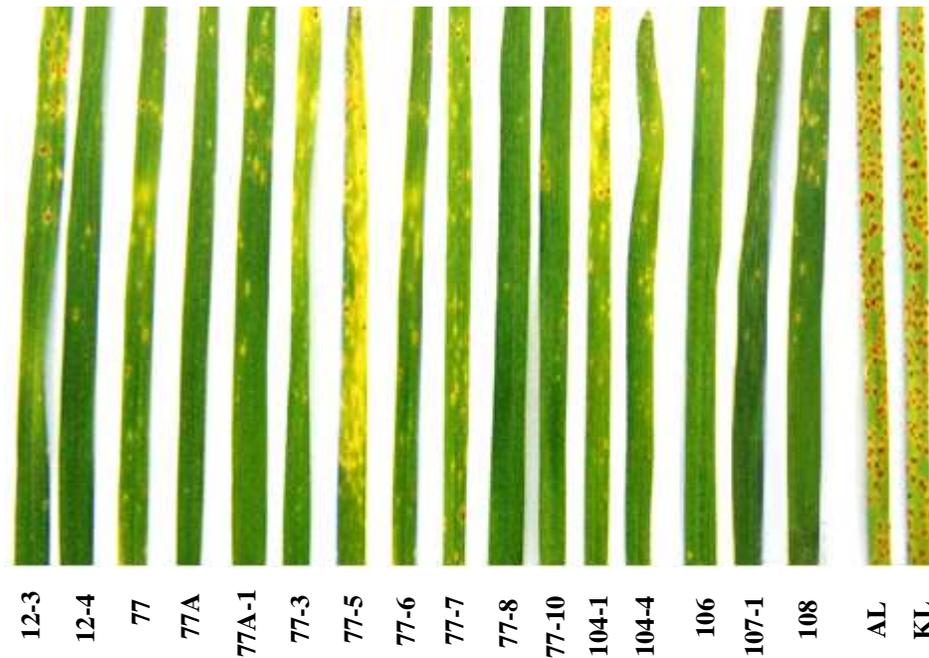


Figure 1 Infection types on Sel.212, Agra Local at seedling stage against leaf rust pathotypes of different groups

Fig.2

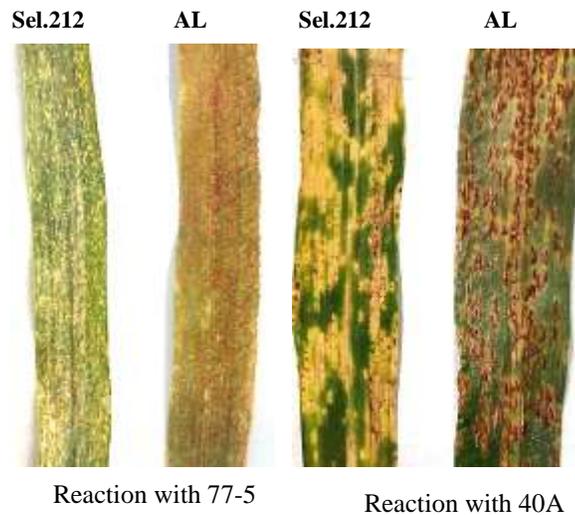


Figure 2 Adult plant response of parental lines, Selection212 and Agra Local against leaf and stem rust pathotypes 77-5 and 40A respectively in field condition

The previous study suggests that Selection212 which is derivative of wheat rye recombinant (Singh, 1991), so far only resistance genes

like *Lr25,26* and *Lr45* for leaf rust have been reported from rye (McIntosh, 1995; 2017). Hence, to exploit the genetic potential of rye

(*Secale cereale*) for rust resistance need more investigation by genetic and molecular studies to identify genetic nature of rust resistance and molecular markers linked to gene of interest. Therefore, Selection212 seems to be a source of broad spectrum resistance for leaf rust and can be used for further investigation by molecular studies, so that resistance can be exploited for marker assisted wheat breeding.

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