5-2019

# Addressing the Challenges Facing Wheat Production: Nebraska and International Breeding Efforts

Sarah Blecha
*University of Nebraska - Lincoln*, sarah.blecha16@gmail.com

# ADDRESSING THE CHALLENGES FACING WHEAT PRODUCTION: NEBRASKA AND INTERNATIONAL BREEDING EFFORTS

by

Sarah Blecha

A Doctoral Document

Presented to the Faculty of

The College of Agricultural Sciences and Natural Resources

In Partial Fulfillment of Requirements

For the Degree of Doctor of Plant Health

Under the Supervision of Professor Gary L. Hein

Lincoln, Nebraska

May, 2019

# ADDRESSING THE CHALLENGES FACING WHEAT PRODUCTION: NEBRASKA AND INTERNATIONAL BREEDING EFFORTS

Sarah Blecha, D. P. H.

University of Nebraska, 2019

Advisor: Gary L. Hein

Bread wheat, *Triticum aestivum* L., provides 20 percent of the global daily calorie intake. It is the third most important food crop, after rice and corn. Biotic challenges significantly reduce wheat yield; chemical control can be a solution but can be cost prohibitive for subsistence farmers. For many farmers, genetic resistance to biotic stresses can be the most cost effective solution.

The International Center for Agricultural Research in the Dry Areas (ICARDA) and the Nebraska Small Grains Breeding Program have been addressing these wheat production challenges. ICARDA is part of an international research consortium to increase wheat yield and tolerance to abiotic and biotic stresses. It focuses on wheat development in the Middle East, Africa, and Asia. Chapter 1 provides a brief overview of how ICARDA breeds for wheat using mega environments, shuttle breeding, and multi-location yield trials.

Many abiotic and biotic stresses decrease wheat yield around the world. Some of the major wheat diseases and incest pests in the ICARDA region include: Septoria tritici blotch, stem rust, stripe rust, Hessian fly, and Sunn pest. A brief review of each of these wheat pests and diseases are provided in Chapter 2.

Currently, there is a less than 1.0 percent annual increase in wheat yield. But to feed the growing population, the annual increase of wheat needs to improve to 1.6

percent. Hybrid wheat could address this yield gap through heterosis. Heterosis, hybrid vigor, in wheat has been estimated to be 3.5 to 15 percent. As hybrid wheat research continues, a transparent and robust SNP panel for hybrid purity testing is required (Chapter 3). Because wheat has a compact floral architecture, researchers and regulators must be able to run a molecular test to confirm if seed is a true hybrid or a female-self. To address this problem, we plan to develop a robust panel of 42 SNPs (two SNPs per chromosome) by finding highly polymorphic regions among parents and breeding lines and testing them within our Nebraska-Texas hybrid genotypes. This study validates two KASP markers (Sr6_1 and Sr6_3) used in the Nebraska and Texas hybrid breeding program. These markers provide KASP markers for both stem rust resistance and hybrid purity testing.

# ACKNOWLEDGEMENTS

Thank you to my committee: Drs. Gary Hein, Sam Wortman, Gerald Adams, and Stephen Baenziger for reviewing my doctoral document and your advice throughout my program. Thank you to Ellen Paparozzi for being my mentor, your guidance through my bachelor's got me to this point of achieving my doctorate. To my professors and staff at UNL, thank you for your instruction both in and out of class. My degrees from UNL would not be possible without your mentorship.

Thank you to my fellow DPH students for their advice and knowledge. Our office conversations provided encouragement and advice when needed. Thanks for all the help in the field and lab from my fellow Wheatie lab group. Our long car rides across Nebraska were a great bonding experience. Thank you to Tadesse, Samira, and everyone at ICARDA for your warm hospitality. I loved my time at ICARDA in Morocco. I appreciate you taking time out of your busy field schedule to show me around.

To all my friends who supported me through grad school, thank you. I appreciate all your help along the way. So many friends gave me advice that guided me along the way. And to my family, thank you for all the support over the years. You saw me through all the rewarding and challenging times. This degree wouldn't be possible without my parents instilling confidence in us and showing us the importance of an education and encouraging us to follow our dreams.

Funding for my DPH was provided by the William J. Curtis Fellowship. The Earle S. Raun Fellowship partially funded my ICARDA internship. Research funding for my work as Dr. Baenziger's lab tech was funded through USDA-ARS and the Nebraska Wheat Board.

## TABLE OF CONTENTS

## LIST OF FIGURES

# CHAPTER 1: INTRODUCTION

The goals for my Doctor of Plant Health (DPH) internships were to 1) broaden and improve my experiences in agronomic production and field research application, 2) gain experience with an international breeding center, and 3) enhance my collaborative skills and cooperative networking. My first internship was distributed through three years working for Dr. Baenziger as a molecular lab technician. I also rated winter wheat trials for disease during the summer, took flowering notes, and helped with harvest and fall planting. The disease ratings were used by Dr. Baenziger for advancing lines. Each year differed in the prevalence of diseases due to weather conditions. Additionally, I trained other graduate students on DNA extraction and the use of Kompetitive Allele Specific PCR (KASP) markers in the lab. A major task each fall was extracting DNA from over 1,500 wheat genotypes to be sent for Genotype-By-Sequencing. Chapter 3 presents an expanded scientific report on developing a single nucleotide polymorphism (SNP) diversity panel for hybrid wheat purity testing using KASP markers, my main research project.

My second internship was with The International Center for Agricultural Research in the Dry Areas (ICARDA) in Rabat, Morocco for six weeks from March 19-April 25, 2018. Spring wheat is the main bread wheat of Morocco due to the hotter and drier climate. By combining the knowledge I gained from the interdisciplinary curriculum available for the DPH program, I was able to integrate knowledge into field evaluation of the bigger picture of improving grain quality. I could diagnose problems in the field not just based on pathology, but also entomology and soil science. Chapter 2 will provide greater detail on the major wheat pests and diseases in Morocco compared to Nebraska.

The major wheat diseases in Morocco are stripe rust and Septoria/tan spot complex and the major insect pests are Hessian fly, Sunn pest, and Russian wheat aphid.

This current chapter provides an overview of ICARDA and how an international center coordinates research between countries. It's important to understand the international collaborative system that oversees wheat breeding efforts before discussing the role ICARDA plays in improving food security. The Consultative Group on International Agricultural Research (CGIAR) is a nonprofit organization that consists of 15 research centers. These centers focus on improving food security through agronomic efforts: breeding, conservation agriculture, integrated pest management, resource management, and social aspects including gender equality and training.

ICARDA and The International Maize and Wheat Improvement Center (CIMMYT) are in the CGIAR system. CIMMYT breeding efforts focus on corn and wheat research. CIMMYT releases wheat varieties in coordination with ICARDA for the region of Central and West Asia and North Africa (CWANA). This region has the highest consumption of wheat in the world. ICARDA breeding efforts focus on spring wheat, durum wheat, winter wheat, barley, chickpeas, lentils, and faba beans. CGIAR provides wheat research an annual funding of $30 million USD and results in a return on investment of $2.2-$3.1 billion based on 2010 data (Lantican et al., 2016). ICARDA moved from Aleppo, Syria to Rabat, Morocco due to civil unrest in 2012 (Yahia, 2012).

These breeding centers are influenced by the societal importance of the crops they study. Morocco is part of the CWANA region, which stretches from Morocco in the west to Ethiopia in the south and to Kazakhstan in the northeast. Wheat is the single most important commodity crop in the CWANA region and accounts for 45% of the

population's daily calorie intake (Tadesse et al., 2016). Each year, about 126 million tons of wheat is produced on an area of 54 million hectares, yet this is below the demand of 164 million tons in the CWANA region (Tadesse et al., 2016). Thus, many of the countries in this region must import wheat as they consume more than they produce. Bread wheat is a staple for Morocco, with the per capita consumption of 216 kg per year (USDA, 2017). In comparison, the American per capita consumption is 60 kg (USDA, 2018). Due to the climate of the CWANA region, spring wheat is the major bread wheat grown.

ICARDA plays a fundamental role in international wheat research because the CWANA region encompasses wheat's center of origin and spans five mega environments (ME). A ME is a region with similar climate, soil, moisture, and major insect pests and diseases (Tadesse et al., 2016). Like other CGIAR centers, ICARDA employs breeding efforts such as MEs, multi-location trials, and shuttle breeding. Breeding for ME allows for more widely adapted crop varieties which can be grown in multiple countries. Additionally, multi-location trials in other countries allows breeding for resistance to a pathogen that might be present in one country but not another, like the stem rust race Ug99. Stem rust is a devastating fungal wheat disease that has multiple strains, but Ug99 is the most virulent strain that overcame many of the previously used resistance genes. Because Ug99 is not present in Morocco, screenings are done at Ug99-positive sites in Njoro, Kenya and Debre Zeit, Ethiopia. Multi-location trials are also employed by the University of Nebraska-Lincoln (UNL) Small Grains Breeding Program, but at the state level within the USA. Some of the varieties that Dr. Baenziger breeds are tall wheats,

which are mostly grown in western Nebraska. Tall wheats are rarely grown in eastern Nebraska due to the higher probability of lodging because of higher rainfall.

Shuttle breeding was first developed by Dr. Norman Borlaug, to speed up the breeding pipeline and get an extra generation of line advancement per year. Breeders still use shuttle breeding today. They grow one generation of the breeding lines in their nursery, and after harvest and quick selection, they ship the seed to another location for field evaluation. The second location needs to be in the opposite season so they are able to gain an extra planting cycle that year. ICARDA does this for their spring wheat, the main nursery is in Morocco and then they send seed the same year to Ethiopia for disease and drought selection. Shuttle breeding is difficult in winter wheat breeding due to its vernalization requirements, potential losses due to winterkilling, and longer growing season.

ICARDA coordinates with the National Agricultural Research Systems (NARS) in countries of the CWANA region to release new wheat varieties. Once ICARDA selects wheat lines they would like to have evaluated for release, they send the seed to NARS. NARS conducts preliminary yield trials, verifies the variety, registers the variety and finally carries out seed multiplication for distribution to farmers (Tadesse et al., 2017). In most developing countries, the public sector locally controls varietal releases through supporting the developmental research as well as the seed multiplication (Tadesse et al., 2016). In the period of 1994-2014, approximately 77% of the wheat varieties released in West Asia and North Africa were from the public sector, while 23% were from the private sector (Lantican et al., 2016). About 50% of wheat varieties grown in the

CWANA region originates from ICARDA/CIMMYT germplasm, either as a released cultivar or part of the pedigree for a cultivar (Tadesse et al., 2016).

Wheat is a self-pollinated crop. This allows the farmer to save seed to use each following year, but the practice delays the adoption of new variety releases. Rain-fed regions tend to experience more farm-saved seed than irrigated regions (Tadesse et al., 2016). The challenge from farm-saved seed is that it slows large scale adoption of improved varieties. Farm-saved seed can also spread disease from one year to the next. Achtar is an old wheat variety that is very susceptible to stripe rust and has moderate drought tolerance. Almost 61% of the seed grown for Achtar comes from non-official sources, including farm-saved seed (Bishaw, Yigezu, Niane, Juárez, & Najjar, 2019). There have been newer stripe rust resistant varieties released since Achtar, but Achtar remains widely grown because it has excellent bread making qualities and societal preference. Although, there was a farmer next to the Marchouch field station that consistently planted the latest ICARDA released varieties. His fields were very uniform and had high yield.

The major spring wheat breeding efforts at ICARDA include selecting for resistance to stripe rust, stem rust, Septoria/tan spot, Hessian fly, Russian wheat aphid, Sunn pest, drought and heat tolerance, and improved bread making qualities. Straw or biomass is a more important trait in Morocco for livestock than it is in Nebraska. Straw strength is not a common trait to breed into wheat lines in Nebraska. Another regional difference is that drought is cyclic in Morocco, occurring every two years. During drought years, the grain yield will significantly decrease, and the wheat is harvested for

livestock feed. When there is a drought, Morocco will import more wheat and flour prices can triple at the grocery store.

ICARDA sends and receives thousands of seeds of various crop varieties each year, which makes it difficult for the Moroccan government to do phytosanitary inspections. To handle the large volume of seed shipments, ICARDA has a Seed Health Laboratory in which they test for bacterial, fungal, and viral pathogens before sending out seed, and after receiving seed. All incoming seed get tested to ensure there are no quarantinable pathogens present, for example the smut and bunt fungi, *Tilletia controversa* and *T. indica*. If such a pathogen is present, those seeds are destroyed. Most outgoing shipments are tested as well, depending upon the destination. Other countries have their own phytosanitary requirements as well. Seed that will be put into long-term storage is tested because seed-borne diseases can decrease seed longevity. The Seed Health Lab conducts ICARDA field visits within Morocco to make sure smuts and other seed-borne diseases are not epidemic in the field. If there is major infection, they will destroy infected plants.

While in Morocco, I attended the Borlaug Global Rust Initiative (BGRI) Technical Workshop. It was a four-day technical workshop with talks from wheat researchers. Most of the presentations were about rust, but some other talks covered breeding techniques and advancements in wheat genomics. I was pleasantly surprised to discover how open and accepting the wheat community is when it comes to sharing research and ideas. Scientists were willing to collaborate and offer advice to troubleshoot issues other researchers are facing. This is what Norman Borlaug wanted, scientists joining together to combat wheat rust on an international scale.

ICARDA hosts an annual Wheat Improvement Course for small grains breeders from Africa, Asia, and the Middle East. ICARDA sends out invitations to universities and other organizations for them to send their small grain breeders. ICARDA hosts 20-30 participants for a paid three-week improvement course. The Improvement Course is a continuation of Norman Borlaug's legacy of training the next generation of plant breeders. During the course, participants learn about traditional breeding, current technologies in breeding, integrated pest management, biotic and abiotic stresses. During the course they also select ICARDA lines in the field for traits of interest that they can incorporate into their breeding program as germplasm. For instance, at the Sidi Al Aidi, Morocco station, they select mainly for drought tolerance. At Marchouch, Morocco, the trainees can select for stripe rust resistant varieties or other agronomic traits such as straw strength. The Wheat Improvement Course happened to coincide with BGRI 2018 being in Morocco, so the whole group was able to attend (ICARDA, 2018).

Finally, I also visited ICARDA's Genetic Resources seed bank. ICARDA is situated in the center of the origin of wheat, and it provides critical access to the genetic diversity present in wheat landraces, wild relatives, and wheat progenitors. One of the most important aspects of any breeding program is the genetic resources available to incorporate into breeding lines. Wild relatives and ancestors provide sources of resistance genes to many abiotic and biotic stresses. When ICARDA was originally in Aleppo, Syria, it served as one of the largest seed banks in the world. But due to civil unrest, it had to move to the Morocco location. Because ICARDA is part of CGIAR, seed from the Genetic Resources bank is freely available to other scientists and breeders around the world. Genetic Resources at ICARDA also has field space in Marchouch to multiply

seeds and record phenotypic notes. Due to the move from Syria to Morocco, ICARDA had to withdraw seed from the Svalbard Global Seed Vault (on the Norwegian island of Spitsbergen) in order to establish its new seed bank location. But in 2018, ICARDA replaced the seed that they had borrowed to replenish the Svalbard Global Seed Vault.

My six week internship at ICARDA provided perspectives on international wheat breeding. Six weeks was not enough time to be fully immersed into each agricultural discipline, but I experienced inclusion in various research areas, including entomology, plant pathology, seed health, genetic resources, and agronomic practices. I could walk into an office and ask questions and they would provide answers and even directly accompany me as advisors and mentors to the polyhouse or field experimental trials at Marchouch. An international wheat research center has the compounded difficulty of collaboration due to language differences, funding challenges, time zone, and climatic differences between countries. Despite these challenges, scientists and breeders have been successful in releasing new wheat varieties to increase food security for people across the world.

**References**

Bishaw, Z., Yigezu, Y.A, Niane, A.A., Juárez R.A.T., & Najjar, D. [Eds.] (2019, February). *Political economy of the wheat sector in Morocco: Seed systems, varietal adoption, and impacts*. International Center for Agricultural Research in the Dry Areas. Retrieved from MELSpace (Monitoring, Evaluation, and Learning Repository/Agricultural Research Repository) website: http://repo.mel.cgiar.org/handle/20.500.11766/8505

ICARDA. (2018, May 6). *Building capacity on wheat improvement*. Retrieved from https://www.icarda.org/update/building-capacity-wheatimprovement

Lantican, M.A., Braun, H.J., Payne, T.S., Singh, R.P., Sonder, K., Baum, M., van Ginkel, M., & Erenstein, O. (2016). *Impacts of international wheat improvement research, 1994-2014.* Mexico, D.F.: CIMMYT.

Tadesse, W., Amri, A., Sanchez-Garcia, M., El-Bouhssini, M., Karrou, M., Patil, S., Bassi, F., Baum, M., & Oweis, T. (2016). Improving wheat production in the central and west Asia and North Africa (CWANA) region. In: Langridge, P. (Ed.), *Achieving sustainable cultivation in wheat* (Vol. 2). doi: 10.19103/AS.2016.0004.39

Tadesse W., Sanchez-Garcia M., Tawkaz S., El-Hanafi S., Sherif A., Tahir I., El Bouhssini M., Nazari K., Sharma R., Bishaw Z., Niane A., Assefa S., Halila H., Amri A., & Baum M. (2017, April 23). *Wheat breeding at ICARDA: Strategies, achievements and prospects.* Retrieved from http://iwgs2017.boku.ac.at/wp/wp content/uploads/abstracts/33/72_13th-IWGS wheat-breeding-at-ICARDA V2_ID33.pdf

United States Department of Agriculture, Economic Research Service. (2018, December 21). *Wheat sector at a glance*. Retrieved from https://www.ers.usda.gov/topics/crops/wheat/wheat-sector-at-a-glance/

U.S. Department of Agriculture, Foreign Agricultural Service, Global Agricultural Information Network. (2017, April 14). *Morocco, Grain and feed annual. Toasted wheat?* (GAIN Report No. MO1703). Retrieved from https://gain.fas.usda.gov/Recent%20GAIN%20Publications/Grain%20and%20Fed%20Annual_Rabat_Morocco_4-14-2017.pdf

Yahia, M. (2012, September 16). Conflict in Syria forces international research centre to move staff. Retrieved from https://www.natureasia.com/en/nmiddleeast/article/10.1038/nmiddleeast.2012.133

# CHAPTER 2: BREEDING EFFORTS FOR WHEAT INSECT PESTS AND DISEASES AT ICARDA AND NEBRASKA

The International Center for Agricultural Research in the Dry Areas (ICARDA) in Rabat, Morocco is an international wheat research center. Scientists and breeders across the globe collaborate to improve understanding of wheat-pest interactions and discover genes for resistance to biotic and abiotic stresses. Breeders work to develop wheat cultivars with improved yield and resistance to these stresses. ICARDA is situated in the region for the center of origin of wheat, providing valuable access to genetic diversity through wild germplasm. Given this location, it is a site for wheat's coevolution with its pests and pathogens. This allows for breeding efforts to focus on gene discovery and wheat plant response to abiotic and biotic stresses.

The major insect pests challenging global wheat production include Hessian fly, Sunn pest, Russian wheat aphid, and wheat stem sawfly (Tadesse, Solh, Braun, Oweis, & Baum, 2016b). On a global scale, the major wheat pathogens include stem/black rust, stripe/yellow rust, leaf/brown rust, Septoria tritici blotch, powdery mildew, and Fusarium head blight (Tadesse et al., 2016a). The focus of this chapter is to provide a short review on a selected few wheat pathogens and insect pests: Septoria tritici blotch, stem/black rust, stripe/yellow rust, Hessian fly, and Sunn pest.

**Septoria tritici blotch**

Septoria tritici blotch (STB) is caused by the fungus *Zymoseptoria tritici* (formerly *Mycosphaerella graminicola* and *Septoria tritici*). STB favors humid, temperate environments and yield loss can reach up to 50% in some fields (McDonald & Mundt, 2016). North Africa faced a devastating STB epidemic in 1968-1969, that drew international attention (Brown, Chartrain, Lasserre-Zuber, & Saintenac, 2015).

Wind dispersed sexual ascospores from the wheat stubble or water-splashed asexual conidia land on the leaves of the wheat seedlings (Ponomarenko, Goodwin, & Kema, 2011). Ascospore germ tubes infect the plant through the stomatal opening and hyphae grow intercellulary (Ponomarenko et al., 2011; O'Driscoll et al., 2014). At 12-20 days after infection, *Z. tritici* becomes necrotrophic and the characteristic yellow and necrotic lesions form on the leaf surface (O'Driscoll et al., 2014). Within the lesions, pycnidia develop near the stomata, and eventually exude conidia that will be water-dispersed to neighboring plants (Ponomarenko et al., 2011). Pseudothecia and pycnidia survive between growing seasons on straw residue (Ponomarenko et al., 2011).

Because STB can go through sexual recombination within the field, resistance to both fungicides and host plant resistance can occur and a single lesion can contain multiple pathogen strains (McDonald & Mundt, 2016). The favored alleles in *Z. tritici* can be dispersed via rain to neighboring plants, and longer distances by the airborne ascospores (McDonald & Mundt, 2016). Due to ascospores being able to infect a neighboring wheat field, strategies should be done on a larger scale than just field scale. McDonald and Mundt suggested a regional management strategy.

Straw or stubble management at the end of the season can reduce the dispersal of ascospores; cover crops can also be planted within stubble to serve as a trap to prevent ascospore dispersal (McDonald & Mundt, 2016). Strobilurin, triazole, propiconazole, succinate dehydrogenase inhibitor, chlorothalonil (McDonald & Mundt), methyl benzimidazole carbamates, and demethylase inhibitor fungicides have been used to control STB, but resistance to several of these classes was reported (O'Driscoll et al., 2014). Rotation of fungicides with differing sites of action would slow resistance.

Fortunately, it is unlikely that ascospores can survive long distance dispersal over oceans (McDonald & Mundt), reducing the spread of epidemics.

In breeding plants for resistance to diseases, qualitative resistance has major genes for resistance, following a gene-for-gene interaction, while quantitative resistance is controlled by many genes and confers partial resistance (Brown et al., 2015). A single R-gene, *Stb6*, is present in many global wheat cultivars, but resistance broke in an Oregon cultivar three years after release (McDonald & Mundt, 2016). Quantitative gene resistance can also be used with major R-genes to slow the breakdown of resistance (McDonald & Mundt). Brown et al. provide a consensus map of chromosomal location for *Stb* genes and markers. There have been 21 *Stb* qualitative genes discovered and a total of 89 regions for qualitative trait loci that are at the seedling stage (27), adult stage (48), and both stages (14) (Brown et al.). Breeding for STB resistance can be difficult because most known *Z. tritici* isolates are now virulent to most of the described *Stb* genes; however, wheat cultivars with qualitative and quantitative resistance to STB remain available (Brown et al.).

**Stem rust, and the Lineage Ug99**

Stem rust or black rust is caused by the fungus *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn. and it is one of the most devastating wheat diseases worldwide, causing yield losses up to 90% (Yu et al., 2014). In 1998, the highly virulent Ug99 (race TTKSK), was discovered in CIMMYT nurseries in Uganda (Singh et al., 2011). Originally, Ug99 overcame the wheat resistance genes *Sr24*, *Sr36* (Yu et al., 2014), and *Sr31* (Singh et al., 2011). Since 1998, Singh et al. (2011) provided seven variants of

Ug99 (TTKSK, TTKSF, TTKST, TTTSK, TTKSP, PTKSK, and PTKST), and the virulence status of their corresponding wheat resistance genes. BGRI identified six additional variants of Ug99 (TTKSF, TTHST, TTKTK, TTKTT, TTHSK, and PTKTK) (BGRI, 2017). The North American race nomenclature system is modified from Roelfs and Martens (1988) and uses a letter-code system based on five sets of four gene wheat differential lines (RustTracker.org, n.d.). With these variants overcoming different resistant genes, it demonstrates the need for more durable *Sr* genes. Food security and sovereignty is threatened by Ug99 because 90% of the world's wheat is susceptible (Singh et al., 2011). A 2007 screening survey of 22 African and Asian countries showed that only 5-10% of the total estimated area of planted wheat hectares provided adequate resistance to Ug99 (Singh et al., 2011).

Leonard and Szabo (2005) provide a complete review of the disease cycle of stem rust. At the end of the growing season, black dikaryotic teliospores form on the wheat plant. These dormant teliospores persist in residual wheat straw until their synchronistic germination of *Berberis* or *Mahonia* species' bud break and new foliation. One or both cells of the teliospore produces a hyphal protrusion, a basidium or promycelium. The mature basidiospores discharge and get carried by air currents to infect the barberry alternate host. Pycnia form on the upper surface of barberry leaves. Pycniospores form within the pycnia and exude from the tip (ostiole) as pycnial nectar. The nectar attracts insects that aid in dispersal. The nectar can also be dispersed by water splash. The pycniospores serve as the male gametes while the receptive hyphae threads of the pycnia are the female gametes. Sexual mating results in genetic recombination and increased genetic diversity, and this increases the potential for the eventual selection of virulent

populations. When compatible mating types come into contact, fusion into a dikaryotic state occurs within the pycnium. A dikaryotic aecium forms beneath the pycnium, ruptures the alternate host leaf's lower epidermis, and forms chains of dikaryotic aeciospores. These aeciospores are forcibly discharged and dispersed via wind to infect wheat plants. Upon successful infection by the aeciospore, a dense mat of fungal hyphae forms in the tissue under the wheat epidermis. From this hyphal mat, sporophores grow and produce masses of single-celled dikaryotic urediniospores. The urediniospores rupture the epidermis of wheat and produce a uredinium (pustule). The urediniospores can be dispersed by air currents and reinfect wheat plants on the stem, leaf sheath, and occasionally the leaf blade (Leonard & Szabo). Primary hosts such as barley, rye, triticale, wheat progenitors and durum wheat (Singh et al., 2011) function as a green bridge that allows for the survival of spores in the uredinial stage to reinfect wheat plants the following growing season. Later in the season, the uredinia switch from producing urediniospores to teliospores that remain dormant until the next season.

Fungicides can control stem rust, but these are cost-prohibitive. Wanyera, Macharia, Kilonzo, and Kamundia (2009) found azoxystrobin + cyproconazole, tebuconazole, and tebuconazole + triadimenol to be most effective at controlling stem rust. Another management strategy is eradication of alternate hosts, but this also is costly. Eradication of the alternate host, barberry, occurred in the US in the 1910s and 1920s. This eliminated the sexual recombination phase of *P. graminis*.

Due to the virulence and devastating yield losses caused by Ug99, many breeders are collaborating to improve resistance through gene discovery, breeding, and spore tracking. In 2014, Yu et al. provided a consensus map of Ug99 stem rust resistance genes.

With this detailed map, molecular biologists and geneticists continue to elucidate the precise location of these genes in order to determine if these resistance genes are unique and to determine their linkage to markers and other genes. Singh et al. (2011) listed eleven other grass species where stem rust resistance genes have been discovered. By elucidating these genes from wheat relatives, scientists can incorporate the gene into wheat germplasm through traditional breeding, introgression, and gene editing.

ICARDA and CIMMYT through BGRI screens for stem rust, Ug99, at Njoro, Kenya and Debre Zeit, Ethiopia (Singh et al., 2011; Tadesse et al., 2016a). These two locations are used because of the high genetic diversity within Ug99 at these two locations in Africa (Singh et al., 2011). Yu et al. (2014) stated that 58 stem rust resistance genes have been numerically designated, and 27 of these were effective or partially-effective against Ug99. Qualitative (seedling resistance) and quantitative (adult plant resistance) genes exist for stem rust resistance. Using qualitative genes led to the 'Boom and Bust' cycles of wheat being resistant to rust for a short time, and then resistance breaking (Yu et al., 2014). Quantitative genes, sometimes referred to as slow rusting genes, confer a high level of resistance when four or five are present in a single line, but each alone confers low resistance (Yu et al., 2014; Singh et al., 2014). Incorporating one or two qualitative genes into elite germplasm is easier than incorporating four or five quantitative genes. An adult plant resistance gene, *Sr2*, is effective against Ug99, but unfortunately, it expresses pseudo-black chaff in the field (Singh et al., 2014). This phenotype is undesirable when expression levels are high because it looks like the bacterial disease- black chaff. The slow rusting gene, *Sr57* (also known as *Lr34/Yr18/Pm38*), has been effective against stem rust for over 60 years (Singh et al.,

2014), proving that minor genes will provide durable stem rust resistance. But this gene alone does not provide complete resistance.

Due to concerns about Ug99, spore tracking is another major endeavor to prepare countries and continents for this rust. So far, Ug99 is only present in eastern Africa and Iran and Yemen in the Middle East. Spore dispersal by air currents could follow a west-east pattern from Africa into the Middle East (Singh et al., 2011). Movement of spores from eastern Africa to southern Africa could be due to the gradual movement on air currents to wheat fields or accidental human movement (Singh et al., 2011). A proposed hypothesis of movement within Africa is spore dispersal from one "island" of wheat to another (Singh et al., 2011). This hypothesis is due to differing growing seasons, as one country is harvesting wheat, another country is planting wheat. The presence of Ug99 in south African countries is concerning with regard to spread to the Americas and Australia, a possibility supported by wind trajectory models and cross-continental rust movements (Singh et al., 2011). BGRI and its partners created RustTracker.org, a Global Cereal Rust Monitoring System (https://rusttracker.cimmyt.org/), to track rust incidence, race, and severity worldwide (Singh et al., 2011) and provides an updated map (Figure 2.1) of Ug99 status (RustTracker.org, 2019).

**Stripe/yellow rust**

Stripe or yellow rust of wheat is caused by the fungus *Puccinia striiformis* f. sp. *tritici* Eriks. (*Pst*) (Chen, Wellings, Chen, Kang, & Liu, 2014). Usually yield loss from stripe rust ranges from 10-70%, but it can be 100% if early infection occurs (Chen, 2005). In 2009, a severe stripe rust epidemic in Morocco was caused by races from the *PstS2*

lineage (Ali et al., 2017). Additionally, seeds affected by severe stripe rust infection have lower vigor and poor emergence (Chen, 2005). Stripe rust races are designated based on virulence or avirulence to wheat cultivars (Chen, 2005).

Stripe rust is considered a cool temperature disease with an optimum range of 7-12ºC, but it is now adapting to warmer climates, with a maximum temperature range of 20-26ºC (Chen et al., 2014). The disease cycle of *Pst* is very similar to that of *Puccinia graminis*. The asexual urediniospores infect the wheat plant, leading to pustule formation in long, narrow stripes on leaves (between veins), leaf sheaths, awns, and glumes (Chen et al.). Later in the season, the sexual teliospores are produced, which germinate to produce a probasidium (Chen et al.). After meiosis, four basidospores are formed that will eventually be discharged from sterigmata on the basidium (Chen et al.). The discharged basidiospores infect *Berberis* spp. or *Mahonia* spp. plants but this rarely occurs in nature (Chen et al.). Unlike *P. graminis*, *Pst* teliospores do not go dormant; and there are only two incidents have been reported of *Pst* on barberry in nature (Chen et al.).

Currently, fungicides are an effective control for stripe rust. Five fungicides to control stripe rust are registered in the US: the triazole propiconazole, the strobilurin azoxystrobin, and combinations propiconazoole + trifloxystrobin, and propiconazole + azoxystrobin (Chen, 2005). Due to the high cost of fungicides, farmers in developing countries cannot afford to spray their wheat.

Host plant resistance through breeding can be a cost effective method to mitigate yield losses. Resistance to stripe rust can either be seedling resistance (all-stage resistance) or adult plant resistance (Chen et al., 2014). Seedling resistance tends to be single gene resistance and resistance breaks fairly quickly while adult plant resistance is

more durable due to polygenic effects (Chen et al.). As of 2014, there are 55 designated stripe rust genes, 41 confer all-stage resistance while 14 confer adult plant resistance (Chen at al.). A review by Chen (2005) provides chromosomal location and plant resistance type for stripe rust resistant genes. The adult plant resistance gene *Yr30* for stripe rust is associated with the adult plant resistance gene for stem rust, *Sr2* (Chen et al.), and the linked genes provide resistance to the two different rusts.

Urediniospores can be dispersed by wind or raindrops leading to epidemics or to the introduction of a new race in a wheat growing area (Chen et al., 2014). Stripe rust was not detected in Australia until 1979 and was probably spread by wind-dispersal to New Zealand the following year (Chen, 2005). Mexico and southern states in the US serve as a source of stripe rust urediniospores that then move north to Canada and the central states of the US (Chen). Stripe rust spores are also being tracked by BGRI on a global scale (https://rusttracker.cimmyt.org/). Models tracking spore dispersal have been developed to predict possible epidemics within the US and Canada (Chen).

**Hessian fly**

Hessian fly, *Mayetiola destructor* Say, is a gall midge in the family Cecidomyiidae, order Diptera. It is present in North Africa, western and central Asia, Europe, and North America (Stuart, Chen, Shukle, & Harris, 2012). Hessian fly can cause a 59% yield loss in the US (Sardesai, Nemacheck, Subramanyam, & Williams, 2005).

The Hessian fly life cycle lasts 28 days and consists of the egg, three larval instars, pupa, and the adult, but only the first two larval instars feed (Stuart et al., 2012). The life cycle is highly synchronized to the growth and development of the wheat host

plant (Stuart et al.). A female adult lays around 200 eggs on the upper surfaces of wheat leaves (Stuart et al.). After hatching, the first larval instar uses the leaf venation as a guide to crawl to the base of the wheat seedling (Stuart et al.). First instar larvae utilize paired, microscopic mandibles to puncture the cell wall to establish a feeding site (Stuart et al.). This feeding site forms into a gall or nutritive tissue (Rani, Pedada, & Singh, 2018) by reprogramming wheat tissue. Second instar larvae imbibe the liquids from the gall. The cuticle of the second instar forms into a puparium, and the third instar develops as a pupae within it. The cuticle sclerotizes, hardens, and turns a dark brown color which lends itself to being termed as a flax seed (Stuart et al.). In Morocco, the third instar larvae can enter a summer diapause that lasts until environmental conditions are favorable in the fall for host development (Lhaloui, El Bouhssini, Otmane, Ouriniche, & Alami, 2016) with cooler temperatures. An adult will eclose from the puparia and live for only a short period of one to four days (Stuart et al.).

Plant response depends upon a compatible or incompatible interaction to Hessian fly feeding. A compatible interaction results in the larval feeding changing the developmental pathways in wheat; cell division and elongation stop in susceptible wheat, causing plants to become stunted, darker green, and resulting in the death of the shoot apical meristem (Stuart et al., 2012). Hessian fly feeding on older wheat plants will cause heads to produce fewer seeds and lower seed weight (Stuart et al.). An incompatible reaction occurs in resistant wheat plants. The full mechanisms and pathways involved in resistant plants are still unknown, but certain plant defenses and responses are known. Immediately after the initial larval feeding, reactive oxygen species are produced within

the plant that triggers plant response of strengthening cell walls by mobilizing membrane lipids and other molecules to prevent larval mouthpart feeding (Stuart et al.).

Hessian fly is present in Morocco and the US, but cultural management strategies differ between Morocco and Nebraska. Fly-free dates, planting after the probable period of fly activity, are often used for determining when to plant winter wheat in the fall in Nebraska. But fly-free dates are not used in Morocco because the delay in planting can cause the wheat to experience severe drought or heat stress during the heading stage.

Interestingly, the gall midge-plant interaction resembles that of plant pathogen-host interactions involving effector-based or gene-for-gene interactions, (Stuart et al., 2012) allowing for discovery and incorporation of plant R genes for breeding resistant cultivars. As of 2016, there are 34 R genes for Hessian fly, all are dominant except *h4* which is recessive (Chen et al., 2016). The first larval instar is the most critical stage for changes to plant development (Stuart et al.) and can be targeted in breeding efforts for resistance by larval antibiosis (Lhaloui et al., 2016). Single R-gene cultivars confer resistance for six to eight years (Stuart et al.), thus the need for multiple genes is critical for long term resistance. Virulent Hessian fly biotypes have increased due to the selection pressure from resistant wheat cultivars (Sardesai et al., 2005). There are 16 Hessian fly biotypes, with different biotypes overcoming different R genes; biotype L is the most virulent and common (MAS Wheat, n.d.; https://maswheat.ucdavis.edu/). Sardesai et al. (2005) discovered a new gene, *H32*, which confers resistance to the virulent Biotype L of Hessian fly. Discovery of new genes is critical for improving resistance, but these resistant genes need to be stacked to extend the time of resistance breakdown.

**Sunn pest**

Sunn pest describes a guild of true bugs in the shield bug and stink bug families, Scutelleridae and Pentatomidae, respectively (Figure 2.2) (Davari & Parker, 2018). *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae) is the most economically important species of Sunn pest for wheat and barley (Davari & Parker). Sunn pest is found in west and central Asia, Eastern Europe, and North Africa (Davari & Parker); fortunately, it is not present in the US.

Yield loss of up to 50-90% in wheat can occur from direct feeding damage from nymphs and adults on wheat stems, leaves, and grain (Davari & Parker, 2018). Feeding on the stems and leaves of young plants causes stunting (Emebiri et al., 2017). But, a secondary issue with Sunn pest feeding leads to grain quality reduction. During feeding, Sunn pest injects proteolytic and amylolytic enzymes which pre-digest endosperm proteins (Malipatil, 2008) and allows it to avoid seed defensive chemicals (Davari & Parker). These enzymes destroy the gluten of the grain resulting in the reduction of baking quality. Only 2-3% grain damage due to these enzymes is needed to ruin an entire batch of flour; contaminated flour fails to rise, burns quickly, and has an unpleasant flavor (Davari & Parker).

Sunn pest has a monovoltine life cycle, one generation per year, with an active and inactive period (Davari & Parker, 2018). The active period is characterized by Sunn pest growth and development on wheat, while the inactive period occurs after the wheat harvest. After 9-10 months, the overwintered adults emerge from the overwintering mountain spots and move to wheat fields. Females lay 70-80 eggs on plant vegetation (Davari & Parker) and eggs hatch after 4-10 days (Malipatil, 2008). There are five instars, the first four feed on leaves, and the fifth instar feeds on developing grain (Davari

& Parker). The newly matured adults migrate back to the mountains after feeding on enough grain to build up fat reserves (Malipatil; Emebiri et al., 2017; Davari & Parker). Adults aestivate underneath trees, pasture plants, and leaf litter (Davari & Parker).

In the Near East and West Asia, around four million acres are sprayed annually to control Sunn pest at a cost of $150 million (Davari & Parker, 2018). Imidacloprid, fenitrothion, trichlorfon, deltamethrin, and lambda cythalothrin are effective chemicals (Davari & Parker). Besides the huge cost and potential for resistance, the reliance on insecticides can have off-target effects on beneficial insects. The emergence rate of the egg parasitoid, *Trissolcus grandis* Thompson, was reduced after applications of fenitrothion and deltanethrin (Davari & Parker). Another management strategy promoted by some countries is the collection of overwintering Sunn pest adults; by removing the adults, fewer eggs are laid, thus reducing the following year's population (El Bouhssini, n.d.).

Breeding efforts for Sunn pest resistance have focused on the vegetative stage of wheat to reduce both the damage caused by the overwintered adults and populations that will damage the grain (Emebiri et al., 2017). A few Iranian wheat cultivars have been reported to being resistant to Sunn pest (Najafi-Mirak, 2012). Sunn pest resistant synthetic hexaploid wheat and landraces have been identified (Emebiri et al.) but further characterization needs to be conducted to determine the resistance mechanism. Emebiri et al. conducted a three-year quantitative trait locus (QTL) study from a susceptible by resistant cross. They found a major QTL between two mapping populations on chromosome 4BS for Sunn pest resistance. The importance of the paper by Emebiri et al. was the development of two SNP markers which can be used by breeding programs.

Without a marker, breeders cannot screen their breeding lines for resistance. Markers are crucial for programs that lack the presence of Sunn pest for natural screening.

**Genetic Resources**

As is the case for all wheat pests and diseases, gene discovery relies on available germplasm of wheat relatives and genome progenitors. *Aegilops tauschii* (DD), *Triticum urartu* (AA), and *Aegilops speltoides* (BB) are wheat's genome progenitors and still function as genetic resources. The CGIAR system also includes major seedbanks within CIMMYT and ICARDA. ICARDA is situated in the center of origin of wheat, providing critical access to wheat landraces, wild progenitors, and ancestors. The ICARDA Genebank has over 73,000 small grain accessions (ICARDA, n.d.b) and has more than 80% of wheat landraces and an extensive *Aegilops* collection (Tadesse et al., 2016a). The Focused Identification of Germplasm Strategy allows breeders and scientists to screen the collection for abiotic and biotic stresses to determine which accessions possibly could provide resistance genes (ICARDA, n.d.a). Through this approach, resistance to powdery mildew, Russian wheat aphid, Sunn pest, and stem rust in wheat has been found (ICARDA, n.d.a). Wheat landraces and synthetic hexaploid wheat were screened for Sunn pest resistance (Emebiri et al., 2017). Over 200,000 wheat accessions from advanced breeding material and germplasm collections were screened for Ug99 (Singh et al., 2011). These targeted screenings of breeding material, landraces, and germplasm collections are critical for determining resistance for wheat pests and diseases.

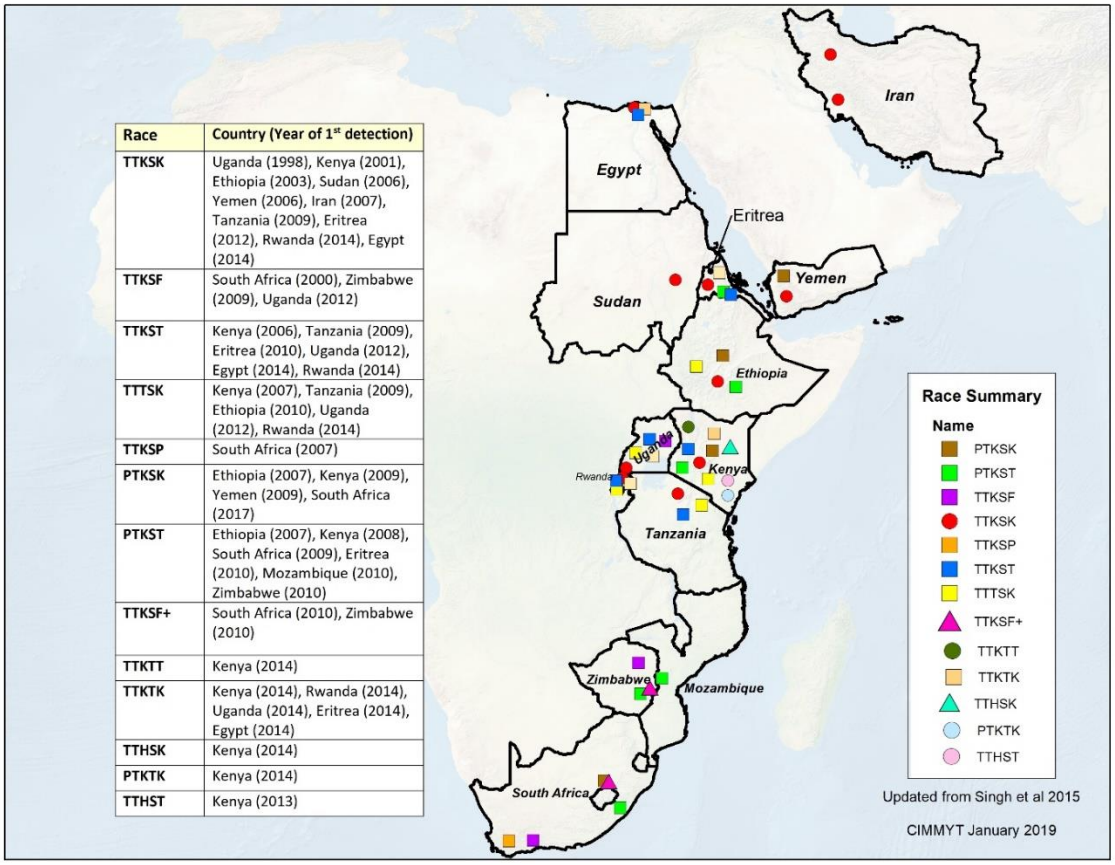
## Difficulties and Challenges

The difficulty with finding relevant articles for these major wheat pests and diseases has been availability. Sunn pest articles are sometimes in languages other than English, making it unavailable for some scientists to read and utilize. Australian biosecurity also ran into the issue of lack of readily available publications in English (Malipatil, 2008). Terminology can be inconsistent or confusing, such as the difference between a wheat genotype and wheat cultivar. A genotype could be considered a breeding line or germplasm source while a cultivar is a released line for commercial use.

## Summary

The main focus of this chapter was breeding strategies for resistance to these major wheat diseases and pests. Chemical control for Septoria tritici blotch, stripe rust, and stem rust exists, but the pathogens develop resistance. The major downside to chemical control is the prohibitive cost for farmers in developing countries. Discovery of novel resistance genes from wheat landraces and wild relatives has been an ongoing area of investigation. Breeding for resistance has usually been incorporation of single R-genes, but resistance breaks quickly. More effort goes into incorporating multiple, minor genes into a single cultivar, and this provides more durable resistance. International research centers have the advantage in breeding for multiple minor genes due to germplasm availability and a large number of breeders.

## Figures




| Race | Country (Year of 1st detection) |
|---|---|
| TTKSK | Uganda (1998), Kenya (2001), Ethiopia (2003), Sudan (2006), Yemen (2006), Iran (2007), Tanzania (2009), Eritrea (2012), Rwanda (2014), Egypt (2014) |
| TTKSF | South Africa (2000), Zimbabwe (2009), Uganda (2012) |
| TTKST | Kenya (2006), Tanzania (2009), Eritrea (2010), Uganda (2012), Egypt (2014), Rwanda (2014) |
| TTTSK | Kenya (2007), Tanzania (2009), Ethiopia (2010), Uganda (2012), Rwanda (2014) |
| TTKSP | South Africa (2007) |
| PTKSK | Ethiopia (2007), Kenya (2009), Yemen (2009), South Africa (2017) |
| PTKST | Ethiopia (2007), Kenya (2008), South Africa (2009), Eritrea (2010), Mozambique (2010), Zimbabwe (2010) |
| TTKSF+ | South Africa (2010), Zimbabwe (2010) |
| TTKTT | Kenya (2014) |
| TTKTK | Kenya (2014), Rwanda (2014), Uganda (2014), Eritrea (2014), Egypt (2014) |
| TTHSK | Kenya (2014) |
| PTKTK | Kenya (2014) |
| TTHST | Kenya (2013) |

**Figure 2.1: Status of the stem rust lineage Ug99 and derived races, provided by RustTracker.cimmyt.org**


**Figure 2.2: Adult Sunn pest at the ICARDA station in Marchouch, Morocco.**

**References**

Ali, S., Rodriguez-Algaba, J., Thach, T., Sorensen, C.K., Hansen, J.G., Lassen, P.,… Hovmoller, M.S. (2017). Yellow rust epidemics worldwide were caused by pathogen races from divergent genetic lineages. *Frontiers in Plant Science, 8,* 1057. doi: 10.3389/fpls.2017.01057

BGRI. (2017, February) *Pathogens*. Retrieved from the Borlaug Global Rust Initiative website: http://www.globalrust.org/pathogens/pathogen-home-page

Brown, J.K.M., Chartrain, L., Lasserre-Zuber, P., & Saintenac, C. (2015). Genetics of resistance to *Zymospetoria tritici* and applications to wheat breeding. *Fungal Genetics and Biology, 79*, 33-41. doi: 10.1016/j.fgb.2015.04.017

Chen, M.-S., Liu, S., Wang, H., Cheng, X., El Bouhssini, M., & Whitworth, R.J. (2016). Genes expressed differentially in Hessian fly larvae feeding in resistant and susceptible plants. *International Journal of Molecular Sciences, 17*(8), 1324. doi: 10.3390/ijms17081324

Chen, W., Wellings, C., Chen, X., Kang, Z., & Liu T. (2014). Wheat stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici*. *Molecular Plant Pathology, 15*(5), 433-446. doi: 10.1111/mpp.12116

Chen, X.M. (2005). Epidemiology and control of stripe rust [*Puccinia striiformis* f. sp. *tritici*] on wheat. *Canadian Journal of Plant Pathology, 27*(3), 314-337. https://doi.org/10.1080/07060660509507230

Davari, A., & Parker, B.L. (2018). A review of research on Sunn pest {*Eurygaster integriceps* Puton (Hemiptera: Scutelleridae)} management published 2004-2016. *Journal of Asia-Pacific Entomology, 21*(1), 352-360. doi:10.1016/j.aspen.2018.01.016

El Bouhssini, M. (n.d.) *Integrated Sunn Pest Management: Approaches for reducing the threat of Sunn pest to wheat harvests.* International Center for Agricultural Research in the Dry Areas. Retrieved from MELSpace (Monitoring, Evaluation, and Learning Repository/Agricultural Research Repository) website: http://repo.mel.cgiar.org/handle/20.500.11766/7700

Emebiri, L.C., Tan, M.-K., El-Bouhssini, M, Wildman, O., Jighly, A., Tadesse, W., & Ogbonnaya, F.C. (2017). QTL mapping identifies a major locus for resistance in wheat to Sunn pest (*Eurygaster integriceps*) feeding at the vegetative growth stage. *Theoretical and Applied Genetics, 130*(2), 309-318. doi: 10.1007/s00122 016-2812-1

ICARDA. (n.d.a). *FIGS in use*. Retrieved from the International Center for Agricultural Research in the Dry Areas website: http://www.icarda.org/figs/figs-in-use

ICARDA. (n.d.b) *ICARDA's Genebank resources & activities: A vital tool in ensuring a food-secure future for dryland communities*. International Center for Agricultural Research in the Dry Areas. Retrieved from MELSpace (Monitoring, Evaluation, and Learning Repository/Agricultural Research Repository) website http://repo.mel.cgiar.org/handle/20.500.11766/7709

Leonard, K.J., & Szabo, L.J. (2005). Stem rust of small grains and grasses caused by *Puccinia graminis*. *Molecular Plant Pathology, 6*(2), 99-111. doi: 10.1111/j.1364 3703.2005.00273.x

Lhaloui, S, El Bouhssini, M, Otmane, R, Ouriniche, S., & Alami, A. (2016). Comparative biology and life cycle of the barley stem gall midge and Hessian fly (Diptera: Cecidomyiidae) in Morocco. *Revue Marocaine de Protection des Plantes, 9*, 17-37.

Malipatil, M. (2008). *Industry biosecurity plan for the grains industry: Threat specific contingency plan: Sunn pest* Eurygaster integriceps. Retrieved from the Plant Health Australia website: http://www.planthealthaustralia.com.au/wp-content/uploads/2013/03/Sunn-pest-CP-2008.pdf

MAS Wheat. (n.d.) *Insect resistance: Hessian fly. Marker Assisted Selection in Wheat*. Retrieved from the Marker Assisted Selection in Wheat website: https://maswheat.ucdavis.edu/protocols/HF/index.htm

McDonald, B.A., & Mundt, C.C. (2016). How knowledge of pathogen population biology informs management of Septoria Tritici blotch. *Phytopathology, 106*(9), 948-955. doi: 10.1094/PHYTO-03-16-0131-RVW

Najafi-Mirak, T. (2012). Evaluation of resistance to Sunn pest (*Eurygaster integriceps* Put.) in wheat and triticale genotypes. *Crop Breeding Journal, 2*(1), 43-48.

O'Driscoll, A., Kildea, S., Doohan, F., Spink, J., & Mullins, E. 2014. The wheat-Septoria conflict: A new front opening up? *Trends in Plant Science, 19*(9), 602-610. doi: 10.1016/j.tplants.2014.04.011

Ponomarenko, A., Goodwin, S.B., & Kema, G.H.J. (2011). *Septoria tritici blotch (STB) of wheat*. (Plant Health Instructor, American Phytopathological Society.) Retrieved from https://www.apsnet.org/edcenter/disandpath/fungalasco/pdlessons/Pages/Septori.aspx. doi:10.1094/PHI-I-2011-0407-01

Rani, D.D., Pedada S., & Singh R. (2018). Hessian fly: New insights to develop a gall nutritive tissue. *Journal of Entomology and Zoology Studies, 6*(1), 720-728.

Roelfs, A.P., & Martens, J.W. (1988). An international system of nomenclature for *Puccinia graminis* f. sp. *tritici*. *Phytopathology, 78*(5), 526-53.

RustTracker.org. (n.d.) Race nomenclature. Retrieved from https://rusttracker.cimmyt.org/?page_id=32

RustTracker.org. (2019, January) Pathotype tracker – where is Ug99? Retrieved from https://rusttracker.cimmyt.org/?page_id=22

Sardesai, N., Nemacheck, J.A., Subramanyam, S., & Williams, C.E. (2005). Identification and mapping of H32, a new wheat gene conferring resistance to Hessian fly. *Theoretical and Applied Genetics, 111*(6), 1167-1173. doi: 10.1007/s00122-005-0048-6

Singh, R.P., Hodson, D.P., Huerta-Espino, J., Jin, Y, Bhavani, S., Njau, P., … Govindan, V. (2011). The emergence of Ug99 races of the stem rust fungus is a threat to world production. *Annual Review of Phytopathology, 49*, 465-481. doi: 10.1146/annurev-phyto-072910-095423

Singh, R.P., Herrera-Foessel, S., Huerta-Espino, J., Singh, S., Bhavani, S., Lan, C., & Basnet, B.R. (2014). Progress towards genetics and breeding for minor genes based resistance to Ug99 and other rusts in CIMMYT high-yielding spring wheat. *Journal of Integrative Agriculture, 13*(2), 255-261. doi: 10.1016/S2095 3119(13)60649-8

Stuart, J.J., Chen, M.-S., Shukle, R., & Harris, M.O. (2012). Gall midges (Hessian flies) as plant pathogens. *Annual Review of Phytopathology, 50*, 339-357. doi: 10.1146/annurev-phyto-072910-095255

Tadesse, W., Amri, A., Sanchez-Garcia, M., El-Bouhssini, M., Karrou, M., Patil, S., Bassi, F., Baum, M., & Oweis, T. (2016a). Improving wheat production in the central and west Asia and North Africa (CWANA) region. In: Langridge, P. (Ed.), *Achieving sustainable cultivation in wheat* (Vol. 2). doi: 10.19103/AS.2016.0004.39

Tadesse, W., Solh, M., Braun, H.-J., Oweis, T., & Baum, M. (2016b). *Handbook: Approaches and strategies for sustainable wheat production*. Location: Beirut, Lebanon. ICARDA.

Wanyera, R., Macharia, J.K., Kilonzo, S.M., & Kamundia, J.W. (2009). Foliar fungicides to control wheat stem rust, race TTKS (Ug99), in Kenya. *Plant Disease, 93*(9), 929-932. doi: 10.1094/PDIS-93-9-0929

Yu, L.-X., Barbier, H., Rouse, M.N., Singh, S., Singh, R.P., Bhavani, S., Huerta-Espino, J., & Sorrells, M.E. (2014). A consensus map for Ug99 stem rust resistance loci in wheat. *Theoretical and Applied Genetics, 127*(7), 1561-1581. doi: 10.1007/s00122-014-2326-7

# CHAPTER 3: DEVELOPMENT OF A SNP DIVERSITY PANEL TO TEST HYBRID PURITY IN WHEAT (*TRITICUM AESTIVUM* L.) USING KASP MARKERS

## Introduction

Bread wheat, *Triticum aestivum* L., is the third most important food crop, providing 20% of the global daily calorie intake (FAO, 2012). To feed the estimated nine billion people by 2050, the yearly wheat yield increase needs to rise from below 1.0% to 1.6% (FAO, 2012). Hybrid wheat can close this yield gap through heterosis, i.e., the increased yield over the best parent, increased yield over the mid parent value, or yield above the best available wheat cultivar. Wheat yield improvements due to heterosis range from 3.5 to 15% (Whitford et al., 2013). Heterosis in wheat is challenging due to its self-pollinating nature and the influence of many gene loci affecting grain yield (Whitford et al.). Heterosis is believed to increase with genetic divergence between parent lines; however, genetically divergent groups are not expected to be present in elite wheat breeding lines (Whitford et al.). Heterotic pools need further research to determine which parents have better recombining abilities that result in increased yields.

Hybrid wheat research was started in the 1950s with investigations of chemical hybridizing agents (CHA) and in the 1960s with cytoplasmic male sterile lines (Cisar & Cooper, n.d.). But hybrid wheat was abandoned in the 1990s, with Cargill being the last company in the US to drop out of hybrid wheat breeding, for reasons of difficulty of the research and the cost of research and commercialization (Cisar & Cooper, n.d.). Now, within the past 10 years, interest in hybrid wheat research has renewed. Some of this is due to more knowledge of other hybrid systems (corn, *Zea mays* L., and rice, *Oryza sativa* L.) along with more extensive characterization of genes and traits.

Hybrid wheat on a large field-scale can be made by using cytoplasmic male sterility, CHA, self-incompatibility, genic male sterility systems, and genetic modification systems (Whitford et al., 2013). Whitford et al. provides an in-depth review

on hybrid wheat production and its challenges. For commercial production of hybrid wheat, parent 1 (female) is crossed to parent 2 (male). The female rows are sprayed with a CHA to induce male sterility. Pollen from the male rows will fertilize the female rows. The resulting $F_1$ seed will then be sold to farmers. Because the seed produced is a hybrid, the farmer would not be able to save the next generation's seed because it would not be uniform. The inability to save seed from a wheat hybrid could pose a problem for farmers that currently save wheat seed from a cultivar to use for planting the following year.

Because wheat is naturally self-pollinated with compacted floral architecture, hybrid production system is complicated. Corn floral architecture allows for easier cross-pollination with the separation of the male tassel and the female silk. Rice is similar to wheat with a compacted and self-pollinated floral architecture. But, rice is diploid and cytoplasmic male sterility has been successful. Complete cytoplasmic male sterility is difficult to achieve in wheat due to a lack of fertility-restoration genes (Whitford et al., 2013), in part due to the complex hexaploid wheat genome. Desired male wheat genotypes have increased anther extrusion and pollen viability. This increases the potential that pollen will cross-pollinate the female rows. Female floral characteristics are harder to investigate because they are concealed within the wheat florets. Desired female traits include increased stigma receptivity to pollen, floral gapping, and stigma hairs would be long and extended outside the floret to better capture pollen. The heritability of these traits is relatively high (Whitford et al.), but is difficult to identify the underlying genes (Boeven et al., 2016). Thus, many programs are looking at increasing the cross-pollination potential of wheat. The male line needs to be shedding pollen at the same time the female plant is most receptive to have a higher seed yield.

The focus of the Nebraska Small Grains Breeding Program has been to optimize a CHA and improve the male and female floral characteristics. Croisor 100 (Sintofen, Asur Seeds) is a plant growth regulator that functions as a CHA, and it is used for hybrid wheat production in Europe (Whitford et al., 2013). This chemical is also being used in the Nebraska Small Grains Breeding Program to make experimental hybrids. Several challenges exist for the CHA system: effective dose, phytotoxicity, and timing of spray application. Croiser 100 is a contact gametocide. Correct spray dosage must be optimized for each genotype. If the applied dose does not have enough CHA activity, the female plants might remain male fertile. If too much CHA is applied, the CHA can be phytotoxic and damage the plant, thus reducing hybrid seed yields. The timing of the application is based on staging the wheat plant. Due to the CHA being a contact gametocide, rainfall can wash the product off the plant, making it ineffective in sterilizing plants, but reapplication can only be done very carefully to avoid phytotoxicity.

It is difficult for consistent hybrid production, with the potential for the seed harvested from the female parent, to be either a true hybrid or a female-self. A molecular tool that can be used to identify true hybrid progeny from female-selfed plants are simple sequence repeat (SSR) markers. SSR markers are routinely used in peanuts (*Arachis hypogaea*) to identify a female-self or hybrid progeny (Gomez et al., 2008). SSR markers utilize PCR to amplify the sequence of interest and gel electrophoresis is used to separate and view the bands. However, gel electrophoresis is laborious and time-consuming, especially for testing thousands of seeds. In comparison, Kompetitive Allele Specific PCR (KASP) allows for high-throughput genotyping to determine purity by eliminating the gel electrophoresis step.

A dependable high-throughput purity testing system that can discriminate between $F_1$ progeny that are true hybrids or female-selfed plants is required in order to facilitate commercial hybrid wheat production. A potential high-throughput purity testing system could be based on the detection of a single nucleotide polymorphism (SNP). A SNP is the difference in one nucleotide between individuals at that specific location. KASP utilizes PCR to amplify the target sequence and then detect the desired SNP with a fluorescent tag. KASP has been used to design markers for pre-harvest sprouting (Lin et al., 2015), leaf rust (*Puccinia triticina*) (Chhetri et al., 2017), and many other important economic traits (Rasheed et al., 2016). Three different primers are used in KASP, two allele-specific forward primers and a common reverse primer. The allele-specific primers differ at the 3' end with either the desired SNP or alternate SNP. One allele-specific forward primer has a 5' FAM fluorescent tag while the other allele-specific forward primer has a 5' HEX fluorescent tag. The presence of the desired SNP or its alternative in the sample will cause a fluorescent signal at either the FAM or HEX tag. The fluorescence is detected by a plate reader and then clustered according to the signal, thus bypassing the need to do gel electrophoresis. The common reverse primer functions as an anchor by annealing to the target sequence flanking the desired SNP.

A major challenge with investigating hybrid wheat is the complexity and size of its genome. The complexity of the wheat genome can be reduced by amplifying genomic coding regions. Genotyping-by-Sequencing (GBS) reduces the complexity of large genomes, such as wheat (Poland, Brown, Sorrels, & Jannink, 2012). GBS reduces the representation of the genome by targeting the sequence flanking restriction enzyme sites (Poland et al.). These researchers developed a two-enzyme approach of a "rare-cutter"

(*PstI*) and "common-cutter" (*MspI*), with barcoded adapters to increase the simplicity of quantification of the library for sequencing. GBS is useful as a genomics selection tool for breeding and association studies. GBS has been used for SNP discovery in breeding programs (Glaubitz et al., 2014), leading to development of KASP markers (Gao et al., 2015).

A robust SNP panel could be used by other wheat breeding programs. This a challenge because our SNP discovery is based upon Nebraska and Texas elite breeding lines. These SNPs might not be present or polymorphic in other breeding programs. The difficulty of developing a SNP diversity panel in wheat is overcoming the complex and repetitive genome. In primer design, it is critical to check the primer sequence against the reference genome to determine the primer is chromosome and genome specific. Otherwise, if the sequence is non-homologous, it could also amplify DNA sequences from the other genomes which would give misleading results. The D-genome is more monomorphic than the A- and B-genomes. Monomorphic SNPs are useless for differentiating parents, which makes hybrid purity testing challenging.

The long-term objectives of this study were to 1) develop a robust SNP diversity panel to test hybrid purity using KASP markers, and 2) have a total of 42 SNP markers, two for each chromosome (one on each distal end of the chromosome).

## Materials and Methods

### *Plant Material*

A total of 150 parental lines were used. One hundred female and 50 male parent elite breeding lines from Nebraska and Texas were used for experimental hybrid development. Male lines were selected based on excellent anther extrusion. Females were

chosen based on being high yielding. Male and female crosses were set up based on synchronization of flowering dates to potentially make hybrids using the balanced incomplete factorial design crossing block.

*DNA Extraction*

Five seeds per genotype were planted in a 96-well plastic greenhouse tray. The 150 parents were grown in the greenhouse for 14 days to the three-leaf stage. The oldest leaf was cut to a length of 4 cm and placed in a 1.2 mL plastic bullet tube on a 96-well plate. In each tube, 3-5 leaves were pooled per genotype for biological replication (depending on germination). Wet leaf tissue was dried down in plastic containers with silica beads. After seven days, the tissue was dry enough for DNA extraction. Two to four zirconia beads were added to each collection tube with dry tissue. Plastic caps were placed on the tubes and tissue was ground to a fine powder using the Qiagen TissueLyzer (GmbH, Germany) at 30 Hz for 6 minutes. DNA extraction was done using the BioSprint 96 DNA Plant Kit and Qiagen BioSprint 96 robot (GmbH, Germany) according to manufacturer's protocols with two minor modifications: 350 µl of RLT buffer was added to dry tissue and 150 µl of autoclaved distilled deionized water was used for the DNA elution step.

*GBS*

DNA was shipped overnight on frozen gel packs to Dr. Poland's lab at Kansas State University for GBS. The restriction enzymes used for GBS were *PstI* and *MspI* to target non-repetitive genome (Poland et al., 2012; Belamkar et al., 2018). Quality control,

SNP calling, and other GBS analyses were performed as previously reported (Belamkar et al.).

*KASP Marker Design and Validation*

Four SNPs for *Sr6* found on the 2D chromosome were chosen based on previous SNP identification within the Nebraska program (Mourad et al, 2018). SNPs were cross-checked with GBS data to confirm no off-target SNPs were within 100 bp upstream or downstream of the desired SNP. Off-target SNPs could create difficulty in primer design and correct primer annealing. KASP primers were designed using PolyMarker (Ramirez-Gonzalez, Uauy, & Caccamo, 2015). Initial input files were formatted according to PolyMarker's website (http://www.polymarker.info/). Fluorescent tail sequences for FAM and HEX were added to the two allele-specific primers. The FAM tail sequence is: 5' GAAGGTGACCAAGTTCATGCT. The HEX tail sequence is: 5' GAAGGTCGGAGTCAACGGATT. Primers were ordered online through IDT (https://www.idtdna.com/pages).

The KASP mixture contained 2.5 µl KASP 2x MasterMix (LGC Genomics, Teddington, UK), 2.5 µl genomic DNA, and 0.07 µl 72x assay mix. The 72x assay mix contained water and 100 µM of each primer: common (reverse), and the two allele-specific forward primers.

The NE13672/Freeman cross is within the hybrid breeding program and was chosen because they are polymorphic at each SNP for each primer set. Two samples of the FAM parent (Freeman), two samples of the HEX parent (NE13672), three NTC

controls (water), and two samples of a synthetic hybrid (a mix of equal quantities of genomic DNA of Freeman and NE13672) were used to validate the four primer sets.

The KASP protocol is modified from LGC Genomics (Teddington, UK) and standardized to the Nebraska Small Grains Breeding Program. The thermocycler was an Eppendorf Mastercycler Pro gradient 384 (Hauppauge, NY, USA). Stage 1 is the Hot-start Taq activation: 94ºC at 15 minutes, 1 cycle. Stage 2 is Touchdown: 94ºC for 20 seconds followed by 65-57ºC dropping 0.8ºC per cycle for 60 seconds, 10 cycles. Stage 3 is Amplification: 94ºC for 20 seconds followed by 55ºC for 60 seconds, 29 cycles. Stage 4 is Cool down or End: 4ºC for seconds, 1 cycle. After amplification, the fluorescence was read by FLUOstar Omega (BMG LABTECH GmbH, Germany). KlusterCaller software (LGC Genomics, MA, USA) was used for initial clustering of data points. Fluorescent values were then exported to create graphs in Microsoft Excel.

**Results and Discussion**

Sr6_1 and Sr6_3 were the two primer sets (Figure 3.1 and Figure 3.2, respectively) that had the clearest clustering of parents and the synthetic hybrid. These two primer sets were successfully validated using the NE13672/Freeman cross in the hybrid breeding program. Both of the graphs for Sr6_1 and Sr6_3 are consistent with LGC manufacturer standard graphs, with clear clusters to separate out parents, the NTC, and synthetic heterozygote (LGC Genomics, Teddington, UK).

The other two markers, Sr6_2 and Sr6_4, had poor clustering characteristics. This result could be due to several different reasons. Wheat has a complex, repetitive genome, and the KASP marker could have amplified an off-target genome or sequence. The

Nebraska and Texas hybrid breeding program consist of winter wheat. Winter wheat could have genomic differences from the reference genome of Chinese Spring (spring wheat), complicating the initial primer design. In the initial GBS SNP calling and discovery, a parent could be called homozygous for a SNP when it is actually heterozygous, affecting clear clusters. The melting temperature of the primer sets could be optimized as the Nebraska breeding program uses a standardized thermocycler protocol.

Sr6_1 and Sr6_3 provide proof of concept for the hybrid breeding program that KASP markers can be developed for high-throughput hybrid purity testing. These two KASP markers allow the Nebraska Small Grains Breeding Program to test experimental hybrids that have parents polymorphic at these two SNPs. Both of these KASP markers were for SNPs on chromosome 2D, so an additional 41 markers are needed to complete the long-term objective of having a panel of 42 markers.

**Conclusion**

Two primer sets (Sr6_1 and Sr6_3) were successfully validated within the Nebraska Small Grains Breeding Program. These two validated primers were trait-associated for stem rust resistance, *Sr6*. These two markers can serve a dual purpose in the breeding program: hybrid purity testing and stem rust resistance selection.

As more GBS data are examined, some markers might not be trait-associated due to ensuring SNP polymorphism between hybrid parents and each chromosome having two SNPs. Robustness of these two primer sets would need to be tested outside the Nebraska

and Texas breeding programs. Development of the SNP diversity panel is ongoing for the Nebraska program, but this study provides two KASP markers for 2D.

In the future, these two KASP markers (Sr6_1 and Sr6_3) will be tested on hybrid seed along with the synthetic heterozygote to validate both the markers and test whether the seed is a true hybrid. This will also provide a way to validate successfulness of the CHA efficacy experiments.

As the wheat hybrid system is optimized for large scale commercial production, a purity testing analysis needs to be implemented. KASP markers allow for high-throughput genotyping that would be used for commercialized hybrid wheat production. Other challenges that need to be addressed in hybrid wheat purity testing is how to detect pollen contamination, how to pool samples, and how to measure percent purity. Pollen contamination would also require knowledge of field set up of the parents. Males that are highly monomorphic at most SNPs in a diversity panel should not be planted close together as the SNP markers would be useless. Experiments can be done on pooling samples compared to taking individual samples.

**Figures**

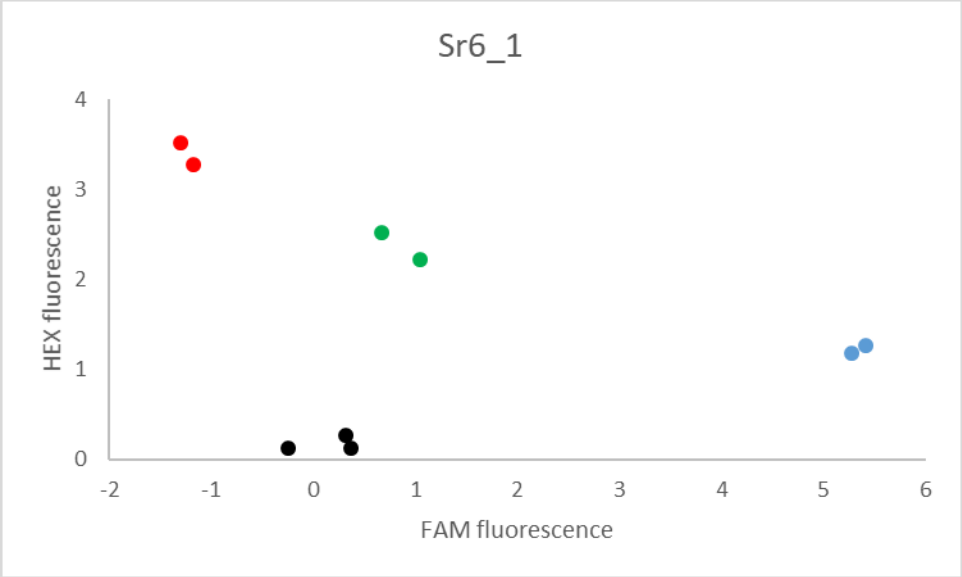



**Figure 3.1: KASP cluster calling for Sr6_1 primer set. The black dots are water (NTC), blue (FAM) are Freeman (T:T), red (HEX) are NE13672 (C:C), and green are synthetic hybrid (T:C). NE13672/Freeman is a cross made in the hybrid breeding program.**

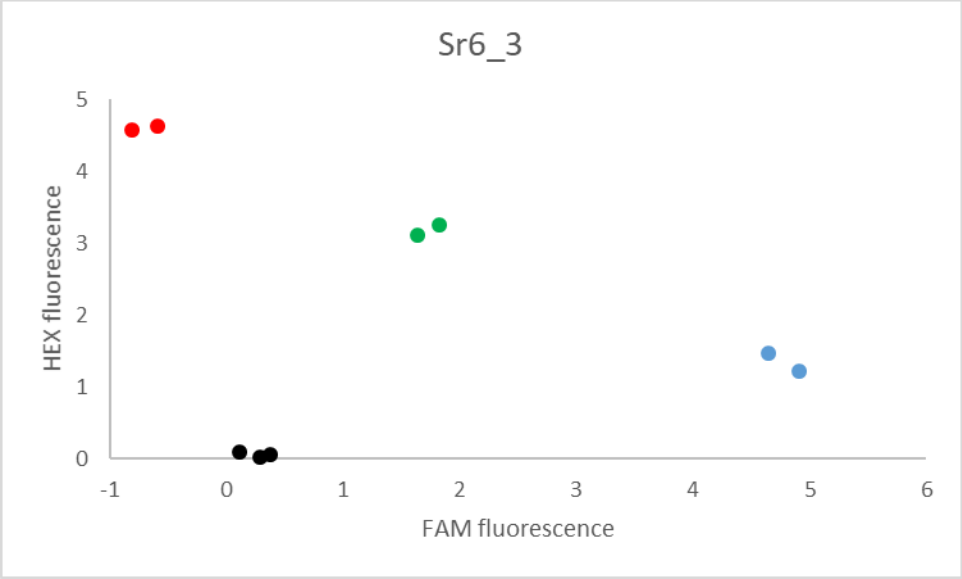



**Figure 3.2: KASP cluster calling for Sr6_3 primer set. The black dots are water (NTC), blue (FAM) are Freeman (A:A), red (HEX) are NE13672 (G:G), and green are synthetic hybrid (A:G). NE13672/Freeman is a cross made in the hybrid breeding program.**

**References**

Belamkar, V., Guttieri, M.J., Hussain, W., Jarquin D., El-basyoni, I., Poland, J., Lorenz, A.J., & Baenziger, P.S. (2018). Genomic selection in preliminary yield trials in a winter wheat breeding program. *G3: Genes, Genomes, Genetics, 8*(8), 2735-2747. doi: 10.1534/g3.118.200415

Boeven, P.H.G., Longin, C.F.H., Leiser, W.L., Kollers, S., Ebmeyer, E., & Würschum T. (2016). Genetic architecture of male floral traits required for hybrid wheat breeding. *Theoretical and Applied Genetics, 129*(12), 2343–2357. doi: 10.1007/s00122-016-2771-6

Chhetri, M., Bariana, H., Wong, D., Sohail, Y., Hayden, M., & Bansal, U. (2017). Development of robust molecular markers for marker-assisted selection of leaf rust resistance gene Lr23 in common and durum wheat breeding programs. *Molecular Breeding, 37*(3), 21. doi: 10.1007/s11032-017-0628-6

Cisar, G., & Cooper, D.B. (n.d.) *Hybrid wheat*. Retrieved from the FAO website: http://www.fao.org/3/y4011e/y4011e0c.htm

FAO. (2012). *Breakout session P1.1 national food security – the wheat initiative – an international research initiative for wheat improvement*. Retrieved from http://www.fao.org/docs/eims/upload/306175/Briefing%20Paper%20(3)-Wheat%20Initative%20-%20H%C3%A9l%C3%A8ne%20Lucas.pdf

Gao, L., Kielsmeier-Cook, J., Bajgain, P., Zhang, X., Chao, S., Rouse, M.N., & Anderson, J.A. (2015). Development of genotyping by sequencing (GBS)- and array- derived SNP markers for stem rust resistance gene *Sr42*. *Molecular Breeding, 35*, 207. doi: 10.1007/s11032-015-0404-4

Glaubitz, J.C., Casstevens, T.M., Lu, F., Harriman, J., Elshire, R.J., Sun, Q., & Buckler E.S. (2014). TASSEL-GBS: A high capacity genotyping by sequencing analysis pipeline. *PLoS ONE, 9*(2), e90346. doi: 10.1371/journal.pone.0090346

Gomez, S.M., Denwar, N.N., Ramasubramanian T., Simpson C.E., Burow G., Burke J.J., … Burow M.D. 2008. Identification of peanut hybrids using microsatellite markers and horizontal polyacrylamide gel electrophoresis. *Peanut Science, 35*(2), 123–129. doi: 10.3146/PS07-109.1

Lin, M., Cai, S., Wang, S., Liu, S., Zhang, G., & Bai, G. (2015). Genotyping-by sequencing (GBS) identified SNP tightly linked to QTL for pre-harvest sprouting resistance. *Theoretical and Applied Genetics, 128*(7), 1385-1395. doi: 10.1007/s00122-015-2513-1

Mourad, A.M.I., Sallam, A., Belamkar, V., Wegulo, S., Bowden, R., Jin, Y., … Baenziger, P.S. (2018). Genome-wide association study for identification and

validation of novel SNP markers for *Sr6* stem rust resistance gene in bread wheat. *Frontiers in Plant Science, 9,* 380. doi: 10.3389/fpls.2018.00380

Poland, J.A, Brown, P.J., Sorrells, M.E., & Jannink, J.-L. (2012). Development of high density genetic maps for barley and wheat using a novel two-enzyme genotyping by sequencings approach. *PLoS ONE, 7*(2), e32253. doi: 10.1371/journal.pone.0032253

Ramirez-Gonzalez, R.H., Uauy, C., & Caccamo, M. (2015). PolyMarker: A fast polyploidy primer design pipeline. *Bioinformatics, 31*(12), 2038–2039. doi: 10.1093/bioinformatics/btv069

Rasheed, A., Wen, W., Gao, F., Zhai, S., Jin, H., Liu, J., … He, Z. (2016). Development and validation of KASP assays for genes underpinning key economic traits in bread wheat. *Theoretical and Applied Genetics, 129*(10), 1843-1860. doi: 10.1007/s00122-016-2743-x

Whitford, R., Fleury, D., Reif, J.C., Garcia, M., Okasa, T., Korzun, V., & Langridge, P. (2013). Hybrid breeding in wheat: Technologies to improve hybrid wheat seed production. *Journal of Experimental Botany*, *64*(18), 5411-5428. doi: 10.1093/jxb/ert333