A look into stem rust resistance breeding

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Abstract

Wheat is one of the largest food commodities globally, but the cultivation of wheat faces many challenges. Among these is the stem rust disease, which historically has caused mass starvation and misery. This thesis explores the stem rust disease in wheat, and the plant breeding for the potential of resistance to stem rust in the future. As well as challenges in the practical, political and social aspects that lengthen the road to progress. The purpose of the thesis is to spread awareness and knowledge about the subject of plant breeding and the spread of high-threat races of stem rust.

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1. Introduction

Bread wheat (*Triticum aestivum*, genome BBAADD) is a hexaploid species that emerged 8,500–9,000 BC through hybridization between a domesticated tetraploid progenitor (genome BBAA), and a diploid carrier of the D subgenome. Not long after its formation in the fertile crescent, it spread throughout much of the world (Levy, 2022). Today more than 2.5 billion people consume wheat based products making it one of the largest single food products worldwide and a staple food of humanity. Wheat production in any setting is constrained by various abiotic (non-biological) conditions like drought, flooding, cold, heat, wind, hail and so on. As well as biotic conditions like diseases caused by bacterial, viral and fungal pathogens. However, when adding the conditions of the current global market brought upon by, among many other hardships, the war between Russia and Ukraine (which, previous to the war, together supplied about 28% of the world's wheat) and subsequent restrictions on trade then the current challenges posed on global production becomes immense (Bentley, 2022). So, because of the current challenging economic environment, more efficient and secure methods of producing wheat are required.

Humans have known that crops become diseased since ancient times and early references to plant diseases go back to 2000 BC, with mentions of rust diseases being made by the Romans later in the fourth century BC (Tronsmo et al. 2020). The agricultural plague that is rust diseases, has likely followed grass crops since their domestication, and among them the stem rust disease (also known as black rust) stands out as one of the most disruptive diseases to human cultivation (Lind, 2017; McIntosh, 2009). It is caused by the heteroecious fungus *Puccinia graminis* f. sp. *tritici* (*Pgt*). It has the capabilities to infect all commonly grown cereals in Sweden, as well as several species of wild grasses like timothy and couch grass (Berlin, 2012). Due to rigorous breeding of wheat throughout the years however, most varieties generally have one or more stem rust resistance genes. Which in combination with the eradication of barberry (*Berberis vulgaris*) during the 20'th century, led to a somewhat false sense of security in Sweden as well as Europe and the US where similar eradication projects were used. But this notion is being challenged by the looming presence of new virulent races of *Pgt* such as the TKTTF race and the Ug99 group of races which threaten wheat production on a large scale, making them one of the biggest pathological threats we are facing today (Patpour, 2022).

Developing new varieties with effective and durable disease resistance against stem rust is therefore more important now than it has ever been, as it has the potential to stabilize yields and reduce economic loss in the continuously unstable world of agriculture. However, breeding programs face many obstacles on their way to results whether it be technological limitations, lackluster funding, poor access to dedicated equipment and facilities, or social opposition. My aim in this thesis is to explore the reality of the field of plant breeding through the lens of stem rust resistance breeding, and elaborate on what challenges and obstacles hinder the advancement of the technology.

2. Purpose and question

Although the thesis aims to explore the field of plant breeding through the lens of stem rust resistance breeding, the purpose also takes on the additional duty of spreading awareness and knowledge about the fields of plant breeding as well as the spread of high-threat races of stem rust. In order to help combat some of the largest challenges that these fields are facing today, namely social opposition brought on by misinformation and lack of knowledge, as well as the lack of funding. This thesis also takes on mainly a global perspective but makes connections to Swedish agriculture as the author as well as the main target group of readers are active in Sweden.

There are three questions posed in this thesis, on which aim and purpose are based. First, how big of a threat is stem rust globally? Second, what obstacles are there in the way of overcoming this threat? And third, what possibilities does plant breeding pose for the future of agriculture?

2.1 Abbreviations

f. sp.	*formae specialis.*
Ug99	Uganda 1999, the place and year of discovery and registration of this race.
Sr31	Stem rust resistant gene 31.
Pgt	*Puccinia graminis* f. sp. *tritici.*
2DL	The second chromosome in the D subgenome, long arm.
TKTTF	An example of the American letter code system for stem rust races.

3. Stem rust - the disease caused by *Puccinia graminis*

3.1 Stem rust, historically

The regions of the eastern Mediterranean sea have allowed man since antiquity to grow and cultivate many plants, with some of the decidedly most important ones being cereals such as oats, barley, rye and wheat. And since antiquity these have suffered from worldly conditions such as drought, insects, weeds and disease (Agrios, 2005). Most families historically grew their crops both for consumption and seed for the next season, making the genetic variety generally low and the crop susceptible to numerous pests and diseases leading to many instances of hunger and survival problems for early farmers (Agrios, 2005; Leonard & Szabo, 2005). During the fourth century B.C. the Romans suffered through hunger so frequently brought upon by rust and other diseases that they dedicated worship to a new addition to their pantheon, the God Robigus whom they also gave a festival at the end of April called Robigalia were they sacrificed red dogs, cows and foxes in order to appease the god so that he would not send rust to destroy their crops (Agrios, 2005; Leonard & Szabo, 2005).

Since the Romans, mentions of the rust diseases remained relatively quiet up until the 17'th century when in 1660 French farmers in the region of Rouen made the connection that barberry plants contributed to the spread of rust diseases (Tronsmo et al. 2020). Legislation was then made that mandated the eradication of barberry plants near fields of wheat cultivation, 200 years before Anton de Bary made public his discovery that stem rust is heteroecious (Tronsmo et al. 2020). Further legal actions were later taken to separate barberry bushes from grain production in other regions, for instance in 1726 in Connecticut, USA, which marked the first of many similar laws in the country (Roelfs, 1982). In 1916 an epidemic of stem rust ravaged many parts of the northern hemisphere including Sweden and the American plains regions of the USA and Canada where it led to a wheat yield reduction of close to 70% compared to 1915 prompting the start of the American barberry eradication program (Roelfs, 1982). The combination of the epidemics of 1916 and the resulting food shortages was also enough for Sweden to adopt its own “barberry law” in 1918. Yet in 1951 an especially severe stem rust epidemic struck and the country lost 20% of its winter wheat and 50% of its spring wheat (Kjellström, 2021). However, the “barberry law” in Sweden was upheld in 1994 (Lind, 2017; Wirsén, 2010).

In modern agriculture stem rust-induced losses may be severe depending on factors like the climate during the year, and since stem rust is favoured by a hot and humid climate the changes brought on by global climate change enhances the prospects for the disease. A modern example of loss is the epidemic that occurred in Ethiopia during the season of 2013-2014 led to up to 92% yield losses compared to the previous year in some regions and harvest yields as low as 0.3 t/ha. This epidemic was primarily caused due to a race of stem rust named TKTTF which proved highly virulent to an otherwise resistant variety named ‘Digalu’ that at the time was resistant to all known races in the highly virulent Ug99 group of *Pgt*, a race group that holds virulence to the vast majority of all wheat varieties grown globally (Olivera, 2015).

The disease had not been deemed a significant threat in Europe since the 1960’s, but that changed in 2016 with the emergence of a new *Pgt* race in Sicily, which caused widespread epidemics in both durum and bread wheat due to this race carrying virulence to a specific resistance gene in wheat named *Sr13b* (Bhattacharya, 2017). Additional outbreaks also followed after 2016 in Central- and West Europe (Patpour et al, 2022). In 2017 an instance of stem rust was discovered on spring wheat in Sweden which was later proven to be genotypically diverse which indicated that barberry had been involved in the outbreak. This in extent meant that stem rust once again had established a more permanent foothold on Swedish wheat than earlier. Although stem rust on other cereals had never disappeared from the country it was all but eradicated on wheat up until this point thanks to a small barberry population and the efficient use of resistant varieties. Further resistance tests also showed that most wheat varieties in the country lacked resistance to this race of stem rust (Kjellström, 2021; Vetenskapsradion nyheter, 2021).

The return is believed to be due in large part to the significant increase of barberry in Sweden since 1994 (Lind, 2017; Patpour et al., 2022). Yet since then, stem rust losses have not been significant in Swedish wheat due to the late appearance of the pathogen. However, a warmer climate in the early season would give stem rust an earlier start which could potentially lead to greater losses. The same could be said about new races emerging with new combinations of the existing virulence genes through sexual reproduction via barberry that is evidently becoming more common in Sweden (Lind, 2017; Vetenskapsradion nyheter, 2021).

3.2 *Puccinia graminis* – the pathogen

3.2.1 Growth conditions

Pgt is favored by hot and humid weather with the optimal conditions for infection being 8-12 hours of dew at a temperature of around 18 ºC, followed by a light rate of 10,000 lux and a temperature increase to 30 ºC with the dew being dried away slowly (Roelf, 1992). It however also thrives in colder climates such as the north of Europe where it can overwinter and spread thanks to the weathering of the teliospores, which in spring then germinates and forms basidiospores that can infect barberry.

3.2.2 Lifecycle

Stem rust also known as black rust due to the dark color of the overwintering teliospores, is caused by the fungus *Puccinia graminis* which belongs to the phylum Basidiomycota, class Urediniomycetes, order Uredinales, and family Pucciniaceae (Leonard & Szabo, 2005). Wheat is attacked by the fungus *Pgt* which is heteroecious with its alternative host being the common barberry (*Berberis vulgaris*) and many other species in the barberry family, which facilitates the fungi's sexual reproduction. The fungus can also infect and reproduce asexually on gramineous hosts of many kinds other than just wheat, including the common couch weed (*Elytrigia repens*) and timothy (*Phleum pratense*) (Leonard & Szabo, 2005). The life cycle of *Pgt* (Fig. 1) consists of five distinct spore stages split between the asexual reproductive stage on the gramineous host and the sexual stage which is completed on berberis. These stages are: basidiospores, pycnidiospores, aeciospores, urediniospores and teliospores.

Basidiospores are one of the haploid spore stages, which typically infect the young tissue of the barberry plant in the spring. As these land on the barberry leaves they germinate and start to develop pycnidium. Haploid pycnidiospores (male gamete) are formed within the pycnium and are secreted together with a nectar to attract insects which alongside rain splashing, help to spread the pycnidiospores to other pycnidia with receptive hyphae (the female gamete) (Hale et al., 2012; Kjellström, 2021). The two mating types are then fused to a dikaryotic stage where hyphae will extend from the pycnium through the leaf to form bell-shaped, diploid aecium on the bottom side of the leaf. These then form aeciospores, thus completing the sexual stage of the fungus, which can then infect the gramineous host.

When infecting the gramineous host, a hyphal mat is developed below the epidermis from which dikaryotic urediniospores meaning that the cells contain two nuclei. These are developed in such amount that it bursts through the epidermis of the infected tissue and creates brown, rust-coloured pustules. In severe infections these pustules can also spread to the leaves and heads of the plant (Leonard & Szabo, 2005). The urediniospore stage makes up the majority of the life cycle in temperate climates as continual uredinial generations produce more urediniospores clonally, that can spread on the wind to infect more grasses and thus repeat the process throughout the season (Singh et al., 2002). Later in the growing season of the host, two-celled telium will start to form from the same hyphal mat as the urediniospores and will start to replace them. These teliospores are dikaryotic, however early in the maturation of the teliospores they go through karyogamy, soon followed by meiosis, but this process is suspended during dormancy (Leonard & Szabo, 2005). The spores also have a thicker and darker cell wall which appears black prompting the name "black rust" in Europe. In relation to this the name "stem rust" is the American name of the disease. These specialized cell walls allow the teliospores to withstand harsh conditions and thus they can overwinter in colder climates unlike any of the other spores in the life cycle. In the spring, remaining meiosis stages are completed resulting in four haploid cell nuclei that later becomes the basidiospores, which are released to infect barberry thus completing the life cycle (Agrios, 2005; Leonard & Szabo, 2005; Leonard & Szabo, 2000).

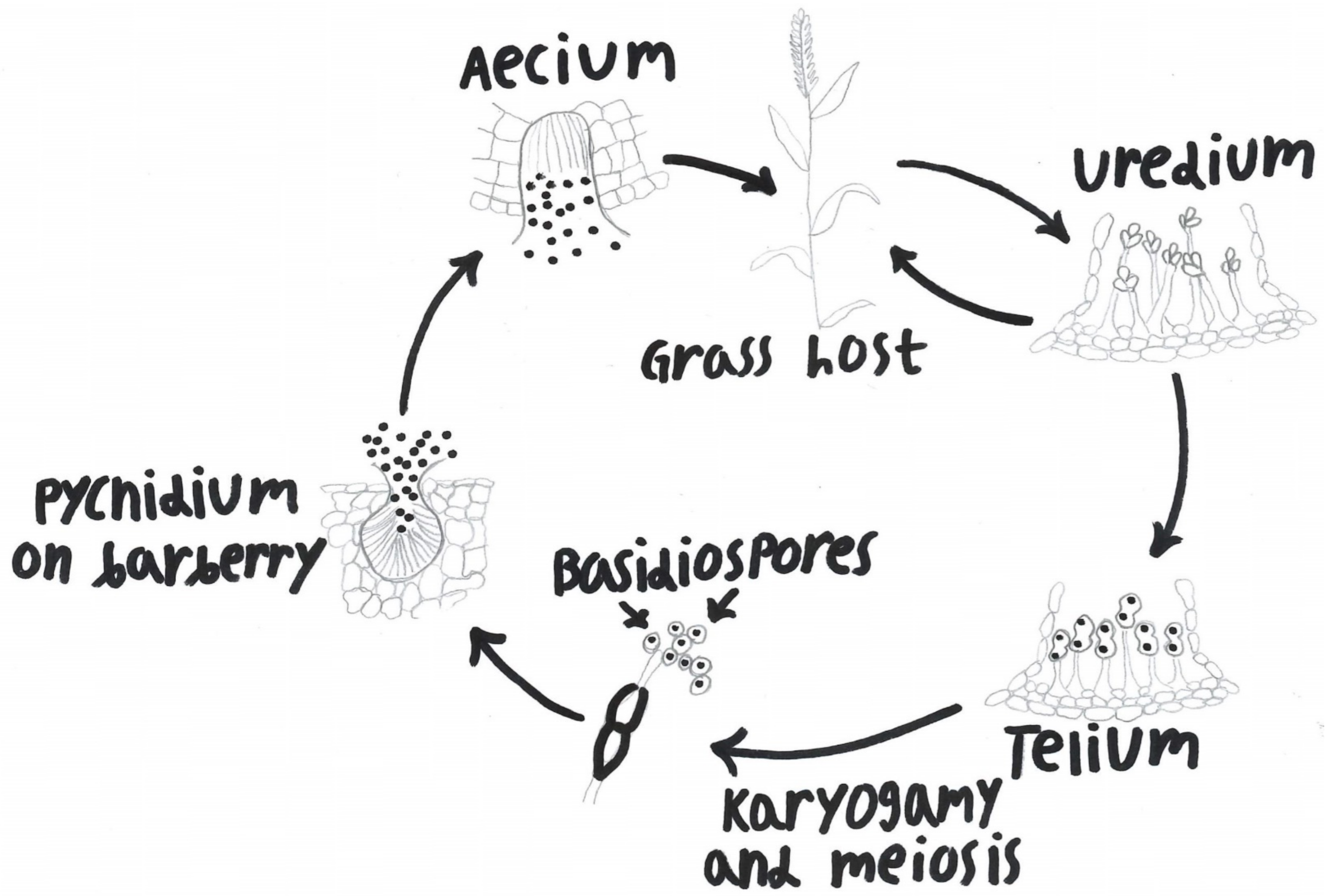

Fig 2. The life cycle of *Puccinia graminis* illustrated by the thesis author. As the grass host gets infected by aeciospores, a hyphal mat and later uredium are formed. Asexually produced urediniospores can then re-infect a new grass host and create additional infection. In the late season telium will start to occupy the same hyphal mat as the uredinium, and soon only the telium will remain, in a dormant stage during winter. In spring the dikaryotic cells of telium go through karyogamy and later meiosis before basidiospores are generated, which travel to the young leaf tissue of the barberry plant and infect it prompting the formation of haploid pycnia. The pycnia later produces flexuous hyphae that penetrate the leaf and forms bell-shaped aecium on the underside which in turn produces aeciospores that can infect the grass host (Leonard & Szabo, 2005).

3.2.3 The emergence of high-threat races

In plant pathology, a race refers to a pathogen's ability to cause disease in its host, and by using a set of differential host varieties with unique combinations of resistance genes it is possible to determine pathogenic races. This is common practice while the naming of the race (nomenclature) that follows varies around the world with many different systems and no one unifying system. In scientific circumstances the most popular nomenclature system for stem rust is based on five-letter sequences as a result of the differential host tests, which results in names like TKTTF or TTKSK (Alpern et. Al., 2020; Rust Tracker, n.d). Another common practice is to name a race based on the name of the infected wheat variety resulting in names like: "Digalu variant". This name-based naming system is practical to use in non-scientific circumstances as a farmer for example, will immediately understand that a particular stem rust race is virulent to the wheat variety in the race-name.

In the 1960s Norman Borlaug produced several semi-dwarf wheat varieties that proved resistant to *Pgt*, and from this programme grew the international maize and wheat improvement center (CIMMYT). During the 1970's CIMMYT started to introduce resistance genes, including the *Sr31* gene derived from rye, into several wheat varieties popular in the Americas, Europe, and Asia (Lidwell-durnin & Lapthorn, 2020). This later led to the near-disappearance of stem rust in the 80's and 90's. Thus, for several decades stem rust was considered less and less of a threat, which consequently led to a large shift in focus and research away from stem rust and onto more relevant diseases at the time such as stripe-, and leaf rust (Lidwell-durnin & Lapthorn, 2020). When a new race of stem rust was discovered on a *Sr31* carrying wheat variety in a plant nursery in Uganda in 1998, it got off to somewhat of a head start in relation to the work being done in combating the disease due to this shift (Singh, 2008).

This new stem rust race was designated Ug99 and became the first race to overcome the *Sr31* resistance gene which at the time had been effective for some 30 years globally. Subsequent additions to the lineage further cemented its wide virulence, overcoming most of the world's existing rust-resistance genes in wheat. In the years following the discovery of Ug99, it spread to many regions and countries along the east- and south coasts of Africa and across the middle east (fig. 2), with the Ug99 group as well as a variety of the TKTTF race being found in Iraq in 2019 (Singh, 2015; Singh, 2008; Nazari, 2021).

This, alongside the possibility of spread of Ug99 into large wheat production areas such as Europe, Ukraine, Kazakhstan and later India and China, makes the threat of severe epidemics very real since most commonly grown varieties of wheat in the world are susceptible (Dixit, 2013; Singh, 2008). The TKTTF "Digalu" race on the other hand, was found in the UK in 2013 and was proven to be virulent to some 80% of all varieties in the country. This, combined with the rapid growth of barberry and evidence of an increasingly humid climate, makes the possibility of a serious stem rust epidemic in the UK a growing threat. (Lewis et al., 2018). All of which is without mentioning the possibility of new "super races” emerging in the areas where, for instance, both the TKTTF race and a Ug99 group member can be found. The combination of these two in particular risks creating a race more aggressive than either of the parents, and today there are several regions and countries where the possibility exists (Vetenskapsradion nyheter, 2021; Singh, 2008; Olivera, 2015; Olivera, 2019). Luckily however breeding efforts during the last two decades have helped to mitigate the spread and overall threat of Ug99 and other virulent races.

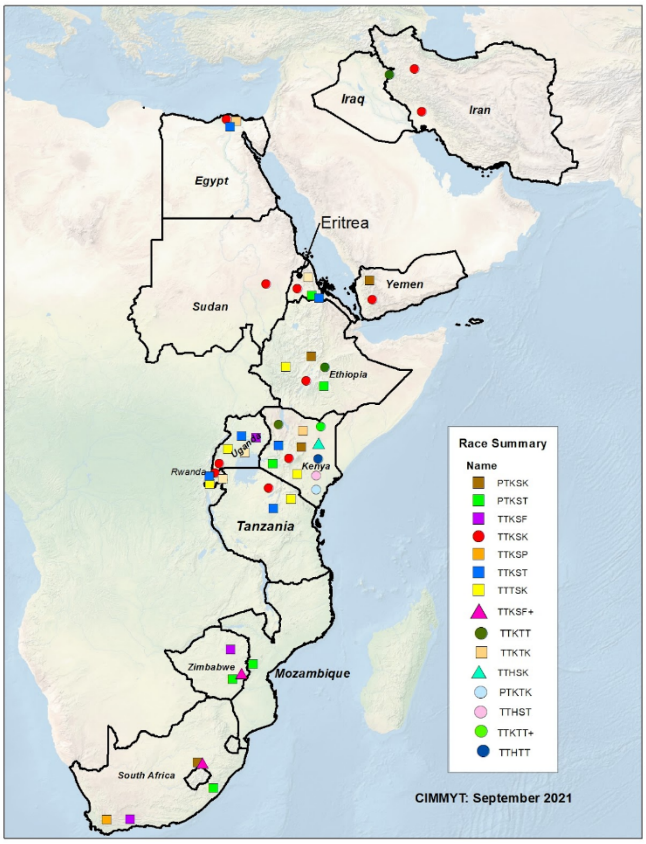

Fig 2. The spread of the Ug99 group of races in September of 2021. CIMMYT. (2021). *Spread of* Ug99 *races*. (Illustration). https://rusttracker.cimmyt.org/?page_id=22 (usage approved by Dave Hodson at CIMMYT) [2023-05-24]

3.2.4 Spread

Puccinia graminis spreads mainly through passive means like wind, using the enormous numbers of spores produced in the uredinio stage. These spores are liberated from their host and picked up by turbulent winds on ground-level, which carry some spores to the linear winds high up in the sky which facilitates the long-distance travel that may carry the spores across oceans and continents to later be deposited either by slowing winds or by rainfall (Nagarajan et al., 1990; Brown & Hovmoller, 2002). This method of dispersal is naturally not very efficient on an individual scale as most of the spores produced most likely will end up on nontarget sites in uncongenial environments or on non-susceptible host, and thus not be able to survive. Yet however inefficient and wasteful this method may inherently be, the random profligacy is in large part compensated for by the truly enormous amount of spores produced per area unit (Nagarajan & Singh, 1990). Single-step pathogen invasions like this are rare in comparison to the more common step-by-step movement and gradual dispersal of spores facilitated by host-to-host dispersal. In some cases through established "puccinia pathways" that are predictable on the basis of host availability and seasonally prevailing winds in the affected regions (Brown & Hovmoller, 2002).

Before exemplifying one such "Puccinia pathway" in the USA, some context is needed: as previously mentioned efforts have been ongoing to some extent in the US to eradicate barberry in favour of wheat production since the start of barrbery eradication programme 1918. This has proven to be an efficient way of controlling the genetic diversity of the disease and thus making it easier for farmers to protect themselves by growing resistant races. However, sometime after the introduction of the programme, it was understood how the continental spread of stem rust epidemics works. Urediniospores overwinter in wheat fields in the southern US and northern Mexico from where they can later spread on the winds up north and infect many of the now more controlled areas. And depending on if the climate of the year in these warmer parts of the continent is favourable to urediniospore production, the urediniospores could arrive in time and in sufficient numbers to cause epidemics in the northern wheat growing fields, bringing the potential for great losses (USDA, 2017; Schumann, 2011).

Other means of transportation aside from wind include animals like birds and of course humans. Humans may prove especially efficient at spreading the spores as global travel carries people and goods across the world. Furthermore, the travel of farming equipment, livestock and material such as straw or clothes carrying the disease also spreads the pathogen (Berlin, 2012). The planting and re-introduction of barberry also contributes greatly to the spread and diversity of the pathogen in certain regions, like the Caucasus mountain region or more specifically the country of Georgia where there is a wide distribution of the plant around large wheat growing areas (Olivera, 2019).

This does not however make Georgia more of a hotspot than Sweden for instance, where the rate of new stem rust races produced is comparable. However, the distribution capabilities of said new races from Sweden into other countries are not comparable to the ones of new races spreading out of Georgia. This has to do mainly with the typical weather pattern in the regions as winds tend to blow north from Sweden into Norway and the Nordic sea. While any wind blowing out of Georgia has the potential to distribute any new races into the neighboring countries such as the European continent, India and Turkey. There is already an example where the *Pgt* race TKTTF was identified in Georgia before it spread into Turkey and later globally (Nagarajan et al., 1990; Rahmatov, 2013; Bentley, 2022; Olivera, 2019).

4. *Pgt* resistance breeding and surveillance

Fungal disease prevention includes methods used in-field such as crop rotation and tillage, but talking about a specific disease like stem rust there are accompanying specific prevention methods. Today there are two predominantly used methods of rust disease management, and those are the application of fungicides and the use of disease resistant varieties. By using more resistant varieties the need for fungicides is reduced and potential consequential loss of resources and environment is prevented which is in accordance with the IPM Directive (2009/128/EC) in the EU and the global sustainability goals 1, 2, 8, 12, 14 and 15 (UN, nd). The yields can at the same time be stabilized by also adding abiotic and various other biotic stress resistances for a changing climate in the breeding process, further saving on resources (Rahmatov, 2016; Cowger et al., 2022). In combination with the previously mentioned spread of high-risk races, the prospect of developing more resistant wheat varieties becomes an attractive solution to more than the initial problem of local disease prevention and yield loss.

4.1 Surveillance

The spread and re-establishment of new virulent races of *Pgt* has been noticeable during the last 25 years since the epidemic in Uganda in 1998 and subsequent registration of the high-threat Ug99 race group, which brought the threat of stem rust to the world's wheat fields back into focus. This prompted new action to be taken and in 2005, the Borlaug Global Rust Initiative (BGRI, part of Cornell University) was established to, among other missions, meet the threat of plant diseases head on. This has also led to the establishment of other organizations that help to track and survey the disease, like for example the Global Rust Reference Center (GRRC) which provides an early warning system for the spread of rust in Europe, and Rusttracker.org (BGRI, 2023a; Hansen, 2022a). Today the surveillance of the spread of the disease is extensive, yet the intensity varies by country and region. Most countries have their own surveillance programmes but they can differ in extension and may not reach all the way out to where they may be needed, leaving certain blind spots globally that may or may not contain large amounts of the disease. When a sample from a plant infected by stem rust is collected it may be race typed (phenotyped and genotyped) domestically or shipped for testing at international institutions like for instance the GRRC in Denmark.

A success-story-example of efficient surveillance would be the early identification of the spread of the “Digalu variant” (TKTTF) in Ethiopia in 2014 thanks to a greatly improved and extensive monitoring and response system. This later led to the identification of TKTTF-resistant races and the distribution of these in at-risk zones in Ethiopia in time for the 2015 planting season. This rapid response thwarted what may have been a major epidemic in parts of Ethiopia in 2015 and 2016 and saved many farmers from potentially great yield and subsequent income losses. Yet the Digalu variant is still spreading throughout the world, and more work like that in Ethiopia will be necessary to prevent new epidemics. As of 2016, this new race was confirmed in nine countries including Turkey, Iran, Lebanon, Egypt, Ethiopia, Georgia, Azerbaijan, Eritrea and Yemen (BGRI, 2023b).

4.2 Breeding

On a global scale, many international organizations that contribute to stem rust resistance breeding with some of the most prevalent being the Borlaug Global Rust Initiative (scientific/breeding network), the Consultative Group for International Agricultural Research (CGIAR) and the International Maize and Wheat Improvement Center (CIMMYT). As well as public organizations such as universities like the Swedish University of Agricultural Sciences (SLU) and several private multinational seed companies that contribute to the wheat germplasm and help to produce resistant wheat seed (Figlan et. al., 2020). The actual work itself can be split into two distinct fields, pre-breeding and breeding. And while both occur in the public sector as well as in the private sector, pre-breeding is generally performed by publicly funded efforts taken in universities for example, most likely due to low profit margins.

Any activity that precedes the development of a new variety and reshuffling of genes by a plant breeder will be termed ‘pre-breeding’. It may for instance entail the finding and mapping of new alleles and other genetic materials in an organism, a plant in this case, to ease the process of the actual breeding that may follow. It could be a certain resistance gene to stem rust, or another disease, that may be suspected to be present in another variety or a wild relative. And if there is an interest to know more about this or possibly move this gene into another variety, then a pre-breeding process might conclude the loci of the single gene in question or even the several genes that make out the resistance and how they correlate. In contrast to this, breeding is the act of creating enhanced varieties with the aim to bring in new functional diversity that may entail higher yield, greater protein quality, shorter stems, greater nutritional value and/or disease or stress resistance among many other possible functions (Raynolds & Braun, 2022). Since plant breeding in general takes a very long time, it is normal for a breeding project to focus on introducing several of these traits and/or other disease resistances, and thus *Pgt* resistance will not always be the main focus of the development of a new variety.

Genetic resistance to a pathogen can be more or less durable, which refers to the amount of time that particular resistance is active before the disease overcomes it and renders it so much less effective that it no longer can be considered effective. In general, resistance to *Pgt* as well as other rust pathogens is commonly characterized as being part of one of the following three general categories. These in themselves depend on factors like the crop growth stage at which the mechanism activates, and the type of resistance gene. 1, race-specific seedling resistance. 2, race-specific Adult Plant Resistance (APR). 3, race non-specific APR, which is also known as slow-rusting or as partial resistance (Lan et al, 2017). Seedling resistance, or as it is also called: all-stage resistance, is resistance that is expressed throughout the entire lifecycle of the plant. While APR is expressed only in the later stages of growth typically after the start of the heading stage in cereals. The race-specific resistance-genes (or simply R-genes, also known as vertical or qualitative resistance) in 1 and 2 can provide relatively high resistance by "specializing" in attaching to specific proteins produced in the pathogen through direct protein-to-protein interaction, and are thus easier to select for when breeding. However, given the swift selection of the pathogen through many generations in one season, R-genes tend to give in to new races of the disease relatively quickly. This is especially true when deployed individually in relation to if they were deployed together with one or more genes for resistance in a process referred to as "pyramiding" (Wang et. al., 2023). This "defeat" of the resistance can then result in a "boom and bust" cycle of high productivity followed soon after by widespread and potentially calamitous disease outbreaks. And for this reason, individual race specific resistance genes are usually not considered durable (Wang & Chen, 2017).

In relation to this, race non-specific genes (horizontal or quantitative) confer only partial yet broad-spectrum resistance against several races of *Pgt*. When these genes are pyramided in new wheat varieties with other minor genes (resistance genes with minor effects), they can present a more genetically complex resistance that pathogen mutations will not overcome effortlessly, making it generally more durable. Several seedling, race-specific resistance genes characterized in wheat are correlated with various degrees of hypersensitive responses through programmed cell death in the host, if the pathogen possesses the corresponding avirulence genes as described in the gene-for-gene hypothesis (fig. 3). Note that the major gene and minor gene corresponds to a monogenic and polygenic resistance gene respectively (Kolmer, 1996; Wang & Chen, 2017; Alemu, 2019).

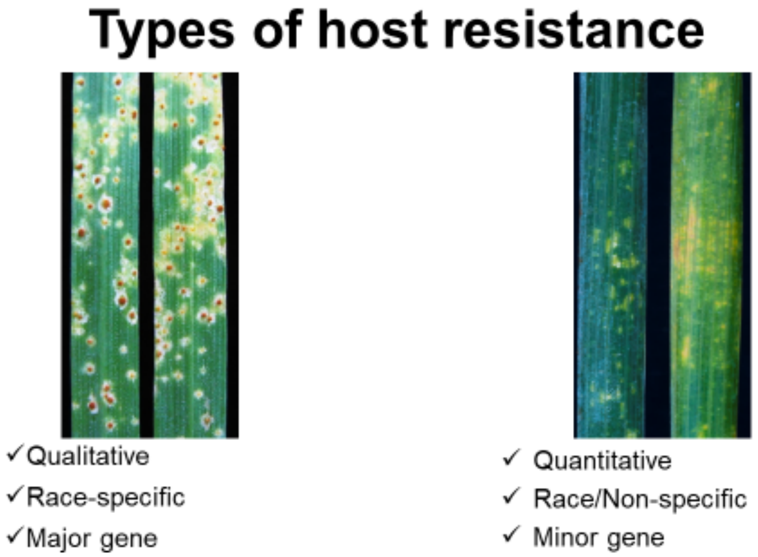

Fig 3. A comparison of plant responses due to different resistance conditions with the less durable type shown on the left. Rahmatov, Mahbubjon. 2023. *Types of host resistance*. Illustration with pictures. (Usage granted by the creator) [2023-04-21]

4.3 Pre-breeding

To further understand the subject pre-breeding as well as how a pre-breeding project might look, here is an example of a study that was performed by Rahmatov et al. (2016) that identified linked molecular markers for a stem rust resistance gene later named *Sr59*. This was done by moving the gene into the wheat genome from the rye genome derived from the rye-wheat substitution line 'SLU238' by the means of a breakage-fusion mechanism and subsequent Robertsonian translocation, which is the breakage of two chromosomes near the centromere and later fusion of two of the halves into one new one. This study emphasizes the utilization of a breakage-fusion mechanism for rye chromatin introgression. It challenges the historical controversy surrounding the more commonly used irradiation technique, indicating that it is not the exclusive method for achieving alien chromatin introgression from the third gene pool. Species within this gene pool hold potential genetic resources for wheat improvement without themselves being able to naturally cross with wheat. The study also suggests that traditional crossing without the *Ph1* gene can yield comparable results in terms of chromatin introgression (Rahmatov et al., 2016; Wang and Chen, 2017).

This translocation is normally prevented between homoeologous due to the presence of a gene named *Ph1* that specifically prevents the translocation of chromatic material between chromosomes. However, in this study the SLU238 line was crossed with a spring wheat mutant line where the *Ph1* gene had been removed (the locus where the gene normally is located is then referred to as *Ph1*b) which allows a Robortsonian translocation to take place. The first generation (F_1

generation) of this crossing (traditional breeding techniques), was selfed and produced a second generation of F_2 seeds that were tested for their seedling response to the most prominent race from the Ug99 group called TTKSK, and later also another stem rust race named TTTTF. 50 F_2 plants were then selected for further study based on both seedling resistance and, a marker haplotype indicative of homozygosity at *Ph1*b, meaning that the plants did not possess the gene (*Ph1*) that would hinder further Robertsonian translocations to occur. As well as a marker haplotype indicative of the presence of both chromosomes 2R from rye and 2D from wheat. This was important in order to know if a movement of chromatic material had occurred from the rye genome to the wheat.

In generation F_3, which was derived from selfed F_2 plants, it was found that only the ones with the chromosome 2R alleles for rye-derived markers were resistant to TTTTF at the seedling stage which would then indicate stem rust resistance, conferred by rye chromatin. Seven of these 146 plants were then selected based on seedling resistance to TTTTF. Marker haplotype indicative of 2R chromatin from rye. After this, a final generation was produced by selfing the seven selected F_3 plants, creating seven families of F_4 plants. Of these at least 20 in each were tested for TTTTF resistance while an additional 5-10 plants of each family were tested for resistance to three other *Pgt* races. In the end, 3 families were found via Fluorescence In Situ Hybridization analysis and marker results, to be homozygous for a T2DS·2RL chromosome meaning that the Robertsonian translocation had been successful. Since no *Pgt* resistance genes had been described from rye chromosome arm 2RL at this point, accession TA5094 was designated as the source of the resistance gene later named *Sr59*.

4.4 New methods and techniques in breeding

The function of any plant breeding strategy is to change the genetic makeup of a plant, whether it be through the means of established methods such as chemical or radiation application to mutate the genome through mutagenesis. Or it could be by more modern gene editing techniques where individual genes can be manipulated using CRISPR/Cas9 for instance. And alongside modern techniques to shorten the generational gap in different crops, the process of developing new varieties has been getting more efficient every year. Yet the greater part of all plant breeding being conducted globally today uses traditional methods of breeding in contrast to the several technologies and methods that have been developed throughout the years, some of which are exemplified in this subchapter (Watson, 2018).

A good first example of modern methods used in breeding is the "speed breeding method". This method utilizes controlled environments to shorten generational time for a given plant as much as possible by maximizing growth time (light period), minimizing rest time (dark period), keeping a consistent temperature during light/dark periods and drying immature seeds in order to shorten the seed to seed cycling. This method can produce four to six generations of wheat or barley in a year compared to the two to three that is normally achieved in greenhouses (Watson, 2018). Another example is “marker selected breeding”, that utilizes genetic markers that attaches to a particular gene or sequence and then “marks” it by “signaling” its location in some way. The marker might be fluorescent or it might be colored a bright shade of blue for instance so it is easily distinguishable during observation. This then eases the process of finding, identifying and/or working with a particular “marked” gene (Wang and Chen, 2017).

In pre-breeding there are several tools that can help speed along a project and save on precious resources. One example of such a tool is GWAS (genome-wide association studies) which aims to make connections between genotypes and phenotypes by scanning DNA or entire genomes, by testing for differences in the allele frequency of genetic variants between individuals. This can be done in order to make a statistical analysis for the risk that any particular individual is the carrier of a certain trait. Which is important in epidemiology studies for instance, in order to account for confounding genetic group differences and thus plan for the possible spread of a disease in said group (Uffelmann et al., 2021). Another example is the mapping of QTL’s (Quantitative Trait Locus), which is a method used to define the general chromosomal position of a gene or gene variants that influence the magnitude of a measurable trait. This is useful in order to know where on a chromosome you would want to look when working with a chosen trait (Miles and Wayne 2008).

5. Challenges posed to plant breeding

Wheat is an essential food source for food and nutrition security worldwide. Even a single year of poor wheat harvests has historically led to social, economic, and political turmoil (WFP, nd). Therefore, successful wheat harvests can have positive consequences for society, such as lower food prices, improved food security, and increased economic activity. Food products derived from it are in several countries a staple food and as a result, disruptions in wheat production does have significant social, economic, and political consequences, as we are witnessing with the COVID-19 pandemic and Russia's invasion of Ukraine. Meanwhile the arms race between human ingenuity and plant pathogen evolution is, and will always be a constant. However, as technology becomes more advanced and new strides in the scientific community are being made, it is not unreasonable to expect more from the scientific world. Yet at the same time these great improvements are only made manifest by overcoming the challenges present in the field of plant breeding. This chapter highlights these and discusses some of the difficulties in the fields of plant breeding and plant pathology.

5.1 Practical challenges

When talking about the practical parts of plant breeding one would be remised not to consider the main challenge in all parts of the breeding process, time. The typical amount of time it takes breeders to produce a new variety of wheat today is somewhere in the span of 7-8 years, followed by 2-3 years of committee variety testing that will determine if the variety is appropriate for release (Jordbruksverket, 2023). This inspection is carried out in all countries that adhere to the International Union for the Protection of New Varieties of Plants (UPOV) (UPOV, nd).

During all this time a pathogen like *Pgt* could not only evolve beyond a targeted mechanism but also spread into new susceptible areas where the new variety may not grow efficiently. Although new technologies like speed breeding, genomic selection and the usage of molecular markers are helping to reduce the time it takes to develop a variety, there will still be constraints on the release and distribution of a new variety. Once a new variety has been developed, and the initial seeds for it have been produced, time is needed to spread the new variety to the relevant areas of cultivation. This process is supported by the farmers that cultivate the new variety since they may help to distribute the variety through trade and communication with their neighbors and colleagues (Ceccarelli, 2015; Aw-Hassan et al., 2008). In general it is very difficult during the breeding process to estimate how the adoption may look or whether it will be adopted at all. This is in large part due to the time it takes wide adoption to occur since it can take 5-6 years before appreciable adoption of a new variety begins (Ceccarelli, 2015). Meanwhile, many aspects related to the adoption may change, such as the agronomic conditions like fertilizer and/or fungicide prices and the spread of pests and disease. Policy measures like the introduction or removal of subsidies or perhaps market demands may also change, making the breeding objectives aimed for at the start of the breeding program outdated (Ceccarelli, 2015). Due to this many breeding projects aim to “solve” general problems, like higher yields, stronger stems and resistance to the ever-present abiotic stresses like water shortage and increasing temperatures. Although the development of disease resistance will still occur if the need is great enough, as in the case of resistance breeding against the Ug99 family of variants as well as other strains with similar virulence (Singh et al., 2008).

Another challenge for breeding plants is the myriad of regulations and rights that a breeder needs to take into consideration in order to proceed with a project. This may for instance include the license needed in the EU in order to conduct work which include plants with patented traits, something that has raised concern in Europe resulting in a restriction of the exclusive right of the patent holder via a ‘limited breeder’s exemption’ in the Unitary Patent System (Louwaars & Jochemsen, 2021). It should also be noted that it is not possible to patent the ‘natural traits’ of plants due to European policy. But, traits created through technical means, like products created from gene editing, are “*protectable when they are sufficiently new, innovative and are usable*”. In addition to this, a large number of process patents have been granted for the basic technology and for many further innovations/applications in plant breeding (Louwaars & Jochemsen, 2021). With this in mind it is not unreasonable to think that further shifts in the breeding section might occur compared to the ones that followed the introduction of transgenic GMOs. And that given the tremendously competitive market for strategic agricultural and food investments globally, the level of uncertainty that exists within the EU may have the potential to divert potential research and development investment away from the EU (Louwaars & Jochemsen, 2021).

Thirdly there is the immediate challenge posed to breeding in the shape of the emerging number of new races of pathogen as well as cereal varieties, and their many inherent traits. Once a suspected infection has occurred it needs to be race-typed and tested on to know what varieties will present effective resistance to it in the future. If the results of a race typing reveals that there is no suitable variety available then it is necessary to develop a new variety (Rahmatov et al., 2016). But in order to do that a screening will have to be performed to find suitable candidates for breeding. Like the earlier example of the development of *Sr59* gene markers. The development of this required firstly a source of resistance to the relevant stem rust race, which could have been found through screening of several wheat-rye introgression lines using molecular markers in order to quickly identify the gene. Once this has been done, a variety of wheat with suitable agronomic traits can be chosen to which this gene will later be transferred (Rahmatov et al., 2016).

Further on, there is at present a near constant lack of funding in the field of plant breeding in the public sector, globally (Raynolds & Braun, 2022). In many regions, the competence of the workforce is sufficient but hindered by the lack of suitable facilities and equipment, like for on-site race typing which hinders a more escalated response to potential disease spread. But somewhat luckily, there is a bright side to this lack of resources as it differs greatly between countries which facilitates the possibility for a network of collaborations between countries, adding to foreign relations and the exchange of information and knowledge (Raynolds & Braun, 2022). It may also add to a growing service providing sector in correlation to the breeding field of work by these companies enabling services like genomic sequencing, variety testing and distribution of resistance varieties in poor regions and countries, which may prove beneficial to the field in the long term.

To further add to the mention of a lack of economic resources it should be mentioned that funding for studies often is hard to come by and that many areas of study are affected by this, like field trials that in general can span over large regions in order to test environmental factors. In the instance of stem rust resistance breeding projects, it is generally easier to find funding as it is such a large concern globally, yet nothing is ever perfect and there is evidently room for further funding to expand the domestic as well as the global efforts to stop threats like Ug99 (Louwaars & Jochemsen, 2021; Stuckenbrock & Grurr, 2023). Out of all the fields in the range of plant breeding for stem rust, one of the fields in most need of funding is surveillance of, and testing of the pathogen in order to keep a continually clear picture of the spread of high-threat races of stem rust globally, like Ug99 in this case. This could then not only give up to date information about the spread and possible new races of the disease, but also help to fuel acknowledgement efforts and funding campaigns in the future. This funding could be in the shape of facility building and/or upgrading, investment in testing equipment in low income countries and regions, and infrastructure and vehicle investment in and around hard-to-access regions of interest, like the east African highlands where the Ug99 group originate (Singh, 2008). A wider research globally also helps to further our understanding of the disease, something that becomes more and more prevalent as fungicides will not be used to the same effect in the future as they are currently (Stuckenbrock & Grurr, 2023).

5.2 Political challenges

The development and use of new techniques in the field of plant breeding may entail great potential in developing new variants. Yet the prospects of any breeding tech are always depending on, not only the scientific success of mentioned technique, but also political policy and social acceptance. In order to exemplify this let's look at a study that was Published by Lessoued et al in 2018. In this study, 374 experts including scientists, regulators, and business professionals with backgrounds and experiences in agricultural biotechnology answered a pair of online surveys that examined the socio-regulatory aspects of uncertainty as it relates to new breeding techniques (Lessoued et al., 2018). In this study there were many interesting results presented based on the experts' opinions on, among others, political intrigue as well as on public opinions and the role these have on the development and adoption of new breeding tech, the public aspects will however be further expanded upon under the next section. The respondents in the study were given a list of factors that might explain innovation-related regulatory uncertainty, and were asked to rank up to five factors they thought were the most limiting to the development of new breeding techniques and the results are shown in Table 1. Adding to the main concern ranked in Table 1, participants were also asked about their belief whether their domestic government would adopt policies in line with their views, and 67% of European respondents answered that they believed it was not likely, which is a testament to the overall rigid EU legislation toward crop biotechnology. They concluded that "*the regulatory uncertainty pertaining to products of new breeding techniques is not due to scientific concerns, but rather political interference in the regulatory approval process*" (Lessoued et al., 2018). This speaks to the difficulties of working in a field so closely dependent on regulations made on such high levels. Something that is in large part affected by public perception of a subject and thus in extension the information presented, and the channels that the public gains said information from.

Table 1. A table showing the frequency of options ranked one to five by respondents to a pair of online surveys. Adapted from: Lassoued et al (2018).

Limiting factors	**Percentage**
Political involvement in regulatory process	24
Unsynchronized approval between countries	20
Inconsistent international standards	19
Incomplete national regulatory rules	17
High regulatory compliance costs	17
Regulatory delays	16
[…]	[…]

5.3 Social challenges

In the social aspect there is immediately a connection to be made back to Ug99 and other *Pgt* races present in countries and regions that are economically challenged or that have poor infrastructure. In these areas communities often have to rely on seeds and innovations from international organizations like CIMMYT or ICARDA. At the same time there might also be opposition against the adaptation of a new variety among farmers due to a preference of using old varieties (Aw-Hassan et al., 2008). Farmers participating in a study conducted in 2008 in Syria described no less than 15 different performance evaluation criteria when testing a new variety of barley, showcasing the number of criteria that could make a variety unattractive to farmers in a certain area (Aw-Hassan et al., 2008).

Even if a variety of wheat were to be entirely immune to all diseases, a farmer might still discard it if factors like yield, degree of lodging or grain size were less attractive compared to other available varieties. Or perhaps a strong local culture dictates a certain social resistance against foreign varieties. In communities like the ones in the study where variety diffusion and spread are at the mercy of farmer-to-farmer interaction and trade, these variations might put a swift stop to the adaptation of a new variety (Aw-Hassan et al., 2008).

Public perception (i.e., the subjective assessment of risks and benefits) is in part the cause of the differences in regulation of the same plant biotechnologies (e.g., CRISPR/Cas9) between western countries in regions such as North America and Europe. In America there is generally a positive attitude towards the safety and benefits of biotech crops (varieties developed using plant biotechnologies), while most Europeans generally have a negative opinion towards the very same (Aerni, 2002; Lessoued et al., 2018). However evidence of a changing perception and consequently legislative actions has emerged in the EU in July of 2023 as the European commission proposed a new regulation that would update the current GMO legislation and de-regulate what they call category 1 NGT-plants. This would mean that plant varieties developed using NGT's to achieve results otherwise achievable through traditional breeding by crossing methods or natural processes, would be regulated aside from the current GMO legislation (The Swedish gene technology advisory board, 2023).

While genetically modified crops historically have been deemed safe, consumer opinion surrounding them has been mixed in many countries and there is a growing crack between the reassuring results of technical risk assessment, and the alarmingly negative public risk perception of agricultural biotechnology (Aerni, 2002). This is fueled by factors like protest organizations in the public debate, lapses in confidence towards institutions that manage technological risks, the association of biotechnology with commercial interests, and a dramatization of risks in mass media which in itself often is the primary source of information to many consumers (Aerni, 2002). In addition to the social uncertainty affected by consumer mindset, there are also the ever-present civil society movements that may question the safety or efficacy of novel technologies thus hampering the adoption of new breeding techniques in favor of public understanding and social acceptance rather than technological aspects. But this is not to say that this is inherently negative, social acceptance is and will always be vital for the adoption of new technologies, but rather it is the lack of public understanding and insight into a subject that can mislead it (Aerni, 2002).

6. Conclusion

There were three questions posed in this thesis, and throughout the text the aim has been to answer these with an in-depth look into *Pgt* resistance breeding. With that in mind, the following section will try to encompass the answers of these questions in a few concluding sentences.

- ***How big of a threat is stem rust globally?***

Although undermined and defeated in recent history, stem rust has come back to prove that it remains a major threat to global wheat production and that it can cause extreme damage and yield losses to susceptible varieties. Even though the disease is under control by use of resistant varieties and fungicide control in the world's major production areas, serious genetic vulnerability exists to new virulent races that could spell disaster in these areas. But active steps are being taken in order to prevent that, like incorporating new effective resistance into wheat breeding programs, as well as conducting surveillance to monitor the movement of the pathogen to know how and when particularly virulent races would spread.

- ***What obstacles are there in the way of overcoming this threat?***

Challenges to the field of plant breeding, and specifically that for stem rust resistance, are ever-present. And while time constraints are naturally difficult to overcome, other big challenges such as opposition, whether they be on a legislative-, public or even cultural level. Funding issues in respect to surveillance and research of, as well as testing facilities for the pathogen. As well as the simply huge amount of both pathogen races and host diversity, can all be addressed and resolved with sufficient effort, attention, and perseverance.

- ***What possibilities does plant breeding pose for the future of agriculture?***

The constant arms race between human ingenuity and pathogen evolution is now, as it has always been, a fact of agriculture worldwide. But unlike any historic precedent we now have tools that can allow all aspects of breeding and pathological work and research, to accelerate to unprecedented levels of progress being made in exceptionally short amounts of time. And in a continually more accepting political environment in the EU, working towards ambitious goals set to counter a negatively changing environmental climate, the field of plant breeding and consequently plant-resistance development is facing an exceedingly exciting yet challenging future.

As *Pgt* continues to spread and evolve, it continues to be threat to wheat production on a global scale. And as new races of the pathogen sow seeds for potential epidemics and destruction, it is easy to gain a sense of worry and to imagine a perilous future for wheat as we know it. But as conveyed in this thesis, the world of stem rust resistance breeding is one strengthened by new and innovative solutions, a dedicated and present workforce, ultimately motivated by the importance of the task at hand.

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