T.C.
NİĞDE ÖMER HALİSDEMİR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF PLANT PRODUCTION AND TECHNOLOGIES

SCREENING INTERNATIONAL SPRING WHEAT LINES FOR THEIR RESISTANCE RESPONSE TO THE CEREAL CYST NEMATODE (CCN) *Heterodera filipjevi*

OSAMEH SALAHDIN ATIYA

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OSAMEH SALAHDIN ATIYA

Master Thesis

Supervisor

Doç.Dr. Halil TOKTAY

August 2019

Osameh Salahdin ATIYA tarafından **Doç Dr. Halil TOKTAY** danışmanlığında hazırlanan **"Screening International Spring Wheat Lines for Their Resistance Response to The Cereal Cyst Nematode (CCN) *Heterodera filipjevi*"** adlı bu çalışma jürimiz tarafından Niğde Ömer Halisdemir Üniversitesi Fen Bilimleri Enstitüsü **Bitkisel Üretim ve Teknolojileri** Ana Bilim Dalı'nda Yüksek Lisans tezi olarak kabul edilmiştir.

(The study titled **"Screening International Spring Wheat Lines for Their Resistance Response to The Cereal Cyst Nematode (CCN) *Heterodera filipjevi*"** and presented by **Osameh Salahdin ATIYA** with the help of supervisor **Doç Dr. Halil TOKTAY,** has been found as Master thesis by the jury at the **Department of Plant Production and Technologies** of Nigde Ömer Halisdemir University Graduate School of Natural and Applied Sciences.)

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ONAY (CONFIRMATION):

Bu tez, Fen Bilimleri Enstitüsü Yönetim Kurulunca belirlenmiş olan yukarıdaki jüri üyeleri tarafından …./…./20.... tarihinde uygun görülmüş ve Enstitü Yönetim Kurulu'nun …./…./20.... tarih ve ..... sayılı kararıyla kabul edilmiştir.

(This thesis has been found appropriate at the date of …./…./20.... by the jury mentioned above who have been designated by Board of Directors of Graduate School of Natural and Applied Sciences and has been confirmed with the resolution of Board of Directors dated …./…./20.... and numbered .....)

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DIRECTOR

THESIS CERTIFICATION

It is certified that I have written this thesis by myself. I further confirm that all information included in this thesis is scientific and is in accordance with the university rules and regulations. Any materials that I have used from external sources as well as help received and all sources used in finalizing this research work and preparing this thesis, all have been acknowledged in the thesis.

Osameh Salahdin ATIYA

ÖZET

BAZI ULUSLARARASI YAZLIK BUĞDAY HATLARININ TAHIL KIST NEMATODU (HETERODERA FILIPJEVI)'NA KARŞI DAYANIKLILIININ BELIRLENMESI

ATIYA, Osameh Salahdin
Niğde Ömer Halisdemir Üniversitesi
Fen Bilimleri Enstitüsü
Bitkisel Üretim ve Teknolojileri Anabilim Dalı

Danışman : Doç.Dr. Halil TOKTAY

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Buğday dünyada üretilen en önemli ürünlerden olup üretimi çok farklı biyotik ve abiyotik faktörler tarafından etkilenmektedir. Tahıl kist nematodları buğdayda önemli zararlar yapan bitki parazitidir. Tahıl Kist nematodları (Heterodera filipjevi) buğdayda %50 ye ulaşan zararlar meydana getirmektedir ve en kolay ve ekonomik mücadele yöntemi de dayanıklı çeşit kullanımıdır. Modern buğday tarımında çeşit seçiminde ana kriter hastalık etmenlerine dayanıklılıktır. Bu çalışmada Uluslararası Mısır ve Buğday Araştırma Merkezi (CIMMYT-Türkiye) tarafından sağlanan 257 uluslararası yazlık buğday hatları Heterodera filipjevi'ye karşı dayanıklılık durumları kontrollü koşullarda (70% bağıl nem, 25°C, ve 16 saat ışıklandırma) iki ayrı denemede uluslararası standart kontrol bitkileri ile testlenmiştir. Test edilen buğday hatları tarla toprağı, kum ve organik madde içeren tüplerde yetiştirilerek her bir bitkiye 250 larva/1 ml şeklinde inokule edilmiştir. Yapılan testler sonucunda 11 hat (4.28%) dayanıklı olarak belirlenmiş, 36 (%14.01) hat ise orta dayanıklı bulunmuştur. Yapılan bu çalışmayla Heterodera filipjevi'e karşı buğday ıslah programlarında kullanılacak yeni genetik kaynaklar belirlenmiştir.

Anahtar Sözcükler: Tahıl Kist nematodları, *Heterodera filipjevi*, Dayanıklılık, dayanıklılık testlemeleri

SUMMARY

SCREENING INTERNATIONAL SPRING WHEAT LINES FOR THEIR RESISTANCE RESPONSE TO THE CEREAL CYST NEMATODE (CCN) *Heterodera filipjevi*

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Wheat is one of the most important crops produced worldwide and its production can be decreased due to various biotic and abiotic factors. Cereal cyst nematodes (CCNs) are plant-parasites that cause significant harm to wheat. The CCN *Heterodera filipjevi* can cause yield losses of up to 50% and the most inexpensive and practical control strategy is the use of resistant hosts. Resistance to biotic stresses such as CCNs is considered the main criteria in modern wheat selection. In this study, 257 international spring wheat lines provided by International Maize and Wheat Improvement Center – Turkey (CIMMYT) that are genotyped in Mexico for drought and heat resistance, were screened for their resistant response to the CCN *Heterodera filipjevi* under controlled conditions (70% relative humidity, 25°C, and a photoperiod of 16 hours) and compared to check cultivars with known resistance. A mixture of sand, field soil, and organic matter (70:29:1 v/v/v) was used as growing media, a 3-day germinated seed of a single line was inoculated with 250 J2/1 ml in a "Cone-tainer" ™ test tube. The experiment was held in 2 separate trials with 3 replication/trail. The results reveal that 11 (4.28%) lines show resistance and 36 (14.01%) lines show moderate resistance response. This study has been able to add new genetic sources of resistance to *Heterodera filipjevi* for future breeding programs.

Keywords: Cereal Cyst Nematodes, *Heterodera filipjevi*, Wheat Resistance, Screening

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SYMBOLS AND ABBREVIATIONS

Symbols	Description
%	Percentage
°C	Degrees Celsius
g	Grams
Kg	Kilogram
sec.	Seconds
µm	Micrometer
mm	Millimeter
cm	Centimeter
ml	Milliliter
L	Liter
v	Volume
RH	Relative Humidity
£	British Pound
US$	United States Dollar

Abbreviations	Description
ddH_2O	Double-distilled Water
H_2O	Water
O_2	Oxygen
CO_2	Carbon dioxide
Na^+	Sodium Ino
NaOCl	Sodium hypochlorite
Diam.	Diameter
DGO	Dorsal Esophageal Gland
LSD	Least Significant Difference
ANOVA	Analysis of Variance
FAO	Food and Agriculture Organization
USDA	United States Department of Agriculture

CHAPTER I

INTRODUCTION

Wheat is one of the oldest cultivated crops in the world and its cultivation can be traced back to about 10000 years ago in the Fertile Crescent area, as part of a widescale transition of the human civilization from hunting and gathering of food sources to settled agriculture. That period is known as the Neolithic Revolution or the First Agriculture Revolution (Shewry, 2009).

Wheat, which is considered to be a universal key food, is a grass that is broadly cultivated for its seeds and byproducts (e.g. straw that is used as animal feed, biofuel, and crafts). *Triticum aestivum* (common wheat), *Triticum durum* and *Triticum spelt* are the most common species of the genus *Triticum* (Shewry, 2009; Mauseth, 2014; Belderok et al., 2000).

It is grown on land more than any other commercial food crop and remains the most vital food grain source for humans. In a recent FAO report, world production of wheat is estimated to be the second most-produced cereal after maize and third in crop production in general (FAOSTAT, 2019).

The Turkish Statistical Institute (Türkiye İstatistik Kurumu "TÜİK") mentioned that wheat is the main cereal cultivated in Turkey. As it is estimated that Turkey produced in 2018 around 20 million tons.

Crops are generally influenced by various abiotic and biotic factors that affect their yield, growth, and metabolism. An example of these factors are drought, heat, cold, salinity, floods, and pathogens that limit crop productivity. In the wheat gene pool, there is an adequate genetic variation that can ensure continuous improvement of wheat adaptation to abiotic stresses (Lawlor and Cornic, 2002; Trethowan and Mujeeb- Kazi, 2008).

Climate change is a challenge facing humanity and effects climate change has been harmful to the agricultural industry. It is projected that countries near the equator will have a reduction in food production (Droogers and Aerts, 2005). The International Water

Management Institute (IWMI) study forecasts that wheat production in South Asia will decline by 50% by 2050 (Fraiture, 2007). Studies indicated that increasing temperatures have negative effects on wheat yields in numerous world regions (Parry, 2004; Asseng, 2015; Liu, 2016; Lobell, 2012; Moore, 2012).

It is going to be a challenge to increase or at least maintain world production of wheat in order to provide future generations with food needed to satisfy demands of the increasing population. Also, it is a current and future challenge to find ways to reduce the impact of factors that decrease the yield of wheat such as plant-parasites along with environmental factors (heat, drought, and pests).

Nematodes are a major group of metazoans within the soil ecosystem and are the most numerous multicellular organisms in it (Bongers and Ferris, 1999). Plant-parasitic nematodes form about 15% of the named nematode species, of which there are more than 4100 species (Wyss, 1997; Decraemer and Hunt, 2006).

Plant-parasitic nematodes that are the cause of agriculture production reduction are regarded to be alarming. Despite their widespread compared to other pests and are commonly very dangerous, stealthy, and costly, there is not enough detailed information or data on their economic impact (Webster, 1987; Vaish, 2017).

A challenge in evaluating the effect of nematodes is that harm caused by their infection is often less evident than that caused by many other pathogens (Vaish, 2017). In 1998, Handoo valued the losses of international crop production due to nematode infection around US$ 80 billion.

The widespread of plant-parasitic nematodes on a majority of vital crops, especially cereal cyst nematodes (CCN) on wheat and their effect on dramatically reducing crop yields, has caught the awareness of governments and international organizations to find methods of management.

The species of CCN avenae complex *Heterodera avenae, Heterodera filipjevi* and *Heterodera latipons* (Rivoal and Cook, 1993; Nicol and Rivoal, 2008; Akar et al, 2009) are considered the most economically important species in West Asia, North Africa, and

the Mediterranean (Nicol et al., 2011). Damage to crops by CCNs is considered the second in importance to damage caused by root-knot nematodes (Jones et al., 2013).

It has been mentioned by Nicole et al. (2011) that the environmental conditions influence the losses that are caused by CCNs and may exceed 90%. In association with other biotic and abiotic factors such as fungal pathogens, water stress, and heat, CCNs can have a synergistic destructive impact (Nicol et al., 2006).

Management methods have been attained by rotation with non-host crops, such as legumes and resistant cultivars. Due to the multi-year survival nature of the cyst that protects the eggs, a crop rotation period of at least 2 years is needed to maintain population densities below economic damage threshold (Bridge and Starr, 2007). So, this might be considered a non-feasible and profitable way of management due to its time and costs in cultivating practices during crop rotation. Host resistance is a desirable alternative because it is less expensive, easy to be used once identified, and it has no environmental toxicity like nematicides, despite successful use of nematicides to control nematodes (Williamson, 2006).

Nilsson-Ehle (1908) bred the first resistant cultivar against nematodes. In Europe and India, resistant cultivars were developed later on, but the most attainment in developing resistant cultivars was in Australia as a project was started in 1978 to screen wheat lines for CCN resistance and tolerance (Lewis, 2009).

Reports by Dababat (2014) and Williamson (2006) that the only enduring method present to control CCN is by the use of resistant cultivars, as it is considered cost-efficient, safe for the environment and user-friendly. In addition, Dababat (2014) stated that globally, the evolution of cultivars with genetic resistance plus genetic tolerance has been accomplished.

Breeding wheat for resistance against CCN started in the 1970s (Brown and Ellis, 1976), and later, Kimber and Feldman (1987) identified novel resistance sources in cultivated and wild wheat relatives. To date, 15 different resistance genes to *Heterodera avenae*, including 11; *Cre1* to *Cre8, CreR, CreY, Cre3S* in wheat and its relatives and *Ha1* to *Ha4*

genes in barley were reported (Bakker et al., 2006; Smiley and Nicol, 2009; Zhai et al., 2008; Moens et al., 2018).

The aim of this study was to screen and evaluate a set of international spring wheat lines for the resistance response to the CCN *Heterodera filipjevi*. This set of spring wheat was selected under heat stress and drought conditions in Mexico by the International Maize and Wheat Improvement Center (CIMMYT) and therefore, it is believed it could be a unique set for cereal nematode resistance.

It was anticipated that this study would provide new resistant spring wheat lines against the CCN *Heterodera filipjevi* which might contain novel sources of resistance and add them as genetic resources for future breeding programs. Another expectation of this research was to provide a base for future research in trying to understand the relationship between resistance and specifically drought and heat tolerance.

CHAPTER II

REVIEW OF LITERATURE

2.1 Wheat

2.1.1 History and evolution

Diploid (genome AA) (einkorn) and tetraploid (genome AABB) (emmer) wheat species were the primitive forms that were cultivated in the southeast of Turkey. Approximately 9000 years ago when hexaploid (genome AABBDD) bread wheat appeared, the cultivation spread to the Near East (Shewry, 2009).

Hexaploid bread wheat represents the majority of wheat that is grown globally, while nearly 5% is tetraploid durum wheat which is mostly found in the Mediterranean area and called pasta wheat. Other species of wheat are grown in insignificant amounts such as einkorn, emmer, and spelt (Shewry, 2009).

Wheat is usually classified by breeders into two groups depending on the growing season, winter wheat and spring wheat. The grouping takes into consideration the chilling requirements, winter hardiness and daylength sensitivity. Spring wheat growing period is between 100-130 days and can be grown in areas of harsh winters and little snow. Heading does not require chilling but it is affected by frost. Winter wheat growing period is between 180-250 days and requires chilling for normal heading (FAO, 2019).

2.1.2 Importance and success

The emergence of historical empires of Babylon, Assyria and in Egypt can be acknowledged to wheat cultivation, as it provided sufficient food to be produced to support cites. In colder climates, individuals' lives were changed, as they were able to survive the European winter by storing grains of wheat for long periods. Due to this fact, wheat can be considered to be the key element in which western civilization was built. In the present day, the area that wheat is cultivated is more than any other crop and the excess of uses have been developed, including flat and steamed bread, leavened bread,

biscuits, cakes, couscous, noodles, pasta, and other industrial uses. (Curtis and Halford, 2014).

During the 20th century when plant breeding based on Mendel's laws of inheritance replaced simple selection by the farmers, crops showed great improvements. Plant breeding alongside the advance and usage of fertilizers, pesticides, and herbicides, noticeably increased the yield of wheat. This development appeared at the right moment to avoid a widespread famine that was on the verge of occurring in Asia (India and Pakistan) in the 1960s (Curtis and Halford, 2014; Evans, 1998).

Norman Borlaug, an American scientist of the International Mazie and Wheat Improvement Center (CIMMYT) in Mexico developed disease-resistant, semi-dwarf wheat varieties, and high yields. These varieties were introduced to India and Pakistan, which remarkably led to the doubling of wheat yield in a period of 5 years between 1965 and 1970. The action of Norman Borlaug prevented a disaster that was about to happen. Alike improvements followed in rice and the expression "Green Revolution" was used to describe Borlaug's accomplishments (Curtis and Halford, 2014).

Since then wheat has shown its supremacy over the temperate world and its lead over other temperate crops not just because of high yield and capability to adapt, but due to the distinctive properties of doughs formed from wheat flours. As these properties allow it to be processed into a variety of products for example bread, pasta, noodles, fermented beverages. (Shewry, 2009)

2.1.3 Production

Wheat is grown on 218.5 million hectares of land, more than any other commercial food crop and remains the most vital food grain source for humans. In the most up-to-date FAO report, world production of wheat is estimated at 771.7 million tons, making it the second most-produced cereal after maize and third in crop production in general (FAOSTAT, 2019).

With the proper agricultural practices maintained such as providing adequate water and nutrients and proper pest and pathogen control, yields of wheat can surpass 10 tons per

hectare. Yet, with poor agricultural practices yield can be low as 2.8 tons per hectare and the world average stands at approximately 3.5 tons per hectare (FAOSTAT, 2019; Shewry, 2009).

Production of wheat has increased significantly over time, while harvested lands reached a peak in 1980 then started to decrease after the introduction of high yielding varieties (Figure 2.1). Asia has the highest production share of wheat at 44% followed by Europe at 33% then the Americans at 16% (Figure 2.2) and the top 10 wheat-producing countries which represent 70 % of total world production (Table 1.1). The top 10 countries of the harvested area of wheat represent 70.5% of the total world harvested lands are also mentioned in Table 1.2. (FAOSTAT, 2019).

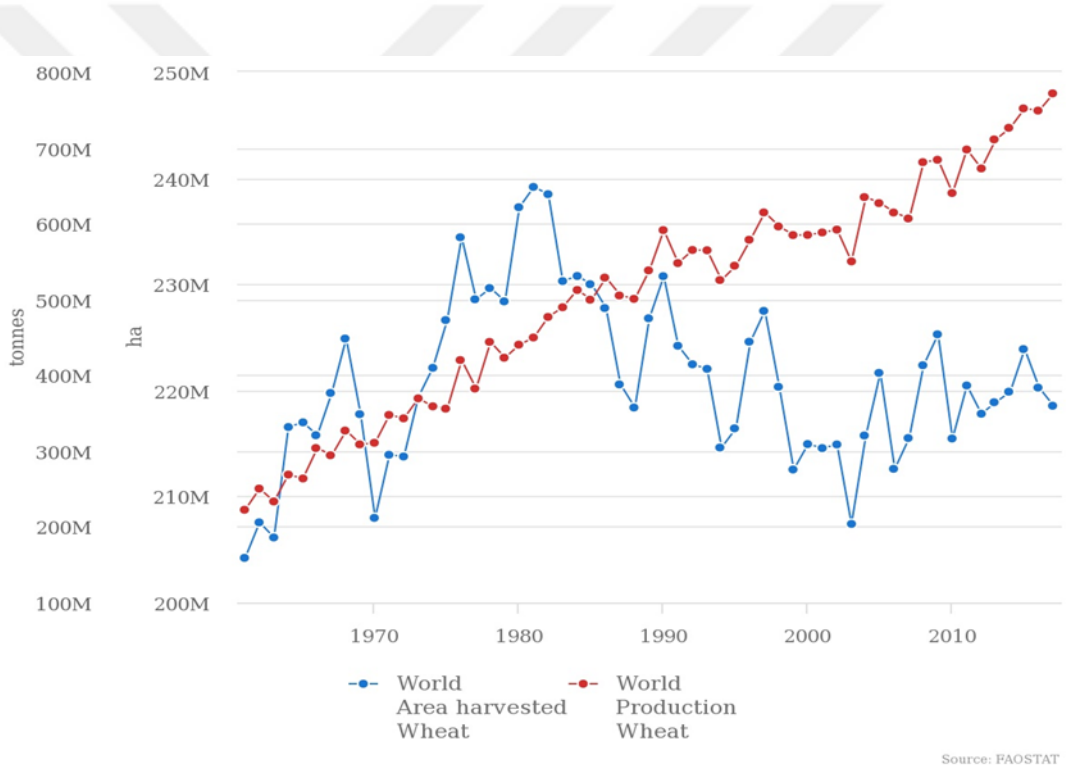

Figure 2.1: Production and Areas Harvested in the World (Total)
(http://www.fao.org/faostat/en/#data/QC/visualize)

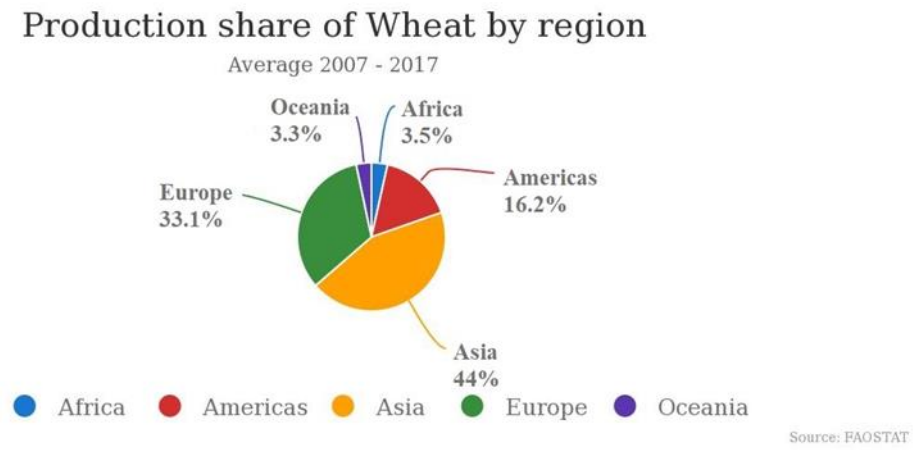

Figure 2.2: Production Share of Wheat by Region
(http://www.fao.org/faostat/en/#data/QC/visualize**)**

Table 2.1: Top 10 Wheat-Producing Countries
(http://www.fao.org/faostat/en/#data/QC)

Countries	Production Percentage (%)	Production in Tones
China	17.4	134,334,000
India	12.8	98,510,000
Russian Federation	11.1	85,863,132
United States of America	6.2	47,370,880
France	4.8	36,924,938
Australia	4.1	31,818,744
Canada	3.9	29,984,200
Pakistan	3.5	26,674,000
Ukraine	3.4	26,208,980
Germany	3.2	24,481,600
Rest of The World	29.75	229,548,103
Total	100	771,718,577

Table 2.2: Top 10 Counties of Wheat-Harvested Areas
(http://www.fao.org/faostat/en/#data/QC**)**

Area	Area Percentage (%)	Area in Hectare
India	14	30,600,000
Russian Federation	12.6	27,517,354
China, mainland	11.2	24,508,000
United States of America	6.9	15,210,680
Australia	5.6	12,191,153
Kazakhstan	5.5	11,911,989
Canada	4.1	9,035,993
Pakistan	4.1	8,972,000
Turkey	3.5	7,662,273
Iran (the Islamic Republic of)	3	6,700,000
Rest of the World	29.4	64,233,626
Total	100	218,543,068

Turkey is the 11th largest wheat producing country in the world, as it represents 2.8% (21 million tons) of global production. In terms of area harvested, Turkey ranks as the 9th highest country representing 3.5% (7.66 million hectares) of the world total. (FAOSTAT, 2019).

According to the Turkish Statistical Institute (Türkiye İstatistik Kurumu "TÜİK"), wheat is the prime cultivated cereal in Turkey followed by barley and oat. The top 10 wheat-producing provinces represent 43.8% of Turkeys' production with Konya and Ankara on top of the list representing 10.2% and 5.5%, respectively (Türkiye İstatistik Kurumu "TÜİK", 2019).

And the total cultivated area of the top 10 provinces represents 42.3% of the total cultivated area with Konya and Ankara on top of the list representing 9.3% and 6.4%, respectively (Türkiye İstatistik Kurumu "TÜİK", 2019).

2.1.4 Nutrition

Wheat importance in the nutritional aspect is it is considered to be a main and source of carbohydrates (energy). Nevertheless, it also contains an abundant amount of other important nutrients such as proteins, fibers, lipids, vitamins, minerals, and phytochemicals that are present in wheat in a minor proportion, which all may have a role in a healthy diet. It contributes to about 20% of the average daily intake of non-polysaccharides (dietary fibers) and contributes to the daily intake of B-complex vitamins (Shewry and Hey, 2015)

Shewry (2009) highlighted that wheat provides as much protein as soybean to human and livestock nutrition, regardless of having a low protein content between 8-15%. In underdeveloped countries, bread, bulgur, noodles, and other products possibly provide a substantial share of protein in the diet, that is why the nutritional importance of wheat protein should not be underestimated. In addition to wheat containing a high starch content, it is considered in the animal feed production to be a source of calories.

2.1.5 Challenges

For more than half a century global human consumption is increasing and meeting this demand is the main challenge that world agriculture is facing. From the period of 1962-2012 wheat consumption has increased by 269-fold in Indonesia, 105-fold in Bangladesh and 37.8-fold in Nigeria but there has been a decrease of wheat consumption in Russia by 33.3% and Ukraine by 44% due to various factors that are not a point of discussion here. Also, demand is rising in countries that are not suitable for wheat production (Curtis and Halford, 2014; Shewry and Hey, 2015).

The global population has tripled from 2.53 billion in 1950 to 7.63 billion in 2018 and is estimated to increase to the area of 9.5-10.5 billion in 2050 which results in the increase of demand, but also the increasing consumption per capita. For example, in China the population doubled between the years 1962-2012, wheat consumption increased 6-folds, so per capita consumption approximately tripled from 29 kg to 92 kg (Curtis and Halford, 2014; Popkin, 2006).

Latest indications continue to point to a reduction in cereal output in 2019/20 and total utilization of all major cereals is likely to continue growing in 2019/20, keeping pace with rising food demand and exceeding world production (Figure 2.3). Wheat production is increasing slightly year to year and trying to stay ahead of global request (Figure 2.4) (FAO, 2019, FAOSTAT, 2019; USDA, 2019).

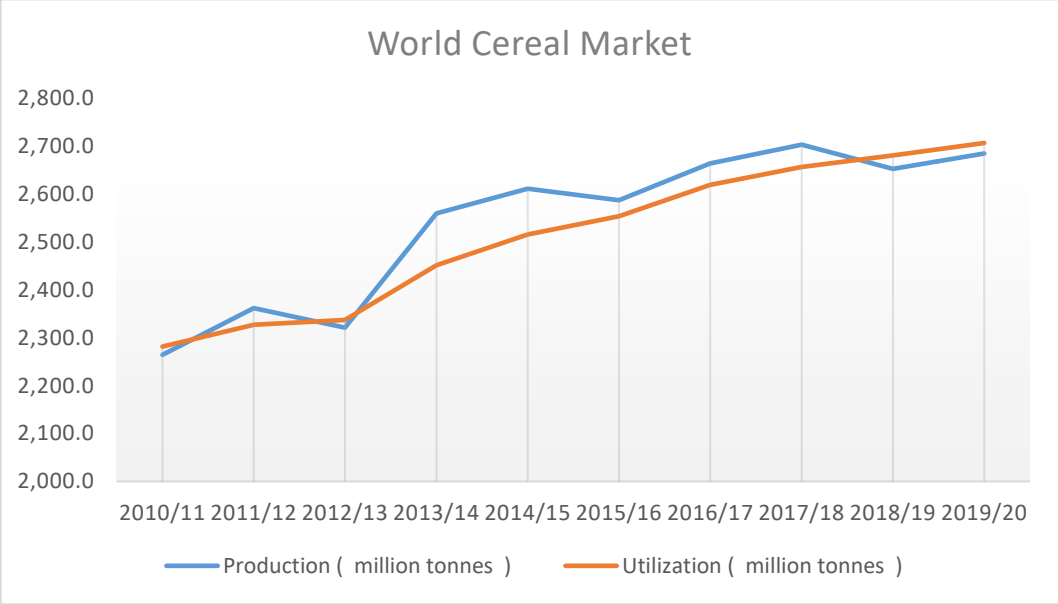

Figure 2.3: World Cereal Production & Utilization (http://www.fao.org/worldfoodsituation/csdb/en/)

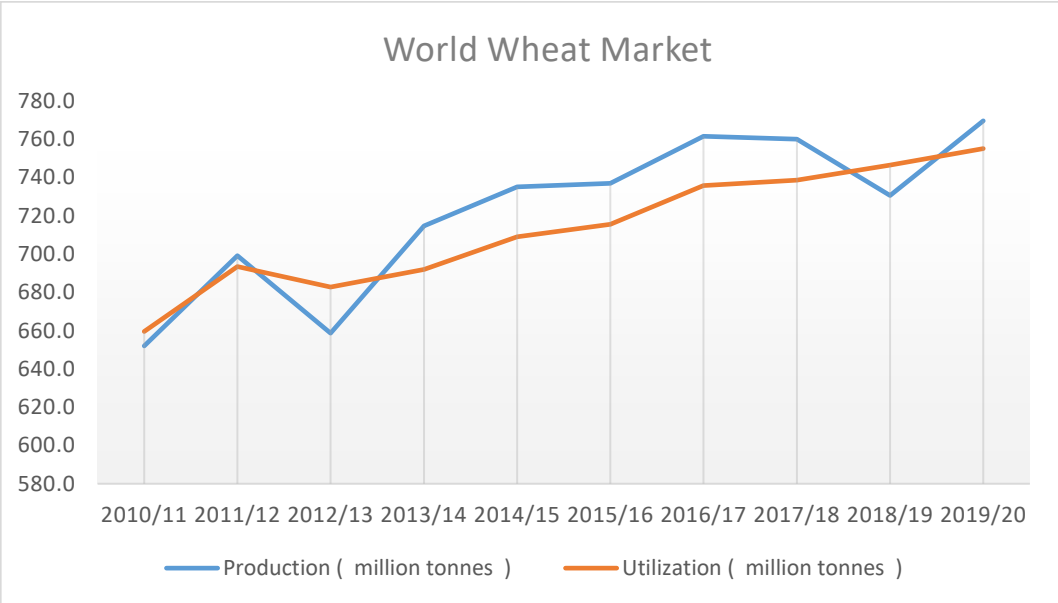

Figure 2.4: World Wheat Production & Utilization (http://www.fao.org/worldfoodsituation/csdb/en/)

With the growing population of the world, the increment of crop production especially increasing wheat yield is a main international priority to feed the people. It has been mentioned in numerous studies that global food production needs to increase by 70-100% as wheat production needs to be increased by at least 50% by the year 2050 to meet global demands; yet current trends are showing steady increase in yield (Godfray et al., 2010; Grassini et al., 2013; Ray et al., 2013; Scott, 2014).

2.1.6 Stresses

The various abiotic stresses (e.g. drought, flood, and temperature) and biotic stresses (e.g. bacteria, fungi, insects, and viruses) can interact together to affect wheat yield and production worldwide. Plant and pathogen interaction is referred to as biotic stresses which notably affect plant health (Hakeem, 2015; Linchtenthaler, 1996).

2.1.6.1 Abiotic stresses

Approximately 91% of the crop production areas are under stresses while only 9% are conducive for production. Abiotic stresses have surfaced as the main challenge for agriculture, such as drought, floods, extreme temperatures, salinity, acidity, nutrient deficiency, and mineral toxicity. Globally, these are considered to be the main reason of crop failure causing an average yield loss of more than 50% (Wang et al., 2007) therefore, causing losses worth hundreds of millions of dollars each year (Bal and Minhas, 2017).

Mickelbart, et al. (2015) referred to abiotic stress as the suboptimal climatic and/or edaphic conditions that harmfully alter cellular homeostasis and eventually weaken growth and health. For terrestrial crop species, the stresses include an excess of water (waterlogging) or shortage (drought), ion toxicity and the deficiency (mineral stresses) and temperature extremes, and tropospheric ozone.

Stress incidents can be classified as temporary stresses (high temperatures during the midday) or chronic stresses (high levels of Na^+ in sodic soil), and the timing of the stress has a relation to the daily cycle and the developmental stage which imposes the impact on viability and yield (Mickelbart, et al., 2015).

Abiotic stresses are considered to have more impact on wheat production more than biotic stresses. As a meta-analysis study projected that with every 2°C increase in temperate and tropical regions there will be a significant loss in wheat yield and related research forecasted a 6% decrease in wheat production (Abhinandan et al., 2018; Challinor et al., 2014; Asseng et al., 2014).

In the early vegetative stages, stresses reduce growth but do not have a significant effect on decreasing the yield of seed crops, opposite to what happens during the reproductive development which stresses noticeably diminish productivity. Generally, abiotic stresses happen in combination or in a sequenced matter, thus to maintain yield preservation fluctuating environments it can require the improvement and development of various mechanisms (Mickelbart et al., 2015).

It is necessary to develop and endorse strategies that will minimize the influence of abiotic stresses. Management of soil and production of crops including breeding new genotypes which can adapt to environmental stresses are the suggested strategies. A major challenge is to facilitate a fast adaptation and mitigation of these strategies without threatening the complex and delicacy of agroecosystems that contain inhabitants that are willing to survive with abiotic stresses (Bal and Minhas, 2017). Mickelbart et al. (2015) reported that plant will express stress tolerance or avoidance by acclimation and adaptation mechanisms that progressed via natural selection.

2.1.6.1.1 Osmotic stress

Osmotic stresses have a strong impact on wheat production and drought that is a worldwide problem which affects any wheat-producing area can cause severe osmotic stress (Daryanto et al., 2016; Oyiga et al., 2016). Roughly 20% of arable farmlands are endangered by soil salinization, which is another source of osmotic stress (Shrivastava and Kumar, 2015).

During all stages of wheat development, there is a possibility of exposure to osmotic stress that will lead to cellular damage. The extent of cellular damage caused during wheat development relies on the intensity and duration of osmotic stress and this can impact the

growth and development processes leading to compromised yield (Sarto et al., 2017; Wang et al., 2003).

Salt stress has a similar effect on wheat as drought stress (Francois et al., 1994). Salinity inhibits leaf growth and tillering by stopping the leaf primordia initiation rates without having an effect on the development stages (Grieve et al., 1993; Grieve et al., 1994). The flag leaf plays an important role in yield and grain filling, as it contributes to 30-50% of seed carbohydrates. Any damage to the flag leaf will have a negative impact on yield. Zheng et al. (2008) reported that osmotic stress caused by drought and ion toxicity can speed the senescence of the flag leaf leading reduction of photosynthetic activity due to the reduction of chlorophyll content (Abhinandan et al., 2018; Abbad et al., 2004; Guóth et al., 2009; Sylvester-Bradley et al., 1990; Farooq et al., 2014; Zheng et al., 2008).

Depending on the ploidy level of wheat cultivars osmotic responses differ. A meta-analysis study conducted on reports based on the tolerance level of 2n, 4n, and 6n cultivars showed that hexaploid cultivars were more tolerant to drought stress than the diploid and tetraploid cultivars (Wang et al., 2017). This is inconsistent with a previous report by Zhang and Kikham (1994) that said that hexaploid wheat showed higher susceptibility than diploid and tetraploid wheat.

2.1.6.1.2 Temperature stresses

Typical crop growth and development is notably controlled by temperature eventually determining yield (Porter and Gawith, 1999; Gray and Brady, 2016). One of the main reasons that wheat is cultivated around the globe is essentially due to its capability to withstand a broad temperature range with upper limits at 47.5°C to lower limits around -17°C (Porter and Gawith, 1999). Climatic events can stimulate other abiotic stresses not restricted to drought and temperature stresses, while deteriorating soils can increase salinity.

2.1.6.1.2.1 Heat stress

Heat stress, which is associated with global warming, has a huge impact on agriculture. Specifically, in wheat, heat stress is known to cause a group of physiological,

biochemical, and morphological changes that affect the growth and development of the crop. It has affected wheat yield causing significant reduction; therefore, the world has been struggling to match record production and to maintain food security (Hafsa Ali et al., 2015).

Between the years 1980-2010, heat stress effect in wheat cropping areas has increased substantially, specifically since the mid-1990s. This delivered not so much compensating but rather more simultaneous yield inconsistencies, provoking the general worry about food security (Zampieri et al., 2017).

High temperatures cause an increase in the atmospheric demand for water and decrease the crops water use efficiency (Ray et al., 2002). The exposure to heat stress results in plant damage by altering the functions of the cellular structures and metabolic processes (Nakamoto and Hiyama, 1999).

Temperatures above 30°C may double germination time, reduce germination percentage, and reduce normal root volume. When temperatures reach above 34°C it affects the grain weight as the duration of grain filling is reduced due to suppression of photosynthesis and directly inhibiting starch biosynthesis (Hafsa Ali et al., 2015; Fokar et al., 1998; Brestic et al. 2014; Telfer at al., 2013).

Wang et al. (2011) reported that exposing wheat to gradually increasing temperatures will permit for a priming phase that improves plant performance and pre-anthesis priming of wheat displayed less crucial post-anthesis damage. Photosystem damage and enzyme strain that affect photosynthesis due to heat leads to reduced yield, negatively affects pollen quality, and shows seed set reduction in wheat (Abhinandan et al., 2018; Hays et al., 2007; Farooq, 2011).

Heat can cause photosynthetic disturbance therefore, inducing early senescence which might be able to reduce assimilates need for grain filling. Machado and Paulsen (2001) noticed hastened senescence and death of wheat plant under greenhouse conditions when subjected to constant temperatures of 30-40°C regardless of the imposed drought. Other effects of high temperature on wheat is; decrease in dry matter which is directly linked to reduction in growth, a negative impact on grain number and filling, the influence on seed

size during kernel filing stage because of higher respiration rates, and 3-4 % loss in yield per 1°C increase in temperature above 15°C (Harding et al., 1990; Machado and Paulsen, 2001; Ihsan et al., 2016; Farooq et al., 2011; Hays et al., 2007).

During anthesis and seed set if there is an occurrence of high temperature, this will lead to a delay in germination and emergence that can affect plant density, which leads to a significant loss in yield loss (Wardlaw et al., 1989; Almansouri et al., 2001). Hence, temperature plays an important role in governing practices such as time of planting and harvesting thus, temperature fluxes during the growing season can cause serious crop losses (Ottman et al., 2012; Yang, Z. et al., 2017).

2.1.6.1.2.2 Cold stress

Winter wheat grown in temperate and arid regions face the risk of cold stress. Cold temperatures modify numerous biochemical processes and can induce membrane damage which affects plant performance negatively. It is important for winter wheat to go through hardening process, as seedlings of the crop gradually acclimate to cold conditions. Priming of wheat also aids to reduce damage and improve stress tolerance through mechanisms such as photosynthetic apparatus preservation (Thakur et al., 2010; Steponkus, 1984; Li et al., 2014). It is also necessary to note that wheat is very sensitive to cold stress during the reproductive phase, as it can affect the number of grains especially if cold stress happens before anthesis (Abhinandan et al., 2018).

Cold stress is generally defined as the plant's response mechanism to freezing temperatures (Gusta and Chen, 1987). Spring wheat in areas close to the equator face more damage due to cold stress shoot and root growth can completely shrink when night temperature falls under 10°C (Xin and Browse 2000). Winter wheat leaves are typically smaller and have less transpiration than spring wheat. The cold temperature will significantly reduce root growth and show a decline in osmotic potential in winter wheat (Fowler, 2001). It has been reported by Abdin et al. (2002) that an adaptive mechanism to survive the low temperature is cold hardening by substantially increasing the level of several proteins i.e. proline and glutathione. Also, Fowler (2001) stated that the expression of proteins, lipids, and sugars double in cold hardened wheat in order to survive low temperatures.

Pollen sterility can be caused by cold stress as it can disrupt gametophyte tissue development. Also, cold stress effects grain filling by reducing nutritional reserves that are diverted to seed development (Ji et al., 2017; Thakur et al., 2010).

2.1.6.1.3 Drought stress

Drought can be defined as the lack of rain or irrigation for a period of time in which the water content of the plant is decreased to a point that will hamper plant processes (Tuberosa, 2012). With all the environmental factors that affect yield, water shortage (drought) is considered the most serious constraint to agriculture, as it is possibly responsible for about 70% of global yield losses (Boyer, 1982). Even though the increase in temperature has a positive effect on crop production in some cool regions, drought will have a negative effect on plant growth and development (Zhang et al., 2018; Lesk, et al., 2016). With low precipitation and increased temperatures, drought is the fundamental limiting factor for crop production (Parry et al, 2007; Daryanto et al., 2017).

A sustainable and economically feasible method to boost crop productivity, reduce crop losses due to drought, and guarantee food security is by the development of cultivars with enhanced drought resistant (Blum, 1988; Bennett et al., 2012).

The effect of drought on agronomical traits of wheat is significant in addition to the duration and harshness of the stress reduce the duration of grain filling thus influencing the degree of yield loss. The intensity and frequency of droughts are projected to rise due to the effect of climate change. Until this point in the 21st century drought frequency and areas affected has increased by 50-200% (Zhang et al., 2018; Zhao and Dai, 2017; Trenberth, 2014).

About 45% of global wheat production is affected by drought as it is considered a major constraint to wheat, affecting its growth, development, and yield. Drought stress causes retard plant growth, inhibits the formation of primary and secondary roots and encourages the formation of stout roots therefore affecting yield (Hakeem, 2015).

Due to the impact of drought stress on wheat, there should be a focus on strategies concerning genetic solutions such as screening wheat germplasm for drought tolerance or

enhanced varieties that use water efficiently and development of water-efficient varieties alongside advanced agronomic practices (Hakeem, 2015).

As a result of natural selection, wheat plants have found diverse responses to reduce damage caused by drought stress and maintain cellular homeostatic. Drought tolerant trait that offers tolerance in one area or year may not show this trait in another location or different year this is why studies on the genetic level are not often performed. The reason for this is because complex theoretical combinations of numerous traits are responsible for tolerance (Yang et al., 2007).

2.1.6.1.4 Waterlogging stress

Waterlogging affects more than 10 million hectares worldwide in environments with excessive rainfall and irrigation. Cultivars that are sensitive to waterlogging can show up to 50% decrease in root mass and around 75% decrease in shoot mass. Roots show an increase in minerals and sugars however shoots will show a decrease in mineral content (Hakeem, 2015).

The effect of waterlogging can be reduced dramatically by applying breeding methods and engineering solutions. In order to attain waterlogging tolerance, it is proposed that a phased procedure is followed. The main step is to integrate adaptive traits from local, national, or international germplasm with known tolerance then the incorporation with other adaptive traits that are relevant to the environment where the is intended to be cultivated (Villareal et al., 2001).

2.1.6.1.5 Mineral stress

Jonathan and Samuel (2004) have defined mineral stress as the suboptimal availability of essential nutrients or toxicity of nutrients or non-nutrient materials such as aluminum, cadmium, sodium, manganese, or some other heavy metals. Mineral stress affects approximately 40 million hectares of wheat globally, principally due to the alkalinity and acidity of the soil. The various reasons for mineral stress according to a study by Ranieri at al. (2005), are the amendment of chemical fertilizers, sludge and sewage irrigation, and atmospheric deposition.

The developed techniques that have been found to manage mineral stress in wheat is usually costly and absurd, but some approaches have been followed to achieve tolerance against mineral stress. One of these approaches is combining between breeding plants with mineral stress tolerance and farming strategies none the less it has been proven that ecological, biological, and economic considerations are more efficient than breeding solutions. Genetic gains can easily be predicted by breeding because single dominant genes are responsible for tolerance. Plant response to mineral stress can be realized by genomics in changing environments (Hakeem, 2015; Sillanpaa, 1990).

2.1.6.2 Biotic stresses

In addition to abiotic stresses that plants encounter, they also face the risk of pathogen infection and attack by herbivorous pests under natural circumstances. Climate change can influence the habitat area of pathogens and pests, as increasing temperatures are acknowledged to ease the spread of the pathogen. Furthermore, plants defense mechanisms have displayed weakness and increased their susceptibility to pathogen infection due to abiotic stresses (Suzuki, et al, 2014; Atkinson and Urwin, 2012; Nicol et al., 2011; Bale et al., 2002).

The interaction between plants and pathogens is referred to as biotic stress and during their life cycle plants health in affected notably as they interact with an extensive diversity of organisms. The infection process requires a form of direct or indirect contact (Peterson, 1974).

Biotic stress is described generally as stress that occurs causing harm to an organism done by other organisms such as bacteria, viruses, fungi, parasites, beneficial and harmful insects, weeds, and cultivated or native plants, precisely in plants it is defined as the stress that negatively affects crops caused by pathogens particularly viruses, bacteria, fungi, nematodes, insects, arachnids, and weeds (Flynn, 2003) Thus, we can understand that biotic stress is broadly defined and depends on those who study it. The biotic stress that is introduced in plants mainly depends on the climate and the targeted species resistance response.

Biotic stresses are thought to be one of the key causes to yield losses in most crops, as there are reports that mention complete yield loss. Some remarkable cases of biotic stress happened in Europe in 1845 when approximately 80% of potato fields were lost sue to *Phytophthora infestans* which causes potato blight. An additional case was in the 1970s when *Helminthosporium maydis* that causes corn leaf blight attacked corn fields which resulted in significant economic losses (Borém, 2012).

Biotic stress is an important cause of preharvest and postharvest losses, as they deny the plant from nutrients resulting in plant vigor reduction and in severe cases the death of the host plant (Singla and Krattinger, 2016). Biotic and abiotic stresses interact to affect wheat yield and production worldwide (Afzal et al., 2015). The primary selection criteria in the modern wheat selection is the resistance to biotic stresses (Todorovska et al., 2009).

Fungi are the chief pathogens that wheat suffers from and are known to cause epidemics however, nematodes mainly stay in the soil and weaken the plant. The main biotics stress groups that are going to be discussed are fungi, bacteria, viruses, and nematodes (Singh, 2017).

2.1.6.2.1 Fungi

The fungi kingdom is considered a large group of eukaryotic organisms which contain members such as fungi, yeast, mold, mildew, and mushrooms. 70% of major plant disease have been reckoned to be caused by fungi, oomycetes, and myxomycetes (da Silva Pereira et al., 2012).

2.1.6.2.1.1 Rusts

Rust diseases are fungal diseases and have been known since long ago to negatively affect wheat production. They are considered the most important disease affecting wheat and it is the disease that scientist have the most knowledge about. Leaf rust or brown rust is the most familiar disease in rust diseases and where ever wheat is cultivated it can be identified due to its adaptation to a broad range of temperatures. Whereas stem rust or black rust has the tendency to arise warm moist regions, while stipe rust or yellow rust

occurs in cool areas. They are known for their inconsistency in avirulence or virulence (McIntosh, 1997; Royo, and Di Fonzo, 2005).

Leaf rust or brown rust is caused by *Puccinia triticina* and is regarded as the most common rust disease in wheat. It is an obligate pathogen that has the capability of creating virulent urediniospores on the condition that tissues of the leaves remain alive. Symptoms on susceptible varieties show large uredinia without causing chlorosis in the host tissue or necrosis (Bolton et al., 2008).

Stem rust or black rust was the most devastating wheat disease until the 1950s and it is caused by *Puccinia graminis* f.sp. *tritici* (Royo and Di Fonzo, 2005). The disease is characterized by the existence of uredinia on the plant, which are brick-red, elongated, blister-like pustules that can be shaken off easily. (Singh, 2008).

Stripe rust or yellow rust is mainly a wheat disease in cool climates with temperatures range between 2- 5°C and caused by *Puccinia striiformis* f.sp. *tritici*. The name comes from the distinctive uredinia stripe that generates yellow-colored urediniospores. Severe losses can occur due to shriveled grains and damaged tillers (Royo and Di Fonzo, 2005).

Common bunt also known as stinking smut and covered smut, is caused by *Tilletia tritici* (syn. *Tilletia caries*) and *Tilletia laevis* (syn. *Tilletia foetida*). It infects spring wheat and winter wheat worldwide (Royo and Di Fonzo, 2005). Symptoms are not simply recognized until close to maturity and are rarely obvious (Wiese, 1987).

Dwarf bunt is caused by *Tilletia controversa* and infects winter wheat also it is limited to areas with snowfall. In disease favorable environments, these two funguses may infect more than 70% of the spikes as dwarf and common bunt decreases approximately 0.8% of the yield for every 1% infection (Hoffmann and Sisson, 1987).

Loose smut is caused by *Ustilago tritici* and can be found wherever *Triticum aestivum* and *Triticum turgidum* are grown. It is a seed-borne pathogen, common in areas with cool and moist climates during the flowering phase of growth. Losses are measured from low to moderate; profit can be reduced by 5-20% with 1-2% infection. (Batts, 1955; Royo and Di Fonzo, 2005).

2.1.6.2.1.2 Rot diseases

Root rot of cereals have been described or mentioned with numerous names such as common root rot, crown rot, foot rot, pink rot, and stalk rot and occur worldwide (Royo and Di Fonzo, 2005; Mergoum et al., 1995). It is caused many fungi such as *Fusarium pseudograminearum* previously known as *Fusarium graminearum, Fusarium culmorum* are the two most stated species of *Fusarium*, *Bipolaris sorokiniana* (syns. *Helminthosporium sativum*), and *Cochliobolus sativus* which are soilborne pathogens found in dryland and rainfed environments (Royo and Di Fonzo, 2005). Seedborne pathogens most likely cause seed blights while soilborne pathogens normally cause crown rot and foot rot (McIntosh, 1997).

Severe infection of rot and crown rot happen under moister-limited conditions and these pathogens should not be underestimated. Symptoms are difficult to identify precisely but the fungi affect roots, crowns, subcrowns, coleoptiles, and stem bases. Under the effect of *Bipolaris sorokiniana*, it is also common for the plant to be stunt, show late death of tillers, premature ripening, and the development of whiteheads or deadheads (Royo and Di Fonzo, 2005).

Powdery Mildew

Powdery mildew is a fungal disease that widely infects wheat all around the world and is most harmful in cool, moist regions such as China, Europe, and Southeast USA. It is caused by *Blumeria graminis* f.sp. *tritici* (nature) and symptoms of the disease are powdery white dots on wheat foliage and as the disease progresses the dots turn gray then black or brown (McIntosh, 1997).

2.1.6.2.2 Bacteria

Controlling bacterial diseases in the plant is problematic specifically after the epidemic has been established. The challenge is demonstrated in the variation of inoculum sources, speedy multiplication of the disease after infection, the appearance of bacteria varieties that have the ability to overcome and nullify certain control strategies, cultivars with low

level of resistance, and the low effect of licensed chemical and biological products (Lopes and Boiteux, 2012).

Phytopathogenic bacterial infection method needs genetic activation of recognition, contact establishment, host colonization, and infection. By the use of contemporary molecular biology and genetic techniques, these complicated actions have been progressively clarified (Lopes and Boiteux, 2012).

Bacterial stripe or commonly known as black chaff or bacterial streak is one of the most important diseases in North and South America, West Asia, and North Africa. It mostly infects wheat varieties whit Mexican origin while landraces from Ethiopian origin are virtually immune (McIntosh, 1997; Tesemma, and Mitiku, 1992). It is caused by *Xanthomonas campestris* pv. *translucens* and symptoms can be found the leaves, leaf sheath, and glumes as thin chlorotic lesions or stripes that have a water-soaked appearance (wheatdoctor.org, 2019a)

Basal glume rot is caused by *Pseudomonas syringae* pv. *atrofaciens* and is believed to have importance in Europe and Ukraine (McIntosh, 1997). Symptoms are found on the leaves, culms, and spikes of wheat as water-soaked lesions that start out small and dark green then develop to dark brown to black (wheatdoctor.org, 2019b).

Bacterial leaf blight is caused by *Pseudomonas syringae* subsp. *syringae* which occurs after heavy rainfall and has been reported in North USA and Canada (McIntosh, 1997).

2.1.6.2.3 Viruses

Viruses can be defined as microscopic, obligatory, intracellular parasites that complete their life cycle of growth and reproduction by relying on the host cell (Shiel Jr., 2019; Boiteux et al., 2012). This close connection between the virus and the host limits the selection of approaches to control viral diseases. according to this situation, the most practical and efficient method of control is genetic resistance to viruses and/or their vectors (Boiteux et al., 2012).

Barley yellow dwarf luteovirus and soil-borne wheat mosaic furovirus are regarded as the two most economically important viruses that affect wheat production. There are also other viruses reported on wheat but little is known regarding their genetic resistance or tolerance (Royo and Di Fonzo, 2005).

Barley yellow dwarf virus is one of the most common and destructive diseases of wheat and is transmitted by at least 23 aphid species and infect over 80 species of grass. Symptoms of the virus are yellowing and dwarfing but are not strongly expressed in wheat when compared to other cereals such as oat and barley (Lister and Ranieri, 1995; Brakke, 1987; Burnett et al., 1995).

Soil-borne wheat mosaic virus can sometimes be a dangerous disease on winter wheat in Europe Japan and USA. the virus is vectored by *Polymyxa graminis* fungus which infects wheat roots but is not destructive (McIntosh, 1997). Symptoms of the virus are most noticeable on young leaves as mild green-yellow mosaic, yellow-green mottling, parallel streaks, and dashes. Sometimes on the leaf tips, necrosis and reddish streaking can take place. (Cowger and Weisz, n.d.).

2.1.6.2.4 Nematodes

Nematodes are microscopic organisms and the most numerous multicellular life form that can be found in all environments. Many of them are beneficial by contributing to nutrient cycling and they feed on soil microorganisms (Vaish, 2017). However, the ones with negative impact are plant-parasitic nematodes, which the majority of these nematode group attack the root cells of the plant and result in economic losses. They are distinguished from other nematodes by a spear-like structure called the stylet which helps them penetrate the cell structure for aiding the nematodes for feeding.

Plant-parasitic nematodes are believed to be a dangerous limitation to plant production and acknowledged to cause substantial harm to wheat crops, alongside many fungal, bacterial, and viral diseases, such nematodes are cereal cyst nematodes (*Heterodera* spp.), root-knot nematodes (*Meloidogyne* spp.), wheat seed gall nematode (*Anguina tritici*), root-lesion nematodes (*Pratylenchus* spp.), stem and bulb nematode (*Ditylenchus* spp.) and stunt nematode (*Tylenchorhynchus* spp.) (Dababat and Fourie, 2018; Vaish,

2017). For this reason, there have been attempts to describe the symptoms that are caused by plant-parasitic nematodes, features of identification and biology. Moreover, there are also efforts to provide significant efficient approaches for nematode management (Vaish, 2017).

Symptoms on plants that are attacked by plant-parasitic nematodes are generally not as severe as symptoms resulting from fungal, bacterial, and viral infection (Vaish, 2017), but in advance stages, symptoms can be more severe than any other source of infection. Stunting, yellowing, and wilting are perhaps the most noticeable symptoms of plant-parasitic nematodes that can be easily mistaken with symptoms of nutritional deficiencies, the existence of hardpan soils, salinity and alkalinity issues, termite infection, and further factors (Smiley, 2016). As the effect of cereal cyst nematodes on cereals is usually underestimated or not recognized by farmers, pest management consultants and researchers (Smiley, 2016).

2.1.6.2.4.1 Cereal cyst nematodes (*Heterodera* spp.)

The CCNs belong to the genus *Heterodera* and get their name from the brown cyst structure that is formed after the death of the mature female (Vaish, 2017). The main species of *Heterodera avenae* complex with economic importance are *Heterodera avenae*, *Heterodera filipjevi*, and *Heterodera latipons* (Smiley, 2016).

The general symptoms on wheat include stunting, yellowing, withering, and wilting. But the key symptom on the root systems is the visibility of swollen, white, round females that later turn into a brown color cyst the enclosures the eggs. The color degree depends on the stage of maturity of the female or cyst (Vaish, 2017). Plant with severe infection are stunted, with light yellowish-green color, thin leaf blades, and few tillers (Dababat and Fourie, 2018). The most effective method of control is the use of germplasm with resistant genes against the cereal cyst nematode (Dababat, 2014).

The cereal cyst nematodes will be discussed in detail below.

2.1.6.2.4.2 Root lesion nematodes (*Pratylenchus* spp.)

The genus *Pratylenchus* contains over 60 species affecting numerous crops globally; with eight species that are acknowledged to parasitize cereals (Handoo et al, 2001; Rivoal and Cook, 2001, Castillo and Volvas, 2001) and *Pratylenchus thornei, Pratylenchus crenatus, Pratylenchus neglectus* and *Pratylenchus penetrans* are found from time to time in coexistence (Smiley and Nicole, 2009; Smiley, 2010).

Pratylenchus thornei is widely studied *Pratylenchus* spp. That parasitizes cereals compared to others and can be found in almost all wheat growing countries. *Pratylenchus neglectus* and *Pratylenchus thornei* are found in more than 90% of dryland crop areas in the Pacific Northwest of the USA. (Dababat and Fourie, 2018).

Infected root systems show water-soaked lesions and may overlap and appear in a green and reddish-brown color. The plans capability to absorb water and nutrients from the soil is radically decreased due to the degrading of the lateral roots and the reduction in root branching and root hairs caused by the *Pratylenchus* spp. (Smiley, 2010). There are no specific above-ground symptoms, as infected plants show chlorosis, stunting, reduced tillering, and overall lack of health which results for the plant to wilt and patches or zones are noticed in infected fields (Smiley, 2010; Vaish, 2017). These symptoms can be easily mistaken with nutrient deficiencies, drought, root disease, barley yellow dwarf disease or infection by other pathogens (Dababat and Fourie, 2018). The penetration of root systems by these nematodes eases the infection by other pathogens such as fungi and bacteria that result in significant yield losses (Smiley, 2010).

When then the environment becomes dry in the late-season infected crops may show premature wilting due to the damaged root systems that are incompetent in absorbing the moisture in the soil. If the is sufficient rainfall, wilting is less likely to occur and the plant may look that they have a nitrogen deficiency. Another indication of nematode infection is the falling wheat yields over multiple years (Vaish, 2017).

The second most important nematode species infecting wheat in terms of economic damage is *Pratylenchus* (Castillo and Vovlas, 2007; Smiley and Nicole, 2009). The species that holds the most economic importance of this group is *Pratylenchus thornei*

that causes yield loses up to 85% on wheat (Dababat and Fourie, 2018). The other two species *Pratylenchus neglectus* and *Pratylenchus penetrans* do not cause the same damage level or have worldwide distribution as *Pratylenchus thornei*. *Pratylenchus neglectus* causes wheat yield losses up to 23% (McDonald and Nicol, 2005) and when present with *Pratylenchus thornei*, they cause yield losses up to 74% (Vanstone et al., 1998).

2.1.6.2.4.3 Root-knot nematodes (*Meloidogyne* spp.)

The root-knot nematode (*Meloidogyne* spp.) especially *Meloidogyne graminicola* has lately appeared as an important pest of wheat and barley that are grown after rice due to the late start of winter and early start of summer in rice-growing areas. This nematode is known to infect crops from nearly all botanical families. It also generates its multiple cycles within the cropping season as a polycyclic pathogen (Vaish, 2017). A number of *Meloidogyne* species attack cereals, such as *Meloidogyne graminicola, Meloidogyne graminis, Meloidogyne kikuyensis* and *Meloidogyne spartinae* in warm climates and *Meloidogyne incognita, Meloidogyne javanica* and *Meloidogyne arenaria* in tropical and subtropical areas (Dababat and Fourie, 2018).

Meloidogyne naasi is considered the most important root-knot nematode that affects cereals in most of Europe and has been found in Chile, Canada, Iran, Maltese islands, New Zealand and Turkey, the USA, and the former USSR. *Meloidogyne artiellia* (British root knot nematode) is known to reproduce on cereals and cause severe damage to legumes. It is found in Algeria, France, Greece, Israel, Italy, Morocco, Russia, Spain, Syria, Tunisia, Turkey, and the UK. *Meloidogyne graminicola* cause severe damage to wheat in rice–wheat. *Meloidogyne chitwoodi* affects cereals in the Pacific Northwest of the USA and is also found in Australia, Mexico, and South Africa. *Meloigogyne graminis* is not widely distributed worldwide and is limited to the southern USA, where it is associated with cereals and turf grasses although of reports finding this nematode in the coastal areas in Germany and the Netherlands (Dababat and Fourie, 2018).

It is common for gall formation on the root systems, especially the root tips on plants infected by the *Meloidogyne* species (Dababat and Fourie, 2018). *Meloidogyne graminicola* tends to lead wheat, barley, and rice to foliar diseases such as foliar blight or

spot blotch of wheat or barley (Vaish, 2017). *Meloidogyne naasi* symptoms are very similar to *Heterodera avenae* symptoms on wheat, with visible areas that show poor growth and yellowing of plants (Dababat and Fourie, 2018).

The economic importance of root-knot nematodes effect on cereals is limited to a couple of species. *Meloidogyne naasi* had a critical effect on wheat yields in Chile and Europe and substantial yield loss of barley in parts of Europe and the USA. *Meloidogyne artielia* was reported on wheat in Greece, Israel, and Italy and *Meloidogyne chitwoodi* was reported on cereals in Mexico and the USA (Dababat and Fourie, 2018)

2.1.6.2.4.4 Wheat gall nematode or ear cockle of wheat (*Anguina tritici*)

Ear cockle disease of wheat caused by *Anguina tritici*, which was reported by Needham in 1743 from England as the first plant-parasitic nematode, was the reason for the disease. The juveniles feed on young leaves and then attack the inflorescence and developing seeds (Vaish, 2017). *Anguina tritici* infects cereals in Western and South Asia, North Africa, Europe, Australia, Brazil, China, Russia, New Zealand, and parts of the USA (Dababat and Fourie, 2018).

Young plants that are infected show minor signs of swelling at the basal part of the stem and the plants appear stunted. Twisting and curling of leaves are observed, which prevent normal ear emergence, that can be easily noticed. The ears are small in size and are dark in color when comparing them to uninfected ears and can be mistaken to be bunt disease (*Tilletia tritici*). Seed galls transition from green to dark brown then black color and become hard. Each seed gall possibly will contain 1000-30000 dormant juveniles that may survive for decades under dry conditions (Vaish, 2017; Dababat and Fourie, 2018).

On the infected ears and leaves a yellow slime/gum-like material is observed and in humid conditions the slime drops on the tissue of the plant converting to a brown color when drying (Dababat and Fourie, 2018). Yellow ear rot of wheat is developed by the association of this nematode with the phytopathogenic bacterium. *Rathayibacter tritici* is vectored by *Anguina tritici* (previously known as *Corynebacterium tritici*) (Maraite et al., 2007) and *Rathayibacter toxicus* which normally does not affect wheat, is vectored by *Anguina funesta* and *Anguina paludicola* (Murray et al., 2017).

All wheat, barley, and rye areas are attacked by *Anguina* species and when associated with the bacteria *Rathayibacter tritici* it can cause a significant negative impact on wheat yields (Maqbool, 1988), with reported losses reaching 30% in Iraq (Stephan, 1988) and 10-30% in China (Chu, 1945).

2.1.6.2.4.5 Stem and bulb nematodes (*Ditylenchus* spp.)

Stem and bulb nematodes (*Ditylenchus* species) are one of the most destructive plant-parasitic nematodes containing numerous species and has numerous hosts. This nematode occurs globally in a wide climatic range but is mainly dominant and devastating in temperate climate areas (Plowright et al., 2002; Vaish, 2017). The species of importance on cereals is *Ditylenchus dipsaci*, mainly on oat and rye and is found in numerous areas such as Africa, Argentina, Australia, Brazil Western and Central Europe, Canada, and the USA (Plowright et al., 2002). Kheiri, 1972 reported in Iran that *Ditylenchus destructor* has been linked to numerous crops such as wheat, potato, tomato, eggplant, soybean, maize, tea, orange, and alfalfa.

General symptoms of the infected plant are basal swellings, dwarfing and twisting of stalks and leaves, internodes and many axillary buds are shortened, which result in the production of an abnormal number of tillers that gives plants a bushy appearance (Dababat and Fourie, 2018). Seriously infected plants will probably die in their early stages resulting in empty spot in the fields and infected plant in later stages fail to produce flower spikes (Kort, 1972).

The economic damage done by *Ditylenchus dipsaci* relies on numerous elements such as host-plant susceptibility, soil infection levels, soil type and weather conditions, and also depends on the extensive intraspecific variation among *Ditylenchus dipsaci* races (Dababat and Fourie, 2018). The nematode holds economic importance on rye and oat, but not on wheat and barley (Sikora, 1988).

2.1.6.2.4.6 Stunt nematode (*Tylenchorhynchus* spp.)

The stunt nematodes belong to the genus *Tylenchorhynchus* (Cobb, 1913) which have been known to be found worldwide. Several *Tylenchorhynchus* species are acknowledged for attacking various agronomical and horticultural plant species (O'Bannon et al., 1991; Vaish, 2017).

The following *Tylenchorhynchus* species have been linked to wheat and barley: *Tylenchorhynchus vulgaris, Tylenchorhynchus eremicolus, Tylenchorhynchus persicus, Tylenchorhynchus indicus, Tylenchorhynchus brassicae,* and *Tylenchorhynchus mashhoodi*. Theses nematodes are considered to be migratory ectoparasitic nematodes, as they feed on the root surface then penetrate the epidermal cells of root hairs and roots but sometimes are endoparasitic feeders restricted to the outer cortical root layers. *Tylenchorhynchus* species have been recorded to feed at the root tip in big groups, causing at this location mechanical breakdown of epidermal, cortical, and undifferentiated vascular tissue (Vaish, 2017).

Clear damage is not observed on crops unless juvenile numbers are greater than 5000 per 1 L soil. Stunt nematodes are particularly susceptible to desiccation, which can lead to a substantial fall in nematode numbers after a dry summer (Vaish, 2017).

2.1.6.2.4.7 Other nematode species

There are other nematodes that have the capability to cause yield loss on cereals but they are not widespread globally and their damage potential is not clarified yet. Such nematodes are *Longidorus elongatus, Merlinius brevidens,* and *Nanidorus/Paratrichodorus* spp. *Paratrichodorus anemones* and *Paratrichodorus minor* (known as *Nanidorus minor*) damage cereal crops in the USA and with sown wheat in early fall in sandy soils that is high susceptible to *Paratrichodorus minor* (Dababat and Fourie, 2018).

2.2 Cereal Cyst Nematodes

2.2.1 Overview

A chief group of the plant-parasitic nematodes with significant economic importance worldwide are the cyst nematodes affecting the world's key crops. The cyst nematodes especially the species within the *Heterodera* and *Globodera* genera are a source of yield loss to various crops such as cereals, rice, potatoes, and soybean that is the reason of their economic importance. The *Heterodera* genera is acknowledged to have the most species, even though other genera contain other cyst nematode species. Formally cyst nematodes were thought to be restricted to temperate climates but it has been known that numerous species occur in tropical and subtropical areas (Evans and Rowe, 1998).

Cyst nematodes are acknowledged to be extraordinary parasites, as they have a specified interaction with a plant that leads to the development of a distinctive feeding structure inside the roots of their host called the syncytium (Jones et al., 2013).

Mulvey (1972) initially classified the cyst nematodes based on their morphological characteristics into five groups and this has established the basis of cyst nematode grouping. The first group contained species that are now been categorized in the genera of *Globodera, Punctodera,* and *Dolichodera*. The second group contained species that are now categorized in the *Cactodera* and *Betulodera* genera. Group 3 is the *Heterodera avenae* complex, group 4 is the *Heterodera schachtii* group and finally, group 5 is the *Heterodera goettingiana* group (Evans and Rowe, 1998). Later on, these groups have been altered due to the developments in molecular analysis. The cyst nematodes were groups into six main clades by Subbotin et al. (2001) on the basis of the analysis of sequences of the internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA) and taking into account morphological characteristics and host specialization. The first clade is formed by *Cactodera* and *Punctodera* species of the *Globodera* genus, the other clades fit the *Avenae, Cyperi, Goettingiana, Humuli, Schachtii* or the *Sacchari* group respectively. The *Heterodera* group can be preliminary separated from other groups depending on the general shape of the cyst as the group has a distinguishing lemon shape of the cyst cone.

Subbotin, et al. (2010a) and Subbotin et al. (2010b) classified the cyst nematodes into eight genera of the *Heteraderinae* subfamily, the genera are *Heterodera*, *Globodera, Cactodera, Dolichodera, Paradolichodera, Betulodera, Punctodera*, and *Vittatidera.* Despite belonging to the *Heteroderinae* subfamily, not all genera are cyst forming; such as *Atalodera, Bellodera, Meloidodera* and *Verutus* genera (Evans and Rowe, 1998).

Relying on only morphological characterization, the previous genus *Heterodera* was divided into the *Heterodera, Globodera, Punctodera* and *Cactodera* genera (Wouts and Baldwin, 1998). Later, the *Dolichodera, Betulodera, Paradolichodera* and *Vittatidera* genera which are represented by one species were added (Turner and Subbotin, 2013). By the year 2017, the cyst nematodes were grouped into eight genera and a total of 121 valid species (Moens et al., 2018).

The nematode genus Heterodera in the family *Heteroderdae* is one of the oldest genera of plant-parasitic nematodes that have been discovered (Schmidt, 1871), after the genera *Anguina* (Scopoli, 1777) and *Tylenchus* (Bastian, 1865). Members of this genus are obligate parasites and different crops are attacked by different species often causing economic loss (Siddiqi, 2000).

The genus *Heterodera* is distinctive among nematode genera because of the phenomenal of the female to change into a tough, brownish color cyst, which protects the eggs that have formed in the female's body (Thorne, 1961). This is what the word Heterodera refers to “different – skin/s” of the female (Siddiqi, 1986).

The whole cyst nematode species are obligatory endoparasites that feed within the roots of their hosts. The body wall of the female turns to a tan color and dries after fertilization and egg formation. This process creates an enduring cyst that contains a great number of embryonated eggs that can survive of a long period of time until the availability of an appropriate host. this enduring characteristic can explain one of the reasons for their economic importance (Moens et al., 2018).

2.2.2 Life cycle

The general life cycle of the cyst nematodes is the same and can be described as the following: subsequent to gastrulation, the nematode embryo grows in length and movement starts inside the egg, then folds develop inside the embryo. The first molt occurs, then the formation of the stylet at the anterior end of the second stage juvenile (J2). The J2 stage is considered the dormant stage of the cyst nematode life cycle and the unhatched J2 can stay inside the cyst for several years depending on the species and the environmental situation. When the appropriate circumstances are met for hatching, the J2 overcome the environmental conditions and makes an opening in the egg using the stylet and exits the cyst by a natural opening called the fenestral region or exits from the neck where the female's head has broken away. This is the end of dormancy and when the active part of the cyst nematode life cycle starts (Perry and Moens, 2006).

The J2 will start searching for a suitable host in the soil depending mainly chemical gradients that the host's root system releases. When the host is found the J2 enters the root system typically exactly behind the root tip. It then migrates to a layer between the endodermis and the phloem called the pericycle and advances to an appropriate feeding site. As soon as the feeding initiates, the J2 molts (second molt) to the third-stage juvenile (J3). The J3 male and female have an advance genital primordial and rectum, also the male has single testis and female has paired ovaries. In order to assist in the rapid growth and developing ovaries, the female shape becomes round or globular. The fourth molt occurs and the female breaks through the root cortex, then the vulva is shaped giving access to the reproductive system, which is retained by egg formation (Perry and Moens, 2006).

Males develop in the root almost as the same rate as females and also appear at the fourth molt wrapped in the cuticle from the third stage. They live for a short period of time, free-living, and non-feeding nematodes. The females secrete sex pheromones to attract the males and might encounter several matings. After that, the development of the embryo in the egg occurs, then the J2 forms while still inside the female's body. The female dies and then the cuticle starts to tan, which becomes a robust protective cyst that enclosers hundreds of eggs, the number of eggs is impacted by environmental condition and species. In the certain *Heterodera* species, the egg sac discharged beyond the cone region. The

cyst separates from the roots when the plant starts to die and stays dormant until a suitable host appears and the life cycle repeats itself (Turner and Evans, 1998). Figure 2.5 represents the life cycle of the CCN *Heterodera avenae* and also represents the General life cycle of CCNs (Yang et al., 2017).

The time that the cyst nematode takes to complete its life cycle from egg to egg differs, it mostly depends on the co-evolution of the species with its host range and the conditions of the environment. Generation numbers per year differ between cyst nematode species, with the rise of soil temperatures the number of generations increases until a certain limit which is based on the species. The majority of temperate cyst nematode species complete one or two generation per year which depends on the growing season length of the host and if the temperature is in the optimal range (Perry and Moens, 2006).

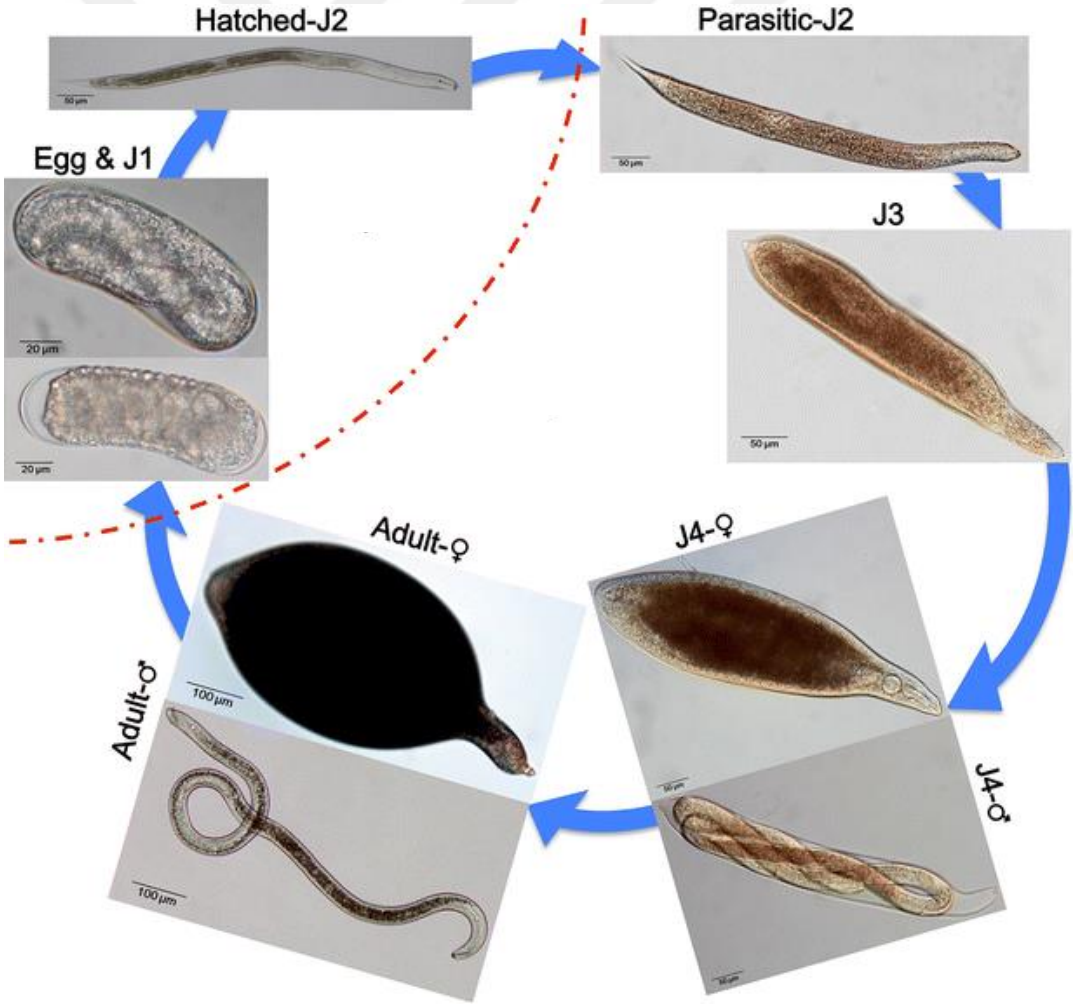

Figure 2.5: Life Cycle of the Cereal Cyst Nematode *Heterodera avenae*, Source (Yang et al., 2017)

2.2.3 Identification

2.2.3.1 Morphology

Similar morphological characteristics are shared by the species of the Heteroderinae family and minor details can set apart the species (Turner and Subbotin, 2013). Classical identification relies on the morphological and morphometric characterization of the cyst and the J2 (Moens et al., 2018).

A significant characteristic of the genera separation is the existence or lack of a vulval cone; as the only genus that the cyst has an easily seen vulval cone is *Heterodera*. The formed opening is called the fenestra and the presence or absence of the fenestra helps in the identification of genera and species (Moens et al., 2018). Subbotin (2010b) has mentioned one of the reasons that the identification within the Heterodera genus is quite problematic, is due to the huge number of species and the variation of morphological and morphometric characters.

The J2 morphology (stylet knobs shape, number of lines in lateral field, number head annules) and morphometrics (body length and width, hyaline tail length, true tail length, stylet length, stylet knob width) also helps in genera and species identification (Moens et al., 2018).

2.2.3.2 Molecular

Biochemical chemical techniques for precise identification have been formed due to the difficulty and considerable time that standard identification consumes, specifically when a sample has more than one species. Fleming and Marks (1982) demonstrated that by using isoelectric focusing (IEF) depending on the basis of different protein profiles the separation of *Globodera rostochiensis* and *Globodera pallida* is possible, and Subbotin et al. (1996) also used this technique to sperate between the *Heterodera* species of the *avenae* group.

DNA based identification methods have quite a few advantages over IEF, as DNA profiles from a limited number of nematodes or even one can be acquired and due to the

results being clear, species are easy to identify. Also, there is no effect on the environment or developmental variation (Subbotin et al., 2013). Many cyst nematodes are analyzed by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and polymerase chain reaction (PCR) techniques with species-specific primers. Species identification of the *Heterodera* genus is possible by using PCR-RFLP of the internal transcribed spacer (ITS) region of the ribosomal RNA (rRNA) gene (Subbotin et al., 1999; Subbotin et al., 2000; Zheng et al., 2000; Madani et al., 2004).

2.2.3.2.1 *Heterodera avenae*

Heterodera avenae was first recorded in 1874 by Kuhn as a parasite in Germany and now it can be found in most of the wheat cultivated areas. It has one generation per year with the hatching of the J2 strongly depending on the temperature. The fenestral region and underbridge can be seen in Figure 2.6

Cyst:

A mature cyst is lemon-shaped with prominent neck and vulval cone. An annulated labial region with 6 continuous lips and a labial disk. Color is dark brown (Subbotin, 2010b).

The general morphometrics of the cyst and J2 as below, which differs between populations (Subbotin, 2010b):

Length: 600-808 µm **Width:** 465-601 µm **Fenestral Length:** 43.2-55 µm **Fenestral Width:** 20.2-24.8 µm **Vulval Bridge Width:** 7.3-12 µm **Vulval Slit Length:** 8.1-10.2 µm **Vulva to Anus Distance:** 55.7-57 µm

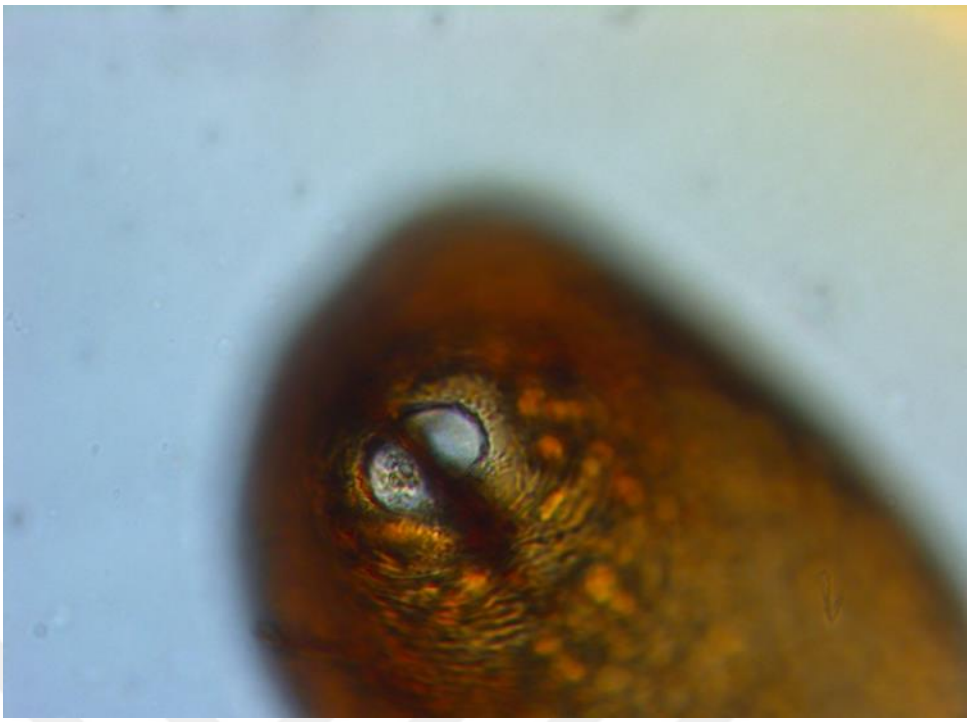

Figure 2.6: The Fenestral Region of *Heterodera avenae*

J2:

Round offset labial region with 2-4 annuli, body annuli distinct. Well-developed stylet with large anteriorly flattened concave basal knobs, with the cone less than half the spear. Rounded median bulb, muscular. Tail 3-4.5 anal body diameter.

Stylet Length: 26-27 µm **Labial region height:** 3.6-4.4 µm **Labial Region Diam:** 8.6-9.7 µm **DGO:** 5.4-5.7 µm **Anterior end to Median Bulb Valve:** 69-77 µm **Anterior end to Excretory Pore:** 108-115 µm **Anterior end to Pharynx:** 117-123 µm **Body Diam. at Mid-body:** 21-24 µm **Body Diam. at Anus:** 15.6-16.3 µm **Tail Length:** 60.5-70 µm **Hyaline:** 38-44.8 µm

2.2.3.2.2 *Heterodera filipjevi*

Heterodera filipjevi when first discovered in 1941 it was thought to be *Heterodera avenae* as they have a large degree of similarity, but the difference can be identified from the fenestral region and underbridge in the cyst (Figure 2.7). Regarding morphology, morphometric and isoelectric focusing (IEF) Subbotin et al. (1996) came to a conclusion

that *Heterodera filipjevi* was discrete in protein patterns from *Heterodera avenae*. One generation develops per growing season (Subbotin, 2010b).

The general morphometrics of the cyst and J2 as below, which differs between populations (Subbotin, 2010b):

Cyst:

A mature cyst is lemon-shaped with prominent vulval cone. Color varies from light to dark brown. Bifenestrate with massive underbridge, thicker in the central part with 2 – 3 arms.

Length: 597-928 µm **Width:** 437-685 µm **Fenestral Length:** 50.7-59 µm **Fenestral Width:** 24.5-30.4 µm **Vulval Bridge Width:** 7.7-13 µm **Underbridge Length:** 74-92 µm **Vulval Slit Length:** 7.3-10.9 µm **Vulva to Anus Distance:** 52-63.4 µm

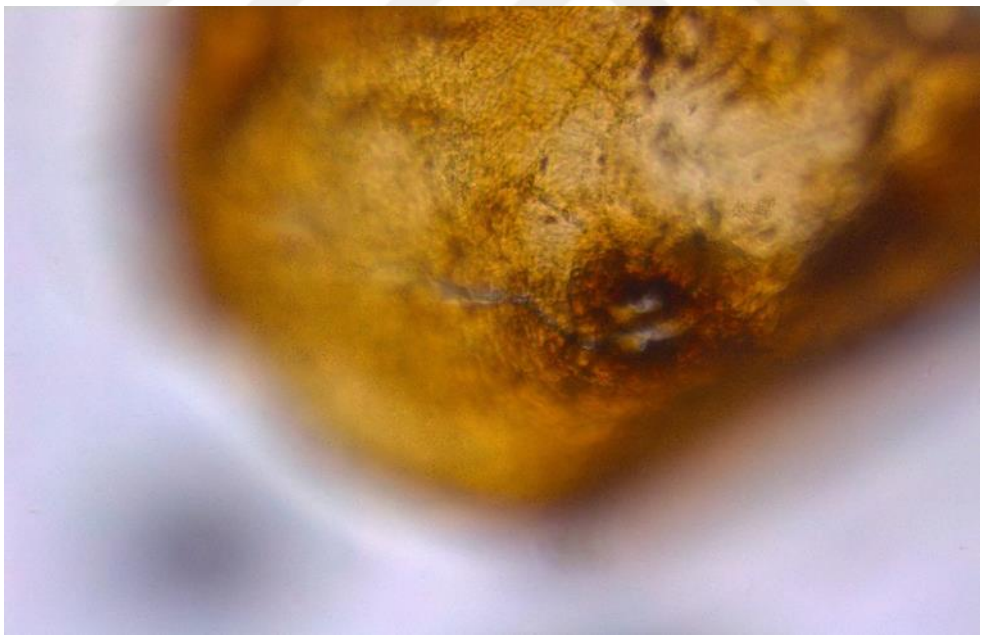

Figure 2.7: The Fenestral Region of *Heterodera filipjevi*

J2:

Offset labial region with 2 faint annuli, prominent labial disk. Well-developed stylet with anteriorly projected knobs, large median bulb. Conical tail and lateral field with 4 incisures.

Stylet Length: 24-26.5 µm **Labial region height:** 3.7-4.3 µm **Labial Region Diam:** 8.2-10.1 µm **DGO:** 5.3-6.2 µm **Anterior end to Median Bulb Valve:** 69.6-80 µm **Anterior end to Excretory Pore:** 94.7-114 µm **Anterior end to Pharynx:** 122-132.2 µm **Body Diam. at Mid-body:** 21-23.3 µm **Body Diam. at Anus:** 14.4-16 µm **Tail Length:** 57.1-62 µm **Hyaline:** 32.8-38.9 µm

2.2.3.2.3 *Heterodera latipons*

Heterodera latipons was first recorded in the early 1960s and similar to *Heterodera avenae* on roots of stunt wheat and barley. It was studied then described by Franklin (1969) as *Heterodera latipons*. It completes one generation per growing season and the J2 is considered sensitive to high temperatures (Subbotin, 2010b).

Cyst:

Lemon shaped cyst; semifenestrate separated by a distance greater than the fenestral width with a distinct fenestral region underbridge (Figure 2.8). Strong underbridge with a thickening in the middle. Color is mid-dark brown.

The general morphometrics of the cyst and J2 as below, which differs between populations (Subbotin, 2010b):

Length: 544-584 µm **Width:** 413-447 µm **Fenestral Length:** 53.5-67 µm **Fenestral Width:** 14.5-21.9 µm **Vulval Bridge Width:** 22.4-33 µm **Vulval Slit Length:** 6-7.9 µm **Underbridge Length:** 89-105 µm **Underbridge Width:** 10.6-11.6 µm

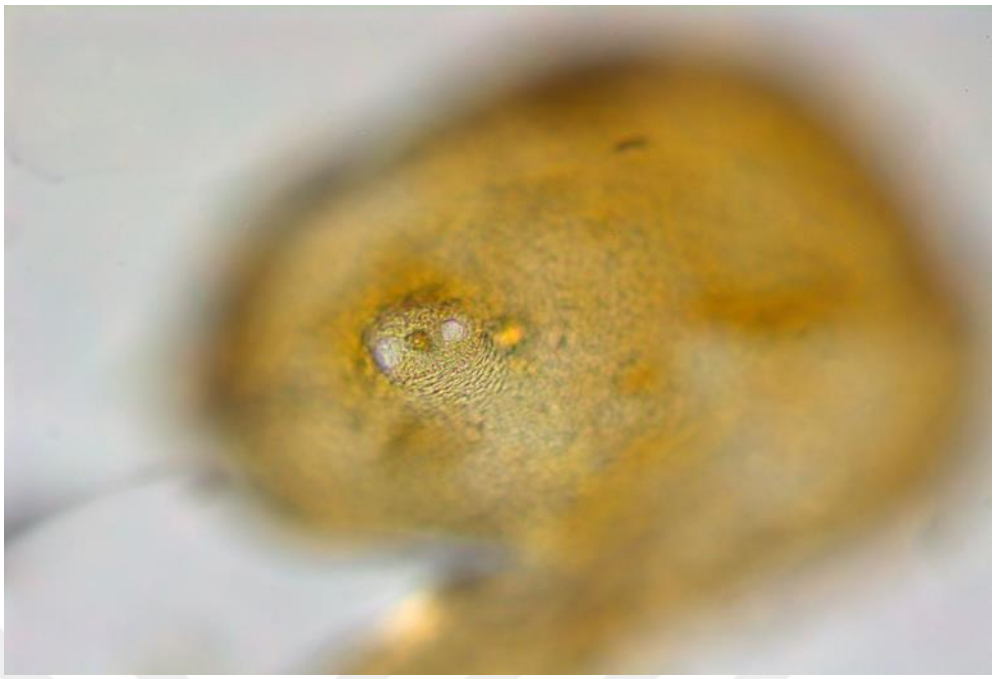

Figure 2.8: The Fenestral Region Underbridge of *Heterodera latipons*

J2:

Slightly curved body, offset labial region with 3 postlabial annuli. Well-developed stylet with anteriorly concave knobs. Distinct hemizonid with the excretory pore opening directly behind or at the same level

Stylet Length: 22 – 26.4µm **Labial region height:** 3.9 – 5.1µm **Labial Region Diam:** 8.4 – 9.6µm **DGO:** 4.6 – 5.5µm **Anterior end to Median Bulb Valve:** 65 – 77.2µm **Anterior end to Excretory Pore:** 97 – 110.6µm **Body Diam. at Mid-body:** 19.8 – 23.8µm **Body Diam. at Anus:** 14.1 – 15.9µm **Tail Length:** 47.8 – 57µm **Hyaline:** 26.5 – 33.7µm

2.2.4 Syncytium

The syncytium is defined as a large multinucleate cell (may consist of up to 250 fused root cells) produced by cell wall breakdown and fusion of protoplasts. the capability of the cyst nematodes to promote the formation of syncytium in the root hairs of the host plant as a method of amending for parasitism is considered impressive. The syncytium is the source of all nutrients that the cyst nematode requires in order to develop to the adult

stage. A single syncytium is induced by a single nematode and it is necessary for this structure to be preserved for a couple of weeks to maintain the feeding of the nematode (Moens et al., 2018).

Moens et al. (2009) reported that there are a few resemblances between the syncytia that are induced by cyst nematodes and the giant cells that are induced by root-knot nematodes. As both are large multinucleated structures with a highly active metabolism that reveals enriched cytoplasm compared to the surrounding tissue. On the other hand, Baldwin et al. (2004) mentioned that through phylogenetic analysis the ability of cyst nematodes and root-knot nematodes to induce these feeding structures has evolved separately.

2.2.5 Hatching

There are substantial differences in the optimal temperature required for the hatching of the cyst nematode species. For species that feed during winter or early spring, low hatching temperature is required. While species that are found in warmer climates, higher hatching temperature is required. The type of soil also affects the hatching rates, as commonly coarse-textured soil encourage hatching followed by the attack of the root systems by the J2. As this type of soil is proper for good aeration and nematode migration. It is noted that at soil field capacity the hatching rate is at maximum, while hatching is limited under drought and waterlogging (Turner and Subbotin, 2013).

According to the nematode species, each of them can hatch over a wide range of temperatures. Hatching of CCN is controlled by diapause, which is a state of prevented development where it does not endure until specific necessities have been fulfilled, even if favorable conditions occur (Toumi et al., 2018).

In terms of *Heterodera filipjevi*, originating from the continental Central Anatolian Plateau of Turkey, it does not show any diapause as the juveniles hatched immediately at the beginning of the winter wheat growing period (Şahin et al., 2009).

2.2.6 Economic importance

As mentioned before the *Heterodera* and *Globodera* genera have economic importance out of the eight genera of the cyst nematode. The species of these genera are found in temperate regions and temperate zones of tropical, sub-tropical and tropical regions. It is evident that these climatic groupings are substantially overlapping (Moens et al., 2018). But here the *Heterodera* genus, which contains the CCNs will be focused on in a bit more detail.

The genus *Heterodera* consists of more than 90 species of nematodes (Subbotin, 2010a; Subbotin, 2010b), and the most economically important species are the CCNs. The CCNs are a complex group of 12 species that are closely related (*Heterodera avenae* Wollenweber, 1924; *Heterodera arenaria* Cooper, 1955; *Heterodera bifenestra* Cooper, 1955; *Heterodera turcomanica* Kirjanova and Shangalina, 1965; *Heterodera latipons* Franklin, 1969; *Heterodera iri* Mathews, 1971; *Heterodera mani* Mathews, 1971; *Heterodera hordecalis* Andersson, 1975; *Heterodera filipjevi* Madzhidov, 1981; Stelter, 1984; *Heterodera aucklandica* Wouts and Sturhan, 1995; *Heterodera spinicauda* Wouts, Schoemaker et al., 1995; *Heterodera pratensis* Gäbler et al., 2000) that attack cereals and grasses (*Poaceae* family), resulting in high crop losses worldwide (Rivoal and Cook, 1993; Nicol and Rivoal, 2008; Yan and Smiley 2010).

The first CCN that was described with noteworthy status was the *Heterodera avenae* (Wollenweber, 1924), followed by the Mediterranean CCN *Heterodera latipons* (Franklin, 1969), then *Heterodera hordecalis* (Andersson, 1974), *Heterodera filipjevi* (Madzhidov, 1981).

The most important species of nematodes in temperate regions that affect cereals is *Heterodera avenae* which is commonly known as the cereal/oat cyst nematode. The nematode has been reported in the majority of the wheat growing countries. It has been reported by Rivoal and Cook (1993) that *Heterodera avenae* infested more than 50% of field of main European cereal growing area. Nicol and Rivoal (2008) reported that annual yield losses are approximately £3 million, while in the USA in the states of Idaho, Oregon, and Washington annual losses in wheat production is around US$ 3.4 million

An additional cyst nematode was mentioned on wheat plants that were stunted, in the Mediterranean region specifically Israel and Libya, Franklin (1969) characterized this nematode as *Heterodera latipons* based on morphological features of the Israel population and is commonly known as the Mediterranean cereal cyst nematode.

Heterodera latipons is widely spread and is fundamentally found in the Mediterranean region and the Middle East, however it was also found in relatively temperate climates of the former Soviet Union, Japan, and Canada (Toumi et al., 2017; Mulvey and Golden, 1983; Subbotin et al., 1996; Momota, 1979; Sewell, 1973). *Heterodera latipons* and *Heterodera avenae* are regularly found together in mixed populations in cereal cropping systems and when compared to *Heterodera avenae* it is thought to cause less damage to cereals (Mor et al., 1992; Mor et al., 2008). Despite of this *Heterodera latipons* was stated in Cyprus to reduce the yield of barley by about 50% and Philis (1988) and Philis (1997) mentioned that the losses are at maximum under harsh drought conditions and monoculture systems. Also, Schölz (2001) noted that in Syria the nematode was more harmful under water stress situations and showed a 20% reduction in barley yield and 30% reduction in wheat yield.

Heterodera filipjevi or commonly known as filipjevi cyst nematode was previously named Gotland strain of *Heterodera avenae*, pathotype 3 of *Heterodera avenae* or race 3 of *Heterodera avenae*. The availability of the nematode is limited to China, Germany, India, Iran, Norway, Poland, Spain, Syria, Sweden, Tajikistan, Turkey, the former Soviet Union, and the USA. Nicol et al. (2006) reported that this nematode causes about 42-50% losses in yield on winter wheat in rainfed conditions. While Hajihasani et al. (2010) reported that in Iran *Heterodera filipjevi* causes approximately 48% yield losses in winter wheat under rainfed conditions. In 2005 Holgado et al. reported that *Heterodera filipjevi* was found on Rye caused damage to the plant.

These three species also create a major limiting biotic factor to cereal production in temperate rain-fed growing regions such as Australia. China, India, Turkey, the USA, and many countries in Europe (Rivoal and Cook 1993; Dixon et al. 2009). Globally, CCNs cause substantial economic yield losses in many countries, especially in dryland cereal systems (Nicol et al., 2003; Subbotin, 2010a; Subbotin, 2010b; Dababat et al., 2015).

CCNs can have a synergistic negative effect in combination with other biotic and abiotic factors, such as fungal pathogens and water stress (Nicol et al., 2006).

The reduction of yield in wheat caused by *Heterodera* species was reported for different countries and regions. Averaging 46% loss of wheat yield caused by *Heterodera avenae* (Meagher, 1972; Ibrahim, 1999; Peng, 2007; Mathur, 1980; Maqbool, 1988; Maqbool, 1992; Holgado, 2003; Hassan 2010; Smiley, 1994; Namouchi-Kachouri, 2007) and averaging 48% loss of wheat yield caused by *Heterodera filipjevi* (Holgado, 2003; Nicol, 2006; Hajihasani, 2010). While in terms of *Heterodera latipons*, the loss in yield for wheat is not well documented but there are some reports that indicate the yield reduction to 50% (Philis, 1988; Philis, 1997; Hajihasani, 2010).

2.2.7 Virulence, pathotype, and race

In nematology, the term virulence means the nematodes capability to reproduce on a resistant plant host, while avirulence is defined as the lack of ability to reproduce. A mixture of virulent and avirulent nematodes are usually found in nematode populations and by the passage of time the ratio may be altered due to two main factors; the selection of the host and vigor of different nematode genotypes (Moens et al., 2018).

Mainly amphimictic plant-parasitic nematodes species are typically showing intraspecific genotypic and phenotypic diversity. Accordingly, before commencing with any breeding program it is wise and sensible to evaluate field population numbers virulence against potential sources of resistance. In case of some cyst nematodes, host races or pathotypes are described as populations that demonstrate intraspecific variation in their responses to host resistances. To help identify which sources of resistant are the most efficient, structures of pathotype and races have been formed (Moens et al., 2018).

Resistant cultivars can help control numerous species of cyst nematodes under the prevailing framework, the interaction of virulence/avirulence nematode genes with the genetic traits in the host plant is called as resistance. So, resistant cultivars reduce the reproduction of a nematode population, but when the population is capable to reproduce and raise its population number on such a cultivar it is recognized as a virulent population

(Moens et al., 2018). Cook and Rivoal (1998) mentioned that genetic variation amongst the virulent population was exhibited as a result of using resistant cultivars.

Identifying pathotypes is based on the interaction between the plant's genetic system and the nematodes genetic system. In the experiments of the effect of virulence phenotype with the differentials of the host plant, these interactions are usually acknowledged. According to this, pathotypes are viewed as a group of individual nematodes with common gene/genes for virulence/avirulence that is different from gene or gene combinations found in other nematode groups (Moens et al., 2018).

The cyst nematodes pathotype schemes have been suggested for the main species of potato cyst nematodes (PCNs) *Globodera rostochiensis* and *Globodera pallida*, CCNs *Heterodera avenae, Heterodera filipjevi* and *Heterodera australis*, and soybean cyst nematode (SCN) *Heterodera glycines*. The bases of these schemes are the reproduction ability or inability of the population on a range of host plant differential within each species (Moens et al., 2018).

Anderson and Anderson (1982) developed a pathotype scheme that portrays the pathotypes of the CCN species *Heterodera avenae, Heterodera filipjevi,* and *Heterodera australis*, that is founded on the multiplication of the species on host differentials of wheat, barley, and oat. The response between barley cultivars and the know resistant genes (*Rha1* 'Ortolan', *Rha2* 'Siri' and 'KVL191', and *Rha3* 'Morocco') have established for the pathotype to be separated into three groups.

Races in nematology is defined as the fields population that has distinct reproduction capabilities on plant lines that carry numerous sources of resistance on resistant cultivars.

Inbreeding programs, an environment of interactions guides the decision to select sources of resistance that are utmost efficient. They are produced by using the availability of host differentials set that represent the variety of resistant sources and the different virulence groups that are represented by nematode populations (Moens et al., 2018).

2.2.8 Screening

Screens (resistant tests) have been established for almost every crop to present phenotypic data for their particular breeding program. The aims for these screens are to find and identify new sources of resistance and also the identification of resistant progeny in segregating populations. Nematode resistance screening depends on the availability of resistant germplasm and advances in breeding programs to produce new genotypes for testing. New genotypes can reveal the genetic complexity of the host, crossing success, crops life cycle, and available resources for screening. The conduction of resistant screens can be done in various ways and all aim to provide outcomes that are efficient, consistent, and reproducible, so that host genotype susceptibility is repeatedly ranked between years and in autonomous exams (Moens et al., 2018).

In order to guarantee that the screening test was effective and to allow comparisons between trials, susceptible and resistant control are added. To reduce error and improve accuracy in the resistant tests it is necessary to have replications. It is essential that the resistant data is strong and sound as decisions are based on it in the nematode management programs that use resistant cultivars, also it is important for genetic studies. Resistant response and nematode reproduction can be dependent on environmental factors, so it is preferable that screen tests are performed under controlled environments (Moens et al., 2018).

Enhanced control and better efficiency are achieved by standardized procedures; in contrast, in order to evaluate important agronomical traits and tolerance assessment, field performance assessment is needed (Moens et al., 2018).

Normally, host response to nematode parasitism is not easily quantifiable, therefore, the number of reproduced nematodes usually determines resistance in nematology. Reproduction index is governed by the new generation of visible females of the surface of the roots, and they are counted before transforming to tanned cysts or if the roots are washed, the remaining females on the roots in addition to ones extracted from the soil are counted. Another method is to count the extracted cysts from the soil and then count the eggs of each cyst to get a measurement of fecundity on separate host genotypes.

Nevertheless, it is labor-intensive and might not be justified and necessary for regular screening of large populations (Moens et al., 2018).

2.2.8.1 Field trials

Fisher (1982) determined after broad experiments with *Heterodera australis*, that it is not likely to precisely evaluate resistance in field trials. Accordingly, Lewis et al. (2009) reported that resistant screening of cultivars is primarily is carried out almost completely under controlled conditions.

Despite that, in several countries it is reported that field trials are used with large numbers of replications and most often, trials are conducted in soils that are naturally infested. Planting cultivars aide by side in replicated strip plots in field trials is used for the comparison of cultivars under high and low nematode stress situations (Smiley et al., 2013; Marshall and Smiley, 2016).

2.2.8.2 Plot trails

It is more efficient and precise to screen cultivars for resistant response under controlled conditions, due to the high variability it is necessary to use a high number of replications (Pinkerton et al., 2011; Pariyar et al., 2016b). This method is very efficient when conducted outdoors during the time that juvenile densities are at the maximum in naturally infested soils.

The duration of the screening is usually around 9 weeks from planting until the assessment of the roots. To avoid saturation of the soil you may allow its surface to become dry. Close and careful supervision of watering must be accounted for, as long as plants do not become severely wilted (Moens et al., 2018).

In this method of screening, to identify genotypes with resistance it is a necessity that white females are counted, either by the ones that are able to be seen on the undamaged root ball surface or the total number of females after washing the roots. The first method allows for a greater number of plants to be examined per day (Lewis et al., 2009; Andersen, 1961).

2.2.8.3 Miniaturized trials

Miniaturized screening test either by using the Petri plate method or test tube method is considered to show the greatest accuracy regarding phenotypic reaction distinction. The techniques are challenging but then again deliver an accurate assessment of cultivar resistance and good quality and clean cyst for succeeding experiments (Moens et al., 2018).

Regarding the Petri plate method, initial experiments are required to regulate the juvenile number needed to produce white females and the assessment test is held on agar in sterile conditions. At the end of the experiment, the plant is considered susceptible if there are 1 or more white females that are swollen (Moens et al., 2018).

In the test tube method, plastic tubes with a 1 mm hole (to allow water and nutrient uptake) in the base are used. The tubes are filled with a growing mixture which differs from researcher to researcher but is generally a mixture of sand, sterilized field soil, and sterilized organic matter. After that, the seed is sterilized and then pregerminated (Moens et al., 2018).

The plants in the tubes are watered and given nutrients (from below) when necessary during the incubation period of 12-16 weeks up until the female nematode mature. After watering is stopped, crops are allowed to dry out naturally for at least a month. The plant is considered resistant to the tested nematode population if approximately 1 cyst or less per plant is found (Moens et al., 2018).

2.2.8.4 Drawbacks

Wild species and genotypes that differ in their genetic background from the susceptible control lines may have numerous genes with little effect on the multiplication of nematodes during the screening test. As there might by substantial difference from the susceptible check regarding the multiplication rates on these plants, either lower or higher (Moens et al., 2018).

Also, to be taken in consideration when evaluating the outcomes of the resistant screens that may have a significant impact on the multiplication of the nematode is the variation among the root systems and the vigor of the plant (Moens et al., 2018).

An important limitation to take into consideration regarding the screening tests, is the difficulty to compare results of the different tests is the lake of a standardized method of testing.

2.2.9 Resistance

Ireholm (1994), Rivoal et al. (2001), and Smiley and Yan (2015) have reported that when a cultivar shows resistance to a certain population of *Heterodera avenae* it does not mean it will show resistance to different *Heterodera avenae* population or species of *Heterodera*. These complications pose a major challenge for managing crop losses by adapting the use of resistant cultivars.

In wheat 11 *Cre* genes have been identified for their resistance to *Heterodera avenae*. It has been reported that some of these genes show effectiveness against *Heterodera australis*, *Heterodera filipjevi*, *Heterodera latipons*, and *Heterodera sturhani* (Seah et al., 1998; Nicol et al., 2003; McDonald and Nicol, 2005; McIntosh et al., 2008; Smiley and Nicol, 2009; Rathjen et al., 1998; Jahier et al., 2001; Rivoal et al., 2001; Lewis et al., 2009; Imren et al., 2013; Smiley and Yan, 2016; Wu et al., 2016;). The *Cre1 – Cre8* genes, *Cre3S*, and *CreR* have been identified and located on chromosomes, up until now the *CreY* gene has yet to be located on a chromosome but has been derived from *Aegilops variabilis* (Moens et al., 2018).

Other undefined sources of resistance have been found in wheat, barley, and oat amongst entries of the International Test Assortment and local genotype collections (McDonald and Nicol, 2005; Smiley et al., 2011; Dababat et al., 2015b; Marshall and Smiley, 2016; Smiley and Marshall, 2016; Wu et al., 2016).

Mainly in wheat, there is rarely a certain or obvious threshold to differentiate between resistance and susceptibility. It is still a mystery on why 1 or few white females form on a highly resistant genotype to a particular nematode population or the significant

difference in phenotypic response in cultivars that have the same single dominant resistant gene. There are limited research papers that review the breeding programs related to the development of resistant cultivars to cereal cyst nematodes (Moens et al., 2018).

2.2.9.1 Successful examples of resistance

A part of a study conducted by Dababat (2019) showed that 28 winter wheat lines from The International Winter Wheat Improvement Program (IWWIP) maintained their resistance to the *Heterodera filipjevi* population of Turkey, while one line showed moderate susceptibility and 6 lines showed susceptibility. The general results of his study found that a key factor that causes reduction in wheat growth and reduction of wheat yield cultivated in Turkey is because of the effect of *Heterodera filipjevi*.

Pariyar et al. (2016b) screened 291 winter wheat accessions from different origin countries which included breeding lines, cultivars and landraces provide by IWWIP for their resistant response to *Heterodera filipjevi* population from Turkey. It was confirmed according to the results that were obtained, Nudakota, Katea, Ekonomka, and Lantian-12 wheat accessions possessed resistance, 16% showed moderate resistance, while the rest of the responses varied between moderately susceptible to highly susceptible.

A part of a different study conducted by Pariyar et al. (2016a) which aimed to screen 161 winter wheat accessions (101 breeding lines, 58 cultivars, and 2 landraces) from the IWWIP were tested for resistant response to *Heterodera filipjevi* population of Turkey. 1% showed resistance and 26% were moderately resistant to *Heterodera filipjevi* while the remaining genotypes ranged in response between moderately susceptible and susceptible. As the study was the first report of QTLs conferring resistance to *Heterodera filipjevi* in wheat as 2 of the QTLs are linked to putative genes known to be involved in abiotic stress.

Yavuzaslanoglu et al. (2016) screened 31 Iranian landraces for their resistant response to *Heterodera filipjevi* population from Turkey, these lines were previously screened for their resistant response to root-lesion nematode *Pratylenchus thornei*. It was found that 1 line was resistant, 5 were moderately resistant, and the remaining lines varied in response between moderately susceptible and susceptible.

Dababat et al. (2014) screened 719 varieties and breeding lines from 25 countries provided by IWWIP from the Facultative and Winter Wheat Observation Nursery for their resistant response to *Heterodera filipjevi* population of Turkey. 114 genotypes showed resistance to *Heterodera filipjevi* and 90 genotypes were moderately resistant. It was noted in the study that the germplasm with the *Cre5* gene present, showed of range of reaction response between resistant or susceptible to *Heterodera filipjevi* so there was no relation the presence of the gene and resistance.

Toktay (2012) screened 42 advanced spring wheat lines provided by CIMMYT – Mexico for their resistant response to Turkish population of *Pratylenchus thornei* and *Heterodera filipjevi*. The lines were screened for the presence of *Cre1* gene. The outcome showed that 5 lines were resistant to *Heterodera filipjevi* and 8 lines were moderately resistant. As the study concluded that there is no relationship between the resistance of *Heterodera filipjevi* population of Turkey and the presence of the *Cre1* gene.

2.2.10 Other control methods

2.2.10.1 Crop rotation

Different crop rotation, which includes a variety of plant families, reduce the population densities of the cereal cyst nematodes (*Heterodera avenae* and *Heterodera filipjevi*) since the hosts selection is restricted to cereal crops (wheat, barley, oat, and triticale) and grass species. Vanstone et al. (2008) mentioned that for the efficient management of CCN in the southern Australian Wheatbelt, two successive seasons of a non-host plant are suggested, so there is incomplete hatching of the CCN, as about 50-90% of the second stage juveniles hatch. Another crop rotation method that has been used since 1950 is the usage of legume pastures for CCN control, as it also can be used as fodder for livestock and it fixes nitrogen in the soil (Meagher and Rooney, 1966).

Oilseed rape (*Brassica napus*), chickpea (*Cicer arietinum*), field pea (*Pisum sativum*), lentil (*Lens culinaris*) and broad bean (*Vicia faba*) are proper crops that can be used in crop rotation. Rotations in Qinghai, China normally includes 1 year planting a susceptible

crop preceded by 1 year planting a nonhost crop such as potatoes, broad bean, or oilseed rape (Riley et al, 2010).

2.2.10.2 Soil cultivation

The population density of cyst nematodes can be affected by the selection of tillage systems and can also have an effect on other plant diseases. Roget et al. (1996) used several tillage practices in field plots to compare the damaging effect of CCNs on roots. It was found that in treatment of 1 cultivation before sowing caused the most severe root damage, whereas in direct-drill plots it was the lowest to show root damage as it did not disturb the soil below the seed, although the in this plot system the occurrence of the take-all root rot disease caused by *Gaeumannomyces graminis* var. *tritici* was the highest.

Ito Araki and Komatsuzaki (2015) reported that during the consecutive cropping of rice, the population density of *Heterodera elachista* under a zero-tillage system was decreased when compared to conventional tillage systems that used a moldboard plow with rotary cultivation, but with the introduction of soybean to the rotation, the nematode population increased.

2.2.10.3 Anaerobic

Blok et al. (2000) described Anaerobic soil disinfection as the promotion of anaerobic conditions in the soil by the integration of fresh organic matter to moist soil and covering it with air-tight plastic for a few weeks. This method causes a decrease in hatching and juvenile viability of plan-parasitic nematodes including cyst nematodes. This is associated with O_2 diminution and the upsurge CO_2 with secondary by-products in the soil, caused by the organic carbon decomposition (Runia et al., 2014; Ebrahimi et al., 2016).

2.2.10.4 Solarization

Solarization is a method that relies on hydrothermal to raise the temperature of the soil by applying a plastic cover over wet soil, which causes numerous physical and chemical alterations within the soil system (Gaur and Perry, 1991). Soluble nutrients such as

ammonium and nitrate-nitrogen concentration are increased due to the decomposition of the organic components.

Solarization wide-range lethal impact affects pests, pathogens, and beneficial microorganisms. Sterilized soil provides a good environment for the recolonization of soil-borne organisms rather than plant-parasitic organisms and this shift in biological balance provides a healthier environment for plant growth (Stapleton, 2000).

Limitations of solarization lies in the economic and environmental consideration regarding cost and discarding of the plastic. For deep soil solarization, soil temperatures need to be greater than 40°C and time needs to be lengthy, so this is a drawback for countries in the Northern hemisphere (Moens et al., 2018).

2.2.10.5 Weed management

Several weed species are reported to be hosts to cyst nematodes and aid in their reproduction, which leads to decreases in the usage effectiveness of nematicides as a management technique. (Thomas et al., 2005). For instance, purple deadnettle (*Lamium purpureum*) serves as a host for SCN which help in its reproduction in field and greenhouse conditions (Venkatesh et al., 2004; Creech et al., 2008). So, it is necessary to identify and eradicate host weed species in order to improve cyst nematode management.

2.2.10.6 Bio-fumigation

There is an increasing interest in the use of biofumigants, as the world has shown a rejection of using chemical control strategies for cyst nematodes management. Biofumigation is usually defined as the management of pest, weed, and diseases by the use of biocidal compounds that are produced from the remains of freshly composted Brassica species (Moens et al., 2018).

Every species of *Brassica* has a distinctive glucosinolate profile in their foliage and roots. Such as Indian mustard primary generates 2-propenyl glucosinolate (Sinigrin), which then hydrolyzes to allyl isothiocyanate (Moens et al., 2018).

Isothiocyanate has shown toxicity to cyst nematode species, for instance, the *in vitro* sensitivity of quiescent and active *Heterodera glycines* J2 to allyl, phenyl, and benzyl isothiocyanate was studied by Schroeder and MacGuidwin (2010). While the compounds have increased mortality and reduced motility to the J2 of *Heterodera glycines*, benzoyl was found to be the most toxic.

Biofumigation is an inherent variability crop protection method as care needs to be taken when choosing the species and cultivar (Moens et al., 2018).

2.2.10.7 Trap crops

Plant species that promote hatching and permit root penetration but stop the completion of the nematode life cycle are called trap crops in nematode management. An example of how this is done, the plant's development of the syncytium might be limited, thus, preventing the development of females. These plants are defined to be a poor host for the nematode that attacks them. Trap crops can be accomplished by the use of resistant cultivars of the main crop (Moens et al., 2018).

Heterodera schachtii population densities can be decreased by intercropping with *Brassica* spp. That are poor hosts for instance oilseed radish (*Raphanus sativus* var. *oleifera*) or white mustard (*Sinapis alba*). Hafez (1994) show that in a field experiment the *Heterodera schachtii* population densities have been reduced by 87-92% succeeding intercropping with oilseed radish cultivars, however, there was a 62-84% reduction when intercropping with white mustard.

2.2.10.8 Plant biomass, oils, and extracts

There have been tests on a large number of plant-based products regarding their possible suppressive impact on cyst nematodes. These plants that are a point of interest are; acacia (*Acacia nilotica*), eucalyptus (*Eucalyptus* spp.), garlic (*Alium sativum*), neem (*Azadirachta indica*), tobacco (*Nicotiana tabacum*) and sweet wormwood (*Artemisia annua*) (Moens et al., 2018).

A drawback in this point is that the bulk of the studies that are examining plant-based products are conducted in vitro or a controlled environment. While being useful, they do not provide a credible field performance indicator (Moens et al., 2018).

There is some limited evidence that neem-based products have the potential to inhibit PCN and SCN nematode population.

2.2.10.9 Biological agents

Biological control is the ability of a living organism or biological control agent to inhibit the density of a pest and/or disease (target organism) population (Eilenberg et al., 2001).

Biological control depends on the natural interactions that are existing within the food web of a healthy and self-regulated soil, where plant-parasitic nematodes are regarded as vital components of the soil as a whole and not as a group of isolated soil organisms (Moens et al., 2018).

Obligate microparasites such as *Pasteuria* spp. efficiently decreases the infestation levels of *Globedera* and *Heterodera* spp. Also, another example of biological control is *Pochonia chlamydosporia* which is a saprophytic fungus with a facultative parasitic capacity on nematodes of economic importance together with *Globodera* and *Heterodera* spp. (Moens et al., 2018).

The bacterial species *Bacillus* has been studied in detail as a possible biological control agent for plant-parasitic nematodes. Some species such as *Bacillus thuringiensis, Bacillus cereus,* and *Bacillus firmus* have shown favorable nematicidal characteristics against cyst nematodes principally the *Heterodera* genus (Geng et al., 2016; Zhang, et al., 2016; Zheng et al., 2016). *Bacillus cereus* (09B18) a plant growth-promoting bacterium isolated from *Heterodera filipjevi,* has shown nematicidal action in field trials, as yields were increased in plots that used *Bacillus* seed-coated seeds (Zhang et al., 2016).

2.2.10.10 Agrochemicals

Nematicides are common chemicals that are used to control nematodes, an expression that usually meaning a substance that is deadly to nematodes. But to be more accurate the term nemastostat is more appropriate to the majority of substances as the regularly paralyze or prevent behavior like feeding, rather than killing directly (Kearn et al., 2015).

In order for the nematicide to take effect, it needs to be applied in the accurate concentration and period, so it can still be preserved in the soil for the nematode to eat up its lipid food reserve before finding a host. The primary objectives of these products are to protect crop yields, stop or reduce nematode reproduction and also avert or decrease nematode-borne virus transmission to the plant (Whitehead, 1968).

There are more than a few target options for chemical control of cyst nematodes. Targets can be the cyst that preserves the eggs in the soil, the J2 that is moving in the soil and then feeding on the plant or within it. Cyst nematode control is complex due to the cyst wall that provides extra protection to the eggs and unhatched J2 (Moens et al., 2018).

Telone II, C-35, C-17, EC and Inline (1,3-dichloropropene; Dow AgroSciences) are licensed chemicals in some states in the USA used for control of *Heterodera* and *Globodera* spp. in a broad range of crops (Moens et al., 2018).

CHAPTER III

MATERIALS & METHODS

3.1 Materials

3.1.1 Plant materials

Two hundred and fifty-seven (257) international spring wheat lines were genotyped and screened for drought and heat resistance by International Maize and Wheat Center – Mexico (CIMMYT – Mexico) were provided by CIMMYT – Turkey, in order to be screened for their resistant reaction to *Heterodera filipjevi* resistance response. The list of the wheat lines used are below (Table 3.1).

Four (4) check cultivars with previous information on their resistant response to the nematode were used, 2 susceptible cultivars (Bezostaja and Kutluk-94) and 2 moderate resistant cultivars (Katea-1 and Sonmez-2001) and in (Table 3.2) is information about the cultivars.

3.2 Methods

3.2.1 Seed selection and sterilization

Two to three representative spikes of a single line were selected and harvested then preserved separately. After that, spikes of each line were threshed and the seeds were kept in small envelopes. A total of 15-25 seeds from each line were used for germination; the surface of the seeds was sterilized before being germinated and transplanted for the experiment according to CIMMYT protocol (Photo 3.1):

1. Seeds were washed with tap water to clean and remove any residue.
2. Then seeds were rinsed in 96% ethanol for 6 minutes.
3. After that, they were rinsed in 4.5% NaOCl for 10 minutes .
4. Finally, the seeds were rinsed about 6 times with sterile distilled water (ddH_2O) to remove residue of ethanol and NaOCl.

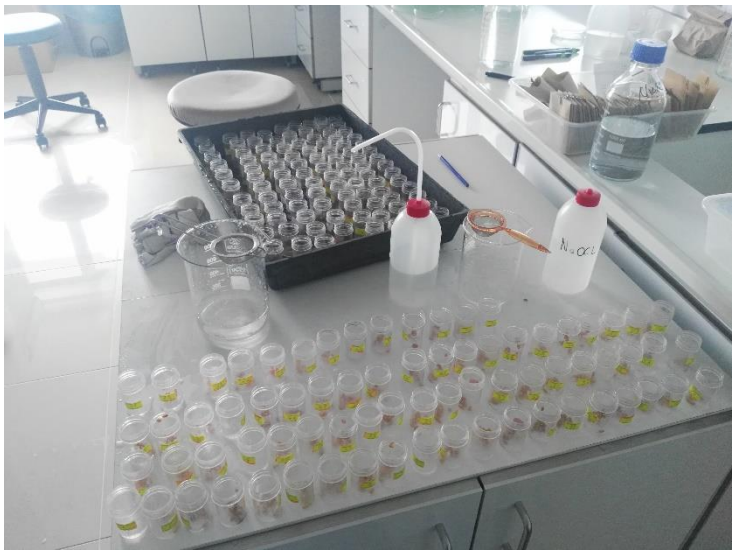

Photo 3.1: Seed Sterilization

3.2.2 Seed germination

To ensure no fungal contamination will occur on the seeds, filter paper and glass Petri plates were autoclaved. Distilled water was used to provide moisture to the seeds during the germination procedure. After that the following steps were followed:

1. Selection similar and healthy-looking seeds from each of the 257 lines and the check cultivars.
2. The required amount of water that was used in the germination was double the amount of total weight of seeds in each Petri plate.
3. In each 9 cm Petri plate, the filter paper was placed inside and half of the amount of recommended water is added.
4. Seeds of a single line were placed in a sole Petri plate and covered with another piece of filter paper and the remaining required amount of distilled water was added. The Petri plates were covered and place in an incubator (Photo 3.2 A).
5. Thee samples were kept in an incubator with no light source for a period of 3 days at 20-22°C at 70-80% RH (Photo 3.2 B).

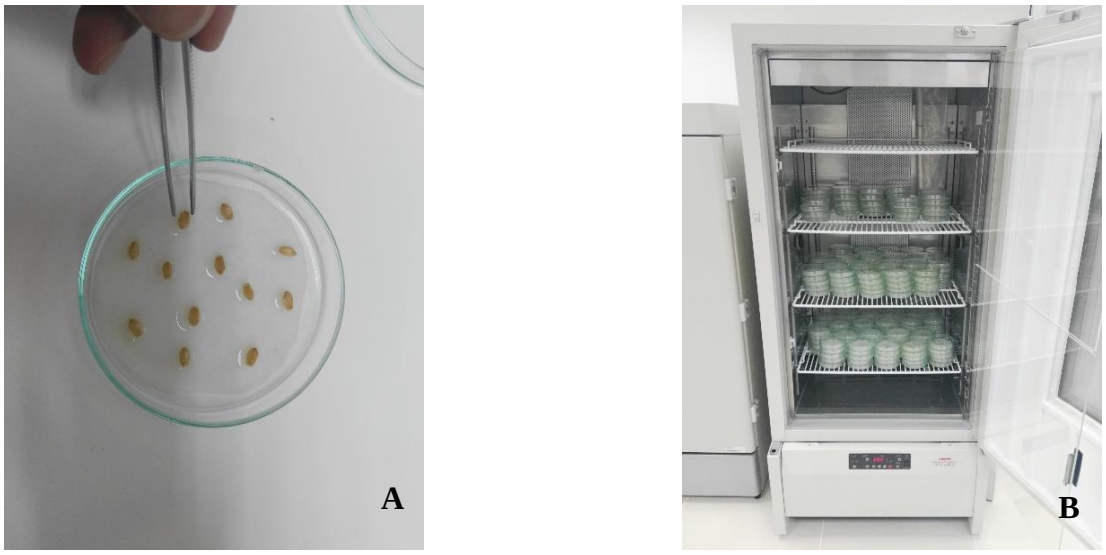

Photo 3.2: A Seed Placement in Petri Plate **B** Incubator

3.2.3 Soil collection and cyst extraction

3.2.3.1 Soil collection

The cysts of *Heterodera filipjevi* were collected from a known infested wheat field in Çiçekdağ district in the province of Kırşehir, Turkey with the following coordination: Latitude 39° 63' 80" N; Longitude 34° 46' 72" E. (Figure 3.1)

Figure 3.1: Kırşehir, Turkey source: https://mapchart.net/turkey.html

3.2.3.2 Cyst collection

Extraction of the cyst from the soil samples was conducted by using a slight modification of Cobb's decanting and sieving method (Cobb, N.A., 1918).

1. Approximately 200 g of soil was added to a 1 L beaker then water was added.
2. The mixture was stirred and left to settle for about 30 sec; in order to let the soil and heavy derbies to settle to the bottom.
3. The mixture was poured over 2 sieves above each other; the top sieve was 850 µm to capture any derbies but allow small particles to pass through, the bottom sieve was 250 µm in order to catch the females and cyst.
4. The process was repeated at least 2 times for each sample (when the water starts to clear) to ensure that all females and cysts were gathered.

3.2.4 Cyst identification

These cyst samples were previously identified by CIMMYT – Turkey by using molecular methods as *Heterodera filipjevi*, but to increase the confidence that they cysts were true to species morphological identification was done by using a light microscope (LEICA DM5500 B) along with imaging software Leica Application Suite (LAS V4.12).

The overall morphological shape of the cyst indicates that it belongs to the genus *Heterodera* (Figure 3.2); Lemon shape, brownish color, and short neck.

Figure 3.2: Cysts of *Heterodera filipjevi*

A total of 20 random cysts were selected to specify the species as their fenestra was examined; fenestra is considered reliable in species identification of the *Heterodera* genus, as each species has a unique fenestra shape (Figure3.3 and Figure 3.4). It was confirmed after examination that the species of the experiment was *Heterodera filipjevi.*

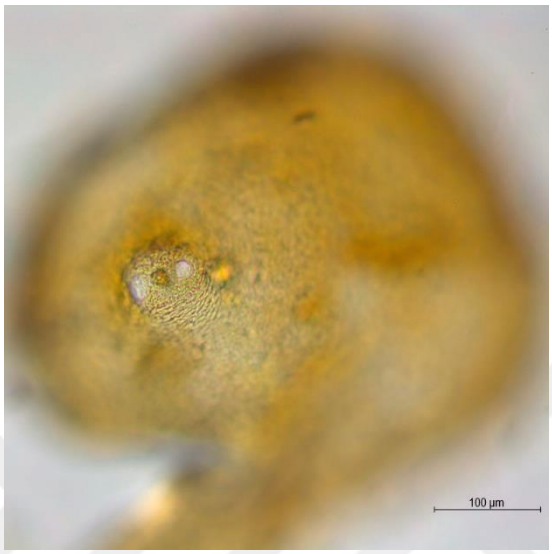

Figure 3.3: *Heterodera latipons*

Figure 3.4: *Heterodera filipjevi*

3.2.5 Cyst collection

In order to estimate how many cysts were needed for hatching to obtain the desired number of juveniles for inoculum, 10 random cysts were cut open and the number of juveniles were counted by using dissecting microscope (LEICA M165 C). The eggs were not counted because of the possibility of them not being viable.

It was estimated that 2515 cysts were needed to be collected, but to ensure that there is a sufficient number of juveniles, triple the required amount of cyst was collected.

Cysts were handpicked from the organic matter residue under a dissecting microscope (Olympus SZ61) which can be seen in (Photo 3.3). Then they were surface sterilized with NaOCl (0.5%) for about 10 minutes. Next, the cysts were rinsed a couple of times with distilled H_2O and prepared for hatching.

Photo 3.3: Cyst Collection

3.2.6 Cyst hatching

Two procedures hatching procedures were used the first was according to CIMMYT – Turkey protocol which follows (Photo 3.4):

1. The surface-sterilized cysts were transferred on a fine mesh (45 µm) in a Petri plate to allow the juvenile to pass, with a medium of distilled H_2O.
2. The Petri plates were stored at 4°C for hatching and checked periodically for hatching.

Photo 3.4: CIMMYT - Turkey Cyst Hatching Method

The second hatching procedure was a miniaturized CCN larval farm similar to CCN larval farm used in Australia which the subsequent steps were followed (Photo 3.5):

1. 200 g of organic matter containing cysts from the washed soil sample was placed on a 45 µm mesh inside a Petri plate.
2. Distilled H_2O was added and the Petri plate was covered.
3. The samples were placed in an incubator at 10°C.
4. After 1 week the water was thrown away and change and it was done again after another week to make sure that the saprophytic nematodes have been extracted.
5. After 1 month the water is checked for hatching and then changed to reduce that possibility of fungal formation.

Photo 3.5: Modified Australian Larval Farm Method

3.2.7 Screening assay of wheat lines

3.2.7.1 Experiment preparation

The tubes were filled with approximately 100 g of a mixture of sand, field soil and organic matter (70:29:1 v/v/v). Field soil and sand was sieved and sterilized for 2 hours on 2 successive days at 110˚C, whereas the organic matter was sterilized at 70˚C for 5 hours. A small piece of cotton was put in the bottom of each tube so that the mixture does not fall out of the opening in the tube and also to increase water uptake (Photo 3.6).

Photo 3.6: Preparation of tubes for transplanting & inoculation

A seed of a single line was transplanted (Photo 3.7), based on its phenotype and with a 2-3 cm radicle, in Ray Leach “Cone-tainer” ™ (RLC4; 2.5 × 16 cm tube) and tested in 3

replicates per trial, the experiment was repeated twice. Check varieties were used as a reference (2 moderate resistant and 2 susceptible) and 6 replicates per trial were used to reduce standard error.

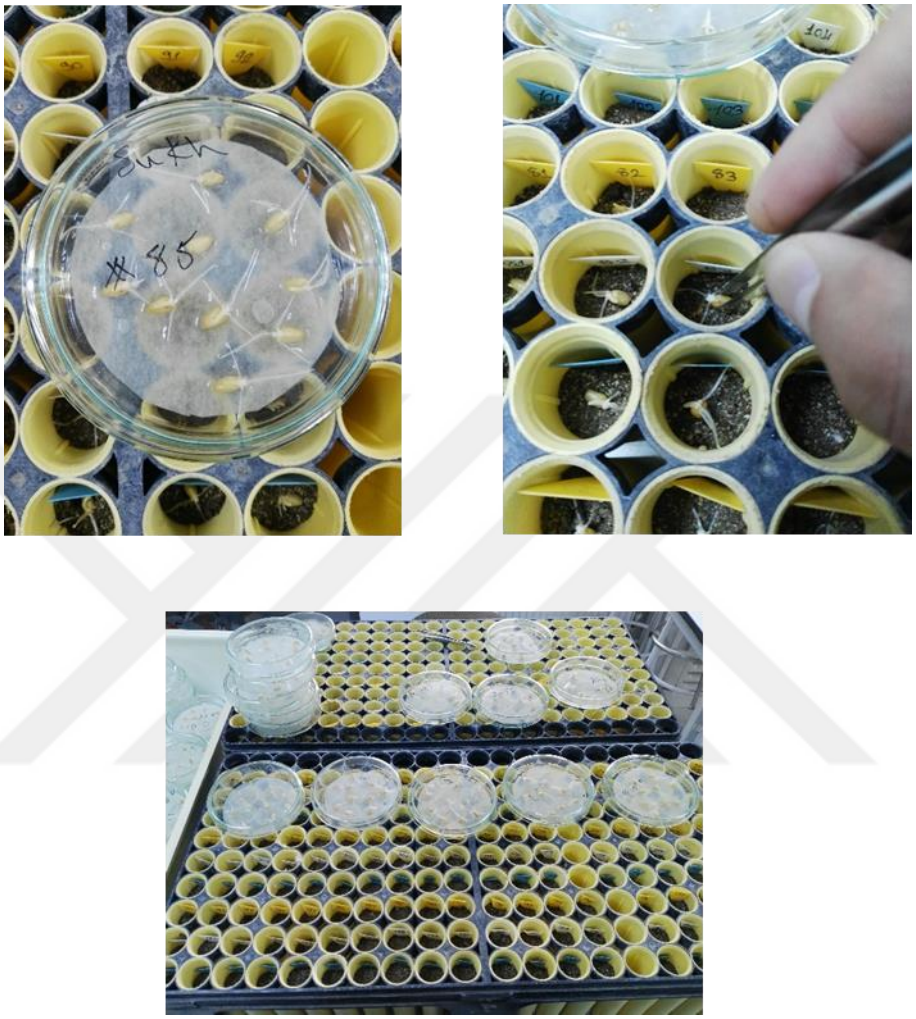

Photo 3.7: Transplanting of germinated seeds

3.2.7.2 Experimental design

The tubes were organized in complete randomized block design in (4) 200 tube rack (RL200; Ray Leach “Cone-tainer” ™ tray) per trial.

The experiment was conducted under controlled conditions in a growth chamber (70% relative humidity, 25°C, and a photoperiod of 16 hours) at the Transitional Zone Agricultural Research Institute in Eskisehir (39.767017°N, 30.403008°E).

3.2.7.3 Juvenile inoculation

After hatching, the J2 were collected in a funnel and then the concentration of the suspension of J2 was adjusted to 250 J2/1 ml (Photo 3.8).

Each tube was inoculated with 250 freshly hatched second-stage juvenile Heterodera filipjevi in 1 ml water into 3 holes of 2 cm depth around the base of the plant 1 day after transplanting.

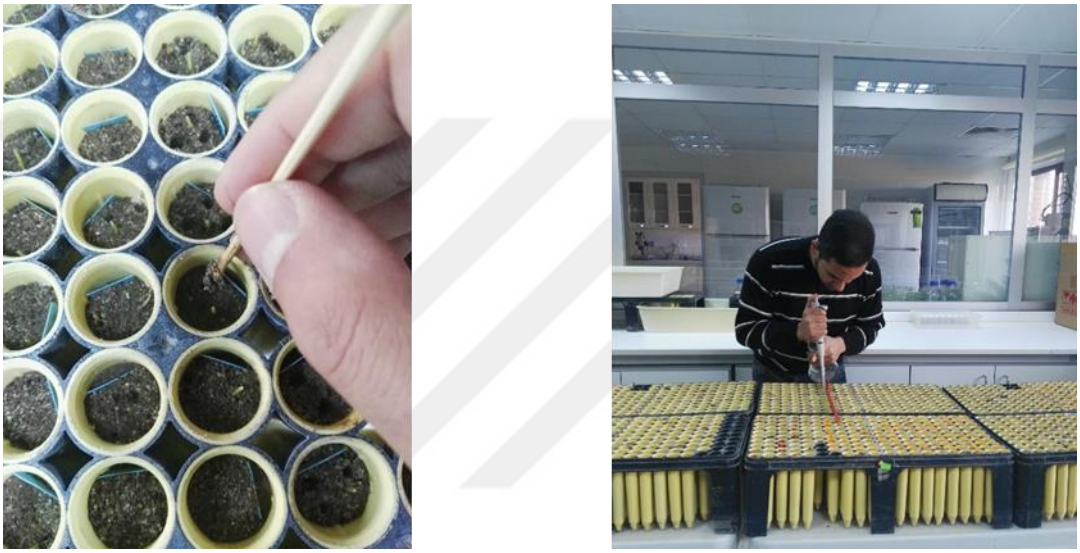

Photo 3.8: Second-Stage Juvenile Inoculation

3.2.8 Plant harvest and assessment

3.2.8.1 Plant harvest and cyst extraction

The plants were uprooted after 14 weeks of inoculation. Soil mixer from a single tube was collected in a beaker filled with water and stirred, the same procedure Cobb's, 1918 decanting and sieving method for cyst extraction from soil was followed but with a couple of modifications. The root system of a single plant was washed gently on the 850 µm sieve to detach any females or cyst left on the root system. This process was repeated 3 times to ensure the gathering of all females and cyst from the soil mixture for counting (Photo 3.9).

Photo 3.9: Wheat Harvest & Washing Roots for Cyst Extraction

3.2.8.2 Assessment protocol

Cysts and females which were collected from the 250 µm sieve were counted under a dissecting microscope (Olympus SZ61). Genotypes were divided into 5 groups based on the mean number of female and cysts per plant and taking to account the reaction of check varieties with known resistance to CCN (Photo 3.10).

Photo 3.10: Cyst Counting for Assessment

The ranking used to classify the lines into the groups of resistance response was similar to the method that Dababat, 2014 used for categorizing the resistant response of the wheat germplasm:

1. R = Resistant (fewer female and cysts/plant than the moderately resistant checks).
2. MR = moderately resistant (as few females and cysts/plant as the moderately resistant checks).
3. MS = moderately Susceptible (significantly more female and cysts/plant than in the moderately resistant check, but not as many as in the susceptible checks).
4. S = Susceptible (as many females and cysts/plant as in the susceptible check and the number of cysts per root system considered damaging).
5. HS = Highly Susceptible (more female and cysts/plant than in the susceptible check).

Table 3.1: The List of Spring Wheat Lines that Were Used in the Study

Entry ID	Cross ID (CID)	Selection ID (SID)	Germplasm ID (GID)	Pedigree (Cross)	Selection History
2	620575	57	7933009	DOY1/AE.SQUARROSA (488)//BAJ #1/3/SUP152	SDSS13Y00006T-0B-0Y-0M-0Y-0B-52Y
3	620575	61	7933013	DOY1/AE.SQUARROSA (488)//BAJ #1/3/SUP152	SDSS13Y00006T-0B-0Y-0M-0Y-0B-56Y
4	620575	62	7933014	DOY1/AE.SQUARROSA (488)//BAJ #1/3/SUP152	SDSS13Y00006T-0B-0Y-0M-0Y-0B-57Y
5	620577	52	7933017	DVERD_2/AE.SQUARROSA (333)//BAJ #1/3/SUP152	SDSS13Y00008T-0B-0Y-0M-0Y-0B-47Y
6	620577	56	7933021	DVERD_2/AE.SQUARROSA (333)//BAJ #1/3/SUP152	SDSS13Y00008T-0B-0Y-0M-0Y-0B-51Y
7	620577	58	7933023	DVERD_2/AE.SQUARROSA (333)//BAJ #1/3/SUP152	SDSS13Y00008T-0B-0Y-0M-0Y-0B-53Y
8	620577	59	7933024	DVERD_2/AE.SQUARROSA (333)//BAJ #1/3/SUP152	SDSS13Y00008T-0B-0Y-0M-0Y-0B-54Y
9	620577	60	7933025	DVERD_2/AE.SQUARROSA (333)//BAJ #1/3/SUP152	SDSS13Y00008T-0B-0Y-0M-0Y-0B-55Y
11	620577	66	7933031	DVERD_2/AE.SQUARROSA (333)//BAJ #1/3/SUP152	SDSS13Y00008T-0B-0Y-0M-0Y-0B-61Y
12	620577	69	7933034	DVERD_2/AE.SQUARROSA (333)//BAJ #1/3/SUP152	SDSS13Y00008T-0B-0Y-0M-0Y-0B-64Y
13	620577	70	7933035	DVERD_2/AE.SQUARROSA (333)//BAJ #1/3/SUP152	SDSS13Y00008T-0B-0Y-0M-0Y-0B-65Y
14	620579	40	7933045	D67.2/PARANA 66.270//AE.SQUARROSA (677)/3/BAJ #1/4/SUP152	SDSS13Y00010T-0B-0Y-0M-0Y-0B-35Y
15	620579	52	7933057	D67.2/PARANA 66.270//AE.SQUARROSA (677)/3/BAJ #1/4/SUP152	SDSS13Y00010T-0B-0Y-0M-0Y-0B-47Y
16	620580	49	7933070	GARZA/BOY//AE.SQUARROSA (695)/3/BAJ #1/4/SUP152	SDSS13Y00011T-0B-0Y-0M-0Y-0B-44Y

17	620581	59	7933077	IG 42134/BAJ #1//SUP152	SDSS13Y00012T-0B-0Y-0M-0Y-0B-54Y
18	620583	34	7933091	H-1624/BAJ #1//SUP152	SDSS13Y00014T-0B-0Y-0M-0Y-0B-29Y
19	620583	36	7933093	H-1624/BAJ #1//SUP152	SDSS13Y00014T-0B-0Y-0M-0Y-0B-31Y
20	620583	37	7933094	H-1624/BAJ #1//SUP152	SDSS13Y00014T-0B-0Y-0M-0Y-0B-32Y
21	620583	39	7933096	H-1624/BAJ #1//SUP152	SDSS13Y00014T-0B-0Y-0M-0Y-0B-34Y
22	620583	42	7933099	H-1624/BAJ #1//SUP152	SDSS13Y00014T-0B-0Y-0M-0Y-0B-37Y
23	620583	48	7933105	H-1624/BAJ #1//SUP152	SDSS13Y00014T-0B-0Y-0M-0Y-0B-43Y
25	620586	48	7933111	INDIA-101/3/FRET2*2/SHAMA//KACHU/4/HUW234+LR34/PRINIA*2//KIRITATI	SDSS13Y00017T-0B-0Y-0M-0Y-0B-43Y
26	620586	59	7933122	INDIA-101/3/FRET2*2/SHAMA//KACHU/4/HUW234+LR34/PRINIA*2//KIRITATI	SDSS13Y00017T-0B-0Y-0M-0Y-0B-54Y
27	620586	66	7933129	INDIA-101/3/FRET2*2/SHAMA//KACHU/4/HUW234+LR34/PRINIA*2//KIRITATI	SDSS13Y00017T-0B-0Y-0M-0Y-0B-61Y
29	620588	9	7933138	H-1311/3/FRET2*2/SHAMA//KACHU/4/HUW234+LR34/PRINIA*2//KIRITATI	SDSS13Y00019T-0B-0Y-0M-0Y-0B-4Y
30	620588	12	7933141	H-1311/3/FRET2*2/SHAMA//KACHU/4/HUW234+LR34/PRINIA*2//KIRITATI	SDSS13Y00019T-0B-0Y-0M-0Y-0B-7Y
31	620588	21	7933150	H-1311/3/FRET2*2/SHAMA//KACHU/4/HUW234+LR34/PRINIA*2//KIRITATI	SDSS13Y00019T-0B-0Y-0M-0Y-0B-16Y
32	620592	65	7933170	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (390)/7/SHA7/VEE#5/5/VEE#8//JUP/BJY/3/F3.71/TRM/4/2*WEAVER/6/SKAUZ/PARUS//PARUS/8/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETO F2001	SDSS13Y00023T-0B-0Y-0M-0Y-0B-60Y
33	620594	29	7933202	INDIA-223/7/SHA7/VEE#5/5/VEE#8//JUP/BJY/3/F3.71/TRM/4/2*WEAVER/6/SKAUZ/PARUS//PARUS/8/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETO F2001	SDSS13Y00025T-0B-0Y-0M-0Y-0B-24Y

34	620594	32	7933205	INDIA-223/7/SHA7/VEE#5/5/VEE#8//JUP/BJY/3/F3.71/TRM/4/2*WEAVER/6/SKAUZ/PARUS//PARUS/8/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETO F2001	SDSS13Y00025T-0B-0Y-0M-0Y-0B-27Y
35	620594	41	7933214	INDIA-223/7/SHA7/VEE#5/5/VEE#8//JUP/BJY/3/F3.71/TRM/4/2*WEAVER/6/SKAUZ/PARUS//PARUS/8/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETO F2001	SDSS13Y00025T-0B-0Y-0M-0Y-0B-36Y
36	620594	53	7933226	INDIA-223/7/SHA7/VEE#5/5/VEE#8//JUP/BJY/3/F3.71/TRM/4/2*WEAVER/6/SKAUZ/PARUS//PARUS/8/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETO F2001	SDSS13Y00025T-0B-0Y-0M-0Y-0B-48Y
37	620595	29	7933229	CHIH95.4.6/7/SHA7/VEE#5/5/VEE#8//JUP/BJY/3/F3.71/TRM/4/2*WEAVER/6/SKAUZ/PARUS//PARUS/8/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETO F2001	SDSS13Y00026T-0B-0Y-0M-0Y-0B-24Y
38	620595	39	7933239	CHIH95.4.6/7/SHA7/VEE#5/5/VEE#8//JUP/BJY/3/F3.71/TRM/4/2*WEAVER/6/SKAUZ/PARUS//PARUS/8/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETO F2001	SDSS13Y00026T-0B-0Y-0M-0Y-0B-34Y
39	620595	57	7933257	CHIH95.4.6/7/SHA7/VEE#5/5/VEE#8//JUP/BJY/3/F3.71/TRM/4/2*WEAVER/6/SKAUZ/PARUS//PARUS/8/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETO F2001	SDSS13Y00026T-0B-0Y-0M-0Y-0B-52Y
40	620598	60	7933318	IG 122727/8/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETO F2001/9/KAUZ//ALTAR 84/AOS/3/PASTOR/4/MILAN/CUPE//SW89.3064/5/KIRITATI	SDSS13Y00029T-0B-0Y-0M-0Y-0B-55Y
41	620600	45	7933333	H-1357/8/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETO F2001/9/KAUZ//ALTAR 84/AOS/3/PASTOR/4/MILAN/CUPE//SW89.3064/5/KIRITATI	SDSS13Y00031T-0B-0Y-0M-0Y-0B-40Y
42	620600	46	7933334	H-1357/8/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETO F2001/9/KAUZ//ALTAR 84/AOS/3/PASTOR/4/MILAN/CUPE//SW89.3064/5/KIRITATI	SDSS13Y00031T-0B-0Y-0M-0Y-0B-41Y
43	620600	49	7933337	H-1357/8/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETO F2001/9/KAUZ//ALTAR 84/AOS/3/PASTOR/4/MILAN/CUPE//SW89.3064/5/KIRITATI	SDSS13Y00031T-0B-0Y-0M-0Y-0B-44Y
45	620600	55	7933343	H-1357/8/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETO F2001/9/KAUZ//ALTAR 84/AOS/3/PASTOR/4/MILAN/CUPE//SW89.3064/5/KIRITATI	SDSS13Y00031T-0B-0Y-0M-0Y-0B-50Y

46	620600	56	7933344	H-1357/8/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETO F2001/9/KAUZ//ALTAR 84/AOS/3/PASTOR/4/MILAN/CUPE//SW89.3064/5/KIRITATI	SDSS13Y00031T-0B-0Y-0M-0Y-0B-51Y
47	620603	53	7933359	IG 42147/6/KAUZ//ALTAR 84/AOS/3/PASTOR/4/MILAN/CUPE//SW89.3064/5/KIRITATI/7/SW89.5277/BORL95//SKAUZ/3/PRL/2*PASTOR/4/HEILO	SDSS13Y00034T-0B-0Y-0M-0Y-0B-48Y
48	620604	65	7933377	IG 42152/6/KAUZ//ALTAR 84/AOS/3/PASTOR/4/MILAN/CUPE//SW89.3064/5/KIRITATI/7/SW89.5277/BORL95//SKAUZ/3/PRL/2*PASTOR/4/HEILO	SDSS13Y00035T-0B-0Y-0M-0Y-0B-60Y
49	620609	48	7933413	DOY1/AE.SQUARROSA (447)/4/PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07/5/NELOKI	SDSS13Y00040T-0B-0Y-0M-0Y-0B-43Y
50	620611	11	7933473	CETA/AE.SQUARROSA (391)/4/PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07/5/NELOKI	SDSS13Y00042T-0B-0Y-0M-0Y-0B-6Y
51	620612	45	7933482	IG 41489/4/PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07/5/NELOKI	SDSS13Y00043T-0B-0Y-0M-0Y-0B-40Y
52	620612	59	7933496	IG 41489/4/PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07/5/NELOKI	SDSS13Y00043T-0B-0Y-0M-0Y-0B-54Y
53	620613	33	7933503	IG 41505/4/PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07/5/NELOKI	SDSS13Y00044T-0B-0Y-0M-0Y-0B-28Y
54	620613	36	7933506	IG 41505/4/PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07/5/NELOKI	SDSS13Y00044T-0B-0Y-0M-0Y-0B-31Y
55	620613	39	7933509	IG 41505/4/PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07/5/NELOKI	SDSS13Y00044T-0B-0Y-0M-0Y-0B-34Y
56	620615	41	7933539	IG 122145/4/PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07/5/NELOKI	SDSS13Y00046T-0B-0Y-0M-0Y-0B-36Y
57	620615	43	7933541	IG 122145/4/PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07/5/NELOKI	SDSS13Y00046T-0B-0Y-0M-0Y-0B-38Y
58	620616	76	7933556	IG 122146/4/PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07/5/NELOKI	SDSS13Y00047T-0B-0Y-0M-0Y-0B-71Y
59	620616	79	7933559	IG 122146/4/PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07/5/NELOKI	SDSS13Y00047T-0B-0Y-0M-0Y-0B-74Y

60	620620	76	7933656	IG 122193/4/PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07/5/NELOKI	SDSS13Y00051T-0B-0Y-0M-0Y-0B-71Y
62	620620	80	7933660	IG 122193/4/PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07/5/NELOKI	SDSS13Y00051T-0B-0Y-0M-0Y-0B-75Y
63	620621	85	7933675	IG 122196/4/PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07/5/NELOKI	SDSS13Y00052T-0B-0Y-0M-0Y-0B-80Y
64	620621	93	7933683	IG 122196/4/PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07/5/NELOKI	SDSS13Y00052T-0B-0Y-0M-0Y-0B-88Y
65	620625	101	7933720	IG 122795/4/PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07/5/NELOKI	SDSS13Y00056T-0B-0Y-0M-0Y-0B-96Y
66	620627	48	7933728	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (890)/6/NELOKI/7/ATTILA*2/PBW65//MURGA	SDSS13Y00058T-0B-0Y-0M-0Y-0B-43Y
67	620629	28	7933741	H-1546/NELOKI/3/ATTILA*2/PBW65//MURGA	SDSS13Y00060T-0B-0Y-0M-0Y-0B-23Y
69	620629	35	7933748	H-1546/NELOKI/3/ATTILA*2/PBW65//MURGA	SDSS13Y00060T-0B-0Y-0M-0Y-0B-30Y
70	620629	36	7933749	H-1546/NELOKI/3/ATTILA*2/PBW65//MURGA	SDSS13Y00060T-0B-0Y-0M-0Y-0B-31Y
71	620629	38	7933751	H-1546/NELOKI/3/ATTILA*2/PBW65//MURGA	SDSS13Y00060T-0B-0Y-0M-0Y-0B-33Y
72	620629	49	7933762	H-1546/NELOKI/3/ATTILA*2/PBW65//MURGA	SDSS13Y00060T-0B-0Y-0M-0Y-0B-44Y
74	620629	54	7933767	H-1546/NELOKI/3/ATTILA*2/PBW65//MURGA	SDSS13Y00060T-0B-0Y-0M-0Y-0B-49Y
75	620629	56	7933769	H-1546/NELOKI/3/ATTILA*2/PBW65//MURGA	SDSS13Y00060T-0B-0Y-0M-0Y-0B-51Y
76	620630	41	7933773	H-1694/NELOKI/3/ATTILA*2/PBW65//MURGA	SDSS13Y00061T-0B-0Y-0M-0Y-0B-36Y
77	620630	44	7933776	H-1694/NELOKI/3/ATTILA*2/PBW65//MURGA	SDSS13Y00061T-0B-0Y-0M-0Y-0B-39Y
78	620630	53	7933785	H-1694/NELOKI/3/ATTILA*2/PBW65//MURGA	SDSS13Y00061T-0B-0Y-0M-0Y-0B-48Y

79	620630	54	7933786	H-1694/NELOKI/3/ATTILA*2/PBW65//MURGA	SDSS13Y00061T-0B-0Y-0M-0Y-0B-49Y
80	620630	60	7933792	H-1694/NELOKI/3/ATTILA*2/PBW65//MURGA	SDSS13Y00061T-0B-0Y-0M-0Y-0B-55Y
81	620631	28	7933794	H-1699/NELOKI/3/ATTILA*2/PBW65//MURGA	SDSS13Y00062T-0B-0Y-0M-0Y-0B-23Y
82	620631	31	7933797	H-1699/NELOKI/3/ATTILA*2/PBW65//MURGA	SDSS13Y00062T-0B-0Y-0M-0Y-0B-26Y
83	620631	33	7933799	H-1699/NELOKI/3/ATTILA*2/PBW65//MURGA	SDSS13Y00062T-0B-0Y-0M-0Y-0B-28Y
84	620631	35	7933801	H-1699/NELOKI/3/ATTILA*2/PBW65//MURGA	SDSS13Y00062T-0B-0Y-0M-0Y-0B-30Y
85	620633	55	7933807	IG 131672/3/ATTILA*2/PBW65//MURGA/4/BORL14	SDSS13Y00064T-0B-0Y-0M-0Y-0B-50Y
87	620633	65	7933817	IG 131672/3/ATTILA*2/PBW65//MURGA/4/BORL14	SDSS13Y00064T-0B-0Y-0M-0Y-0B-60Y
88	620633	75	7933827	IG 131672/3/ATTILA*2/PBW65//MURGA/4/BORL14	SDSS13Y00064T-0B-0Y-0M-0Y-0B-70Y
89	620634	51	7933829	INDIA-38/3/ATTILA*2/PBW65//MURGA/4/BORL14	SDSS13Y00065T-0B-0Y-0M-0Y-0B-46Y
90	620634	53	7933831	INDIA-38/3/ATTILA*2/PBW65//MURGA/4/BORL14	SDSS13Y00065T-0B-0Y-0M-0Y-0B-48Y
91	620634	58	7933836	INDIA-38/3/ATTILA*2/PBW65//MURGA/4/BORL14	SDSS13Y00065T-0B-0Y-0M-0Y-0B-53Y
92	620634	59	7933837	INDIA-38/3/ATTILA*2/PBW65//MURGA/4/BORL14	SDSS13Y00065T-0B-0Y-0M-0Y-0B-54Y
94	620634	63	7933841	INDIA-38/3/ATTILA*2/PBW65//MURGA/4/BORL14	SDSS13Y00065T-0B-0Y-0M-0Y-0B-58Y
95	620634	65	7933843	INDIA-38/3/ATTILA*2/PBW65//MURGA/4/BORL14	SDSS13Y00065T-0B-0Y-0M-0Y-0B-60Y
96	620635	64	7933844	INDIA-50/3/ATTILA*2/PBW65//MURGA/4/BORL14	SDSS13Y00066T-0B-0Y-0M-0Y-0B-59Y

97	620635	65	7933845	INDIA-50/3/ATTILA*2/PBW65//MURGA/4/BORL14	SDSS13Y00066T-0B-0Y-0M-0Y-0B-60Y
98	620635	66	7933846	INDIA-50/3/ATTILA*2/PBW65//MURGA/4/BORL14	SDSS13Y00066T-0B-0Y-0M-0Y-0B-61Y
99	620635	67	7933847	INDIA-50/3/ATTILA*2/PBW65//MURGA/4/BORL14	SDSS13Y00066T-0B-0Y-0M-0Y-0B-62Y
100	620635	68	7933848	INDIA-50/3/ATTILA*2/PBW65//MURGA/4/BORL14	SDSS13Y00066T-0B-0Y-0M-0Y-0B-63Y
101	620635	71	7933851	INDIA-50/3/ATTILA*2/PBW65//MURGA/4/BORL14	SDSS13Y00066T-0B-0Y-0M-0Y-0B-66Y
102	620635	72	7933852	INDIA-50/3/ATTILA*2/PBW65//MURGA/4/BORL14	SDSS13Y00066T-0B-0Y-0M-0Y-0B-67Y
103	620637	70	7933864	TXL92.8.1/3/ATTILA*2/PBW65//MURGA/4/BORL14	SDSS13Y00068T-0B-0Y-0M-0Y-0B-65Y
104	620637	72	7933866	TXL92.8.1/3/ATTILA*2/PBW65//MURGA/4/BORL14	SDSS13Y00068T-0B-0Y-0M-0Y-0B-67Y
105	620638	44	7933881	LOCAL RED/AE.SQUARROSA (223)//BORL14/3/COPIO	SDSS13Y00069T-0B-0Y-0M-0Y-0B-39Y
106	620638	45	7933882	LOCAL RED/AE.SQUARROSA (223)//BORL14/3/COPIO	SDSS13Y00069T-0B-0Y-0M-0Y-0B-40Y
107	620638	57	7933894	LOCAL RED/AE.SQUARROSA (223)//BORL14/3/COPIO	SDSS13Y00069T-0B-0Y-0M-0Y-0B-52Y
108	620638	60	7933897	LOCAL RED/AE.SQUARROSA (223)//BORL14/3/COPIO	SDSS13Y00069T-0B-0Y-0M-0Y-0B-55Y
109	620638	61	7933898	LOCAL RED/AE.SQUARROSA (223)//BORL14/3/COPIO	SDSS13Y00069T-0B-0Y-0M-0Y-0B-56Y
110	620638	67	7933904	LOCAL RED/AE.SQUARROSA (223)//BORL14/3/COPIO	SDSS13Y00069T-0B-0Y-0M-0Y-0B-62Y
111	620638	70	7933907	LOCAL RED/AE.SQUARROSA (223)//BORL14/3/COPIO	SDSS13Y00069T-0B-0Y-0M-0Y-0B-65Y
113	620640	50	7933939	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (628)/5/BORL14/6/COPIO	SDSS13Y00071T-0B-0Y-0M-0Y-0B-45Y

114	620640	51	7933940	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (628)/5/BORL14/6/COPIO	SDSS13Y00071T-0B-0Y-0M-0Y-0B-46Y
115	620640	52	7933941	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (628)/5/BORL14/6/COPIO	SDSS13Y00071T-0B-0Y-0M-0Y-0B-47Y
116	620640	56	7933945	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (628)/5/BORL14/6/COPIO	SDSS13Y00071T-0B-0Y-0M-0Y-0B-51Y
117	620640	58	7933947	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (628)/5/BORL14/6/COPIO	SDSS13Y00071T-0B-0Y-0M-0Y-0B-53Y
118	620641	47	7933957	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (630)/5/BORL14/6/COPIO	SDSS13Y00072T-0B-0Y-0M-0Y-0B-42Y
119	620641	48	7933958	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (630)/5/BORL14/6/COPIO	SDSS13Y00072T-0B-0Y-0M-0Y-0B-43Y
120	620641	50	7933960	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (630)/5/BORL14/6/COPIO	SDSS13Y00072T-0B-0Y-0M-0Y-0B-45Y
121	620641	52	7933962	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (630)/5/BORL14/6/COPIO	SDSS13Y00072T-0B-0Y-0M-0Y-0B-47Y
122	620641	53	7933963	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (630)/5/BORL14/6/COPIO	SDSS13Y00072T-0B-0Y-0M-0Y-0B-48Y
124	620641	56	7933966	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (630)/5/BORL14/6/COPIO	SDSS13Y00072T-0B-0Y-0M-0Y-0B-51Y
125	620641	57	7933967	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (630)/5/BORL14/6/COPIO	SDSS13Y00072T-0B-0Y-0M-0Y-0B-52Y
126	620641	61	7933971	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (630)/5/BORL14/6/COPIO	SDSS13Y00072T-0B-0Y-0M-0Y-0B-56Y
127	620641	62	7933972	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (630)/5/BORL14/6/COPIO	SDSS13Y00072T-0B-0Y-0M-0Y-0B-57Y
128	620641	63	7933973	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (630)/5/BORL14/6/COPIO	SDSS13Y00072T-0B-0Y-0M-0Y-0B-58Y
129	620641	65	7933975	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (630)/5/BORL14/6/COPIO	SDSS13Y00072T-0B-0Y-0M-0Y-0B-60Y
130	620641	67	7933977	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (630)/5/BORL14/6/COPIO	SDSS13Y00072T-0B-0Y-0M-0Y-0B-62Y

131	620642	78	7933986	D67.2/PARANA 66.270//AE.SQUARROSA (1085)/3/BORL14/4/COPIO	SDSS13Y00073T-0B-0Y-0M-0Y-0B-73Y
132	620642	82	7933990	D67.2/PARANA 66.270//AE.SQUARROSA (1085)/3/BORL14/4/COPIO	SDSS13Y00073T-0B-0Y-0M-0Y-0B-77Y
133	620642	84	7933992	D67.2/PARANA 66.270//AE.SQUARROSA (1085)/3/BORL14/4/COPIO	SDSS13Y00073T-0B-0Y-0M-0Y-0B-79Y
134	620642	85	7933993	D67.2/PARANA 66.270//AE.SQUARROSA (1085)/3/BORL14/4/COPIO	SDSS13Y00073T-0B-0Y-0M-0Y-0B-80Y
135	620645	40	7933999	IWA8612416/BORL14//COPIO	SDSS13Y00076T-0B-0Y-0M-0Y-0B-35Y
136	620645	45	7934004	IWA8612416/BORL14//COPIO	SDSS13Y00076T-0B-0Y-0M-0Y-0B-40Y
137	620645	51	7934010	IWA8612416/BORL14//COPIO	SDSS13Y00076T-0B-0Y-0M-0Y-0B-46Y
138	620645	53	7934012	IWA8612416/BORL14//COPIO	SDSS13Y00076T-0B-0Y-0M-0Y-0B-48Y
139	620645	54	7934013	IWA8612416/BORL14//COPIO	SDSS13Y00076T-0B-0Y-0M-0Y-0B-49Y
140	620645	55	7934014	IWA8612416/BORL14//COPIO	SDSS13Y00076T-0B-0Y-0M-0Y-0B-50Y
141	620646	97	7934022	IWA8611400/BORL14//COPIO	SDSS13Y00077T-0B-0Y-0M-0Y-0B-92Y
142	620646	111	7934036	IWA8611400/BORL14//COPIO	SDSS13Y00077T-0B-0Y-0M-0Y-0B-106Y
143	620646	117	7934042	IWA8611400/BORL14//COPIO	SDSS13Y00077T-0B-0Y-0M-0Y-0B-112Y
144	620646	118	7934043	IWA8611400/BORL14//COPIO	SDSS13Y00077T-0B-0Y-0M-0Y-0B-113Y
145	620647	76	7934047	T.DICOCCON PI94624/AE.SQUARROSA (454)//COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00078T-0B-0Y-0M-0Y-0B-71Y
146	620647	90	7934061	T.DICOCCON PI94624/AE.SQUARROSA (454)//COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00078T-0B-0Y-0M-0Y-0B-85Y

147	620647	91	7934062	T.DICOCCON PI94624/AE.SQUARROSA (454)//COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00078T-0B-0Y-0M-0Y-0B-86Y
148	620648	107	7934074	T.DICOCCON PI94625/AE.SQUARROSA (372)//COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00079T-0B-0Y-0M-0Y-0B-102Y
149	620648	110	7934077	T.DICOCCON PI94625/AE.SQUARROSA (372)//COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00079T-0B-0Y-0M-0Y-0B-105Y
150	620648	111	7934078	T.DICOCCON PI94625/AE.SQUARROSA (372)//COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00079T-0B-0Y-0M-0Y-0B-106Y
151	620648	112	7934079	T.DICOCCON PI94625/AE.SQUARROSA (372)//COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00079T-0B-0Y-0M-0Y-0B-107Y
152	620648	113	7934080	T.DICOCCON PI94625/AE.SQUARROSA (372)//COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00079T-0B-0Y-0M-0Y-0B-108Y
153	620648	117	7934084	T.DICOCCON PI94625/AE.SQUARROSA (372)//COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00079T-0B-0Y-0M-0Y-0B-112Y
154	620648	118	7934085	T.DICOCCON PI94625/AE.SQUARROSA (372)//COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00079T-0B-0Y-0M-0Y-0B-113Y
155	620650	69	7934091	IG 41620/COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00081T-0B-0Y-0M-0Y-0B-64Y
156	620650	83	7934105	IG 41620/COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00081T-0B-0Y-0M-0Y-0B-78Y
157	620652	70	7934116	PERSIA-7/COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00083T-0B-0Y-0M-0Y-0B-65Y
158	620652	71	7934117	PERSIA-7/COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00083T-0B-0Y-0M-0Y-0B-66Y
159	620652	74	7934120	PERSIA-7/COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00083T-0B-0Y-0M-0Y-0B-69Y
160	620652	76	7934122	PERSIA-7/COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00083T-0B-0Y-0M-0Y-0B-71Y
161	61665	-1	5410961	EMPTY PLOT	
162	620652	78	7934124	PERSIA-7/COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00083T-0B-0Y-0M-0Y-0B-73Y

165	620652	84	7934130	PERSIA-7/COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00083T-0B-0Y-0M-0Y-0B-79Y
166	620652	86	7934132	PERSIA-7/COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00083T-0B-0Y-0M-0Y-0B-81Y
167	620653	79	7934141	PERSIA-21/COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00084T-0B-0Y-0M-0Y-0B-74Y
168	620653	80	7934142	PERSIA-21/COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00084T-0B-0Y-0M-0Y-0B-75Y
169	620653	82	7934144	PERSIA-21/COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00084T-0B-0Y-0M-0Y-0B-77Y
170	620653	86	7934148	PERSIA-21/COPIO/3/KACHU #1/KIRITATI//KACHU	SDSS13Y00084T-0B-0Y-0M-0Y-0B-81Y
171	620654	67	7934154	CETA/AE.SQUARROSA (850)/3/KACHU #1/KIRITATI//KACHU/4/PBW343*2/KUKUNA*2//FRTL/PIFED	SDSS13Y00085T-0B-0Y-0M-0Y-0B-62Y
172	620654	69	7934156	CETA/AE.SQUARROSA (850)/3/KACHU #1/KIRITATI//KACHU/4/PBW343*2/KUKUNA*2//FRTL/PIFED	SDSS13Y00085T-0B-0Y-0M-0Y-0B-64Y
173	620654	74	7934161	CETA/AE.SQUARROSA (850)/3/KACHU #1/KIRITATI//KACHU/4/PBW343*2/KUKUNA*2//FRTL/PIFED	SDSS13Y00085T-0B-0Y-0M-0Y-0B-69Y
174	620655	63	7934180	CETA/AE.SQUARROSA (872)/3/KACHU #1/KIRITATI//KACHU/4/PBW343*2/KUKUNA*2//FRTL/PIFED	SDSS13Y00086T-0B-0Y-0M-0Y-0B-58Y
175	620655	69	7934186	CETA/AE.SQUARROSA (872)/3/KACHU #1/KIRITATI//KACHU/4/PBW343*2/KUKUNA*2//FRTL/PIFED	SDSS13Y00086T-0B-0Y-0M-0Y-0B-64Y
176	620657	115	7934191	CETA/AE.SQUARROSA (895)/3/KACHU #1/KIRITATI//KACHU/4/PBW343*2/KUKUNA*2//FRTL/PIFED	SDSS13Y00088T-0B-0Y-0M-0Y-0B-110Y
177	620657	120	7934196	CETA/AE.SQUARROSA (895)/3/KACHU #1/KIRITATI//KACHU/4/PBW343*2/KUKUNA*2//FRTL/PIFED	SDSS13Y00088T-0B-0Y-0M-0Y-0B-115Y
178	620657	125	7934201	CETA/AE.SQUARROSA (895)/3/KACHU #1/KIRITATI//KACHU/4/PBW343*2/KUKUNA*2//FRTL/PIFED	SDSS13Y00088T-0B-0Y-0M-0Y-0B-120Y
180	620661	102	7934240	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (460)/5/KACHU #1/KIRITATI//KACHU/6/PBW343*2/KUKUNA*2//FRTL/PIFED	SDSS13Y00092T-0B-0Y-0M-0Y-0B-97Y
181	620661	104	7934242	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (460)/5/KACHU #1/KIRITATI//KACHU/6/PBW343*2/KUKUNA*2//FRTL/PIFED	SDSS13Y00092T-0B-0Y-0M-0Y-0B-99Y

182	620661	105	7934243	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (460)/5/KACHU #1/KIRITATI//KACHU/6/PBW343*2/KUKUNA*2//FRTL/PIFED	SDSS13Y00092T-0B-0Y-0M-0Y-0B-100Y
183	620662	87	7934260	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (477)/5/KACHU #1/KIRITATI//KACHU/6/PBW343*2/KUKUNA*2//FRTL/PIFED	SDSS13Y00093T-0B-0Y-0M-0Y-0B-82Y
184	620662	92	7934265	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (477)/5/KACHU #1/KIRITATI//KACHU/6/PBW343*2/KUKUNA*2//FRTL/PIFED	SDSS13Y00093T-0B-0Y-0M-0Y-0B-87Y
185	620662	96	7934269	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (477)/5/KACHU #1/KIRITATI//KACHU/6/PBW343*2/KUKUNA*2//FRTL/PIFED	SDSS13Y00093T-0B-0Y-0M-0Y-0B-91Y
186	620662	102	7934275	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (477)/5/KACHU #1/KIRITATI//KACHU/6/PBW343*2/KUKUNA*2//FRTL/PIFED	SDSS13Y00093T-0B-0Y-0M-0Y-0B-97Y
187	620663	52	7934294	GARZA/BOY//AE.SQUARROSA (278)/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/QUAIU #1	SDSS13Y00094T-0B-0Y-0M-0Y-0B-47Y
188	620665	63	7934321	GARZA/BOY//AE.SQUARROSA (281)/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/QUAIU #1	SDSS13Y00096T-0B-0Y-0M-0Y-0B-58Y
189	620665	67	7934325	GARZA/BOY//AE.SQUARROSA (281)/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/QUAIU #1	SDSS13Y00096T-0B-0Y-0M-0Y-0B-62Y
190	620667	74	7934353	DOY1/AE.SQUARROSA (415)/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/QUAIU #1	SDSS13Y00098T-0B-0Y-0M-0Y-0B-69Y
191	620669	84	7934377	LOCAL RED/AE.SQUARROSA (220)/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/QUAIU #1	SDSS13Y00100T-0B-0Y-0M-0Y-0B-79Y
192	620669	92	7934385	LOCAL RED/AE.SQUARROSA (220)/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/QUAIU #1	SDSS13Y00100T-0B-0Y-0M-0Y-0B-87Y
193	620670	86	7934390	LOCAL RED/AE.SQUARROSA (222)/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/QUAIU #1	SDSS13Y00101T-0B-0Y-0M-0Y-0B-81Y
194	620674	34	7934395	JAL95.4.3/VORB//ROLF07	SDSS13Y00105T-0B-0Y-0M-0Y-0B-29Y
196	620678	59	7934409	H-1491/ROLF07//NAVJ07	SDSS13Y00109T-0B-0Y-0M-0Y-0B-54Y
197	620678	78	7934428	H-1491/ROLF07//NAVJ07	SDSS13Y00109T-0B-0Y-0M-0Y-0B-73Y
198	620678	79	7934429	H-1491/ROLF07//NAVJ07	SDSS13Y00109T-0B-0Y-0M-0Y-0B-74Y

199	620680	63	7934440	IG 41243/NAVJ07//KACHU	SDSS13Y00111T-0B-0Y-0M-0Y-0B-58Y
200	620680	69	7934446	IG 41243/NAVJ07//KACHU	SDSS13Y00111T-0B-0Y-0M-0Y-0B-64Y
201	620683	105	7934479	H-1601/NAVJ07//KACHU	SDSS13Y00114T-0B-0Y-0M-0Y-0B-100Y
202	620683	109	7934483	H-1601/NAVJ07//KACHU	SDSS13Y00114T-0B-0Y-0M-0Y-0B-104Y
203	620683	111	7934485	H-1601/NAVJ07//KACHU	SDSS13Y00114T-0B-0Y-0M-0Y-0B-106Y
204	620684	103	7934502	MEX94.30.10/NAVJ07//KACHU	SDSS13Y00115T-0B-0Y-0M-0Y-0B-98Y
205	620684	107	7934506	MEX94.30.10/NAVJ07//KACHU	SDSS13Y00115T-0B-0Y-0M-0Y-0B-102Y
206	620685	73	7934516	ARLIN/AE.SQUARROSA (283)//KACHU/3/BAJ #1	SDSS13Y00116T-0B-0Y-0M-0Y-0B-68Y
207	620685	88	7934531	ARLIN/AE.SQUARROSA (283)//KACHU/3/BAJ #1	SDSS13Y00116T-0B-0Y-0M-0Y-0B-83Y
208	620687	106	7934557	CROC_1/AE.SQUARROSA (436)//KACHU/3/BAJ #1	SDSS13Y00118T-0B-0Y-0M-0Y-0B-101Y
209	620687	116	7934567	CROC_1/AE.SQUARROSA (436)//KACHU/3/BAJ #1	SDSS13Y00118T-0B-0Y-0M-0Y-0B-111Y
210	620687	118	7934569	CROC_1/AE.SQUARROSA (436)//KACHU/3/BAJ #1	SDSS13Y00118T-0B-0Y-0M-0Y-0B-113Y
211	620687	128	7934579	CROC_1/AE.SQUARROSA (436)//KACHU/3/BAJ #1	SDSS13Y00118T-0B-0Y-0M-0Y-0B-123Y
212	620687	129	7934580	CROC_1/AE.SQUARROSA (436)//KACHU/3/BAJ #1	SDSS13Y00118T-0B-0Y-0M-0Y-0B-124Y
213	620687	134	7934585	CROC_1/AE.SQUARROSA (436)//KACHU/3/BAJ #1	SDSS13Y00118T-0B-0Y-0M-0Y-0B-129Y
215	620687	142	7934593	CROC_1/AE.SQUARROSA (436)//KACHU/3/BAJ #1	SDSS13Y00118T-0B-0Y-0M-0Y-0B-137Y

216	620687	144	7934595	CROC_1/AE.SQUARROSA (436)//KACHU/3/BAJ #1	SDSS13Y00118T-0B-0Y-0M-0Y-0B-139Y
217	620687	150	7934601	CROC_1/AE.SQUARROSA (436)//KACHU/3/BAJ #1	SDSS13Y00118T-0B-0Y-0M-0Y-0B-145Y
218	620688	74	7934610	AE.SQUARROSA (1029)/DVERD_2//KACHU/3/BAJ #1	SDSS13Y00119T-0B-0Y-0M-0Y-0B-69Y
219	620689	92	7934627	CROC_1/AE.SQUARROSA (176)//KACHU/3/BAJ #1	SDSS13Y00120T-0B-0Y-0M-0Y-0B-87Y
220	620689	97	7934632	CROC_1/AE.SQUARROSA (176)//KACHU/3/BAJ #1	SDSS13Y00120T-0B-0Y-0M-0Y-0B-92Y
221	620689	105	7934640	CROC_1/AE.SQUARROSA (176)//KACHU/3/BAJ #1	SDSS13Y00120T-0B-0Y-0M-0Y-0B-100Y
222	620690	59	7934658	GAN/AE.SQUARROSA (206)//KACHU/3/BAJ #1	SDSS13Y00121T-0B-0Y-0M-0Y-0B-54Y
223	620690	64	7934663	GAN/AE.SQUARROSA (206)//KACHU/3/BAJ #1	SDSS13Y00121T-0B-0Y-0M-0Y-0B-59Y
224	620690	84	7934683	GAN/AE.SQUARROSA (206)//KACHU/3/BAJ #1	SDSS13Y00121T-0B-0Y-0M-0Y-0B-79Y
225	620690	88	7934687	GAN/AE.SQUARROSA (206)//KACHU/3/BAJ #1	SDSS13Y00121T-0B-0Y-0M-0Y-0B-83Y
226	620692	127	7934723	D67.2/PARANA 66.270//AE.SQUARROSA (448)/3/KACHU/4/BAJ #1	SDSS13Y00123T-0B-0Y-0M-0Y-0B-122Y
227	620693	54	7934725	D67.2/PARANA 66.270//AE.SQUARROSA (506)/3/KACHU/4/BAJ #1	SDSS13Y00124T-0B-0Y-0M-0Y-0B-49Y
228	620694	78	7934760	INDIA-59/KACHU//BAJ #1	SDSS13Y00125T-0B-0Y-0M-0Y-0B-73Y
229	620694	84	7934766	INDIA-59/KACHU//BAJ #1	SDSS13Y00125T-0B-0Y-0M-0Y-0B-79Y
230	620694	96	7934778	INDIA-59/KACHU//BAJ #1	SDSS13Y00125T-0B-0Y-0M-0Y-0B-91Y
231	620695	92	7934788	INDIA-107/KACHU//BAJ #1	SDSS13Y00126T-0B-0Y-0M-0Y-0B-87Y

232	620695	106	7934802	INDIA-107/KACHU//BAJ #1	SDSS13Y00126T-0B-0Y-0M-0Y-0B-101Y
233	620695	108	7934804	INDIA-107/KACHU//BAJ #1	SDSS13Y00126T-0B-0Y-0M-0Y-0B-103Y
234	620695	113	7934809	INDIA-107/KACHU//BAJ #1	SDSS13Y00126T-0B-0Y-0M-0Y-0B-108Y
235	620696	80	7934835	IG 41242/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/QUAIU #1	SDSS13Y00127T-0B-0Y-0M-0Y-0B-75Y
236	620698	51	7934841	IG 41474/NAVJ07//KACHU	SDSS13Y00129T-0B-0Y-0M-0Y-0B-46Y
239	620699	76	7934848	IG 41506/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/QUAIU #1	SDSS13Y00130T-0B-0Y-0M-0Y-0B-71Y
240	620699	79	7934851	IG 41506/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/QUAIU #1	SDSS13Y00130T-0B-0Y-0M-0Y-0B-74Y
241	620699	80	7934852	IG 41506/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/QUAIU #1	SDSS13Y00130T-0B-0Y-0M-0Y-0B-75Y
242	620699	83	7934855	IG 41506/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/QUAIU #1	SDSS13Y00130T-0B-0Y-0M-0Y-0B-78Y
243	620699	88	7934860	IG 41506/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/QUAIU #1	SDSS13Y00130T-0B-0Y-0M-0Y-0B-83Y
244	620710	72	7934893	IG 41735/NAVJ07//KACHU	SDSS13Y00141T-0B-0Y-0M-0Y-0B-67Y
245	620710	73	7934894	IG 41735/NAVJ07//KACHU	SDSS13Y00141T-0B-0Y-0M-0Y-0B-68Y
246	620710	83	7934904	IG 41735/NAVJ07//KACHU	SDSS13Y00141T-0B-0Y-0M-0Y-0B-78Y
247	620718	10	7934909	IG 43238/NAVJ07//KACHU	SDSS13Y00149T-0B-0Y-0M-0Y-0B-5Y
248	620726	52	7934939	IG 107128/4/PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07/5/NELOKI	SDSS13Y00157T-0B-0Y-0M-0Y-0B-47Y
249	620726	55	7934942	IG 107128/4/PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07/5/NELOKI	SDSS13Y00157T-0B-0Y-0M-0Y-0B-50Y

250	620729	39	7934982	IG 122139/NAVJ07//KACHU	SDSS13Y00160T-0B-0Y-0M-0Y-0B-34Y
251	620729	43	7934986	IG 122139/NAVJ07//KACHU	SDSS13Y00160T-0B-0Y-0M-0Y-0B-38Y
252	620736	39	7934992	IG 122627/6/KAUZ//ALTAR 84/AOS/3/PASTOR/4/MILAN/CUPE//SW89.3064/5/KIRITATI/7/SW89.5277/BORL95//SKAUZ/3/PRL/2*PASTOR/4/HEILO	SDSS13Y00167T-0B-0Y-0M-0Y-0B-34Y
253	620736	42	7934995	IG 122627/6/KAUZ//ALTAR 84/AOS/3/PASTOR/4/MILAN/CUPE//SW89.3064/5/KIRITATI/7/SW89.5277/BORL95//SKAUZ/3/PRL/2*PASTOR/4/HEILO	SDSS13Y00167T-0B-0Y-0M-0Y-0B-37Y
254	620736	43	7934996	IG 122627/6/KAUZ//ALTAR 84/AOS/3/PASTOR/4/MILAN/CUPE//SW89.3064/5/KIRITATI/7/SW89.5277/BORL95//SKAUZ/3/PRL/2*PASTOR/4/HEILO	SDSS13Y00167T-0B-0Y-0M-0Y-0B-38Y
255	620736	46	7934999	IG 122627/6/KAUZ//ALTAR 84/AOS/3/PASTOR/4/MILAN/CUPE//SW89.3064/5/KIRITATI/7/SW89.5277/BORL95//SKAUZ/3/PRL/2*PASTOR/4/HEILO	SDSS13Y00167T-0B-0Y-0M-0Y-0B-41Y
256	620736	48	7935001	IG 122627/6/KAUZ//ALTAR 84/AOS/3/PASTOR/4/MILAN/CUPE//SW89.3064/5/KIRITATI/7/SW89.5277/BORL95//SKAUZ/3/PRL/2*PASTOR/4/HEILO	SDSS13Y00167T-0B-0Y-0M-0Y-0B-43Y
257	620746	59	7935021	IG 122738/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/QUAIU #1	SDSS13Y00177T-0B-0Y-0M-0Y-0B-54Y
258	620746	64	7935026	IG 122738/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/QUAIU #1	SDSS13Y00177T-0B-0Y-0M-0Y-0B-59Y
260	620747	52	7935047	IG 122740/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/QUAIU #1	SDSS13Y00178T-0B-0Y-0M-0Y-0B-47Y
261	620748	27	7935051	IG 122741/NAVJ07//KACHU	SDSS13Y00179T-0B-0Y-0M-0Y-0B-22Y
262	620748	32	7935056	IG 122741/NAVJ07//KACHU	SDSS13Y00179T-0B-0Y-0M-0Y-0B-27Y
263	620748	33	7935057	IG 122741/NAVJ07//KACHU	SDSS13Y00179T-0B-0Y-0M-0Y-0B-28Y

264	620749	38	7935064	IG 122743/NAVJ07//KACHU	SDSS13Y00180T-0B-0Y-0M-0Y-0B-33Y
265	620759	76	7935072	PERSIA-88/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/QUAIU #1	SDSS13Y00190T-0B-0Y-0M-0Y-0B-71Y
266	620759	94	7935090	PERSIA-88/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/QUAIU #1	SDSS13Y00190T-0B-0Y-0M-0Y-0B-89Y
267	620768	44	7935101	H-1659/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/QUAIU #1	SDSS13Y00199T-0B-0Y-0M-0Y-0B-39Y
269	620768	49	7935106	H-1659/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/QUAIU #1	SDSS13Y00199T-0B-0Y-0M-0Y-0B-44Y
270	620777	51	7935151	IWA 8602098/NAVJ07//KACHU	SDSS13Y00208T-0B-0Y-0M-0Y-0B-46Y
271	620783	93	7935164	IWA8612134/NAVJ07//KACHU	SDSS13Y00214T-0B-0Y-0M-0Y-0B-88Y
272	620785	121	7935181	IWA8614378/NAVJ07//KACHU	SDSS13Y00216T-0B-0Y-0M-0Y-0B-116Y
273	620785	124	7935184	IWA8614378/NAVJ07//KACHU	SDSS13Y00216T-0B-0Y-0M-0Y-0B-119Y
274	620785	126	7935186	IWA8614378/NAVJ07//KACHU	SDSS13Y00216T-0B-0Y-0M-0Y-0B-121Y
275	620785	128	7935188	IWA8614378/NAVJ07//KACHU	SDSS13Y00216T-0B-0Y-0M-0Y-0B-123Y
276	620785	130	7935190	IWA8614378/NAVJ07//KACHU	SDSS13Y00216T-0B-0Y-0M-0Y-0B-125Y
277	620786	68	7935209	IWA8612701/6/KAUZ//ALTAR 84/AOS/3/PASTOR/4/MILAN/CUPE//SW89.3064/5/KIRITATI/7/SW89.5277/BORL95//SKAUZ/3/PRL/2*PASTOR/4/HEILO	SDSS13Y00217T-0B-0Y-0M-0Y-0B-63Y
278	620786	80	7935221	IWA8612701/6/KAUZ//ALTAR 84/AOS/3/PASTOR/4/MILAN/CUPE//SW89.3064/5/KIRITATI/7/SW89.5277/BORL95//SKAUZ/3/PRL/2*PASTOR/4/HEILO	SDSS13Y00217T-0B-0Y-0M-0Y-0B-75Y

Table 3.2: The List of Check Cultivars that Were Used in the Study

Checks	Origin	Pedigree	Accessions status	Resistance
Bezostaya	Russia	LUT17/SRS2	Cultivar	S
Kutluk-94	Turkey	KRASNODARSKAYA//INIA-66/LILIFEN/3/CALIBASAN	Cultivar	S
Katea-1	Turkey	KHEBROS/BEZOSTAYA-1	Cultivar	MR
Sonmez-2001	Turkey	BEZOSTAYA-1//BEZOSTAYA-1/TEVERE/3/KREMENA/LOVRIN-29/4/KATYA-1[3669]	Cultivar	MR

CHAPTER IV

RESULTS

4.1 Screening Assay

The outcome of this study found that 4.28% of the screened wheat lines were resistant (R), 14.01% are moderately resistant (MR), 28.02% are moderately susceptible (MS), 30.74% are susceptible (S), and 22.96% of the lines are highly susceptible (HS) (Table 4.1). the calculation of some descriptive statistics parameters (mean, standard error (SE), and standard deviation (SD)) was determined and compared the results with the data of the check cultivars for the evaluation of wheat line resistant response to *Heterodera filipjevi* (Table 4.2)

The mean number of *Heterodera filipjevi* females and cysts that have formed on the moderately resistant check cultivars Katia-1 and Sonmez-2001 ranged between 3.16-5 females and cysts per plant which confirmed their resistance, and the susceptible cultivars Bezostaya and Kutluk-94 ranged between 7.5-10 females and cysts per plant which confirmed their susceptibility.

A total of 11 (4.28%) lines showed less females and cysts count per plant (> 3.16) than the moderately resistant check cultivars (Katia-1 and Sonmez-2001), thus they were group as resistant (R) lines with the line 265 showing the least females and cysts formation among the 11 resistant lines.

36 (14.01%) lines showed similar female and cyst count as the MR check cultivars (Katia-1 and Sonmez-2001) that were in the range of 3.16-5 females and cysts per plant, so they were grouped as moderately resistant lines.

A total of 72 (28.02%) of the lines had notably higher cyst count than the MR check cultivars but lower than the susceptible check cultivars (Bezostaya and Kutluk-94) that were more than 5 and less than 7.5 females and cysts per plant, thus they have been grouped as moderately susceptible (MS) lines.

A total of 79 (30.74%) lines showed similar cyst count as the susceptible cultivars (Bezostaya and Kutluk-94) that were in the range of 7.5-10 females and cysts per plant, therefore they were grouped as susceptible lines.

The remaining 59 (22.96%) lines had notably higher count of females and cysts than the susceptible check cultivars (Bezostaya and Kutluk-94) more than 10 females and cysts per plant, thus they were considered as highly susceptible lines.

4.2 Resistant Groups

Hence, the resistance response to *Heterodera filipjevi* in our experiment was divided into 5 groups according to the mean number of females and cysts formed on the plant's root system compared to the check cultivars as following:

1. R = Resistant (< 3.16 females and cysts/plant).
2. MR = moderately resistant (3.16-5 females and cysts/plant).
3. MS = moderately Susceptible (5< - <7.5 females and cysts/plant).
4. S = Susceptible (7.5-10 females and cysts/plant).
5. HS = Highly Susceptible (≥ 10.1 females and cysts/plant).

4.2.1 Hatching of cysts

Both hatching protocols that were followed were successful to an extent in the hatching of second-stage juveniles. The most important outcomes are summarized in (Table 4.1).

Table 4.1: Comparison of Hatching Protocols

Comparison Points	CIMMYT – Turkey Protocol	Modified Larval farm – Australian method
Preparation	Cysts need to be collected from OM, Surface sterilization of the cysts before placing in Petri plates for hatching.	Cyst collection and sterilization is not required beforehand. OM is directly placed mesh or permeable bag when placing in Petri Plate.
Incubation Temperature	4°C	10°C
Start of Hatching	After 3 months	After 1.5 months
Peak Hatching	After 7 months	After 6 months

Table 4.2: Resistant Response Groups of the Spring Wheat Lines

Entry ID	Mean	SE	SD	Resistant Response	Grouping Order
265	0.40	3.1064	0.5477	R	1
210	1.00	0.3651	0.8944	R	1
70	2.17	1.1377	1.3292	R	1
203	2.33	0.8433	1.2111	R	1
264	2.33	0.3333	0.8165	R	1
54	2.60	2.5560	2.4083	R	1
49	2.80	2.8255	2.2804	R	1
263	2.83	1.1377	1.7224	R	1
42	3.00	1.3166	1.7889	R	1
251	3.00	1.1832	1.8974	R	1
254	3.00	1.5055	1.4142	R	1
128	3.17	0.6540	1.6021	MR	2
Katea-1 (MR)	3.17	1.1081	2.7142	MR	2
81	3.20	2.7618	2.2804	MR	2
69	3.33	0.8028	1.9664	MR	2
157	3.33	2.0276	2.0656	MR	2
225	3.33	1.4063	1.5055	MR	2
250	3.33	1.5846	2.0656	MR	2
257	3.33	0.8819	2.1602	MR	2
57	3.50	1.4083	2.3452	MR	2
256	3.50	1.2845	1.6432	MR	2
208	3.67	2.0276	4.9666	MR	2
261	3.67	1.2293	2.5033	MR	2
Sonmez-2001 (MR)	3.80	0.9695	2.1679	MR	2
87	3.83	1.6816	2.2286	MR	2

227	3.83	0.6009	1.4720	MR	2
174	4.20	3.5839	2.4900	MR	2
143	4.33	1.0853	2.6583	MR	2
219	4.33	1.6262	2.7325	MR	2
127	4.50	1.5420	2.9496	MR	2
144	4.50	1.8930	3.0166	MR	2
145	4.50	1.6882	2.7386	MR	2
168	4.50	1.6073	2.8810	MR	2
169	4.50	1.9621	2.8107	MR	2
189	4.50	1.3354	2.8107	MR	2
199	4.50	1.1762	2.8810	MR	2
239	4.50	1.2845	3.1464	MR	2
247	4.50	1.4776	2.8810	MR	2
273	4.50	0.9574	2.3452	MR	2
Sonmez-2001 (MR)	4.50	0.8466	2.0736	MR	2
160	4.67	0.9545	2.3381	MR	2
170	4.83	1.9221	3.3714	MR	2
215	4.83	1.8151	4.4460	MR	2
229	4.83	1.0462	2.5626	MR	2
246	4.83	1.9565	4.7924	MR	2
278	4.83	0.9458	2.3166	MR	2
165	5.00	1.5275	2.7568	MR	2
166	5.00	1.1255	2.7568	MR	2
221	5.00	1.8074	3.0332	MR	2
241	5.00	2.0976	5.1381	MR	2
Katea-1 (MR)	5.00	1.2910	2.5820	MR	2
20	5.17	1.9734	2.6394	MS	3
29	5.17	0.9458	2.3166	MS	3

30	5.33	1.6055	3.1411	MS	3
114	5.33	1.4757	3.6148	MS	3
137	5.33	1.9090	3.0111	MS	3
244	5.33	1.1450	2.8048	MS	3
148	5.50	1.6073	3.1464	MS	3
159	5.50	1.5438	3.7815	MS	3
184	5.50	2.6426	6.4730	MS	3
262	5.50	1.0567	2.5884	MS	3
271	5.50	1.8930	3.7815	MS	3
110	5.60	3.8333	3.8471	MS	3
47	5.67	1.3081	3.2042	MS	3
181	5.67	1.4530	3.5590	MS	3
234	5.67	1.5635	3.8297	MS	3
88	5.83	1.9221	3.7639	MS	3
99	5.83	1.5147	3.7103	MS	3
216	5.83	1.7013	4.1673	MS	3
248	5.83	1.6816	3.6009	MS	3
270	5.83	2.4278	5.9470	MS	3
272	5.83	2.3154	4.0208	MS	3
33	6.00	2.6583	6.5115	MS	3
41	6.00	2.6077	6.3875	MS	3
67	6.00	3.3764	8.2704	MS	3
202	6.00	2.3523	4.1473	MS	3
222	6.00	1.6125	3.9497	MS	3
204	6.17	1.3764	3.3714	MS	3
206	6.17	1.7401	4.2622	MS	3
218	6.17	2.0562	3.9707	MS	3
220	6.17	1.7013	4.1673	MS	3

275	6.17	1.9394	3.3116	MS	3
34	6.33	2.6415	6.4704	MS	3
60	6.33	1.7256	4.2269	MS	3
78	6.33	1.7638	4.3205	MS	3
130	6.33	1.8915	3.4448	MS	3
23	6.50	2.6926	4.5497	MS	3
64	6.50	3.2736	8.0187	MS	3
77	6.50	1.8394	4.5056	MS	3
155	6.50	0.4282	1.0488	MS	3
158	6.50	2.4049	4.5056	MS	3
274	6.50	1.5221	3.7283	MS	3
124	6.67	1.4981	3.6697	MS	3
146	6.67	2.1705	3.7238	MS	3
211	6.67	2.7162	6.6533	MS	3
249	6.67	3.6301	8.8919	MS	3
277	6.67	1.8012	4.4121	MS	3
39	6.83	3.0813	7.5476	MS	3
82	6.83	2.1357	4.4008	MS	3
109	6.83	2.9935	7.3326	MS	3
139	6.83	2.2123	3.8687	MS	3
217	6.83	1.4004	3.4303	MS	3
236	6.83	2.1357	4.3089	MS	3
253	6.83	2.0562	5.0365	MS	3
18	7.00	2.1134	3.6878	MS	3
45	7.00	2.3805	4.6904	MS	3
51	7.00	1.8439	4.5166	MS	3
52	7.00	2.5033	2.9665	MS	3
55	7.00	3.3961	4.8166	MS	3

56	7.00	1.6330	4.0000	MS	3
62	7.00	1.2649	3.0984	MS	3
97	7.00	1.3166	3.2249	MS	3
84	7.17	1.8151	4.4460	MS	3
90	7.17	3.0046	7.3598	MS	3
98	7.17	2.1820	4.6224	MS	3
106	7.17	1.5794	3.8687	MS	3
197	7.17	0.9458	2.3166	MS	3
245	7.17	3.4488	8.4479	MS	3
255	7.17	1.8871	3.3116	MS	3
74	7.33	2.6791	4.8854	MS	3
83	7.33	1.4757	3.6148	MS	3
154	7.33	2.3333	4.5019	MS	3
267	7.33	1.2824	3.1411	MS	3
131	7.50	2.1409	5.2440	S	4
136	7.50	1.9621	4.8062	S	4
205	7.50	1.5438	3.7815	S	4
223	7.50	1.3844	3.3912	S	4
226	7.50	1.0567	2.5884	S	4
243	7.50	1.9451	4.7645	S	4
Bezostaya (S)	7.50	1.3229	2.6458	S	4
37	7.67	1.1450	2.8048	S	4
38	7.67	1.7638	4.3205	S	4
119	7.67	3.6209	8.8694	S	4
201	7.67	3.1163	5.1251	S	4
231	7.67	1.7256	4.2269	S	4
4	7.83	1.7401	4.2622	S	4
9	7.83	3.3308	8.1588	S	4

11	7.83	1.5366	3.7639	S	4
149	7.83	1.4472	3.5449	S	4
182	7.83	2.1972	5.3821	S	4
212	7.83	0.9804	2.4014	S	4
228	7.83	2.7978	5.0365	S	4
242	7.83	2.0562	5.0365	S	4
31	8.00	1.3416	3.2863	S	4
40	8.00	1.9833	4.8580	S	4
76	8.00	4.4045	10.7889	S	4
150	8.00	2.6957	5.0596	S	4
178	8.00	3.5870	5.3666	S	4
196	8.00	1.8797	4.6043	S	4
27	8.17	2.2423	5.4924	S	4
58	8.17	3.0267	4.7081	S	4
108	8.17	3.1243	5.5287	S	4
200	8.17	3.2395	5.7067	S	4
207	8.17	2.3010	5.6362	S	4
209	8.17	3.0705	5.0365	S	4
240	8.17	1.3764	3.3714	S	4
2	8.33	3.0948	3.0768	S	4
48	8.33	1.9944	4.8854	S	4
102	8.33	2.6541	5.6451	S	4
118	8.33	1.9090	4.6762	S	4
16	8.50	2.1718	5.3198	S	4
32	8.50	2.0777	5.0892	S	4
53	8.50	2.9972	5.3198	S	4
113	8.50	3.6856	9.0277	S	4
134	8.50	3.2939	5.9245	S	4

162	8.50	2.4597	4.5497	S	4
167	8.50	2.4049	5.8907	S	4
224	8.50	2.7172	5.6833	S	4
142	8.67	2.7769	5.4283	S	4
269	8.67	3.0732	5.5015	S	4
22	8.83	2.5221	5.1153	S	4
36	8.83	2.7739	5.6362	S	4
59	8.83	3.2906	5.3821	S	4
75	8.83	2.0562	5.0365	S	4
115	8.83	2.3298	5.7067	S	4
135	8.83	2.9373	5.8109	S	4
252	8.83	2.9935	5.8452	S	4
17	9.00	2.6957	5.4037	S	4
129	9.00	3.3367	5.8992	S	4
171	9.00	2.2657	5.5498	S	4
183	9.00	2.6833	5.5136	S	4
276	9.00	2.4221	5.9330	S	4
43	9.17	1.8871	4.6224	S	4
153	9.17	3.0046	5.7067	S	4
177	9.17	2.0235	4.9565	S	4
266	9.17	1.8151	4.4460	S	4
Kutluk-94 (S)	9.25	4.3084	8.6168	S	4
12	9.33	2.3617	5.7850	S	4
19	9.33	2.7528	3.4448	S	4
193	9.33	1.9090	4.6762	S	4
141	9.50	2.0936	5.1284	S	4
Kutluk-94 (S)	9.50	2.3629	4.7258	S	4
147	9.67	2.7406	6.7132	S	4

190	9.67	2.1396	5.2409	S	4
233	9.67	2.7162	6.6533	S	4
13	9.83	3.4968	6.5243	S	4
25	9.83	3.1136	6.6156	S	4
46	9.83	2.2423	5.4924	S	4
186	9.83	2.7008	6.6156	S	4
198	9.83	1.8514	4.5350	S	4
235	9.83	2.4822	6.0800	S	4
5	10.00	3.0441	5.1769	S	4
72	10.00	3.3066	6.0663	S	4
105	10.00	4.1553	10.1784	S	4
230	10.00	3.5870	6.7231	S	4
Bezostaya (S)	10.00	2.1213	4.2426	S	4
14	10.17	3.4392	5.9470	HS	5
79	10.17	1.6210	3.9707	HS	5
103	10.17	2.9145	5.6362	HS	5
258	10.17	3.0921	6.6758	HS	5
92	10.33	3.0185	6.5320	HS	5
172	10.33	1.7256	4.2269	HS	5
188	10.33	2.2608	5.5377	HS	5
192	10.33	3.3632	6.4395	HS	5
21	10.50	2.4049	5.8907	HS	5
120	10.50	1.2845	3.1464	HS	5
122	10.50	3.2532	5.2440	HS	5
3	10.67	2.7528	6.7429	HS	5
65	10.67	2.5777	6.3140	HS	5
95	10.67	2.7162	6.6533	HS	5
125	10.67	2.8480	6.9761	HS	5

175	10.67	3.1269	6.7725	HS	5
35	10.83	0.8333	2.0412	HS	5
50	10.83	3.8937	6.1455	HS	5
63	10.83	2.7008	6.6156	HS	5
180	10.83	2.7008	6.6156	HS	5
191	11.00	3.7417	7.4027	HS	5
111	11.17	3.2906	6.4005	HS	5
176	11.17	2.8568	6.9976	HS	5
71	11.33	3.3830	7.0899	HS	5
138	11.33	2.6791	6.5625	HS	5
173	11.33	3.9044	7.3937	HS	5
232	11.33	3.0185	7.3937	HS	5
94	11.50	4.0062	7.8677	HS	5
107	11.50	2.9749	7.2870	HS	5
117	11.50	3.5000	5.9245	HS	5
96	11.67	3.3830	7.3121	HS	5
140	11.67	3.2728	8.0166	HS	5
6	11.83	3.2804	8.0353	HS	5
26	11.83	2.6002	6.3692	HS	5
66	11.83	3.4777	6.6156	HS	5
80	11.83	2.7131	6.6458	HS	5
121	12.00	3.2762	8.0250	HS	5
132	12.00	3.0659	7.5100	HS	5
185	12.00	1.9664	4.8166	HS	5
156	12.17	2.0883	5.1153	HS	5
260	12.33	3.8701	8.1158	HS	5
152	12.50	3.5940	6.1237	HS	5
116	12.67	4.8212	7.8145	HS	5

133	12.67	2.9627	7.2572	HS	5
161	12.67	3.2830	8.0416	HS	5
213	12.67	3.2007	7.8401	HS	5
85	13.17	3.7896	6.3692	HS	5
104	13.17	2.3010	5.6362	HS	5
151	13.17	3.9868	7.7567	HS	5
194	13.33	4.9103	9.1360	HS	5
15	13.50	3.3936	8.3126	HS	5
89	13.50	4.8768	9.3113	HS	5
187	13.50	4.0641	7.6877	HS	5
126	13.67	3.3731	8.2624	HS	5
101	14.00	3.4157	8.3666	HS	5
100	15.00	3.4641	8.4853	HS	5
7	15.50	3.4034	8.3367	HS	5
91	15.67	6.0203	10.7455	HS	5
8	17.50	5.1494	10.0150	HS	5

CHAPTER V

DISCUSSION

One of the most effective and desirable methods to control of cereal cyst nematodes especially *Heterodera filipjevi* is the use of resistant lines to prevent yield loses, due to its low cost, easiness, and acknowledged as not being harmful to the environment (Dababat et al., 2014; Williamson, 2006). Different control methods can be used but have limitations. On the other hand, the complete mechanism of resistance is still an enigma with fragmentary knowledge on plant immunity to plant-parasitic nematode (Moens et al., 2018). There are very few reports related to wheat-nematode interaction.

There are sources of resistance to several of the significant plant-parasitic nematodes of agriculture. Nevertheless, dedication to breeding programs, continuous efforts by breeders and nematologists, and resistant sources that are capable of introgression into the desirable agronomic germplasm are required for integration of resistance into economically feasible crops effective implementation by the corresponding sectors (Moens et al., 2018).

Specific genes for the resistance of *Heterodera filipjevi* are yet to be identified despite some *Cre* genes have shown success against the nematode such as; *Cre8* and *CreR* showed resistance (Imren et al., 2012) and Toktay et al. (2012) screened some resistant wheat lines containing *Cre1* gene which showed different resistance response to *Heterodera filipjevi*. It has been recognized that some of the 11 *Cre* genes that are known as a resistant source in wheat to *Heterodera avenae* can show resistance to *Heterodera filipjevi* and have shown success against other cereal cyst nematodes (CCNs) (Moens et al., 2018).

There are some assumptions in the scientific community that there might be a strong connection between drought and heat tolerance with CCN resistance, but with no solid proof or published work to support this hypothesis. Kimber and Feldman (1987), mentioned that wheat varieties showing resistance or tolerance have shown to provide resistance against a wide range of biotic and abiotic stresses.

This study has managed to find 11 resistant and 36 moderately resistant spring wheat lines, as this screened wheat set originates from a diverse genetic background. Different screening studies done on wheat accessions originating from different sources have shown resistance to *Heterodera filipjevi*. Such studies have been done by Dababat (2019), Dababat et al. (2014), Pariyar et al. (2016a), Pariyar et al. (2016b), Yavuzaslanoglu et al. (2016) and Toktay (2012).

It is difficult to truly compare the result of this study to other similar or related studies despite using the same experimental setup as there are a lot of variables between the experiments. In order to understand the point of differences, this study can be summed upped as following; 250 second-stage juvenile (J2) were used to inoculate 2-day old germinated seedlings that were transplanted in "Cone-tainer" ™ with a mixture of sand, field soil and organic matter (70:29:1 v/v/v). then they were transferred to a growth chamber (70% relative humidity, 25°C, and a photoperiod of 16 hours). The plants were harvested after 14 weeks and after assessment of the number of formed females and cysts/plant for the genotypes and the moderate resistant and susceptible cultivars, then simple descriptive statistics were preformed (SE, SD, and Mean). After that the lines were divided into 5 groups as following: Resistant (< 3.16 females and cysts/plant), Moderately resistant (3.16-5 females and cysts/plant), Moderately Susceptible (5.1-7.4 females and cysts/plant), Susceptible (7.5-10 females and cysts/plant), and Highly Susceptible (≥ 10.1 females and cysts/plant).

One of the main reasons for the difficulty in comparing is due to different categorizing of the resistant response groups that rely on the average number of formed females and cysts on the root system per plant and comparing them to the check wheat accessions with known resistance. Such as Pariyar et al. (2016b) and Yavuzaslanoglu et al. (2016) have used a different arrangement of the average number of formed females and cysts/plant assigned to the resistant groups with reliance on the check wheat accessions response. Zhang et al. (2012) even used a different method of sorting which relied on the relative resistance index (RRI); RRI = [1 – (the mean number of white females per plant on a tested line/the mean number of white females per Wenmai 19 check plant).

Also, a restriction is in where the experiments are held, Dababat (2019) conducted his experiment under field conditions, Hajihasani et al. (2010) experiment was conducted in

pots under field conditions, while Zhang et al. (2012) conducted his experiment in greenhouse conditions. Although studies like Pariyar et al. (2016a), Pariyar et al. (2016b), and Toktay (2012) conducted their study in a growth chamber under a controlled condition like this study, so it can be a point of similarity.

Additionally, there are differences in some of the used methodology; this study used 250 J2 to inoculate 2-day old germinated seedling, while Yavuzaslanoglu et al. (2016) used only 100 J2 for inoculation for 7-day old germinated seedling and Toktay (2012) used 200 J2 that were inoculated half during transplanting and the other half after 24 hours.

Another limitation is the differences in the method of analysis of the data. Dababat (2019) transformed his data then used analysis of variance (ANOVA), Yavuzaslanoglu et al. (2016) transformed the data prior to using ANOVA with the calculation of LSDs and Toktay (2012) studies used ANOVA for analyzing the data.

Despite these difficulties in comparison due to reason mentioned above, it is possible to compare an important an important aspect which is the percentage of resistant lines found in these studies. Generally, when screening wheat accessions for their resistant response almost all studies have obtained a low percentage of resistant accessions from the total screening. This study managed to find a total of 4.28% resistant lines from 257 lines, Dababat (2019) study tested resistant lines that were obtained from previous screening of thousands of wheat accessions. Pariyar (2016a) found only 1% resistant accessions from a total of 161 accessions and in another study by Pariyar (2016b) also only 1% of resistance of wheat accessions from a total of 291 accessions.

So, when proceeding with these types of studies it is expected to find a very low percentage of resistance among the screened accessions. When comparing the results of this study in terms of percentage of resistance accessions found with the other studies, this study is considered to have a noticeably high percentage success.

The studies mentioned above including this study, have all been able to find resistant and moderately resistant sources of wheat germplasm from a diverse origin to *Heterodera filipjevi*, with one study by Dababat (2019) using the same location of our nematode population. As mentioned, it is actually hard to compare results but the end goal of each

of these studies and this study is the same, all have been successful in finding wheat accessions with resistance to the *Heterodera filipjevi* and adding them as genetic resources.

The reason if our current finding is the possibility that resistant and moderately resistant lines may contain a source of *Cre* genes; like *Cre1* as in Toktay et al. (2012) study, *Cre8* or *CreR* in Imren et al. (2012) study, *Cre5* as in Dababat et al. (2014) study, or the same QTLs Pariyar et al. (2016a) has identified or even due to the presence of new sources of resistance. This matter cannot be confirmed in this study but if future analysis is conducted then maybe a clearer idea will be formed for why these specific lines showed a resistant response to *Heterodera filipjevi*.

Hatching of the CCNs can occur in a wide temperature range which strongly depends on their origin and the species (Toumi et al., 2018). *Heterodera filipjevi* originating from the continental Central Anatolian Plateau of Turkey does not show any diapause (Şahin et al., 2009) and this was also noticed in this study; second-stage juvenile were observed when checked.

Two methods of hatching were tried as each method used a different temperature, one was 4°C and the other was 10°C. At 4°C hatching started after 3 months and peak hatching was after 7 months and at 10°C hatching started after 1.5 months and peak hatching was after 6 months. The findings in this study of the hatching of Heterodera filipjevi at different temperature were similar to the findings of Şahin et al. (2009).

During this study 2 hatching produces were followed to ensure that an adequate amount J2 was acquired. The observation and outcomes of the hatching protocols acknowledged that the CIMMYT – Turkey method was more laborious and time-consuming in the initial phases. Individual cysts need to be collected from the organic matter and then cyst surface sterilization is needed after that cysts are placed on a mesh in a Petri plate and checked for hatching.

The modified Australian larval farm method does not need to collect cysts for the organic matter as it is placed on mesh bag or permeable plastic bag; in case of this study, the organic matter was placed on a heavy-duty tissue paper.

Some of the drawbacks in the second method which in the first method was not encountered is the monitoring of the Petri plates. The water for the first 3-4 times needs to be thrown because of the presence of saprophytic nematodes. Then every couple of days to a week the water in the Petri plates need to be checked for hatching and changed to ensure no fungal contamination will occur.

CHAPTER VI

CONCLUSION

The main objective of our study was to evaluate the resistant response of drought and heat tolerant spring wheat lines to the cereal cyst nematode *Heterodera filipjevi*. This is not the first study to screen wheat accessions, but it is the first to evaluate the resistant response of the nematode to wheat genotyped with drought and heat tolerance. This study also aims to establish a base for future research in trying to understand the relationship between nematode resistance and drought and heat tolerance.

This study managed to add an additional 11 wheat lines with resistance and 36 lines with moderate resistance to Heterodera filipjevi as genetic resources for future wheat breeding programs. This might be good for helping advance resistance to cereal cyst nematodes in general but specifically to improve resistance against *Heterodera filipjevi*. It is expected that this study can provide supplementary data with previous work by Pariyar et al. (2016a) for future studies concerned in finding resistant genes to *Heterodera filipjevi*.

Although resistant and moderately resistant lines to *Heterodera filipjevi* were found, it should be noted that further assessment these lines is recommended to fully verify their resistant and moderately resistant status. Also, it is recommended that these lines should be screened for resistance response to other *Heterodera* species, mainly *Heterodera avenae* and *Heterodera latipons* for the line to obtain a wider range of resistance.

The protocols that were followed (related to hatching temperatures) for the hatching of *Heterodera filipjevi* of the Turkish population have concurred Şahin et al. (2009) study. Both CIMMYT – Turkey and Modified Larval farm – Australian method have their benefits and drawbacks, but when using the later method, it is recommended to use a mesh bag or permeable plastic bag instead of heavy-duty tissue paper as it is easier to manage.

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