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Inducing novel resistance gene in wheat towards stem rust to improve food security

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ABSTRACT

Bread wheat (*Triticum aestivum*) is one of the three most important cereal worldwide that provides a major source of daily protein and nutrition for humans. Unfortunately, this crop yield capacity is affected by both biotic and abiotic stresses. Stem rust is one of the most globally devastating wheat diseases of wheat that caused by fungal pathogen *Puccinia graminis* f. sp. *tritici* (*Pgt*). This diseases can decrease up to 100% yield loss on susceptible varieties. The emergence of important races of pathogen specially the Ug99, increases the need for more genetic diversity in this crop. Unfortunately, wheat has a narrow genetic bottleneck, whereas wild relatives such as Rye (*Secale cereale*) can increase its genetic limitations and has served as an excellent source of genetic variability for improving bread wheat against stresses. Therefore, many wheat-rye introgression lines have been developed at the Swedish University of Agricultural Sciences (SLU) to increase genetic diversity for wheat improvement. Some of these introgression lines have shown good resistance to important races of stem rust.

PREFACE

Plant breeding is an essential technique which provides a better solution when biotic and abiotic issues occur and increase food security. By implementing this technique, it is possible to develop varieties that are more adapted to harsh environmental changes. Wheat-alien introgression lines, created from the traditional crossing of wheat/rye, increase the narrow genetic of wheat and increase its genetic diversity through donating numerous acceptable genes.

This introductory paper discus the possibilities to explore resistance in wheat-rye introgression lines against stem rust, introgression of these resistance genes into adapted wheat cultivars and secure their agronomic and quality performance.

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Importance of wheat

Wheat (*Triticum* spp.) belongs to the grass family (Poaceae = Gramineae), and is (together with maize and rice) a major staple crop that provides necessary calories for the major part of the world's population (FAO, 2021). Wheat is the world's third crop in relation to production volume (after rice and maize), the second-most-consumed crop (after rice), and the most traded crop (FAO, 2018). In total, wheat contributes around 20% of all calories utilized for human nutrition (Shiferaw *et al.*, 2013; D'Odorico *et al.*, 2014). Wheat is also an important source of proteins, minerals, B vitamins, micronutrients, trace elements and dietary fibers (Johansson *et al.*, 2014; Johansson *et al.*, 2020a; Shewry and Hey, 2015; Topping, 2007). Wheat flour is used to make bread, biscuits, confectionery, noodles etc. Wheat is also used for animal feed, ethanol processing, wheat beer, etc.

The predicted climate change and the increase in the world population are two of the most important challenges in the 21st century (FAO, IFAD, UNICEF, WFP, and WHO, 2019). According to FAO (2021). The present global population is about 7.9 billion people, with more than 9 billion predicted by 2050 (FAO, 2009). The capacity to achieve superior wheat yield is influenced by the impact of biotic and abiotic stresses. The anticipated rise in temperature by the climate change, as well as the predicted increases in frequent hot and dry conditions and heavy rainfall incidents, are expected to have a detrimental impact on wheat production (Shukla *et al.*, 2019). Wheat diseases are already reducing the worldwide yield production by 10–28% (Bockus *et al.*, 2001; Figueroa *et al.*, 2018; Savary *et al.*, 2019). The globally most important fungal diseases of wheat, caused by biotrophs (obligate parasites), include the three rusts (Stem rust, Stripe rust and Leaf rust), powdery mildew, and the bunts and smuts; whereas, those caused by hemibiotrophic (facultative parasites) include *Septoria tritici* leaf blotch, *Septoria nodorum* blotch, spot blotch, tan spot, and Fusarium head blight (Dean *et al.*, 2012). Negative effects on wheat yield from these diseases can be reverted by management practices, fungicide applications, and genetic resistance. Even though chemical control is an effective disease prevention tool, it may hampering the environment, and average crop losses have not decreased during the last half-century, whereas pesticide usage has nearly doubled (Oerke and Dehne, 2004). Utilizing resistance through crop breeding is an effective, dependable, and environmentally friendly method that can be combined with other management activities to increase wheat yield and ensure food security.

Wheat Rusts

Rust pathogens have had a major impact on global wheat production since the domestication of the crop, and these pathogens continue to pose a threat to the global wheat supply (Roelfs *et al.*, 1992). Global annual losses due to wheat rust pathogens are estimated to be between US$ 4.3 and 5.0 billion. (P. Pardey, University of Minnesota, unpublished-2020).

Rust are obligate biotrophic fungi, meaning that their growth and development are entirely reliant on nutrients obtained from cells of a living host (Cummins and Hiratsuka, 2003; Duplessis *et al.*, 2012). The ability of various rust species to infect specific hosts differ, reflecting their biological differences and classifies them into formae specials (ff. spp.) (Eriksson, 1894). Thus, three different wheat rust diseases are present, all caused by members of the Basidiomycete family, genus Puccinia. The three wheat rust species are named P. *graminis* f. sp. *tritici* (Pgt) known as stem rust, P. *striiformis* f. sp. *tritici* (Pst) known as stripe rust rust and P. *triticina* (Pt) known as leaf rust (McIntosh *et al.*, 1995). Fig.1.

(A-*Pgt*) (B-*Pst*) (C-*Pt*)

Fig. 1: Symptoms of wheat rust diseases caused by *Puccinia graminis* f. sp. *tritici* (A), *Puccinia striiformis* f. sp. *tritici* (B) and *Puccinia triticina* (C). *Photo by: Mehran Patpour (A), Mahboobeh Yazdani (B, C).*

Table 1: Current and historical importance of wheat stem, stripe and leaf rusts for the epidemiological zones (Saari and Prescott, 1985 with modification).

Zone	Stem rust		Stripe rust		Leaf rust	
	Current [a]	Historical	Current	Historical	Current	Historical
			Africa			
North	Local	Major	Local	Local	Major	Major
East	Major	Major	Major	Major	Local	Local
Southern	Local	Major	Local	Rare	Local	Local
			Asia			
Far East	Local	Major	Major	Major	Local	Local
Central	Minor	Minor	Local	Local	Major	Major
South	Minor	Major	Local	Local	Local	Major
Southeast	Minor	Minor	Rare	Rare	Major	Major
Middle east	Minor	Minor	Local	Local	Major	Major
West	Local	Major	Major	Major	Local	Local
Australia	Local	Major	Local	Rare	Local	Local
			Europe			
East	Minor	Major	Local	Local	Major	Major
West	Minor	Major	Major	Major	Local	Major
North America	Minor	Major	Local	Local	Major	Major
South America	Local	Major	Local	Local	Major	Major

[a]Major = severe losses without the cultivation of resistant varieties; Minor = usually occurs, but of little significance; Local = only occurs in a small part of the region, losses in these areas may be occasionally severe if susceptible varieties are grown; Rare = not present, rarely seen, or as in Australia and New Zealand, recently introduced.

Due to the fact that all the three rust fungi are biotrophic, for survival, they all need an alternate host beside their primary host which is wheat. In Table 2, primary and alternate hosts, as well as symptoms and generally accepted environmental conditions needed by the three rust diseases are summarized (Roelfs *et al.*, 1992).

Table 2: The rust diseases of wheat, their primary and alternate hosts and symptoms (Roelfs *et al.*, 1992, Jin *et al.*, 2010, with modification).

Disease	Pathogen	Primary hosts	Alternate hosts	Symptoms
Stem rust	*Puccinia graminis* f. sp. *tritici*	Bread and durum wheats, barley, triticale	*Berberis vulgaris*	Isolated uredinia on upper and lower leaf surfaces, stem and spikes
Stripe rust	*Puccinia striiformis* f. sp. *tritici*	Bread and durum wheats, triticale, a few barley varieties	*Berberis vulgaris*	Systemic uredinia on leaves and spikes and rarely on leaf sheaths
Leaf rust	*Puccinia triticina*	Bread and durum wheats, triticale	*Thalictrum, Anchusa, Isopyrum, Clematis*	Isolated uredinia on upper leaf surface and rarely on leaf sheaths

The present introductory paper is focusing primarily on stem rust and therefore the following sections will only treat knowledge related to stem rust.

Biology of stem rust

Symptoms

Erumpent pustules on the stems and leaf sheaths are the most common symptoms of stem rust in wheat (Fig. 2). Each pustule is the result of an infection by a single rust spore. Symptoms of the initial infections do not appear until 7-10 days after infection. The fungal mycelium, which has been developing inside the plant tissue, then masses directly underneath the epidermis and begins producing thousands of spores, which burst the epidermis and emerge as powdery, rust-colored urediniospores. Each urediniospore has the potential to produce a new infection that will cause similar damage on the same plant or another wheat plant. Within a few weeks, multiple cycles of infection, sporulation, and re-infection can cause devastating epidemics in wheat fields.

Fig. 2: Symptom of disease on stem, *Photo by: Mehran Patpour*

Life cycle

The life cycle of *Puccinia graminis* f. sp. *tritici* is complicated and consist of five different spores: basidiospores → pycniospores → aeciospores → urediniospores → teliospores (Roelfs, 1985). The fungus is heteroecious; which means that the fungus requires two unrelated host plants. As shown in Table 2, the primary host of the stem rust fungi is wheat, while the most common secondary host is *Berberis vulgaris*. The sexual part of the life cycle occurs on the secondary host, while the asexual part occurs on the primary host (Leonard and Szabo, 2005). When the climate is warm and humid, the wheat acts as a green bridge or major inoculum source to begin a new cycle of the stem rust wheat disease in the following fall. Aeciospores are the predominant source of primary inoculum for wheat stem rust in places with cold winters (Leonard and Szabo, 2005).

The life cycle start with the germination of overwintered teliospores in a suitable environment (Roelfs, 1985) and creation of basidiospore (Fig. 3). These spores infect young leaves of common barberry (*Berberis vulgaris*) or other susceptible *Berberis*, *Mahonia*, or *Mahoberberis* species (Rodriguez-Algaba *et al.,* 2014). The resulting infections on barberry create specialized infection structures called pycnia, which are essential for the fungus sexual stage (Fig. 4).

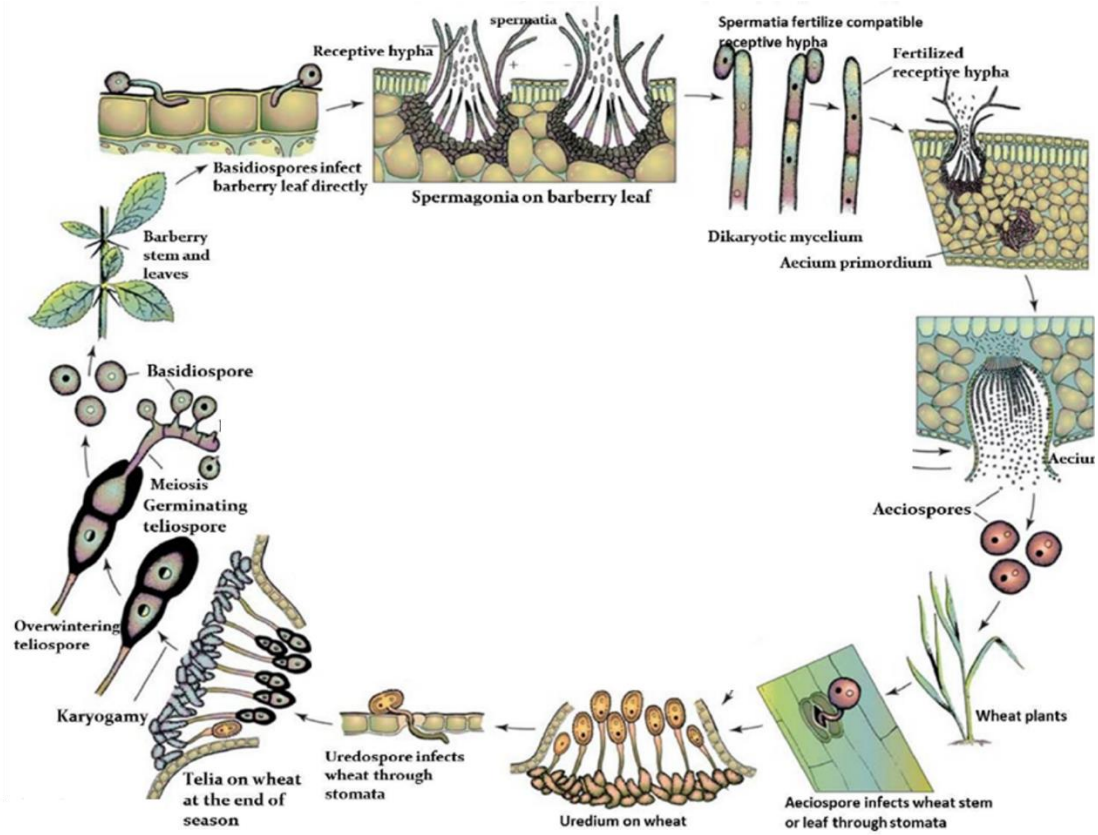

Fig. 3: Life cycle of *Puccinia graminis* f. sp. *tritici* (Agrios, 2005)

On barberry, P. *graminis* completes its sexual part (Anikster *et al.*, 1999), in which one hyphae from pycnium mate with spores of another pycnium and the fertilized structure develops into an aecium (Craigie, 1927; Fig. 4). Aeciospores from the aecium infect wheat and the asexual or repetitive part of *Pgt* starts with creation of uridium and urediniospores, which can infect other nearby wheat plants or even move by wind to another continent and make infection in those areas. At the end of the season when the condition is no longer suitable for establishment of pathogen, urediniospores change to black teliospores that can survive for 13 years in the soil (Leonard and Szabo, 2005).

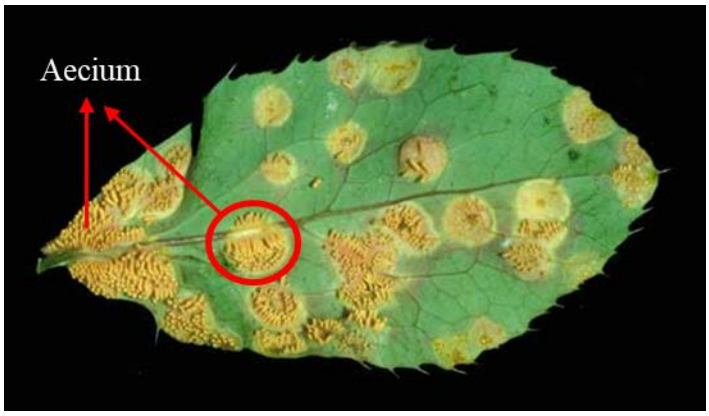

Fig. 4: Fertilized structure of aecium on *Berberis vulgaris, Photo by: USDA website*

Economic Importance of stem rust

Stem rust is a threat to wheat production and food security in wheat producing areas all over the world where the fungi is present and no resistance wheat genotypes are available (Chaves *et al.*, 2013). Historically, stem rust has been a major threat in most wheat production countries of United States of America, Australia, South Africa, Middle East, and Europe (Saari and Prescott, 1985).

In the United States of America, epidemics of the disease has been reported from 1904, 1916, 1954, 1965, 2015, 2016 and 2017 (Roelfs, 1985; Saunders *et al.*, 2019). The worst outbreak of stem rust in the United States occurred in 1935, when 50 percent of the wheat production in North Dakota and Minnesota was totally destroyed (Leonard, 2001).

In Australia, spectacular epidemics are well known as these occurred by widespread sowing of susceptible varieties in the 1973 (Roelfs, 1985); this even resulted in a 40% total grain failure in specific years (Roelfs, 1985). Stripe and leaf rusts are the most widespread rust fungi in European countries. That is because the alternate host of stem rust (barberries) was eradicated in the twenty century, and the environmental conditions for stem rust occurrence were not ideal (Stakman, 1923; Saunders *et al.*, 2019).

In South Africa, stem rust caused by *Puccinia graminis* f. sp. *tritici* has been a major constraint for the wheat production (Pretorius *et al.*, 2007). A new stem rust race, Ug99 (TTKSK) emerged in East Africa in 1999 (Pretorius *et al.*, 2000). This new race created epidemics in Kenya and Ethiopia, and thereafter three Ug99 variants occurred in South Africa (Visser *et al.*, 2009), which accelerated the introduction of new resistance varieties in the country. The occurrence of Ug99 was also reported from Iran in 2009 (Nazari *et al.*, 2009). The Ug99 and its variants

have been reported as having the potential to spread globally, i.e. posing a direct threat to one-quarter (50 million hectares) of the world's wheat supply which is present in west Asia (Singh *et al.*, 2008).

The emergence of new variants of important races such as the Ug99 (TTKSK) and other virulent races to a significant number of genotypes previously reported to contain genes with stem rust resistance, has increased global awareness of the threat of stem rust and highlighted the importance of introducing new resistance varieties.

Favorite condition for stem rust occurrence

Long-distance movement of stem rust spores have been reported if suitable wind conditions are prevailing, e.g. across the North American Great Plains (Roelfs, 1985), from Australia to New Zealand, and on exceptional incidents to a distance of around 8000 km between southern Africa and Australia (Luig, 1985). In the case of long-distance dispersion, spore penetration on crops in a new region are often correlated with rain showers (Singh *et al.*, 2008). The stem rust urediniospores, which are the ones infecting the wheat, are relatively tolerant to atmospheric conditions if their moisture content is moderate (20–30%) (Roelfs *et al.*, 1992). Table 3 shows the minimum, optimum, and maximum temperatures for urediniospore germination (Roelfs *et al.*, 1992).

Table 3: Environmental conditions required for stem rust (Roelfs *et al.*, 1992).

Stage	**Temperature (°C)**			**Light**	**water**
	Minimum	Optimum	Maximum		
Germination	2	15-24	30	Low	Necessary
Sprout	-	20	-	Low	Necessary
Appressorium formation	-	16-27	-	None	Necessary
Penetration	15	29	35	High	Necessary
Growth	5	30	40	High	None
Sporulation	15	30	40	High	None

Urediniospores germinate in 1–3 hours, once they are exposed to free moisture at a variety of temperatures (Singh *et al.*, 2008), and 6–8 hours is needed for the full infection process (Singh *et al.*, 2008).

Race typing of stem rust

In 1922, Stakman and Levine (1922) published the first key on physiologic races of *Puccinia graminis* f. sp. *tritici*. In principal, the physiologic races were at that time built on the 12 isogenic differential hosts. Later, these lines were expanded to 20 sets, including: ISr5-Ra (*Sr5*), Cns_T_mono_deriv (*Sr21*), Vernstein (*Sr9e*), ISr7b-Ra (*Sr7b*), ISr11-R (*Sr11*), ISr6-Ra (*Sr6*), ISr8-Ra (*Sr8a*), CnSr9g (*Sr9g*), W2691SrTt-1 (*Sr36*), W2691Sr9b (*Sr9b*), BtSr30Ws (*Sr30*), Combination VII (*Sr13+Sr17*), ISr9a-Ra (*Sr9a*), ISr9d-Ra (*Sr9d*), W2691Sr10 (*Sr10*), CnsSrTmp (*SrTMP*), LcSr24Ag (*Sr24*), Benno Sr31/6*LMPG (*Sr31*), VPM 1 (*Sr38*) and McNair 701 (*SrMcN*) (Jin *et al.*, 2008, Roelfs *et al.*, 1993; Roelfs and Martens, 1988). The infection patterns from a stem rust infection is categorized into four classes, with 0, 1, and 2 indicating host resistance and 3 and 4 indicating host susceptibility (Table 4, Fig. 5). Correspondence between race name and virulence on common stem rust differential lines described in table 5.

Table 4: Description of infection types (Roelfs, 1985).

Infection Type		Symptoms
0	Resistant	No uredia or other macroscopic sign of infection
;	Resistant	No uredia, but hypersensitive necrotic or chlorotic flecks of varying size present
1	Resistant	Small uredia often surrounded by necrosis
2	Resistant	Small to medium uredia often surrounded by chlorosis or necrosis
3	Susceptible	Medium-sized uredia that may be associated with chlorosis or rarely necrosis
4	Susceptible	Large uredia without chlorosis or necrosis

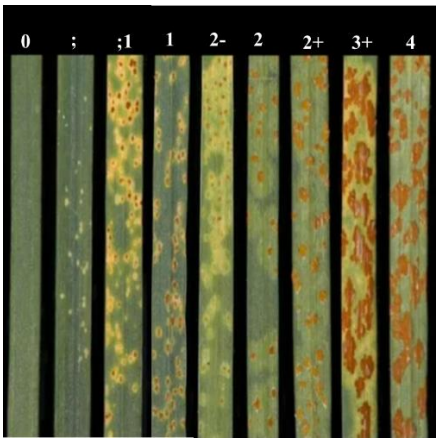

Fig. 5: Infection type of of *Puccinia graminis* f. sp. *Tritici* (Stakman *et al.*, 1962).

IT = 0 (no uredia); IT = 0; (fleck); IT = 1 (small uredia); IT = 2 (small to medium uredia); IT = 3 (medium uredia without chlorosis or necrosis) IT = 4 (large uredia without chlorosis or necrosis).

Table 5: Correspondence between race name and virulence on common stem rust differential lines (Hovmøller *et al.*, 2020).

Virulence corresponding to NA diffrentials 1-20 (Main gene indicated)																				
Race name	**Sr5**	**Sr21**	**Sr9e**	**Sr7b**	**Sr11**	**Sr6**	**Sr8a**	**Sr9g**	**Sr36**	**Sr9b**	**Sr30**	**Sr17**	**Sr9a**	**Sr9d**	**Sr10**	**SrTmp**	**Sr24**	**Sr31**	**Sr38**	**SrMcN**
LKMNC	+	-	-	-	-	+	+	+	+	-	-	+	+	-	+	-	-	-	-	+
RFCNC	+	+	-	+	-	-	+	+	-	-	-	+	+	-	+	-	-	-	-	+
RFCPC	+	+	-	+	-	-	+	+	-	-	-	+	+	-	+	+	-	-	-	+
TKKTF	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	-	-	+	+
TKTTF	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+
TTKSK	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+
TTKST	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+
TTKTK	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+
TTKTT	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
TTRTF	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	+	+
TTTTF	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+

Wheat stem rust resistance gene

There are approximately 58 specific stem resistance genes known to date (McIntosh *et al.*, 1995; Hafeez *et al.*, 2021). A number of these genes have their origin from alien wheat relatives and have been introduced to wheat by different methods (Table 6). All known and designated genes, with the exception of *Sr2* stages (Singh *et al.*, 2008), *Sr55* (Moore *et al.*, 2015), *Sr57* (Krattinger *et al.*, 2009) are race specific and are expressed in both seedling and adult plant. A race specific gene is defined by the presence of a gene-for-gene interaction between the host plant resistance gene and the pathogen's avirulence genes. The majority of the stem rust resistance genes acts by enabling the development of only midsized uredinia, through surrounding it with necrosis or chlorosis, thereby reducing sporulation (McIntosh *et al.*, 1995).

Table 6: The sources and origins of stem rust resistance genes (Singh *et al.*, 2011).

Source/Origin	*Sr Gene*
Aegilops comosa	34
Aegilops sharonensis	62
Aegilops speltoides	32, 39, 47
Aegilops tauschii	33, 45, 46
Aegilops ventricosa	38
Secale cereale	27, 31, 50, 59, $1RS^{Amigo}$, Satu
Thinoporum elongatum	24, 25, 26, 43
Thinoporum intermedium	44
Triticum aestivum	5, 6, 7a, 7b, 8a, 8b, 9a, 9b, 9f, 10, 15, 16, 18, 19, 20, 23, 28, 29, 30, 41, 42, 48, 49, TMP
Triticum araraticum	40
Triticum comocum	34
Triticum monococcum	21, 22, 35, 60, Tm5
Triticum timopheevi	36, 37
Triticum turgidum	2, 9d, 9e, 9g, 11, 12, 13, 14, 17
Triticum ventricosum	38

Important races of stem rust

Long-term breeding efforts, including those carried out by international organizations, e.g. International Maize and Wheat Improvement Center (CIMMYT) have resulted in new resistant wheat varieties, thereby reducing the importance of stem rust, which was considered to be no longer a threat to the world wheat supply in the 1980ies (Saari and Prescott, 1985). As a result, attention was mostly focused on stripe and leaf rust (Singh *et al.*, 2008). However, in 1998, a new race of stem rust was identified in Uganda, which showed virulence to *Sr31*. This race was designated Ug99 in the year 1999 (Pretorius *et al.*, 2000). The East African highlands are a known ‘‘hot-spot’’ for the evolution and survival of new rust races (Saari and Prescott, 1985). Wanyera et al. (2006) used the North American naming scheme to give the novel stem rust race the name TTKS (Roelfs and Martens, 1988), and more recently as TTKSK, as a fifth set of differentials has been added, to further expand the characterization (Jin *et al.*, 2008). Thereafter, a variant of Ug99 (TTKST) was detected in 2006 in Kenya, which showed virulence also to *Sr24* (Singh *et al.*, 2008). Since then, so many previously resistance stem race races known to be ineffective. Table 7 shows the effectiveness and ineffectiveness of stem rust resistance in the Ug99 race group.

Table 7: Efficacy and inefficacy of stem rust resistance to Ug99 race group (www.globalrust.org and USDA-ARS Cereal Disease Laboratory).

Race	Resistance gene to stem rust		country
	effective	**ineffective**	
TTKSK (Ug99)	*Sr1RS^{Amigo}, 2*, 13a,*, 14a, 22, 24a, 25a, 26, 27a , 28a, 29*, 32, 33*, 35, 36a, 37, 39, 40, 43, 44, 45, Tmp, Sr59*	*5,6,7a, 7b, 8a, 9a, 9b, 9d, 9e, 9f, 9g,10, 11, 12, 15, 16, 17, 18, 19, 20, 21, 23, 30, 31, 34, 38, 41, 42, Wld-1*	Uganda, Kenya, Ethiopia, Sudan, Yemen, Iran
TTKSF	*Sr1RS^{Amigo}, 2*, 13a,*, 14a, 22, 24a, 25a, 26, 27a , 28a, 29*, 31, 32, 33*, 35, 36a, 37, 39, 40, 43, 44, 45, Tmp, Sr59*	*5,6,7a, 7b, 8a, 9a, 9b, 9d, 9e, 9f, 9g,10, 11, 12, 15, 16, 17, 18, 19, 20, 23, 30, 31, 41, 42, Wld-1*	South Africa, Zimbabwe
TTKST (Ug99 + *Sr24*)	*Sr1RS^{Amigo}, 2*, 13a,*, 14a, 22, 25a, 26, 27a , 28a, 29*, 32, 33*, 35, 36a, 37, 39, 40, 43, 44, 45, Tmp, Sr59*	*5,6,7a, 7b, 8a, 9a, 9b, 9d, 9e, 9f, 9g, 10, 11, 12, 15, 16, 17, 18, 19, 20, 21, 23, 24, 30, 31, 34, 38, 41, 42, Wld-1*	Kenya
TTTSK (Ug99 + *Sr36*)	*Sr1RS^{Amigo}, 2*, 13a,*, 14a, 22, 24a, 25a, 26, 27a , 28a, 29*, 32, 33*, 35, 37, 39, 40, 43, 44, 45, Tmp, Sr59*	*5,6,7a, 7b, 8a, 9a, 9b, 9d, 9e, 9f, 9g, 10, 11, 12, 15, 16, 17, 18, 19, 20, 21, 23, 30, 31, 34, 36, 38, 41, 42, Wld-1*	Kenya
TTKSP (Ug99 progenitor + *Sr24*)	*Sr1RS^{Amigo}, 2*, 13a,*, 14a, 22, 24a, 25a, 26, 27a , 28a, 29*,31a, 32, 33*, 35, 36a, 37, 39, 40, 43, 44, 45, Tmp, Sr59*	*5,6,7a, 7b, 8a, 9a, 9b, 9d, 9e, 9f, 9g, 10, 11, 12, 15, 16, 17, 18, 19, 20, 21, 23, 30, 34, 38, 41, 42, Wld-1*	South Africa
PTKST	*Sr1RS^{Amigo}, 2*, 13a*, 14a, 21, 22, 25a, 26, 27a , 28a, 29*, 32, 33*, 35, 36a, 37, 39, 40, 42, 43, 44, 45, Tmp, Sr59*	*5,6,7 a, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9f, 9g, 10, 11, 12, 15, 16, 17, 18, 19, 20, 23, 24, 30, 31, 34, 38, 41, Wld-1*	Kenya, South Africa
PTKSK	*Sr1RS^{Amigo}, 2*, 13a,*, 14a, 21, 22, 24a, 25a, 26, 27a , 28a, 29*, 32, 33*, 35, 36a, 37, 39, 40, 42, 43, 44, 45, Tmp, Sr59*	*5,6,7a, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9f, 9g, 10, 11, 12, 15, 16, 17, 18, 19, 20, 23, 30, 31, 34, 38, 41, Wld-1*	Kenya, Ethiopia

Since the emergence of Ug99, additional novel races of stem rusts have emerged. One such example is the "Sicily race" (TTRTF), which is now widespread in Italy, Spain, Tunisia, Iran, and Sweden (Hovmøller *et al.*, 2022). The TTRTF was first identified through wheat stem rust collections made in 2014 from Akhalkalaki, Georgia (Olivera *et al.*, 2019), and is virulent to 23 *Sr* genes (IT 3 or higher) including: *Sr5*, *Sr6*, *Sr7a*, *Sr7b*, *Sr8a*, *Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr9g*, *Sr10*, *Sr11*, *Sr13b*, *Sr17*, *Sr21*, *Sr35*, *Sr36*, *Sr37*, *Sr38*, *Sr44*, *Sr45*, *SrTmp*, and *SrMcN* (Patpour *at al.*, 2020). This race caused severe epidemics of wheat stem rust on durum wheat in Italy in 2016 and 2017 (Patpour *et al.*, 2018), and has also been reported present in Georgia (2014),

Hungary (Olivera *et al.*, 2019), Egypt (Esmail and Szabo, 2018) Ethiopia (Tesfaye *et al.*, 2019), Eritrea and Iran (Patpour *et al.*, 2020). TTRTF is a significant threat to the wheat production in affected areas because of its wide virulence spectrum, which involves also virulence to *Sr13b* in durum wheat and higher-than-normal IT for *Sr50* (Patpour *et al.*, 2020). The frequency of the most important stem rust races from 2011-2019 is mapped in figure 6.

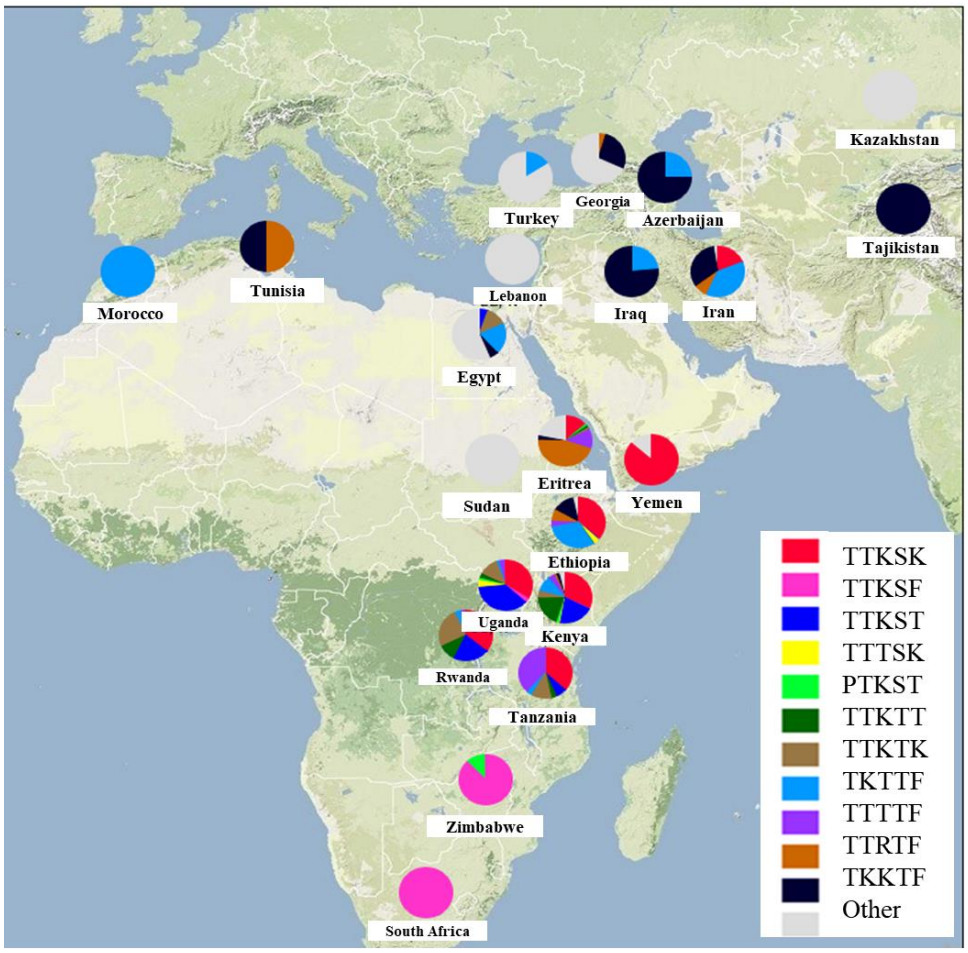

Fig 6: Frequency of stem rust races from 2011-2019 (Source: Global Rust reference center).

Current distribution of Ug99

As mentioned previously, the race TTKSK (Ug99) is a devastating race of *P. graminis* f. sp. *tritici* that was first detected in Uganda in 1998 (Pretorius *et al.*, 2000), and which shows virulence to gene *Sr31*. Due to the fact that most varieties bred at CIMMYT holds the *Sr31* as a resistance gene towards stem rust, breaking this resistance is detrimental for wheat production in large areas of Africa, Asia and Central America (Singh *et al.*, 2008). This race also has

virulence to a wide range of resistance genes including: *Sr1RS*^{Amigo}, *Sr2*, *Sr13a* , *Sr14a*, *Sr22*, *Sr24a*, *Sr25a*, *Sr26*, *Sr27a* , *Sr28a*, *Sr29*, *Sr32*, *Sr33*, *Sr35*, *Sr36a*, *Sr37*, *Sr39*, *Sr40*, *Sr43*, *Sr44*, *Sr45* and *SrTmp* (Pretorius *et al.*, 2012, Patpour *et al.*, 2015). Currently, the pathogen has evolved into several variants with similar DNA fingerprints but slightly different avirulence and virulence profiles (Szabo, 2007; Jin *et al.*, 2009; Singh *et al.*, 2011). Thus these variants are the results of single step mutations and they are therefore designated as TTKSF, TTKST, TTTSK, TTKSP, PTKSK, PTKST, and TTKSF+ (Pretorius *et al.*, 2012). Wind irradiation and rain deposition have equipped stem rust uredospores to long-distance migration (Rowell and Romig, 1966; Singh *et al.*, 2006), and can also be unintentionally spread as spores through transportation. At present the variants of Ug99 have thereby spread throughout Africa and developed themselves in the Middle East (Figure 7; Singh *et al.*, 2006).

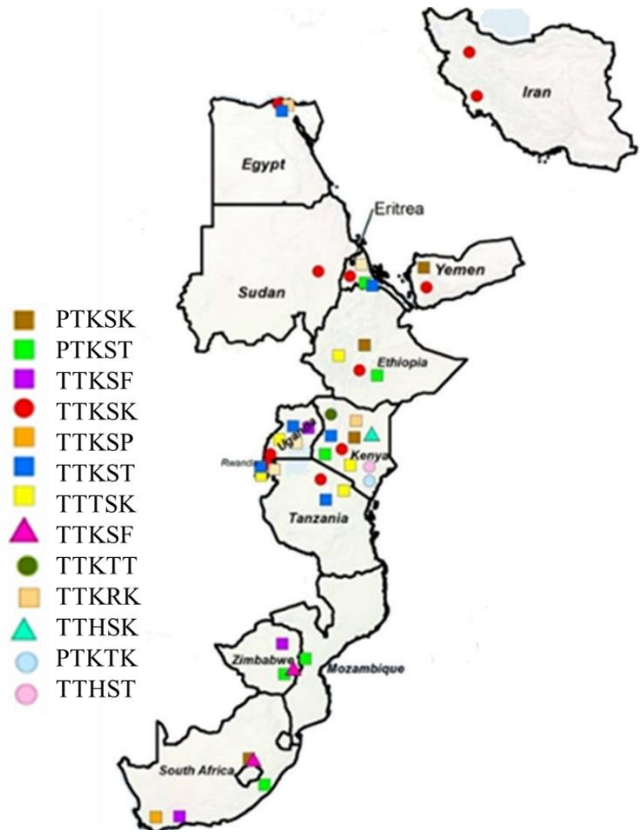

Fig 7: Distribution of important races of stem rust; Ugg99 distribution marked with red dot -2019 (Source: WheatrustTraker.org)

Disease management strategies

A) Resistance

There are two types of host resistance: qualitative and quantitative resistance. Race-specific, monogenic (major genes), and hypersensitive resistance are also terminology used to characterize qualitative resistance. They express at the seedling stage and are often referred to as all-stage resistance. Quantitative resistance, on the other hand, is race non-specific resistance, slowing rust, polygenic (minor genes), durable resistance, which can only be expressed in adult plants (Adult Plant Resistance, APR) (Van der Plank, 1968).

1- Seedling resistance or all stage resistance:

Approximately 58 stem rust resistance genes (*Sr*) at seedling stage have been identified in wheat (McIntosh *et al.,* 1995; Hafeez *et al.,* 2021). Disease mechanism is based on the gene for gene theory, in which a pathogen's avirulence gene is matched to a host's resistance gene (Flor, 1955). Then, the effector-triggered immunity (ETI) will start between pathogen and host which will trigger a cascade of recognition molecules (Jones and Dangl 2006). Seedling resistance genes generally encode immunological receptors of the nucleotide binding site-leucine rich repeat (NBS-LRR) type (Periyannan *et al.,* 2013; Saintenac *et al.,* 2013), while APR genes encode kinase-START and ABC transporters (Periyannan *et al.,* 2013; Saintenac *et al.,* 2013; Fu *et al.,* 2009) and non-ABC transporter (Moore *et al.,* 2015). Wheat NBS-LRR proteins interact chemically and structurally to promote rust pathogen resistance and perform different functions in avirulence recognition (Jones and Dangl, 2006). Disease resistance proteins signal downstream components when pathogen molecule activity is detected, as a result the defensive reaction is produced (Jones and Dangl 2006). The widespread adoption of these genes may boost the pathogen population's selection pressure to overcome resistance. Currently, the durability of race specific resistance is very low and some of these seedling resistance genes have been overcome by the new pathogen races.

2- Adult plant resistance

Adult plant resistance will only be expressed after the plant is fully grown. It implies that plants may be vulnerable as seedlings, but as they mature, they will become resistant. They form of resistance that delays in disease progression known as slow-rusting resistance.

Varieties that have APR show modality resistance response to stem rust at field. They also known as high temperature adult-plant resistance (HTAP) which will be expressed in the fully grown plant stage at higher temperatures (Chen and Line, 1995). APR's general strategy is to extend the latent period and reduce sporulation. These genes show durable resistance and can be very effective in an epidemics. Adult plant resistance is frequently confers resistance to a variety of pathogen races (Krattinger *et al.,* 2009; Herrera-Foessel *et al.,* 2010; Moore *et al.,* 2015). They often combine a multi resistance of all rusts pathogens like: Sr55/Yr46/Lr67, Sr57/Yr18/Lr34 and Sr58/Yr29/Lr46 APR genes (McFadden, 1930; Fu *et al.*, 2009; Herrera-Foessel *et al.*, 2010; Yang *et al.,* 2013; Lan *et al.,* 2014). Environmental conditions (light, temperature and duration of dew), host (plant fertilizers, growth stage), pathogen (first inoculation) can affect the expression of APR gens.

3- Durable resistance

The term "durable resistance" refers to a type of resistance that can last for a long period in commercial varieties under favorable stem rust conditions (Johnson, 1984). In general, APR genes are more persistent than seedling genes and can last for a longer time (Ayliffe *et al.,* 2008; Rouse *et al.,* 2014; Brown, 2015). The best example is *Sr31* which was durable in all stage of plant growth for decays and has overcome by the new variant of Ug99 (Pretorius *et al.,* 2000; Singh *et al.,* 2008). Pleiotropic genes can be used in the development of durable resistance. *Sr2/Yr30/Lr27* and *Sr57/Yr18/Lr34* are two instances of long-lasting resistance that have been combined with other major and minor resistance genes to obtain optimal rust resistance levels (Singh *et al.,* 2011; Ellis *et al.,* 2014).

B) Gene pyramiding

Gene pyramiding, or the accumulation of resistance genes, produces more persistent resistance than a single resistant gene. This is based on the idea that pathogenicity mutations in pathogens are uncommon and only happen through chance (Schafer and Roelf, 1985). If the possibility of mutation for one gene is 10-6, then the possibility of mutation for two genes is 10-12 and for 3 genes is 10-18. When additional genes are

added to the gene pyramid, the number of mutations that can occur at the same time decreases significantly. Gene pyramiding requires the availability of several effective genes for breeding that have not yet been defeated by the disease. Gene pyramidization efforts are boosted by increasing the accessibility of molecular markers for stem rust resistance genes (Olson *et al.,* 2010, Liu *et al.,* 2010).

How to breed for resistance

The most common way of transferring genes through recombination and selection is crossing and backcrossing between varieties with useful features. Wheat lines with agronomic, disease resistance, and other important qualities will be chosen to transfer genes using this approach. This strategy is used to develop the majority of CIMMYT lines and varieties. The use of this strategy to develop resistance to devastating fungal diseases including stem, stripe, and leaf rusts is still seen to be the most cost-effective and environmentally friendly approach for wheat breeding (Ellis *et al.,* 2014). However, new selective tools such as markers, genomic selection (GS), genome-wide association mapping (GWAS) etc. enhance wheat breeding projecs. Furthermore, transgenic wheat has aided the development of significant resistance sources (Mondal *et al.,* 2016).

Alien material and how to use them for resistance

Many important traits for wheat improvement have been found in rye, including the resistance genes *Sr27*, *Sr50*, *Sr59 Sr1RS^{Amigo}*, *SrSatu*, *Lr25*, *Lr45*, *Pm7*, and others (Knott, 1989; The *et al.*, 1991; Marais and Marais, 1994; McIntosh *et al.*, 1995; Friebe *et al.,* 1996, Rahmatov *et al.,* 2016a, Rahmatov *et al.,* 2016b). They include various important traits and can be utilized as a foundation for long-term resistance and to improve the quality of bread wheat (Johansson *et al.,* 2014; Johansson *et al.*, 2020b).

The majority of desirable traits can be transferred on through a traditional crossing program. Wild relatives, landraces, and close relatives of wheat, on the other hand, provide a unique source of new genetic variants for adoption into modern wheat varieties (Molnár-Láng *et al.*, 2015). Successful transfers and utilization of alien resistance genes *Sr24* and *Sr26* from *Agropyron elongatum* (*Thinopyrum ponticum*), *Sr31* located in the 1BL.1RS translocation from

‘‘Pektus’’ rye (*Secale cereale*) and an undesignated gene on 1AL.1RS translocation from ‘‘Insave’’ rye, *Sr36* from T. *timopheevi* and *Sr38* from T. *ventricosum* further reduced stem rust incidence in various countries around the world in 1970s and 1980s. The foreign resistance gene *Sr31* has been employed extensively in agriculture since the 1980s in spring, facultative, and winter wheat breeding programs all over the world (Singh *et al.*, 2008). Since 1BL.1RS translocation contained resistance genes for all three rusts and powdery mildew on the same translocation (*Sr31/Yr9/Lr26/Pm9*), its usage was initially linked to higher grain yields and resistance to all three rusts and powdery mildew.

Method to bread for resistance

Molecular markers

A molecular marker is any type of molecular data that can indicate a selective distinction between two living organisms. Molecular markers are useful tools to develop the genetic structure of quantitative and qualitative traits as well as the gene sites that influence those (William *et al.*, 2003). Many studies have been conducted utilizing molecular markers to give resistance in essential crops such as wheat, corn, rice, barley, potatoes, and sugar beets. Significant degree of polymorphism, dominant inheritance, relative abundance on the genome, pleiotroph, uniform distribution on the genome, easy availability (without cloning), easy and rapid measurement, high repeatability, and acceptable cost are all requirements for an optimal molecular marker. Molecular markers, according to Tanksley (1983), are useful in distinguishing five inherit traits i.e. 1) Genotypes may be identified at every plant tissue and cellular level using molecular loci; 2) At molecular marker loci, a high number of naturally occurring alleles can be discovered.; 3) Different alleles of a molecular marker are not associated to negative effects; 4) alleles at most molecular/loci are co-dominant- for the purpose of distinguishing all possible genotypes in a segregating population; 5) There are rarely epistatic or pleiotropic effect- as a result, a huge number of segregating markers in a single population may be tracked.

Molecular markers can be divided into two categories 1) Isozyme markers and 2) DNA based markers.

Markert and Moller (1959) coined the term "isozymes," and this class of markers is used to characterize the several molecular structures of bands that can be seen for the same enzyme. Genetic variation, linkage/genetic mapping, and the discovery of QTLs may all be done with

DNA-based markers. Molecular approaches can identify and visualize DNA sequences and/or segments associated to a gene locus and/or morphological or other plant traits. DNA based markers can be classified in the following groups (Paterson *et al.*, 1991; Jones *et al.*, 1997; Gupta, *et al.*, 1999; Qi *et al.*, 2004; Xu, 2010; Nadeem *et al*., 2017):

1) Hybridization based markers (e.g. RFLP)
2) PCR-based molecular markers (e.g. RAPD, SSR)
3) Molecular markers based on PCR followed by hybridization (RAPD/MP-PCR)
4) Sequencing and DNA chip based markers (SNPs)
5) Diversity array technology (DArT) is a novel type of DNA markers which employs a microarray hybridization
6) Expressed Sequence Tags (EST)
7) The Kompetitive allele-specific PCR (KASP) genotyping assay

1- Hybridization based markers (e.g. RFLP)

RFLP was the first molecular marker technique and the only marker system based on hybridization. Individuals of same species exhibit polymorphism as a result of insertion/deletions (known as InDels), point mutations, translocations, duplications and inversions. The initial stage in the RFLP process is to isolate pure DNA. This DNA is mixed with restriction enzymes obtained from bacteria, which are utilized to break DNA at specific loci (known as recognition sites). As a result, a large number of pieces of various lengths are produced. The separation of these fragments is accomplished using agarose or polyacrylamide gel electrophoresis (PAGE), which results in a variety of bands. Each band indicates a different length fragment. The major reasons of variance in the RFLP pattern include base-pair deletions, mutations, inversions, translocations, and transpositions. As a result of these changes, recognition sites insert or deletions, resulting in fragments of different lengths and polymorphism. The restriction enzymes will not cut the fragment if a single base-pair variation occurs in the recognition site. However, if a point mutation occurs on one chromosome but not the other, the marker is shown to be heterozygous, as both bands are present. (Madhumati, 2014).

2- PCR-based molecular markers (e.g. RAPD, SSR)

Kary Mullis, an American biologist, invented the polymerase chain reaction (PCR) in 1983, and since then it has become a widely used tool in molecular plant breeding. PCR is a fundamental procedure that involves synthesizing a specific piece of DNA repeatedly, resulting in massive amounts of a single DNA sequence (Saiki *et al.*, 1985). Random amplified polymorphic DNAs (RAPDs), a type of PCR-based DNA marker, can also be modified into sequence characterized amplified regions (SCARs). The other sorts of molecular markers that are frequently practiced include: Amplicon Length Polymorphisms (ALPs), Amplified Fragment Length Polymorphisms (AFLPs), Cleaved Amplified Polymorphic sequences (CAPs), DNA Amplification Fingerprinting (DAF), Inter Simple Sequence Repeat amplification (ISSR), Simple Sequence Repeats (SSRs) or microsatellites, and Sequence-Tagged Ssites (STS). Two most important one are SSR and SNP markers, as they are now frequently utilized in wheat breeding for mapping purposes.

2-1- Simple sequence repeats (SSRs)

Microsatellites (also known as simple sequence repeats, or SSRs) are small DNA sequence motifs that are repeated in sequence. These markers have a number of benefits: i) Each locus has an distinct identity and is codominant; ii) They are commonly polymorphic at the population level, due to changes in the amount of repetitions; and iii) they are easily tested by PCR. However, in order to develop species-specific primers into the flanking regions of the repeat motif, sequence information is necessary.

A locus' polymorphism is determined by the number of repetitions, which increases or reduces the locus' length, and is often confirmed by comparing PCR-fragment length. Microsatellites have been used to assess the genetic diversity of a population (Liu *et al.*, 2010). SSR markers are considered a marker of preference since they are co-dominant, have a high level of repeatability, and can be employed effectively in plant mapping research (Tautz, 1989).

3- Molecular markers based on PCR followed by hybridization (RAPD/MP-PCR)

RAPD/MP-PCR technique was developed by Williams *et al*. (1990) and Welsh and Mcclelland (1990) independently. Amplification of genomic DNA is achieved by PCR using single, short (10 nucleotide) and random primer. Amplification occurs during PCR when two hybridization

sites are similar and move in opposing directions. The length and size of both the target genome and the primer are completely dependent on the amplified fragments (Jiang, 2013). The selected primer should have minimum 40% GC content, as a primer having less than 40% GC content will probably not withstand the annealing temperature (72°C) where DNA elongation occurs by DNA polymerase (Williams *et al.* 1990). The PCR product is subsequently separated in an agarose gel stained with ethidium bromide for visualization (Welsh and Mcclelland 1990). Polymorphism between primer binding sites can be determined by validating the presence or absence of certain bands in the electrophoresis (Jiang, 2013). The quantity and quality of DNA, PCR buffer, magnesium chloride concentration, annealing temperature, and Taq DNA are all crucial parameters that impact the reliability of RAPD markers (Wolff *et al.*, 1993).

4- Sequencing and DNA chip based markers (SNPs)

SNPs are single base-pair polymorphisms that occur in an individual's genomic sequence. SNPs may be transversions (C/G, A/T, C/A or T/G) or transitions (C/T or G/A) on the basis of the nucleotides substitution. Single base changes, such as SNPs that are insertion/deletions (InDel) in a single base, are common in mRNA. The smallest unit of heredity is a single nucleotide base, therefore SNP can provide the simplest and the most number of markers. SNPs are present in abundance in plants and animals and the SNP frequency in plants ranges between 1 SNP in every 100–300 bp (Xu , 2010). SNPs are extensively dispersed across the genome, with varied rates in the coding and non-coding regions of genes, as well as between two genes known as intergenic region (Xu , 2010). Based on various methodologies of allelic identification and detection platforms, a significant variety of SNP genotyping methods have been created. Among these, RLFP (SNP–RFLP) is the simplest and easiest method and the CAPS marker technique also can be applied in the SNP detection. If one allele possesses restriction enzyme binding sites while the other alleles do not, digestion will result in fragments of various lengths. SNPs are identified by analyzing sequencing data that has been deposited in databases. Various genotyping assays for SNPs have been developed based on a variety molecular processes. Among them, primer extension, invasive cleavage, oligonucleotide ligation and allele-specific hybridization are most important (Sobrino *et al.*, 2005). SNPs are the most appealing markers for genotyping due to a variety of modern high-throughput genotyping technologies such as NGS, GBS, and chip-based NGS, as well as allele-specific PCR (Agarwal *et al.*, 2008).

5- Diversity array technology (DArT)

DArT is a technology that allows for the genotyping of polymorphism loci that are dispersed throughout the genome. Microarray hybridization method is very repeatable. No preceding sequencing information is required for the finding of loci for a trait of interest (Jaccoud *et al.*, 2001; Wenzl *et al.*, 2004). The most significant advantage of this technology is its high throughput and inexpensive. A single-reaction experiment can genotype thousands of genomic sites to find polymorphic markers with this method. Genotyping can be done with as little as 50–100 ng of genomic DNA. The scoring and identifying of markers are both done on the same platform. There is no need for particular genotyping tests after the identification of a marker, except to begin assembling polymorphic markers into an array of a single genotype. Genotyping arrays containing polymorphic markers are commonly used for genotyping (Huttner *et al.*, 2004).

6- Expressed Sequence Tags (ESTs)

Short DNA sequences that match to a segment of a complementary DNA (cDNA) molecule that may be expressed in a cell at a certain time are known as ESTs. ESTs are currently being employed as a quick and easy way to profile genes expressed in different tissues, cell types, and developmental stages (Adams *et al.*, 1991). ESTs are single-read sequences generated from cDNAs that are normally unedited and automatically processed. The process of discovering genes using ESTs is divided into four parts: 1) The construction of cDNA libraries and single-pass sequencing of (randomly) selected clones, 2) EST quality check the removal of vector and low quality sequences, 3) The alignment of ESTs to identify the number of represented genes and 4) The annotation of these genes (Viralkumar *et al.*, 2017).

7- The Kompetitive allele-specific PCR (KASP) genotyping assay

The KASP assay uses a new homogeneous fluorescent genotyping system. It is able to deliver high levels of flexibility in generating data sets from 1 SNP to thousands of SNPs (Robinson and Ganske, 2012). KASP has been used for many years to accelerate research into improving genetics of animals (Robinson and Ganske, 2012) and plants (Delannay *et al*., 2012; Ribaut *et al.*, 2010). The mechanism of action of KASP depend on a unique florescence primer tail sequence at the 5′ end of FAM or HEX. In the first round of PCR, only the correct allele-specific primer binds and its 5′ tail is incorporated into the PCR product. On the second round, the

reverse primer releases a sequence complementary to the 5′ tail of the allele-specific sequence. This allows for the secondary fluorophore labeled oligo to bind. This releases fluoresce color. As PCR continues, generation of signal increases. After accomplishment of PCR, the fluorescent signal can be read and a genotype determined by qPCR (Smith and Maughan, 2015).

Table 8 described the advantages and disadvantages of different genetic markers for studying resistance in stem rust and the most available stem rust markers are listed in table 9.

Table 8: Advantages and disadvantages of different genetic markers (Nadeen *et al.*, 2017 with modification).

Markers	Advantages	Disadvantages
RFLPs	Co-dominant No need of prior sequence information	Time consuming High quantity of pure DNA needed Expensive Time consuming
SSRs	Co-dominant marker Less quantity of DNA is required High reproducibility	High developmental cost Presence of more null alleles Occurrence of homoplasy
RAPD	Easy to use Less quantity of DNA is required Polymorphic	Dominant Highly purified DNA is required. Low reproducibility. Not locus-specific
SNP	Cost effective Widely distributed in genome No need of prior sequence information High reproducibility Co-dominant marker	High developmental cost
DArT	Cost effective High throughput Highly polymorphic Prior sequence information not needed High reproducibility	Dominant marker High developmental cost
ESTs	Highly polymorphic A quick and easy way to profile genes High reproducibility	High developmental cost
KASP	Cost effective Lower genotyping error rate more flexible than other methods	Need of prior sequence information High developmental cost

Table 9: List of available stem rust markers (Rahmatov, 2013 with the modifications)

Gene/ QTLs	Chromosome	Marker	Type	Sequence or Primer Pair	Reference
Sr1A^{Amigo}	1AL/1RS	Xbarc1048	Xbarc1048	F 5' ACGTGGTAATTAGTTGGGAGTCTGTA 3' R 5' TGACAACCCCCTTTCCCTCGT 3'	Yu *et al.*, 2009; Saal and Wricke, 1999
		SCM9	SSR	F 5' TGACAACCCCCTTTCCCTCGT 3' R 5' TCATCGACGCTAAGGAGGACCC 3'	
		Xbarc028	SSR	F 5' CTCCCCGGCTAGTGACCACA 3' R 5' GCGGCATCTTTCATTAACGAGCTAGT 3'	
Sr2	3BS	Xqwm533	SSR	F 5' GTTGCTTTAGGGGAAAAGCC 3' R 5' AAGGCGAATCAAACGGAATA 3'	Hayden *et al.*, 2004
		stm598tcac		F 5' GTTGCTTTAGGGGAAAAGCC 3' R 5' TCTCTCTCTCTCTCACACACAC 3'	
		Xgwm389	SSR	F 5' ATCATGTCG ATCTCCTTGACG 3' R 5' TGC CAT GCACATTAGCAGAT 3'	Röder *et al.*, 1998
Sr6	2DS	Xwmc453	SSR	F 5' ACTTGTGTCCATAACCGACCTT 3' R 5' ATCTTTTGAGGTTACAACCCGA 3'	Tsilo *et al.*, 2009; Yu *et al.*, 2009
		Xcfd43	SSR	F 5' AACAAAAGTCGGTGCAGTCC 3' R 5' CCAAAAACATGGTTAAAGGGG 3'	
Sr9a	2BL	Xgwm47	SSR	F 5' TTGCTACCATGCATGACCAT 3' R 5' TTCACCTCGATTGAGGTCCT 3'	Röder *et al.*, 1998
Sr13	6AL	Xwmc580	SSR	F 5' AAGGCGCACAACACAATGAC 3' R 5' GGTCTTTTGTGCAGTGAACTGAAG 3'	Simons *et al.*, 2011
		Xdupw168	SSR	F 5' CGGAGCAAGGACGATAGG 3' R 5' CACCACACCAATCAGGAACC 3'	
Sr15	7AL	STS638	STS	F 5' GCGGTGACTACACAGCGATGAAGCAATGAAA 3' R 5' GCGGTGACTAGTCCAGTTGGTTGATGGAA	Neu *et al.*, 2002
Sr17	7BL	wPt5343	DArT	F 5' TATTCTACAACGCTCCATCC R 5' CGCATGCAANCCATACCTTT	Crossa *et al.*, 2007; Yu *et al.*, 2009
		wPt0600	DArT	F 5' AGCTCGTACAATGGTGG R 5' CATGAAATAAGCTGCCACTT	
Sr19	2BS	wPt9402	DArT	F 5' ATTTTATATTGCCGTGCCAG R 5' ATGGCCAGCACGATAGAGAG	Crossa *et al.*, 2007; Yu *et al.*, 2009
Sr22	7AL	cfa2123	SSR	F 5' CGG TCTTTGTTTGCTCTAAACC 3' R 5' ACC GGC CATCTATGATGAAG 3'	Yu *et al.*, 2010
		cfa2019	SSR	F 5' GACGAGCTAACTGCAGACCC 3' R 5' CTCAATCCTGATGCGGAGAT 3'	
		Xbarc121	SSR	F 5' ACTGATCAGCAATGTCAACTGAA 3' R 5' CCGGTGTCTTTCCTAACGCTATG 3'	
Sr24	3DL	Xbarc71	SSR	F 5' GCGCTTGTTCCTCACCTGCTCATA 3' R 5' GCGTATATTCTCTCGTCTTCTTGTTGGTT 3	Mago *et al.*, 2005; Yu *et al.*, 2010
		Sr24#12	AFLP	F 5' CACCCGTGACATGCTCGTA 3' R 5' AACAGGAAATGAGCAACGATGT 3'	
Sr25	7DL	BF145935	EST	F 5' CTTCACCTCCAAGGAGTTCCA C 3' R 5' GCGTACCTGATCACCACCTTGAAGG 3'	Ayala-Navarrete *et al.*, 2007
		Gb		F 5' CAT CCT TGG GGA CCT C 3 R 5' CCA GCT CGC ATA CAT CCA 3	Yu *et al.*, 2010

Gene/ QTLs	Chromosome	Marker	Type	Sequence or Primer Pair	Reference
Sr26	6AL	Sr26GSPF	PPT	F 5' GGAATACTCGAATACCAGGCCAT 3' R 5' CCTTAGAGCTTATGGTCCGGTA 3'	Zhang *et al.*, 2021
		Sr26GSPR	PPT	F 5' TTGCCACTGTGAACATGTTTATAGAT 3' R 5' AACGGTGACATTGTACAAATATCTA 3'	
Sr28	2BL	wPt7004-PCR	DArT	F 5' CTCCCACCAAAACAGCCTAC 3' R 5' AGATGCGAATGGGCAGTTAG 3'	Rouse *et al.*, 2012;
		wmc332	SSR	F 5' CATTTACAAAGCGCATGAAGCC 3' R 5' GAAAACTTTGGGAACAAGAGCA 3'	
Sr31	1BL/1RS	1B-159		F 5' AGCGCAGATAATGTTTGAACC 3' R 5' AAGTCGAAACCACAGTTATC 3'	Mago *et al.*, 2004;
		Iag95	STS	F 5' CTCTGTGGATAGTTACTTGATCGA 3' R 5' CCTAGAACATGCATGGCTGTTACA 3	Mago *et al.*, 2002;
		wpt8949	DArT	F 5' TGGGATGCGAGAATATCCGG R 5' TGCGATGCCTAAAGCCTCTC	Crossa *et al.*, 2007; Yu *et al.*, 2009
		wpt1328	DArT	F 5' GCGCCGGTCGGACAGACCGG R 5' GAACTACTAATTACTGTACA	
Sr32	2AS, 2B	STM773	SSR	F 5' AAACGCCCCAACCACCTCTCTC R 5' ATGGTTTGTTGTGTTGTGTGTAGG	Somers *et al.*, 2004; Yu *et al.*, 2009
		Xbarc55	SSR	F 5' GCGGTCAACACACTCCACTCCTCTCTC 3' R 5' CGCTGCTCCCATTGCTCGCCGTTA 3'	
Sr33	1DS	Abc156	STS	F 5' TTACGGGATCAAAGCTGAGGC R 5' GACAAGCAACACCAACCAAGC	Mago *et al.*, 2002; Yu *et al.*, 2009
Sr35	3AL	Xcfa2170	SSR	F TGGCAAGTAACATGAACGGA R ATGTCATTCATGTTGCCCCT	Yu *et al.*, 2009; Zhang *et al.*, 2010
		Xwmc559	SSR	F ACACCACGAATGATGTGCCA R ACGACGCCATGTATGCAGAA	
		Xcfa2076	SSR	F CGAAAAACCATGATCGACAG R ACCTGTCCAGCTAGCCTCCA	
		Xwmc169	SSR	F TACCCGAATCTGGAAAATCAAT R TGGAAGCTTGCTAACTTTGGAG	
Sr36	2BS	Xgwm319	SSR	F 5' GGTTGCTGTACAAGTGTTCACG 3' R 5' CGGGTGCTGTGTGTAATGAC 3'	Tsilo *et al.*, 2008; Yu *et al.*, 2010
		Xwmc477	SSR	F 5' CGTCGAAAACCGTACACTCTCC 3' R 5' GCGAAACAGAATAGCCCTGATG 3'	
		Xstm773-2	SSR	F 5' ATGGTTTGTTGTGTTGTGTGTAGG 3' R 5' AAACGCCCCAACCACCTCTCTC 3'	
Sr39	2B	Sr39#22r		F 5' AGAGAAGATAAGCAGTAAACATG R 5' TGCTGTCATGAGAGGAACTCTG	Mago *et al.*, 2009
		Be500705		F 5' ATCTGTGGCAGTGTGCTCCT R 5' TCCTGCAAATGCTTGTCGTT	
		Sr39#50s		F 5' CCAATGAGGAGATCAAAACAACC R 5' CTAGCAAGGACCAAGCAATCTTG	
Sr40	2BS	Xgwm344,	SSR	F 5' CAAGGAAATAGGCGGTAACT 3' R 5' ATTTGAGTCTGAAGTTTGCA 3'	Yu *et al.*, 2009; 2010
		Xwmc661	SSR	F 5' CCACCATGGTGCTAATAGTGTC R 5' AGCTCGTAACGTAATGCAACTG	
		Xgwm374	SSR	F 5' ATAGTGTGTTGCATGCTGTGTG 3' R 5' TCTAATTAGCGTTGGCTTGCC 3'	
		Xwmc474	SSR	F 5' ATGCTATTAAACTAGCATGTGTCG R 5' AGTGGAAACATCATTCCTGGTA	

Gene/ QTLs	Chromosome	Marker	Type	Sequence or Primer Pair	Reference
Sr44	7DS	Wpt2565	DArT	F 5' TACTTTGATTTGGTCAGTTG R 5' TCGCGACCAAGCTCTACAAT	Crossa *et al.*, 2007
		Cdo475	RFLP	F 5' GACACATTGACCGCATCTTA R 5' CCTTCACCTCGCTCCCTACC	Yu *et al.*, 2009
Sr45	1DS	Xwmc222	SSR	F 5' AAAGGTGCGTTCATAGAAAATTAGA R 5' AGAGGTGTTTGAGACTAATTTGGTA	Yu *et al.*, 2009
		Xcfa2158	SSR	F 5' TTTCGTCTTCAAAATGCACTG R 5' TGGTAGCTTACAAAGGTGCG	
Sr50 (R)	1DL/1RS	AW2-5		F 5' GAATCCCATTGTTCAGCAAGT 3' R 5' TAGCACTCCAGCAGACTCCAC 3'	Anugrahwati *et al.*, 2008
		CI2F	RFLP	F 5' AGGGTCACACAGGCAATCTAA 3' R 5' CATTCTGGTTTTCCGCAGCAAC 3'	Mago *et al.*, 2004
		1B-159		F 5' AGCGCAGATAATGTTTGAACC 3' R 5' AAGTCGAAACCACAGTTATC 3'	
		1B-267		F 5' GCAAGTAAGCAGCTTGATTTAGC 3' R 5' AATGGATGTCCCGGTGAGTGG 3'	
		Xmwg060	STS	F 5' CAACGATACAACAGGCTCAA R 5' CTGGATAGAGAAGCCATGGA	
Sr52	6AS	BE497099-STS	STS	F 5' TTCGCTCCACCAGGAGTCTA 3' R 5' GTGTCTCGCCATGGAAGG 3'	Qi et al, 2011; Röder *et al.*, 1998
		WMS570/ Xgm570	SSR	F 5' TCGCCTTTTACAGTCGGC 3' R 5' ATGGGTAGCTGAGAGCCAAA 3'	
Sr59	2DS.2RL	KASP_2RL_c25837	KASP	A1 5' TAGTGTTTTGCTCGACCACTGTC 3' A2 5' GTTAGTGTTTTGCTCGACCACTGTT 3' C1 5' CACCAAACACTACCCACACCATCTA 3'	Rahmatov *et al.*, 2016
		KASP_2RL_c21825		A1 5' ACATTTCGGTTGGTATTGATTCTAACG 3' A2 5' ACATTTCGGTTGGTATTGATTCTAACC 3' C1 5' CCAGCCATGAAGAAAATAACAATTCGAGAT 3'	
		KASP_2RL_c20194		A1 5' CCAGCTAGGACAAACTTTGCCTAAA 3' A2 5' CAGCTAGGACAAACTTTGCCTAAG 3' C1 5' CTTGTGGGCGCTCGTGGCTTT 3'	
Sr60	5A^{m}S	gwm154	SSR	F 5' TCACAGAGAGAGAGGGAGGG 3' R 5' ATGTGTACATGTTGCCTGCA 3'	Chen *et al.*, 2018
		gwm415		F 5' GATCTCCCATGTCCGCC 3' R 5' CGACAGTCGTCACTTGCCTA 3'	
		gwm156		F 5' CCAACCGTGCTATTAGTCATTC 3' R 5' CAATGCAGGCCCTCCTAAC 3'	
		gwm186		F 5' GCAGAGCCTGGTTCAAAAAG 3' R 5' CGCCTCTAGCGAGAGCTATG 3'	
Sr61	6E	*Sr61GSPF*	PPT	F 5' AACCAACAATTCGATGACACAAGG 3' R 5' CGCCTCTAGCGAGAGCTATG 3'	Zhang *et al.*, 2021
		Sr61GSPR	PPT	F 5' CGATATCTACGTGCATTTGATTTACG 3' R 5' CGCCTCTAGCGAGAGCTATG 3'	
Sr62	1BL/1DL	*C11837_STS-4*	STS	F 5' CGTGCCTATTCTGTCTGTACC 3' R 5' CACATACTGACTTTCCTCTCAAA 3'	Yu *et al.*, 2022
		C69317_STS	STS	F 5' TATGCACAACGGAAGCCTTC 3' R 5' TGCCAATCAATTTCACGAGATCC 3'	
		C122784r_KASP	KASP	A1 5' GTCAGTTGTCCAAATGCACCA 3' A2 5' GTCAGTTGTCCAAATGCACCT 3' C1 5' GGTAACCCCACGAGACGATAA 3'	

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