

## RESISTANCE POTENTIAL OF PAKISTANI WHEAT LANDRACES (*TRITICUM AESTIVUM* L.) AGAINST STRIPE RUST (*PUCCINIA STRIFORMIS*) AND KARNAL BUNT (*TILLETIA INDICA*)

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### Abstract

Wheat is the staple food crop of Pakistan. Heavy losses in crop yield are caused by stripe rust and other foliar diseases. Karnal bunt (*Tilletia indica*) has also shown damage to wheat crop recently. The infested grains become unsuitable for human consumption. The current study was carried out to exploit indigenous landraces for stripe rust and Karnal bunt resistance for crop improvement. This two year study determined the field based partial resistance against the stripe rust in 204 wheat landraces based on average coefficient of infection (ACI). The average coefficient of infection showed that out of 204 landraces, 47 landraces showed resistance, 38 showed moderate whereas 119 were found to be highly susceptible against stripe rust. The same landraces were artificially inoculated at booting stage to determine their resistance against Karnal bunt. Only one landrace was found resistant against Karnal bunt, 13 moderately susceptible, 60 susceptible and 102 highly susceptible. Although the landrace found to be resistant against Karnal bunt was highly susceptible against stripe rust. Only one landrace was identified to be highly resistant against stripe rust and moderately resistant against Karnal bunt. Most of the landraces were highly susceptible against Karnal bunt. This emphasizes the need to explore more germplasm against Karnal bunt for the identification of resistant genotypes to be used in breeding programs.

**Key words:** Landraces, Wheat, Yellow rust, *Tilletia indica*, Pakistan.

### Introduction

Wheat (*Triticum aestivum* L) is one of the most commonly grown crop and is a staple food to meet the increasing demand of the ever-increasing human population. About 44% (95Mha) area of wheat sown worldwide is located in Asia. China, India and Pakistan are chief wheat producing countries in Asia (Singh *et al.*, 2004). Wheat crop is vulnerable to a number of fungal, bacterial and viral pathogens. Wheat rusts have emerged as most important fungal diseases in Asia and other wheat growing regions on globe. Stripe rust (*Puccinia striiformis*), leaf rust (*Puccinia recondite*) and stem rust (*Puccinia graminis*) are three main rusts of wheat (Singh *et al.*, 2004). These rusts are accountable for causing heavy financial losses along with deteriorating the quality of grains. The intensity of damage depends on the genetic potential of germplasm to combat rust. The damages due to susceptible varieties might go up to 80% (Eversmeyer & Browder 1974; Kolmer, 1996; Beard *et al.*, 2007). Rust pathogens have the ability to multiply and mutate rapidly and are easily dispersed through the air from one field to the other (Singh *et al.*, 2005). Because of the airborne nature of the disease and health hazards associated with pesticides in staple food, the chemical control of rust is not advisable. In developed countries, planting of resistant cultivars is the most preferred wheat management strategy (Khan, 1987; Roelf *et al.*, 1992).

Among the three rust pathogens, stripe rust is a leading risk not only for wheat in Pakistan, but also in remaining parts of the world (McIntosh, 1980; Singh *et al.*, 2004; Shah *et al.*, 2010). Wheat is grown on an area of 13.3 m ha in Pakistan, China and India, out of which 24.8 m ha is

susceptible to stripe rust (Singh *et al.*, 2004). The yield losses because of stripe rust range between 10 to 70% subjected to cultivar susceptibility, earliness of initial infection, disease development rate and its duration (Chen, 2005). The annual losses due to rust in Pakistan are estimated to Rs. 1500 million (Ahmad *et al.*, 2002). Historically, rust epidemics with intensity above 20% have been reported in 1973, 1978, 1995 and 2003 (Aqil & Hussain, 2004). During these epidemics the extensively cultivated varieties like Pirsabak-85, Pak-81 and Inquilab-91 were severely attacked by stripe rust rendering them susceptible (Ahmad, 2004). During 2004-2005, stripe rust epidemics in upper Punjab and NWFP (Khyber Pakhtunkhwa) posed serious threats due to the cultivation of susceptible varieties (Afzal *et al.*, 2010). This emphasizes that continued identification of resources resistant to stripe rust is important for sustainable production.

Karnal bunt is another important disease of wheat triggered by a smut fungus *Tilletia indica*. This disease was first reported in Karnal, a place in the Northwest of India (Munjil, 1975). It is one of the major diseases which causes quality as well as monetary losses. Karnal bunt spores are found to travel long distances through the smoke of burnt biomass. These spores are observed to travel through countries and continents and are found to be susceptible after reaching other places (Sims & Mims, 2004). The susceptibility of fungus is comparatively low in old land races of emmer wheat, but is very high in all new and improved cultivars. The most susceptible cultivars are observed to be Italian emmer wheat cultivars (Riccioni *et al.*, 2006). Emergence of Karnal bunt is a huge threat to grain industry. It does not owe direct losses in yield, but there are many quarantine rules which may hamper international transport of damaged grains (Singh

*et al.*, 2007). Export of infected seeds may lead to a proliferation of seed borne pathogens in different countries and even in different continents (Singh, 2005). Quarantine regulations on Karnal bunt resulted in extreme economic losses (Werkneh *et al.*, 2008). The quality and quantity of gluten, which is an important component of bread is observed to be deteriorated by Karnal bunt infection, which may increase the economic losses (Gopal & Sekhon., 1988). Karnal bunt has a seed borne, soil borne and airborne nature, due to which it can be diagnosed in three pathogenic forms. These pathogenic forms are teliospores, sporidial and mycelial. The pathogens occur in infectious or proliferative entities at various stages during its life cycle (Kumar *et al.*, 2008). Karnal bunt is globally quarantined disease and the pathogen of disease shows high variability due to its heterothallic nature (Aggarwal *et al.*, 2010).

Wheat germplasm resources include landraces, varieties and its wild weedy relatives (Skovmand *et al.*, 2002). Among these, wheat landrace is an important genetic resource offering resistance against biotic and abiotic stresses. The genetically diverse landraces provide a great source for resistance against disease causing pests and pathogens (Harlan, 1992), improved grain quality (Chaparzadeh *et al.*, 2008), various morphological, agronomic traits and yield stability (Strelchenko *et al.*, 2008). This genetically diverse germplasm confers resistance to stripe rust (Van Dijk *et al.*, 1988; Zhang, 1995), leaf rust (Van Ginkel & Rajaram, 1992) and stem rust (Hare, 1997; McIntosh *et al.*, 1998). One of the most widely used variety 'Inqilab-91' has become susceptible to stripe rust resulting in heavy yield losses (Qamar *et al.*, 2014). Therefore landraces are of immense significance in identifying resistant genetic resources that can be introduced in wheat breeding programs for developing disease resistant varieties (Mahmoud *et al.*, 2015). The genetically diverse landraces have a disease buffering effect that limits the wide spread of pathogens. Therefore, exploitation of landraces is highly essential for the identification of new resources of resistance. In Pakistan, numerous landraces have now totally vanished and been substituted by new wheat varieties. The assembly of landraces has directed the need to characterize the collected germplasm to distinguish valuable traits related to yield and resistance to biotic and abiotic threats. Therefore, the present study was conducted to identify Pakistan wheat landraces with potential resistance against stripe rust.

## Material and methods

**Screening wheat landraces against stripe rust:** A panel of 204 landraces collected from different parts of Pakistan were screened for adult plant resistance to stripe rust during 2012-13 and 2013-14 at National Agriculture Research Center, Islamabad. For screening, each entry was planted in five meter rows with a 30cm space among each other. Morocco; a universal susceptible to rust diseases was used as a control. In order to improve the inoculum pressure, a row of morocco was grown after every 20<sup>th</sup> entry within the landraces to be screened. Artificial inoculations, with the mixture of field collections complemented with an inoculum of known

virulence were carried out for stripe rust in NARC Islamabad during both seasons. Observations on plant's response of stripe rust were recorded according to Loegering, (1959). Rust intensity was noted according to modified Cobb's Scale as a percentage of rust infection on landraces (Peterson *et al.*, 1948). The response of the variety denotes the kind of infection and was recorded as shown in Table 1. In order to calculate the coefficient of infection (CI), the response value was multiplied with the intensity of infection in percent. Average coefficient of infection was derived from the sum of the CI value of each genotype divided by number of years.

**Testing wheat landraces against Karnal Bunt:** The same set of landraces was screened for Karnal Bunt resistance during the crop cycle 2013-2014. To prepare the inoculum, diseased kernels carrying Karnal bunt spores were shaken for a few seconds in water/tween-20 solution, centrifuged at a speed of 3000 rpm and filtered through a mesh to eliminate the seed residues. The filtered material was surface sterilized by using 0.5% sodium hypochlorite and centrifuged for two minutes. Teliospores were washed with sterile distilled water, plated on 1.5% water agar and kept at room temperature. The germinating teliospores after 5 to 8 days were shifted to potato-dextrose agar. After nine days fungal colonies were scratched the medium and shifted to another potato dextrose agar plate. The colonies were cut into small cubes and positioned on the lid of autoclaved glass petri plate. Double distilled water was added to the bottom of each plate and spore concentration was measured after every 24 hours. This sporidia suspension was then used for boot inoculation. The inoculated plants of each genotype were tagged. The inoculated heads of each genotype were harvested at maturity. These were then hand threshed and total number of healthy grains and infested grains were counted for estimating the percentage of infested grains. The susceptible and resistant genotypes were evaluated using the infection ranking scale of Aujla *et al.*, (1989) as shown in Table 2.

**Table 1. Field response of landraces against stripe rust.**

Reaction value	Observation	Response
No Disease	0	0
Resistant	R	0.2
Res. – Mod. Resistant	RMR	0.3
Moderately Res. – Mod. Sus	MRMS	0.4
Moderately Susceptible	MS	0.6
Mod. Sus – Susceptible	MSS	0.8
Susceptible	S	0.9

**Table 2. Rating scale used to determine the level of resistance/susceptibility against Karnal bunt.**

Disease rating scale	% Grain infection	Resistance/Susceptibility category
0	No infection	Highly resistant
1	1% or less bunted grains	Resistant
3	1.1-2% of bunted grains	Moderately resistant
5	2.1-5% of bunted grains	Moderately susceptible
7	5.1-10% of bunted grains	Susceptible
9	More than 10% of bunted grains	Highly susceptible

## Results and Discussion

Wheat landraces usually represent collections from diverse geographical areas, which act as a great source of new rust resistant genes for developing novel and genetically diverse disease resistant germplasm (Sthapit *et al.*, 2014). Evaluation of partial resistance in the field is very important in developing countries as they provide the advantage of screening hundreds of genotypes together. Different measures are used for assessing partial resistance, which confers durable and longlasting resistance. This can be coefficient of infection (CI) (Pathan & Park, 2006), Relative Resistance Index (RRI) (Afzal *et al.*, 2009), infection rate (IR) (Broers *et al.*, 1996), final rust severity (FRS) (Parlevliet, 1985), area under rust progressive curve (ARUPC) (Wilcoxson *et al.*, 1975). Partial resistance was determined through coefficient of infection (CI) and average coefficient of infection (ACI). It is a commonly used parameter by most of the researchers for the assessment of stripe rust (Shah *et al.*, 2003). On the basis of ACI values of 0-20, 21-40, 41-60, landraces were divided into three groups possessing better, moderate and low level of resistance. It was observed that 47 landraces exhibited a better level of resistance against stripe rust (ACI 0-20) whereas 38 landraces showed a moderate level of resistance (ACI 21-40) (Table 3). Landraces exhibiting a better level and moderate level of resistance belong to different areas of Pakistan. Landraces were from Swat (accession no. 18837, 18799, 12045, 18830, 18835, 18667, 18833, 12249, 18836, 18932, 12258), Chitral (accession no. 18896, 18858, 18873, 18894, 11810, 18850), Mansehra

(accession no. 18788, 12021, 18793, 12238, 12240), Gilgit (11599, 11597, 11807), Chakwal (18693, 18671), Shrkru (11766, 11799), Dir (12058, 12266, 18843), Abbottabad (12108, 12233, 12103), Chagi (11303, 11338, 11341), Buner (12244), Malakand (12104), Muzaffarabad (11435), Islamabad (12076), DI Khan (18913) and Pishin (11274) were having a better level of resistance (Table 4). Whereas landraces from Chitral (18849, 18888, 18861, 18863), Swat (18812, 18838, 11793, 18811), Muzaffarabad (11423, 11424, 11425, 11435), DI Khan (11393), Chilas (11796), Ziarat (11332, 12144), Nowsehra (12074), Sibi (11236), Chakwal (18679), Lakimarwat (18926), Dir (18842, 18846), Islamabad (12077), Mastung (11783, 12123), Malakand (11787), Mansehra (12034, 12031), Pishin (11200), Gilgit (11571), Abbottabad (12235, 18776), Chagai (11342, 11312), Tank (18925), Awaran (11521) were found to be moderately resistant (Table 5). Most of these landraces belong to khyber Pakhtunkhwa province of Pakistan. The remaining 119 landraces were found to be susceptible. In wheat landraces stripe rust resistance was investigated by Loladze, (2006) and observed that 45% (74 accessions of the total germplasm) evaluated were found to be resistant at adult stages. Bux *et al.*, (2012) investigated the disease response of 115 Pakistani wheat landraces to yellow rust at seedling and adult plant stage. Afzal *et al.*, (2009) studied the resistance in 188 wheat breeding lines against stripe rust. He observed 150 genotypes responded with  $RRI \geq 7 \leq 9$  and were in the desirable range. While 28 cultivars with an RRI value of  $>5 < 7$  were placed in an acceptable range whereas 10 cultivars with an RRI value of  $<5$  were placed in the undesirable range.

**Table 3. Landraces possessing better and moderate level of resistance against stripe rust.**

ACI	Landraces
0-20 (Better Resistance)	18896, 18858, 11599, 18788, 12244, 18837, 12108, 18799, 11303, 18873, 11800, 12045, 18830, 18894, 18835, 11597, 8667, 11338, 12021, 18833, 18693, 11766, 12233, 11274, 18671, 11341, 12066, 12258, 12104, 12249, 12058, 11810, 18793, 12240, 11435, 18836, 12103, 18850, 11807, 12266, 11799, 12076, 18843, 18932, 12238, 18913
21-40 (Moderate Resistance)	18849, 11393, 11423, 11796, 11797, 11332, 12074, 11236, 18679, 18926, 11200, 18812, 11424, 12144, 18842, 12077, 11783, 11787, 12034, 11571, 18838, 11793, 11425, 12235, 18888, 11342, 11312, 18925, 18776, 18861, 12031, 18811, 11521, 12123, 18846, 18863

**Table 4. Location of landraces possessing better level of resistance against stripe rust.**

ACI	Location	Landraces
0-20 (Better Resistance)	Swat	18837, 18799, 12045, 18830, 18835, 18667, 18833, 12249, 18836, 18932, 12258
	Chatral	18896, 18858, 18873, 18894, 11810, 18850
	Mansehra	18788, 12021, 18793, 12238, 12240
	Gligit	11599, 11597, 11807
	Chakwal	18693, 18671
	Skardu	11766, 11799
	Dir	12058, 12266, 18843
	Abbottabad	12108, 12233, 12103
	Chagi	11303, 11338, 11341
	Buner	12244
	Malakand	12104
	Muzaffarabad	11435
	Islamabad	12076
	DI Khan	18913
	Pishin	11274

**Table 5. Location of landraces possessing moderate level of resistance against stripe rust.**

ACI	Location	Landraces
21-40 Moderate Resistance	Chitral	18849, 18888, 18861, 18863
	Swat	18812, 18838, 11793, 18811
	Muzaffarabad	11423, 11424, 11425, 11435
	DI khan	11393
	Chilas	11796
	Ziarat	11332, 12144
	Nowsehra	12074
	Sibi	11236
	Chakwal	18679
	Lakimarwat	18926
	Dir	18842, 18846
	Islamabad	12077
	Mastung	11783, 12123
	Malakand	11787
	Mansehra	12034, 12031
	Pishin	11200
	Gilgit	11571
	Abbottabad	12235, 18776
	Chagai	11342, 11312
	Tank	18925
	Awaran	11521

**Table 6. Resistance and susceptibility of wheat landraces collected from different regions of Pakistan against Karnal Bunt.**

Susceptibility/Resistance category	Accession	Location
Resistant (Less than 1% infection)	18844	Dir
	12114	Kharan
	11601	Gilgit
	18836	Swat
	18932	Lakki Marwat
	11185	Kharan
	11797	Chilas
Moderately Susceptible (2.1–5%) bunted grains	11799	Skardu
	18896	Chitral
	12069	Mardan
	11560	Qila Saifullah
	11425	Muzaffarabad
	18894	Chitral
11313	Chagai	

Wheat yield and production is limited by several biotic and abiotic stresses. Seed borne diseases like Karnal bunt affect wheat quality and yield. Wheat with more than 3% bunted seeds is not considered good for human consumption (Mehdi *et al.*, 1973). Most of the cultivars sown in Pakistan are high yielding but because of unidirectional choice and standard, the genetic base is narrowed down and it is quite dangerous to cultivate these varieties over a large area. This has resulted in onset of new rust pathotypes making these cultivars susceptible (Yasmeen *et al.*, 2013). Therefore, it is extremely important to recognize resistant genetic resources from landraces to be used in wheat breeding programs to develop improved varieties with a broader genetic base (Mahmoud *et al.*, 2015).

The screening of these landraces against Karnal bunt indicated that none of them was highly resistant; only one landrace was resistant with less than 1% of bunted grains. 13 genotypes were moderately susceptible, 60

were susceptible and 102 were highly susceptible. The landrace (04295) with less than 1% of bunted grains was found to be susceptible to stripe rust. Four landraces (18896, 18894, 11799, 18932) that were resistant to stripe rust were moderately sensitive to Karnal bunt. One landrace (11836) was found to be stripe rust resistant and moderately resistant to Karnal bunt. Two landraces (11797, 11425) were moderately resistant to stripe rust and moderately susceptible to Karnal bunt (Table 6). All the remaining landraces with better and moderate level of resistance against stripe rust were susceptible to Karnal bunt. This shows that in comparison with stripe rust, these landraces are more susceptible to Karnal bunt. There is an alarming situation in Punjab, regarding Karnal bunt in the past two to three decades. Resistance to Karnal bunt is absent in the available commercial cultivars (Anonymous, 2005). Arif *et al.*, (2013) screened advanced wheat lines and currently used commercial cultivars against Karnal bunt and observed that all of the commercial wheat cultivars were highly susceptible. Various commercial cultivars like Inqilab-91, Uqab-2000, BK-2002, Manthar-03, Lasani, Sahar-06, Fareed-06 and Faisalabad-85 were found susceptible by Ullah *et al.*, (2012).

The study concludes that of the 204 wheat landraces tested, 47 landraces showed better resistance, 38 were moderate and 119 were highly susceptible against stripe rust. Only one landrace was found resistant against Karnal bunt, 13 were moderately susceptible, 60 were susceptible and 102 were highly susceptible. The landrace found to be resistant against karnal bunt was highly susceptible against stripe rust. Only one landrace was identified which was resistant against stripe rust and moderately resistant against Karnal bunt. Most of the landraces were highly susceptible against Karnal bunt. These findings demonstrate narrow resistance potential of these landraces against Karnal Bunt whereas stripe rust resistance is promising. New genetic resources need to be explored for Karnal bunt resistance for crop improvement.

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