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# CURRENT STATUS OF THE OCCURRENCE AND DISTRIBUTION OF (*PUCCINIA TRITICINA*) WHEAT LEAF RUST VIRULENCE IN PAKISTAN

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**Abstract**

Leaf rust caused by *Puccinia triticina* Eriks & Henn., is a serious fungal wheat disease of global occurrence. In order to determine its presence and virulence distribution within Pakistan, a trap nursery comprising of 39 isogenic wheat lines and 12 commercial bread wheat varieties carrying different *Lr* genes were planted and evaluated at 5 locations over 2 consecutive crop cycles; 2004-05 and 2005-06. The study objectives were to identify the naturally prevailing leaf rust virulences. Entries with leaf rust genes *Lr9, Lr19 and Lr28* were resistant at all locations. Leaf rust genes *Lr1, Lr2a, Lr2b, Lr2c, Lr3, Lr3ka, Lr3bg, Lr10, Lr11, Lr12, Lr14b, Lr15, Lr16, Lr17, Lr18, Lr20, Lr21, Lr23, Lr24, Lr25, Lr26,* Gatcher (*10, 27+31*), *Lr29, Lr30, Lr32, Lr33, Lrb* and *Lr23+* indicated presence of virulence at most of the locations. The genes *Lr13, Lr22a, Lr34* and *Lr35* possessed virulence at Karachi and Nawabshah. Partial virulence was observed on genes *Lr36* and *Lr37* at three locations. Majority of the commercial wheat varieties in Sindh showed susceptibility against leaf rust. Utilization of this data for wheat improvement coupled with national varietal and gene deployment is discussed.

## Introduction

The occurrence of rust diseases in cultivated cereals has significantly influenced the development of human civilization (Roelfs *et al.,* 1992). Wheat rusts have historically been one of the major biotic stress production constraints in Asia and globally (Singh & Rajaram, 1991). Leaf rust caused by *Puccinia triticina* Eriks & Henn., is a serious wheat production hazard (Mclntosh *et al.,* 1995). It is the most destructive and devastating disease due to its time of appearance, nature of attack, regular occurrence and prolonged growing season that is prevalent for its development in the wheat growing areas of the world (Khan *et al.,* 1997). Leaf rust can reduce total yield by about 1.0% for each 1.0% increase in the pathogens infection capacity (Khan *et al.,* 1997). In 1973 leaf rust intensity ranged from 40-50% with 100% infection occurring on susceptible wheat varieties (Hassan *et al.,* 1973). The severe 1978 leaf rust epidemic in Pakistan resulted in an estimated national loss of US$ 86 million due to a 10% yield loss (Hussain *et al.,* 1980).

Leaf rust is a polycyclic fungal pathogen with a capability to change its virulent nature faster than the release of new wheat varieties (Chaudhry *et al.,* 1996; Khan, 1987). Thus wheat varieties cannot prolong their field resistance life (Khan, 1987). Due to airborne nature of the disease, use of chemicals is neither economical nor feasible on a large scale. Use of resistant varieties however, is the most economically practical way to control the disease. Thus consistent with global trends, resistant cultivars developed by

pyramiding effective *Lr* genes have significantly reduced losses caused by rusts in Pakistan as well (Khan, 1987).

Genetics of resistance and pathogenicity in host / pathogen relationships is the key element in development of resistant cultivars. Field surveys are equally important for monitoring the distribution of current pathotypes and virulence factors caused by *P. triticina*. Observations and monitoring at the field level helps greatly in knowledge of new virulence pathogen combinations (Welling *et al.,* 1996). Previously pathogen virulences had been reported on the basis of seedling tests. Very few attempts were made to obtain field data for evaluating virulence presence in rust populations (McIntosh *et al.,* 1995).

The objective of this study was to identify the prevalence of leaf rust virulences and study the natural effectiveness of *Lr* genes in different agroclimatic zones of Pakistan. For getting detailed information leaf rust trap nurseries consisting of 39 isogenic lines and 12 commercial bread wheat varieties carrying different *Lr* genes were planted in different agroclimatic zones/hot spot locations of leaf rust. The generated data would become the source for formulating the wheat breeding course nationally.

## Materials and Methods

A trap nursery (consisting of 51 lines) specially designed for leaf rust comprising of near isogenic wheat lines and commercial bread wheat varieties was planted at 5 Pakistan locations, 3 in Punjab and 2 in Sindh. The planting was carried out over 2 years in each November (2005, 2006) and the nursery evaluated each crop cycle in 2005 and 2006. The 5 locations represented different agro-ecological zones and "hot spots" where conditions are most favorable for leaf rust infection and development. Each nursery line was planted in an unreplicated single meter row length where rows were 30 cm apart. Two rows of the most rust susceptible spreader (Morocco) were planted around the nursery as borders. In addition, one row of this susceptible check (Morocco) was also planted at every 15$^{th}$ test entry. Observations were recorded on natural occurrence and the first appearance of leaf rust infection on the susceptible check Morocco. The leaf rust data was recorded according to the modified Cobb's scale as described by Peterson *et al.,* 1948 .

## Results and Discussion

From the leaf rust severity and infection type data (Table 1) we conclude that leaf rust resistance genes *Lr2c, Lr3, Lr3bg, Lr10, Lr12, Lr14a, Lr14b, Lr16, Lr18, Lr20, Lr23, Lr24, Lr25, Lr26, Lr10, 27+31, Lr29, Lr30, Lr32, Lr33* and *Lrb* gave susceptible reactions demonstrating that *Lr* genes were virulent under natural field conditions at all 5 test locations. The lines containing genes *Lr1, Lr2a, Lr2b, Lr2c, L3ka, Lr12, Lr15, Lr17* and *Lr21* showed virulence at 4 locations except Sialkot. Virulence for *Lr13, Lr22a, Lr34* and *Lr35* was detected at Karachi and Nawabshah, while *Lr23+* was ineffective at Karachi, Nawabshah and Bahawalpur. No virulence was observed on *Lr9, Lr19, Lr28, Lr36* and *Lr37* genes. The commercial varieties Inqilab91, Bhakkar-2002, Bakhtawar-92, Tatara, Fakhr-e-Sarhad, TJ-83, Sarsabz, Marvi-2000 and AS-2002 were observed to be susceptible at Karachi, Nawabshah and Bahawalpur whereas Auqab-2002 and GA-2002 showed susceptibility at 2 locations i.e., Karachi and Nawabshah (Table 2).

**Table 1. Field response of known isogenic lines and commercial varieties during 2004-05 and 2005-06 crop cycles.**

| S. No. | Genes | Varieties | Karachi | | Nawabshah | | Bahawalpur | | Faisalabad | | Sialkot | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2004-05 | 2005-06 | 2004-05 | 2005-06 | 2004-05 | 2005-06 | 2004-05 | 2005-06 | 2004-05 | 2005-06 |
| 1. | Lr22b | THATCHER | 40S | 50S | 20MSS | 30S | 20MSS | 10MSS | 20MSS | 20S | 10S | 20S |
| 2. | Lr1 | RL6003 | 40S | 60S | 5S | 30S | 60MSS | 60S | 70MSS | 5S | 30MRMS | 20MRMS |
| 3. | Lr2a | RL6016 | 30MSS | 70S | 5S | 30S | 70MSS | 40S | 5S | 5S | 5MRMS | 20RMR |
| 4. | Lr2b | RL6019 | 40S | 70S | 10MSS | 30S | 60S | 60S | 5S | 5S | 30MRMS | 10MSS |
| 5. | Lr2c | RL6047 | 40S | 80S | 10S | 30S | 20S | 70S | 10S | 20S | 50MSS | 30S |
| 6. | Lr3 | RL6002 | 40S | 80S | 10S | 50S | 80S | 70S | 50S | 50S | 70S | 50S |
| 7. | Lr3ka | RL6007 | 30S | 50S | 50S | 70S | 90S | 60S | 5S | 5S | TMR | 40R |
| 8. | Lr3bg | RL6042 | 40S | 50S | 5S | 20S | 80S | 70S | 5S | 5S | 40MSS | 30MSS |
| 9. | Lr9 | RL6010 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10. | Lr10 | RL6004 | 40MSS | 40MSS | 50S | 70S | 70S | 60S | 80S | 80S | 70S | 60S |
| 11. | Lr11 | W976 | 40S | 50S | 60S | 70S | 80MSS | 80S | 60S | 70S | 60S | 80S |
| 12. | Lr12 | RL6011 | 40S | 50S | 40S | 70S | 90S | 80S | 40S | 70S | 70S | 70S |
| 13. | Lr13 | MANITOU | 20MSS | 50MSS | 60S | 50S | 70MRMS | 40MRMS | TR | 60R | 5MRMS | 60R |
| 14. | Lr14a | RL6013 | 30S | 50S | 70S | 70S | 60S | 70S | 50S | 50S | 30S | 20S |
| 15. | Lr14b | RL6006 | 20S | 50S | 60S | 70S | 40S | 10S | 30S | 30MSS | 20MSS | 20MSS |
| 16. | Lr15 | RL6052 | 40MSS | 50S | 60S | 60S | 20MSS | 10MSS | 40S | 40S | 10MRMS | 60R |
| 17. | Lr16 | RL6005 | 40MSS | 50S | 60S | 60S | 60S | 60S | 60S | 80S | 30MSS | 40MSS |
| 18. | Lr17 | RL6008 | 40S | 50S | 10S | 30S | 80S | 70S | 70S | 30S | 5MRMS | 20MRMS |
| 19. | Lr18 | RL6009 | 30S | 60S | 5S | 30S | 60S | 60S | 70S | 70S | 20MSS | 20MSS |
| 20. | Lr19 | RL6040 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 21. | Lr20 | Thew | 70S | 80S | 20S | 20S | 70S | 80S | 60S | 30S | 40MSS | 30MSS |
| 22. | Lr21 | RL6043 | 20MSS | 30S | 20S | 20S | 70S | 60MSS | 20S | 60MRMS | 10RMR | 20RMR |
| 23. | Lr22a | RL6044 | 20MSS | 10MS | 30S | 50S | 50MRMS | 30RMR | 40MRMS | 40MRMS | 0 | 0 |
| 24. | Lr23 | RL6012 | 30S | 20S | 40S | 50S | 70S | 80S | 10S | 50S | 20MSS | 30MSS |
| 25. | Lr24 | RL6064 | 40S | 50S | 40S | 50S | 50S | 70S | 50S | 50S | 10MSS | 10MSS |
| 26. | Lr25 | TRANSEC | 60S | 60S | 5S | 50S | 50S | 80S | 60S | 60S | 20S | 20S |

**Table 1. (Cont'd.).**

| S. No. | Genes | Varieties | Karachi | | Nawabshah | | Bahawalpur | | Faisalabad | | Sialkot | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2004-05 | 2005-06 | 2004-05 | 2005-06 | 2004-05 | 2005-06 | 2004-05 | 2005-06 | 2004-05 | 2005-06 |
| 27. | Lr26 | RL6078 | 40S | 60S | 10S | 20S | 70S | 80S | 30MSS | 0 | 10MSS | 10MSS |
| 28. | Lr10, 27+31 | GATCHER | 40S | 50S | 5S | 30S | 80S | 90S | 20S | 30S | 30S | 20S |
| 29. | Lr28 | CS2D-2M | TR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30. | Lr29 | RL6080 | 30S | 40S | 60S | 60S | 50S | 50S | 50S | 50S | 20MSS | 5MSS |
| 31. | Lr30 | RL6049 | 60S | 40S | 60S | 70S | 60S | 70S | 30S | 30S | 10MSS | 5MSS |
| 32. | Lr32 | RL5497 | 60S | 60S | 5S | 70S | 50S | 50S | 60S | 60S | 10MSS | 10MSS |
| 33. | Lr33 | RL6057 | 70S | 30S | 10S | 20S | 80S | 80S | 20S | 0 | 10S | 20S |
| 34. | Lr34 | RL6058 | 20MSS | 20S | 5MSS | 10S | 60MRMS | 40MRMS | 0 | 0 | 0 | 0 |
| 35. | Lr35 | RL5711 | TMS | TMS | 0 | 10MS | TMR | 0 | 0 | 0 | 0 | 0 |
| 36. | Lr36 | E84018 | 5MRMS | 10MRMS | 5MR | 10MRMS | 10MRMS | 5MRMS | 0 | 0 | 0 | 0 |
| 37. | Lr37 | RL6081 | 10MRMS | 20MRMS | 0 | 30MRMS | 20MRMS | 30MRMS | 0 | 0 | 0 | 0 |
| 38. | LrB | RL6051 | 60S | 60S | 30S | 40S | 40S | 80S | 10S | 50S | 10S | 10S |
| 39. | Lr23+ | GAZA | 40MSS | 90S | 80S | 50S | 70S | 90S | 10MRMS | 0 | 10MR | 10R |
| 40. | Inqilab | | 20MSS | 20S | 0 | 30S | 5MRMS | 5MSS | 0 | 0 | 0 | 0 |
| 41. | BhakKar | | 40MSS | 60S | TMS | 10MS | 60MRMS | 30MSS | 0 | 0 | 0 | 0 |
| 42. | Bakhtawar | | 40MSS | 10S | 0 | 30S | 30MRMS | 5S | 0 | 0 | 0 | 0 |
| 43. | Tatara | | 20MSS | 20MSS | TMR | 20S | 5MRMS | 30S | 0 | 0 | 0 | 0 |
| 44. | F/S * | | 30MSS | 80S | 0 | 10MS | 20MRMS | 50S | 0 | 0 | 0 | 0 |
| 45. | TJ 83 | | 30MSS | 60S | TMS | 30S | 50MRMS | 20S | 0 | 0 | 0 | 0 |
| 46. | Sarsabz | | 40MSS | 50S | 0 | 30S | 50MRMS | 20MSS | 0 | 0 | 0 | 0 |
| 47. | Marvi | | 5MSS | 60S | 0 | 10S | 50R | 50S | 0 | 0 | 0 | 0 |
| 48. | Auqab | | TMS | 40MSS | 0 | TR | 0 | 5MRMS | 0 | 0 | 0 | 0 |
| 49. | GA-2002 | | 0 | 50S | 0 | 30MS | 0 | 0 | 0 | 0 | 0 | 0 |
| 50. | AS-2002 | | 10MSS | 30MSS | TMS | 50S | 0 | 5MSS | 0 | 0 | 0 | 0 |
| 51. | Morocco | | 100S | 100S | 100S | 100S | 100S | 100S | 80S | 80S | 70S | 70S |

* F/S = Fakhr – e - Sarhad

**Table 2. The leaf rust virulence spectrum of known isogenic wheat lines across the five testing Pakistan locations.**

| S. No. | Location | Isogenic lines |
|---|---|---|
| 1. | Karachi | 1, 2a, 2b, 2c, 3, 3ka, 3bg, 10, 11, 12, 13, 14a, 14b, 15, 16, 17, 18, 20, 21, 23, 24, 25, 26, 10,27+31, 29, 30, 32, 33, 34, 35, B, 23+ |
| 2. | Nawabshah | 1, 2a, 2b, 2c, 3, 3ka, 3bg, 10, 11, 12, 13, 14a, 14b, 15, 16, 17, 18, 20, 21, 23, 24, 25, 26, 10,27+31, 29, 30, 32, 33, 34, 35, B, 23+ |
| 3. | Bahawalpur | 1, 2a, 2b, 2c, 3, 3ka, 3bg, 10, 11, 12, 14a, 14b, 15, 16, 17, 18, 20, 21, 23, 24, 25, 26, 10,27+31, 29, 30, 32, 33, B, 23+\| |
| 4. | Faisalabad | 1, 2a, 2b, 2c, 3, 3ka, 3bg, 10, 11, 12, 14a, 14b, 15, 16, 17, 18, 20, 21, 23, 24, 25, 26, 10,27+31, 29, 30, 32, 33, B\| |
| 5. | Sialkot | 2c, 3, 3bg, 10, 11, 12, 14a, 14b, 16, 18, 20, 23, 24, 25, 26, 10,27+31, 29, 30, 32, 33, B\| |

The field data of isogenic lines suggests a similar pattern of rust appearance and distribution under natural conditions. Lines carrying resistance genes *Lr1, Lr2a, Lr2b, Lr2c, Lr3, L3ka, Lr3bg, Lr12, Lr16, Lr21, Lr23, Lr24, Lr25, Lr30, Lr32* and *Lr33* were susceptible at all locations indicative of the availability of the pathogens virulence naturally. During the 2003-04 crop cycle *Lr3bg* had no virulence at Sialkot, Faisalabad, Bahawalpur and Tandojam (Unpublished data). During the 2004-05 and 2005-06 cycles however, it expressed virulence at all test locations thus providing evidence that over three years virulence change has occurred.

*Lr9* is located on chromosome 6B. It was first transferred to Chinese Spring wheat from *Aegilops umbellulatum* (Sears,1956) and the translocation 47 became known as 'Transfer" (Sears, 1961). Virulence for *Lr9* was found in USA in 1971, four years after its use in soft red winter wheats (Shanner *et al*., 1972). Its virulence then was also observed in Brazil and Argentina. Huerta-Espino (1992) found virulence in isolates from Italy, Burundi and Pakistan though the overall frequency was very low. This gene is postulated in the Pakistan variety 'Marvi'; Rattu, 2006. *Lr9* has not been widely deployed despite its effectiveness (McIntosh *et al*., 1995). In Pakistan no virulence exists and thus its usage potential for wheat improvement is high.

*Lr13* was originally reported as a gene for adult plant resistance although it was always clear that the onset of resistance occurred at a relatively early growth stage (Dyck *et al*., 1966). The virulence to *Lr13* is decreasing (Khan *et al*., 2002). Its decline in the field is probably due to removal of the cultivation of susceptible wheat varieties like WL711, Yecora, Blue Silver, Pavon 76 and Punjab 81 that carry the *Lr13* gene.

*Lr19* is located in chromosome 7AL in the translocation line 7A/Ag #12 (Eizenga, 1987). Despite the excellent level of protection provided by *Lr19* and lack of virulence in Pakistan this gene has not been widely utilized because the translocation line is associated with yellow flour pigment. A mutant with lighter yellow color was subsequently produced (Knott, 1980). This was further improved upon by recombining different wheat lines that carried *Lr19, Bdv2* and had white flour color (Singh *et al*., 2001). *Lr19* is linked with *Sr25* (McIntosh *et al*., 1976). Occasional trapping of *Lr19* (Khan *et al*., 2002) indicates its presence in nature but no virulence was observed during this study duration.

Special mention of *Lr26* despite its susceptibility is essential since this figures significantly in Pakistani wheat cultivars. The virulence to *Lr26* appears every year and wheat varieties carrying *Lr26* continue to be cultivated globally since the T1BL.1RS translocation that it is associated with has exceptional agronomic/yield advantages. Veery 'S' from CIMMYT has been a major donor parent of this translocation source that includes Kavkaz (Rajaram *et al*., 1983). Due to the high frequency of these translocation wheat lines in the international cultivation sphere, *Lr26* based cultivars also dominate within our germplasm (Khan *et al.,* 2002). Isogenic lines show susceptibilty when present in single dosage, but when used in pyramided combinations they can contribute towards resistance. The cultivar 'Auqab' possesses leaf rust resistance genes *Lr10* and *Lr26* and susceptibility for this variety was picked at only two locations. Separately *Lr10* and *Lr26* show virulence at all the locations but in combination form express resistant. Thus the structuring of gene combinations should remain a continuous exercise since in a dynamic system everlasting durability around a good combination cannot be realized. *Lr26* is completely linked with *Sr31* and *Yr9* and the 1RS arm has a *Secale cereale* cv. Petkus origin.

*Lr28* is located on chromosome 4AL (McIntosh *et al*., 1982) having an *Ae. speltoides* origin. The gene is not postulated in any commercial variety of Pakistan. Though Huerta-Espino (1992) found virulence among isolates from Pakistan its significant presence has not been observed in the country over the last 10 years (Chaudhry *et al*., 1996; Rattu, 2006).

*Lr34* on chromosome 7D is completely linked with *Yr18* (Singh, 1992a) and a gene for leaf tip necrosis (Singh, 1992b). Because of *Lr34*'s wide spread resistance effectiveness under field conditions and due to its interactive effects (German & Kolmer, 1992) it has been selected in many wheat breeding programmes for its resistance contribution. Virulence for *Lr34* under our conditions reportedly appeared at the end of March and beginning of April (Chaudhry *et al*., 1995). The presence of *Lr34* has been associated with giving interesting side effects on stripe and stem rust resistances. Mclntosh (1992) found that all near isogenic lines of Thatcher with *Lr34* were significantly more resistant than Thatcher to stripe rust in the field. Thatcher lines with *Lr34* were also reported to be more resistant to stem rust than Thatcher alone (Dyck, 1987). Locational variation for resistance in lines with *Lr34* was reported by Singh & Rajaram (1991) from tests in Mexico where a higher infection level was observed at El-Batan than in Obregon.

*Lr36* located on chromosome 6BS from *Ae. speltoides* is not exploited worldwide (McIntosh, 1995) in agriculture and has potency. *Lr37* on chromosome 2AS is closely linked with *Yr17* and *Sr38* (Bariana & McIntosh, 1993) and expresses as adult plant resistance. It is considered to be highly effective in Australia under field conditions (Bariana, 1991) with extended potential here.

The virulence for leaf rust resistance genes *Lr9, Lr19, Lr28, Lr36* and *Lr37* was not observed during this study across all test locations. Hence these genes are effective in Pakistan conditions and hold priority for their incorporation in national wheat breeding programmes. Chaudhry *et al*., (1996) had earlier reported that *Lr9, Lr19* and *Lr28* gave complete resistance around a 'zero' reaction score against all prevailing leaf rust virulences based upon three years of testing. Occasional trapping of *Lr19* (Khan *et al*., 2002), does indicate its natural presence and may cause a problem if varieties are evolved or cultivated widely with this gene alone. Its virulence has been reported in India. This gene may however be used in different combinations for durable resistance. Similar is the recommendation for *Lr28* around a durability perspective where its utilization in gene combinations would be advantageous.

Most of our commercial varieties showed susceptibility at Karachi, Nawabshah and Bahawalpur. The wheat varieties Tatara and Fakhr-e-Sarhad are widely grown in Northern areas of Pakistan but the susceptibility for these varieties is available at Karachi, Nawabshah and Bahawalpur which suggests that if these varieties get planted in Sindh or southern Punjab they will show susceptiblity. Such a varietal spread should be discouraged in order to secure varietal performance around leaf rust resistance. Tatara and Bakhtawar carry *Lr26* (Rattu, 2006) and Sarsabz grown in Sindh has *Lr16*; all showing susceptibility. Susceptibility has also been observed on Inqilab 91 that carries *Lr10, Lr27 + Lr31* (Rattu, 2006) at most of the test locations. This variety probably with a genetic resource input of *Lr10, Lr27 + Lr31* (Mirza *et al*., 2000, Rattu, 2006) gives an alarming situation within Pakistan as it still occupies a substantial production area. It is important that the future directions for breeding for leaf rust resistance exploits genetic resources with genes for which either virulence is lacking, or use those possessing some

susceptibility (eg. *Lr26)* in combinations with minor genes as accessible to ensure durability of resistance. It is also crucial that gene postulation efforts of our wheat leading varieties are vitalized in order to provide better direction in targeting national breeding objectives.

## Conclusion

Virulence for *Lr9, Lr19* and *Lr28* was not observed at any of the test locations. Genes Lr36 and Lr37 expressed partial virulence and also have potency for exploitation. Varieties possessing these genes are recommended for deployment in permutation combinations as resistance sources in wheat breeding programmes to integrate their genetic diversity in national germplasm. This approach will assist in generation of future resistant cultivars around appropriate gene combinations thereby providing durable resistance outputs for wheat productivity.

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