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REVIEW ARTICLE

Leaf Rust: A Serious Disease of Wheat in Pakistan

Iram Naurin*, Ramsha Qaisar, Saood Jahan, Saqib Ali, and Aiman

ABSTRACT

Wheat is one of the major crops and also staple food of many areas. It fulfils the basic nutrition requirements of people. Demand of food in directly proportional to increasing number of populations. But most of the time yield is decreased due to attack of diseases. Wheat leaf rust is a common disease of wheat and cause 50% losses in yield. Leaf rust is cause by fungi named *Puccinia triticina.* Pathogen mostly attack on leaf blade and cause damage. Pathogen spores travel through wind and can spread on wide area. Many molecular studies have been for identification of resistant gene in cultivated and wild species to boost up the immunity of cultivated species against disease. For the purpose of genetic dissection scientist used molecular techniques like MAS and gene pyramiding, they used markers for identification of resistant genes. Scientists got success in their findings and identified many resistant gene in wild and cultivated varieties of wheat. Use of these genes will be helpful in breeding programs for the development of resistant varieties and to make increase in production.

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INTRODUCTION

Wheat is among top five cereal crops around the world (Klarquist et al., 2016). Pakistan is among top producers of wheat in world as well as wheat is staple food crop of Pakistan (FAOSTAT, 2016). In the whole world more than 90% of wheat production comes from bread wheat (*Triticum aestivum*) and it is utilized to manufacturing of bakery products. The rest, approximately less than 10% of wheat production comes from *Triticum durum* also called durum wheat and it is used to manufacture pasta and macaroni (Pena, 2002).

Among the biotic stresses, diseases are the major cause of yield losses. Around 81 bacterial, 40 fungus and 32 viral diseases are known to occur on wheat (Bonjean and Angus, 2001). Rust is major and economically important disease in case of fungal diseases. Pathogens that cause wheat rust were reported in dated back 1300BC in Italy and considered as oldest pathogen. More than 2000 years ago it considered as a serious disease in Greece and Italy (Kislev, 1982; Roelfs et al., 1992). In the start of 20th century rust spread in form of epidemics and then it initiates the study of life cycle of pathogens, genetics of disease resistance and genetics of parasite-plant interaction (Mclntosh, 1995; Carver, 2009; Bolton et al., 2008). These fungi mostly attack on stems and leaves of specific verities and genera (Agrios, 2005).

Low temperature, rain fall and humidity are suitable factors for the occurrence of stripe rust (Khan et al., 1998; Salman et al., 2006). Stripe rust arranged yellow pustules in a line appear on leaf (Hussain et al., 1996). Heavy losses in wheat yield were reported in the past due to attack of stripe rust, 50% loss in yield were reported in susceptible varieties. It may cause annual loss of prouction around 2.2 million tons (Yaqoob, 1991; Hafiz, 1986).

Black rust is caused by *Puccinia graminis*. Shiny dark black spores are formed on leaves at near of stems. Wet and humid warm season is most suitable for disease development and spores spreading. If the crop is susceptible then stem or black rust can cause 100% losses in terms of broken and damaged stems and leaf as well as shriveled wheat grains (Waqar et al., 2018).

Wheat leaf rust is still a most important threats that cause significant losses in wheat as well as lower the grains quality (Carver, 2009; Agrios, 2005; Carena, 2009; Singh et al., 2011). Leaf rust cause 50% losses in wheat yield (Anonymous 1992). In this paper our main focus on leaf rust, although there are many papers available about wheat leaf rust, in this review we will give the latest updates in all aspects of disease, its life cycle, mode of action, how to diagnose and on molecular aspects of disease.

General overview of Brown Rust or Leaf Rust

Most common type of rust is brown rust. It is widespread around the world (Roelfs et al., 1992; Carver, 2009). Brown rust mostly attacks on leaf blades and if favorable conditions are available then it can attack also on glumes and leaf sheaths (Huerta-Espino et al., 2011; Roelfs et al., 1992). Brown rust is more common in world than other two types (Bolton et al., 2008; Roelfs et al., 1992; Carver, 2009). Spreading of urediniospores depends upon the wind's direction. It can become epidemic in wheat growing regions in every year (Huerta-Espino et al., 2011; CABI, 2012). *Lr1* to *Lr79,* 79 loci showing resistance against *Puccinia triticina* have been demonstrated in wheat (Singh et al., 2013; Park et al., 2014; Herrera-Foessel et al., 2014; Qureshi et al., 2018).

Causal agent nomenclature and Taxonomic identification

Puccinia triticina caused leaf rust in wheat. It forms urediniospores only on live tissues of leaf. These spores cause infection. It completes its life cycle on primary and secondary hosts. Cultivated and wild emer wheat, *Triticum durum, Triticum aestivum*, *Ae. cylindrical*, *Ae. speltoides* and triticale are known as primary hosts. *Isopyrum fumaroides* and *Thalictrum speciosissimum* are known as secondary hosts of pathogen (Bolton et al., 2008). The urediniospores travel through wind and they can affect host up to several miles as a result it become epidemics in a country and some times more than one country (Bolton et al., 2008; Roelfs et al., 1992). A diagram has been shown for quick and easy understanding about life cycle of *Puccinia triticina*. (**Figure 1**).

Distribution of disease in Pakistan

The central and southern areas of Pakistan are most suitable for growth of pathogen and disease development (Khalil and Jan, 2003). Agarwal et al., (2003) described cold weather with warm climate are most favorable for development of disease. Now leaf rust becoming serious threat in northern areas and KPK due to change in climatic conditions. In a study the surveillance of three years showed Punjab wheat production effect more with disease than other areas (Khan et al., 2020).

Symptoms of leaf rust

Brown rust mostly attack on leaves but awns and glumes also get infected. Symptoms appear on upper surface of infected leave, from a circular shape to oval shape spot that is yellow in color. After sometimes it turns into a pustule that is orange in color and having halo surrounding of yellow color (**Figure 2**). A huge number of spores produced, when pustules dislodged an orange color dust spread on Equipment's, hands and on leaf. These can be dislodged by wind or rain contact. Later on, black color spores produced and results come in mixture of orange and black lesions. These lesions develop on head of seed and do not turn into erumpent pustules again. This is a distinguish feature from stem rust.in ten days, a single spore can produce thousands new spores in this way leaf rust

cause more damage in short time (Marsalis and Goldberg, 2016).

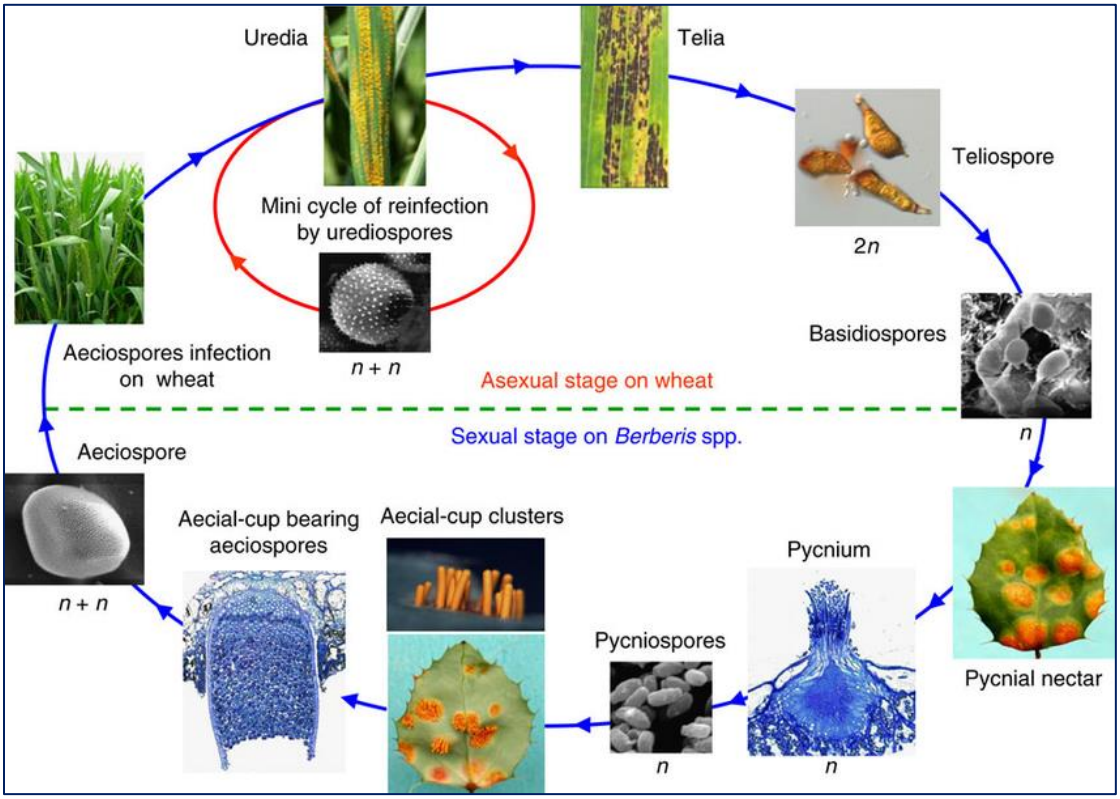

Figure 1. Life cycle of *Puccinia triticina* (Adapted by Zheng et al., 2013).

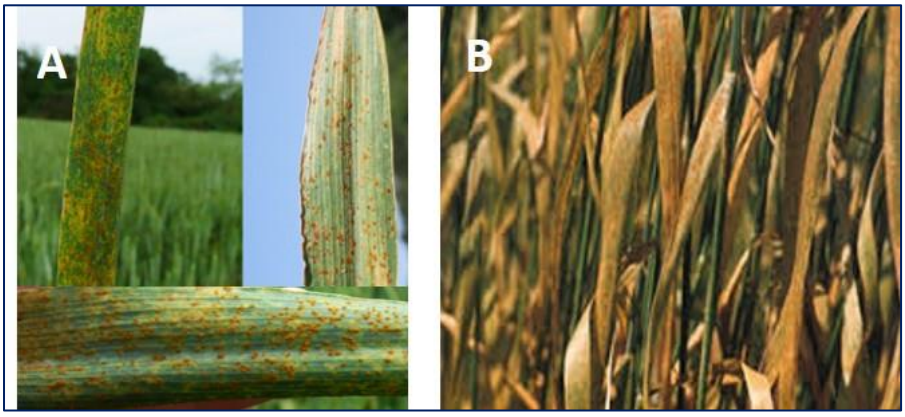

Figure 2. Leaf rust. A), Closet view of leaf rust (orange pustules with halo yellow surrounding); B), Damage field with leaf rust.

Disease cycle

Allen (1926) was the first demonstrated the development and infection process of *P. triticina* in wheat with help of cytological studies. Urediniospores spread with help of rain or wind on leaves. When moisture is available spores developed into germ tube. For the germination they need 100 percent humidity, 20°C with 4-8 hours of light (Zhang et al., 2003; Zhang and Dickinson, 2001; Hu and Rijkenberg, 1998). Thigmotropic response controlled the growth of germ tube of *P. triticina*. The germ tube

grows continually until it encountered by a stoma (Dickinson, 1969). When germ tube encountered to a stomata, elongation of the tube become stop and there it makes an appressorium. After 24 hour of inoculation appressorium formation occurs (Zhang et al., 2003). From urediniospores 2 nuclei moves inside the appressorium and mitosis occur. A septum form and isolates appressorium and germ tube. After appressorium formation stomata become close (Caldwell and Stone, 1936). The fungus get entry inside the stoma by means of a penetration peg that is originate from appressorium. A substomatal vesicle forms inside the stoma and another mitosis cycle occur (Wynn and Staples, 1981). From this vesicle an infected hypha developed directed to the mesophyll cells (Allen, 1926). Here, haustorial mother cell delimited by septum. In between 12-24 hours haustorial mother cell forms, after appressorium penetration.it has 3 nuclei (Hu and Rijkenberg, 1998). It adheres cell wall tightly (Allen, 1926). Haustorium formed inside the host cell when peg penetration formation occurs in the area of host and haustorial mother cell connection. The haustorium is feeding source of fungus. The extrahaustorial membrane separated the fungus from host cell and uptake of nutrients occur through extrahaustorial membrane. The extrahaustorial membrane also act as envelop for haustorium (Szabo and Bushnell, 2001; Panstruga, 2003; Mendgen et al., 2000; Bushnell and Rowell, 1981). From three nuclei two nuclei disintegrated and haustorium has only one nucleus (Allen, 1926). More hyphae formed after haustorial formation and they get connection to other host tissues. Later on, fungal branching network results into a mycelium. Symptoms of disease shown in seven to ten days after inoculation when uredinia produced by mycelium. Uredinia has dikaryotic urediniospores that are in orange-red color and release after breakage of epidermis by uredinia. After that release leaves of infected plants looks rusty (Schafer, 1987). A schematic diagram has been shown for quick and easy understanding of disease development process (**Figure 3**).

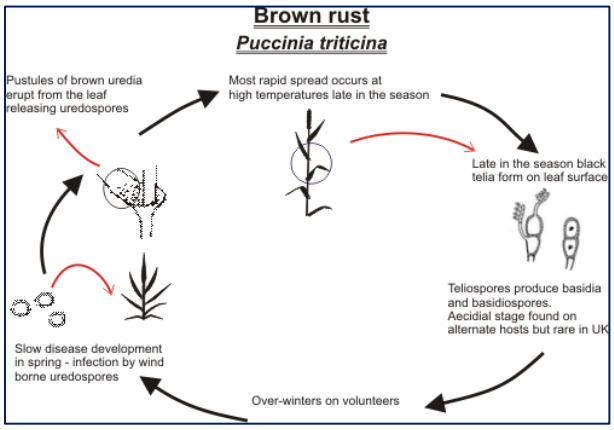

Figure 3. Wheat leaf rust disease cycle. (Source: http://www.agrilearner.com/wheat-disease/)

Favorable factors for disease

Temperature from 15˚C-25˚C with free moisture and high RH (relative humidity) favorable for development of leaf rust. For germination of urediniospores 20˚C is optimum temperature (Table 1). When these conditions are present, infection occur only in 6-8 hours. Epidemic of leaf rust is directly proportional to time. It spread more in windy and dry days with cool nights when there is dew also available there (Singh et al., 2002). Figure 4, illustrates in season-wheat leaf Rust cycle. It requires moisture to germinate and works at 100% humidity.

Table 1. Conditions required for brown rust development (Singh et al., 2002).

			Temperature ˚C		
Rust stage	Free moisture	Light	minimum	maximum	Optimum
Sporulation	None	High	10	25	35
Growth	None	High	2	25	35
Penetration	Essential	No effect	10	20	30
Appressorium	Essential	None	5	15-20	30
Germ ling	Essential	Low	5	15-20	30
Germination	Essential	Low	2	20	30

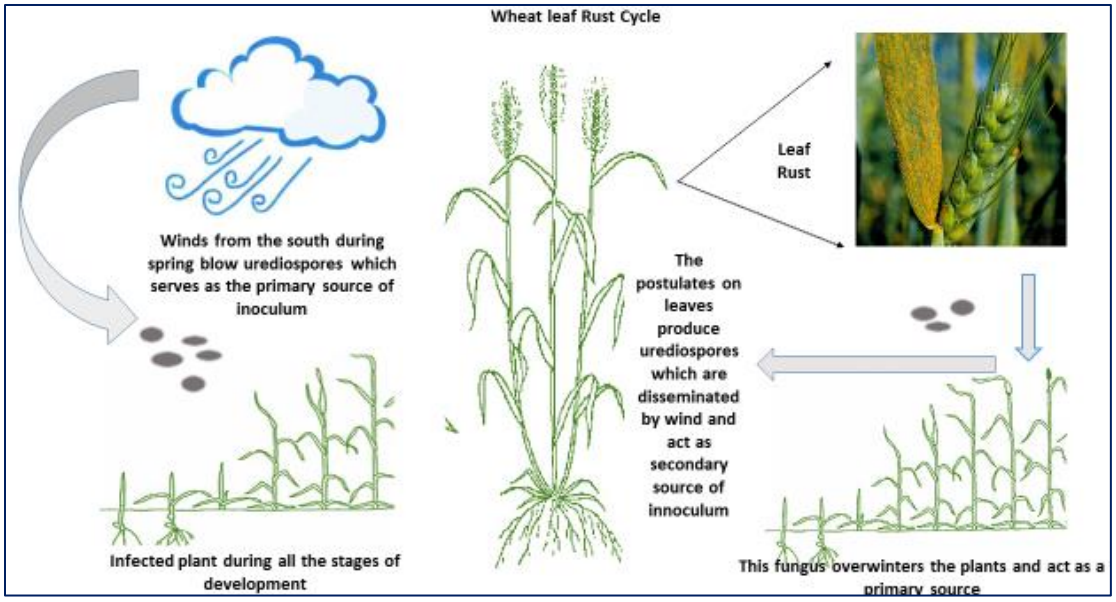

Figure 4. In season wheat leaf rust cycle (adapted by Salgado et al., 2017)

DISEASE MANAGEMENT – HOST PLANT RESISTANCE

The effective leaf rust resistant genes in several germplasm in the across the world is summarized in Table 2.

Lr34 and Lr46 (Slow rusting genes)

More than the 60 wheat *Lr* resistance genes and QTLs have identified. Most of the Lr genes are the race-specific. As the leaf resistant pathogen (*Puccinia triticina*) races are continually evolving therefore the resistance provided by the resistant genes is short lived. In order to obtain good resistant cultivars which can perform well in high disease pressure several ''slow-rusting'' genes are needed. Wheat-breeders can use these slow-rusting genes complexes to in compliment to these race-specific rusting genes because slow rusting genes are more likely to be dependent on the environment. And availability of many molecular markers can greatly facilitate the process of pyrimidazation. Among many of the rust resistant genes a small group genes. The gene *Lr34* was firstly reported in 1966, in cultivar Frontana present on short arm of chromosome 7D. A study described when *Lr34* was cloned it was as same as the gene *Pm38, Ltn1* and *Yr-18* and also codes for putative ABC transporter (Spielmeyer et al., 2005).

Table 2. Sources of resistance genes in wheat lines

Genes	Source of resistant gene	Chromosome location	Wheat Lines	References
Lr19	*Triticum turgidum*	7EL	Recombinant lines-Ag 1-22 and Ag 1-23	Prins et al. (2001)
Lr21	*Ae. Tauschii*	-	KS94U215	Huang and Gill (2001)
Lr22a	*Ae. Tauschii*	2DS	Canadian cultivar AC Minto	Hiebert et al. (2007)
Lr25 & Lr29	*Agropyron elongatum*	7DS	RL6080	Procunier et al. (1995)
Lr32	*Ae. Tauschii*	3DS	BW196R	Thomas et al. (2010)
Lr35	*A. speltoides*	2S	Bread wheat	Labuschagne et al. (2002)

Lr37	*Common wheat*	2AS	Bread Wheat	Labuschagne et al. (2002)
Lr39	*Aegilops tauschii*	2D	KS86WGRC02	Raupp et al. (2001)
Lr46	-	1BL	'Pavon 76' (PI 519847), Parula (PI 520340).	William et al. (2003)
Lr47	*A. speltoides*	7A	*T. astievum*	Dubcovsky et al. (1998)
Lr50	*T. armeniacum*	2BL	KS96WGRC36	Brown et al. (2003)
Lr51	*Triticum speltoides*	IB	Bread wheat	Helguera et al. (2005)
Lr67	Common wheat accession PI250413	4D	Backcross line-RL6077	Herrera et al. (2014)
Lr68	Brazilian cultivar Frontana	2BL	CIMMYT's spring bread wheat Parula	Herrera et al. (2012)
Lr75	-	1BS	Swiss winter bread wheat cultivar "Forno"	Schnurbusch et al. (2004)

GENE PYRAMIDING

Gene pyramiding is a method in which we strive to acquire all desirable/favorable genes in a single plant. For this purpose, DNA markers are used to isolate all of the desirable genes through a process known as marker-assisted breeding method. This molecular approach helps to access the identity of entire gene progeny. The most important goal of gene pyramiding is to shape a desirable plant consisting of homozygous genes in any respective gene loci (Servin et al., 2004; Malav et al., 2016).

Approaches of gene pyramiding

Gene pyramiding approach has following steps or techniques i) the first one is known as pedigree method in which we gather all goal genes in root genotype (unmarried genotype) ii) second step is known as fixation step in which the primary goal is to introduce all goal genes into homozygous kingdom for example to derive best genotype from root genotype. Nodes in the tree indicate intermediate genotypes and their range can vary. Advanced pyramiding can also be used in subsequent pass. Conventional method includes three breeding methods of gene pyramiding i) pedigree method, ii) Backcross method and iii) recurrent-selection method while molecular method stimulating gene pyramiding includes i) MAS and ii) transgenic method. Gene pyramiding play a vital role in modern agriculture. This technique is used to develop plants with resistant genes which confers long lasting resistance or tolerance to abiotic and biotic stress factors. Durable or multiple disease-resistance against specific and different races of pathogens can also be accomplished through gene pyramiding. Also used to improve existing high yielding cultivars (Ribaut and Hiosington, 1998; Ribaut et al., 2001; Malav et al., 2016). Schematic representation of gene pyramiding (**Figure 5**).

Marker assisted selection (MAS) breeding to target the leaf rust resistance genes

It is useful for traits like drought tolerance, disease like rust resistance (Hare, 1979). In Hungary, the four winter wheat cultivars contain the resistance genes and they are integrated with marker assisted selection. Further using backcross program, they produced BC generations by incorporated resistant genes in wheat varieties.

DNA markers and identification of leaf rust resistant genes

The *Lr14a* is a gene showed by their amplified bands and resistance response. The (SSR) and Amplified markers differentiate the durum wheat with (*Lr14a)* from durum ('Altar C84') as well as common wheat from isogenic line with (*Lr14a)*. (Lr14a) should increase the efficiency of durum wheat in combination with other leaf resistant genes (Herrera-Foessel et al., 2008). For their mapping and resistant responses, several genes reduce their losses. (Lr9) which is a leaf resistant gene showed complete linkage by three (RAPD) and one (RFLP) marker. Schematic representation of Marker assisted based

gene pyramiding for resistance genes were presented in Figure 5.

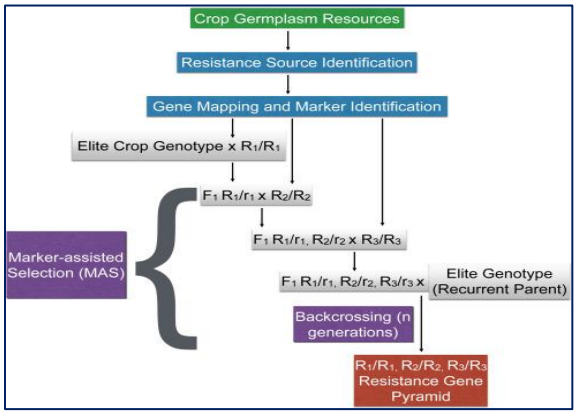

Figure 5. Schematic representation of wheat gene pyramiding for leaf rust resistance (adapted from Hartman et al., 2016)

Genetic analysis of leaf rust resistant genes

Almost one hundred years ago, two wheat cultivars Malakof and Webster studied to check the resistant for rust of leaf (Mclantosh et al., 2003). These two cultivars with genes designated *Lr1* and Lr2 having resistance against the leaf rust (Ausemus et al., 1946).

Leaf rust resistant genes derived from *Triticum aestivum*

There are eighty-one resistance genes, identified from *Triticum aestivum* by various researchers which includes *Lr1* (Roelfs et al., 2000), *Lr 2*, *Lr2a, Lr 2c, Lr3, Lr 4, Lr6, Lr8,* (Mclntosh et al., 2003), gene Lr7 (Wisniewska et al., 2003; Mclntosh et al., 2003), Lr 20 (Mclntosh *et al*., 2003), Lr 22b, Lr23, Lr27 and Lr30 (Nelson et al., 1997), Lr3, Lr 40 and Lr33 (Mclntosh et al., 2003), and Lr46 (Wisniewska et al., 2003). Mclntosh et al. (2003) were reported *Lr48* gene and *Lr52, Lr49, Lr68, Lr67* genes were identified by Shahin et al. (2015).

CONCLUSION

Many resistant cultivars have been developed worldwide from the available effective resistant genes and by incorporation of resistant genes from the associated species in to wheat genome backgrounds and through the marker assisted based gene pyramiding. Multidisciplinary research is being done to determine underlying genetic bases of many virulence and resistance genes and the complex genes interactions among them in order to future understands the interaction between wheat system to *P. triticina* on the way to especially durable and effective genetic control of leaf rust disease in the coming future.

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