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Mapping QTLs for phenology and grain yield related traits in bread wheat

THESIS

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CYNTHIA ORTIZ ROBLES

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Thesis advisors:

G.Beatriz Xoconostle Cázares, PhD.

Sukhwinder Singh, PhD.

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DEDICATED TO

MY FAMILY

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Dated: **(Cynthia Ortiz Robles)**

Department of Biotechnology and Bioengineering
CINVESTAV- MÉXICO

ABSTRACT

Wheat (*Triticum aestivum* L.) is the most widely grown staple food crop and is important for global food security. One fifth of the total calories consumed by the world population come from wheat. FAO estimates that global agricultural production must increase by 60 percent from 2005/07 to 2050 to feed a world population estimated at 9 billion. Under this situation, grain yield is the most important trait in wheat breeding which in ultimately depends on the yield components including- number of grains per spike (NGS) and 1000-kernel weight (TKW).

The yield and yield components are controlled by multiple genes and are influenced by environment; therefore, it is important to have analytical methods to understand the genetic component of these quantitative traits. In this study, we use Genotyping by sequencing (GBS) and SSR molecular markers to validate the results. Progress in molecular marker technology and the development of quantitative trait analysis software have permitted researchers to construct genetic maps in wheat to identify and estimate the effects of quantitative trait loci (QTL) associated with important agronomic traits including yield and its components. Genetic modification of varieties is a preferred alternative. Genomics assisted approaches offer promise of fast track precision breeding in wheat.

The aim of this research is to detect quantitative traits (QTLs) associated with the phenology and wheat grain yield in a bi-parental population derived from the cross between a synthetic and elite line (BOTNO / AE .SQUARROSA (666) // Kachu). This population was subjected to GBS for an indepth genetic characterization in order to identify genomic regions associated with yield/yield components.

Stripe rust, caused by *Puccinia striiformis*, is yield constraint in wheat. New sources of durable resistance are needed to minimize the yield losses due to it. A major effect rust resistance QTL explaining up to 45% phenotypic variance was found to be contributed from “Kachu” in the RIL population. Further analysis revealed this QTL to be contributed from a segment of *Triticum ventricosum* on chromosome 2NS translocated at short arm of bread wheat chromosome 2AS in “Kachu”. QTL position was confirmed using *T. ventricosum* specific primer VENTRIUP-LN2 which is linked to the gene *Yr17*. This marker can be efficiently utilized in breeding wheat varieties for higher

yield under biotic stress situation. Two QTLs were identified associated with days to heading (**DTH)** on chromosomes 5A and 2A explaining phenotypic variances up to 8.05% and 20.60% respectively. Two QTLs were identified associated with height **(HT)** on chromosomes 2A and 4B explaining phenotypic variance up to 11.69% and 25.28 respectively. For thousand kernel weight **(TKW)** one QTL were identified in chromosome 2A which explained phenotypic variance up to 16.49%. Two QTLs were identified associated with grain per spike **(GS)** on chromosomes 2A and 4B. The first QTL explained phenotypic variance up to 24.0%, the second up to 19.05%.

Identified genomic regions are being introgressed in to the popular but susceptible wheat varieties through marker assisted breeding for enhancing yellow rust resistance, incorporating yield components and thereby improving grain yield ultimately.

CONTENTS

List of Figures

List of Tables

1. INTRODUCTION

1.1 Justification

Wheat is a staple food crop of world. Genetic yield potential increment of wheat at global level is below 1% which is insufficient to meet the demands of growing population across the world (Ray et al. 2013). Wheat yields are stagnating in 37% of global areas (Ray et al. 2013). Mexico is one of the populous countries in world, so yield increment becomes very important. Knowing the fact that yield potential increase in past decades has not been substantial, biotic and abiotic stress mitigation becomes very important. Drought stress, high temperatures during growth stages of plants and multitude of biotic stresses (yellow rust being most devastating) are the principal factors hampering grain yields in wheat belts.

After green revolution paradigm shift in wheat yields was due to the introduction of rust resistance genes in cultivars. Also plant scientists developed several high yielding varieties which helped in reducing food security problem in developing countries. This yield improvement is associated with genetic improvement in yield potential and biotic and abiotic stress adaptation (Rana, 2013).

In due course of development synthetic wheat has played a key role. To enhance the effectiveness of transferring elite genes from the two ancestral species to common wheat, scientists have replicated the pathway of natural origin of hexaploid wheat to create synthetic hexaploid wheat (SHW). Synthetic wheat is produced by the hybridization of tetraploid with diploid (wild relatives), the resultant hexaploid synthetic wheat works as a genetic bridge between wild and cultivated wheat. Synthetic hexaploid wheat has been verified as a valuable source of resistance or tolerance to biotic and abiotic stresses (Rana, 2013). Rust resistance brought to elite cultivated varieties from wild relatives/synthetics. Yellow rust caused by *Puccinia striiformis* is currently most devastating biotic stress hampering wheat yields in Mexico as well as other parts of world.

Control of this disease can be achieved through the timely use of fungicides although this can be expensive to resource poor farmers, and ineffective if not completed in a timely fashion. Genetic resistance is a more effective way to control the disease as, once the resistant variety is sown; least input will be required by the farmer in relation to disease control. Disease resistance genes help in mitigating the yield losses, however, for an increase in the yield potental of plant yield component should be improved strategically.

Efforts focusing genetic improvement for drought and heat environments as well as yellow rust resistance can help significantly in enhancing productivity. A multifaceted approach is urgently required to enhance wheat productivity as well as to minimize the losses caused by diseases to combat the situation. In order to meet the global wheat demand by 2050, the yields need to be increased by 2.5% per annum. This is not possible without thorough genetic amelioration of the plant. Yield components need to be improved and pyramided to the adapted varieties so as to improve overall wheat yields.

The most important pre-requisite in this process would be a thorough and good quality phenotypic characterization of target populations developed through cross of adapted cultivars and exotics (synthetics, wild, landraces, etc). This information, combined with molecular tools, will establish the genotype-phenotype relationship, and thus the identification of genetic regions (genes) that provide substantial genetic gains in terms of improved yield components. In addition, resistance to biotic/abiotic stresses can be incorporated to mitigate the yield losses.

1.2 Objetives

General objective

Identification of genomic regions associated with phenology and grain yield related traits in wheat mapping population derived from *Botno/Ae. Squarrosa//* Kachu cross.

Particular Objectives

- Record phenotypic data associated with grain yield related traits and severity of disease caused by yellow rust.
- Construction of linkage maps using Genotyping by sequencing (GBS) and simple sequence repeat (SSR) markers.
- QTL analysis using phenotypic and genotypic data.

1.3 Hypothesis

Synthetic and Elite lines with better performance and their characteristics are governed by genes that can be incorporated into new lines to enhance grain yield in wheat. These features are associated with DNA sequences, and can be identified by QTL analysis using phenotype and markers.

2. LITERATURE REVIEW

2.1 Origin and Taxonomy of Wheat

Wheat (*Triticum spp.*) is one of the most important crop in the world. Over 200 million hectares (ha) of wheat are cultivated worldwide (Reynolds, 2010). The origins of wheat trace back to the fertile half crescent located in Western Asia. Some of the earliest remains of the crop have been found in Syria, Jordan, and Turkey and date back almost 9,000 years. (Belderok, 2000). Subsequently it distributed throughout the Mediterranean basin, until reaching Italy and Spain around the year 7000 a.a. (Mac Key, 2005).

Hexaploid wheat (Triticum aestivum L.) (2n=6x=42) has a large genome size of about 17,300 Mb which is approximately 35 times and 110 times larger than that of rice (Oryza sativa L.) and Arabidopsis, respectively (Hussain and Rivandi, 2007). Common wheat originated from two independent polyploid crossing events. Hexaploid wheat is an allopolyploid (AABBDD) formed first through hybridization of *Triticum urartu* (2n=2x=14, AA) with an unknown source of the B genome, despite speculation tending toward Aegilops speltoides (2n=2x=14, BB), and subsequently hybridization with Aegilops tauschii (2n=2x=14, DD) (Daud and Gustafson, 1996; Devos and Gale 1997). Current commercial wheats belong to the species Triticum aestivum (hexaploid, 2n = 42, AABBCC genome) considered as bread wheat wich it is used in the production flour for bread, cookies and pastries and Triticum durum (tetraploid, 2n = 28, AABB genome) known as a durum wheat which main commercial product is pasta and its derived products (Carrillo, 2006).

2.2 The importance of wheat

Wheat is a globally important crop, it has been consume by man for centuries and is also used as animal feed. Wheat is is a cereal grass family and is ranked third when it comes to the total production volume. The global amount of wheat produced came to about 724 million tons in crop year 2014/2015. The main producing countries are the European Union: 155 million tons, China: 130 millions tons and India: 89 million tons. (FAO, 2015). With a projected world population of 9 billion by 2050, it is expected that demand for wheat will increase by 60% so global wheat grain production must increase 2% annually to meet the requirement of consistently increasing world population (Rosegrant and Agcaoili, 2010). One fifth of the total calories of the world's population comes from wheat and also it provides more nourishment (calories & proteins) for humans than any other single food crop (FAO, 2013), making wheat an important component of food security

Código de campo cambiado

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worldwide. Accounting for a fifth of humanity's food, wheat is the second only to rice which provides 21% of the food calories and 20 %of the protein for more than 4.5 million people in 94 developing countries (Braun, 2010).

Table 1. Wheat production: leading producers. FAO, 2015.

	2014	**2015 estim.**	**2016 F´´cast**	**Change: 2016 over 2015**
	millions tonnes			%
European Union	157.1	160.5	154.0	-4.0
China	126.2	130.2	129.0	-0.9
India	95.9	86.5	89.0	2.9
Russian Federation	59.7	61.8	62.5	1.2
United States	55.1	55.8	54.4	-2.6
Canada	29.4	27.6	28.9	4.7
Pakistan	26.0	25.5	25.5	-0.1
Ukraine	24.1	26.5	22.0	-17.1
Australia	23.1	24.2	24.5	1.2
Turkey	19.0	22.6	22.0	-2.7
Kazakhstan	13.0	13.7	13.5	-1.8
Argentina	13.9	11.3	14	23.9
Iran Islamic Rep. of	10.6	11.5	12.5	8.7
Egypt	9.3	9.0	9.0	0.0
Other countries	67.5	67.3	63.3	-5.9
World	729.8	734.1	724.0	-1.4

*Countries listed according to their position in global production (average 20014-2016)

The total Mexican wheat production for 2015/2016 is forecast at 3.7 million metric tons, place in 31° of the world's producers. Sonora continues to be the main wheat producing state with approximately 46 percent of total wheat production, followed by Baja California, which contributes 16 percent, and Guanajuato with 15 percent. (Grain and Feed Annual Mexico-MX5011, 2015)

2.3 Importance of grain yield in wheat production

Grain yield is a key economic driver of successful wheat production (Xianshan Wu, 2012). Future progress in wheat production will depend on improvement in grain yield. Future yield gain has also been challenged by global climate change, diminishing natural resources, rising prices for fertilisers and pesticides, and competition for arable land (Reynolds, 2012). Grain yield improvement is the ultimate goal for most wheat breeding programs across the world. Although grain yield is a complex trait with low heritability and highly influenced by genotype and environment interaction, high

yielding commercial varieties of many crops including wheat have been developed through direct selection for grain yield even if the relationship of yield with its component traits has already been established. Quantitative traits provide the greatest challenge for making genetic improvement because plant breeders have little information on the number, location, and contribution of each gene to the final expression of the trait (Koebner and Snape 1999; Mohan, 1997).

2.3.1 Yield components

To understand the causes of variation in final grain yield, its components must be studied along with the growth of the crop. To achieve genetic gain for wheat yield improvement, a first step is to understand its determination at genetic and physiological levels. Yield reflects the interaction of the environment with all growth and development processes that occur throughout the life cycle. Given its complexity, yield component approaches have long been used to dissect this trait. That is, yield can be divided into a number of relatively simpler components either numerically or physiologically (Slafer, 2007).

Several studies have been reported on the genetic control of yield and its component traits. Crop yield is directly and multiply determined by yield-component traits such as number of spikes per plant (NSP), number of grains per spike (NGS), one-thousand grain weight (TGW), and indirectly affected by other yield-related traits, e.g. plant architecture (Shi J., 2009). Some others authors said that grain yield can be dissected into different number of component traits such as number of spikes per plant (NSP), number of grains per spike (NGS), one-thousand grain weight (TGW), total number of spikelets per spike (TNSS), number of sterile spikelets per spike (NSSS), spike length (SL), density of spikelets per spike (DSS) and plant height (PH), (Xianshan Wu, 2012).

Understating the genetic basis of yield and yield component traits is critical for crop improvement. Some yield associated traits are less environmentally sensitive and have higher heritabilities than grain yield (Cuthbert JL, 2008). These component traits are also under QTL control and the effects of individual QTLs on phenotypic variation are relatively small (Yano and Sasaki 1997). Genetic improvement of wheat plants has played a central role in boosting yield since the Green Revolution by changes in plant architecture (e.g. height) and increases in resistance to biotic and abiotic stresses. It is believed that this trend will continue, and that more efforts are needed to develop novel varieties with even higher yield and quality.

2.3.2 Thousand-kernel weight

Thousand-kernel weight is one of the three main yield components of wheat. It has a high and consistent heritability value. Thousand-kernel weight is also phenotypically the most stable yield component (Sun X.Y, 2009), and the effects of most genes affecting thousand kernel weight additive. Hence, early generation selection for thousand-kernel weight is most likely effective (Wang, 2012).

2.3.3 Spike characters

Grain yield is under big influence of spike properties, and interdependence and correlation between spike length and spikelet number per spike (MARTINČIĆ, 1996). Spikelet number affects the total number of kernels per unit area. The more spikelets per spike, the more kernels per spike, which may influence the final kernel number. Also long spikes with high spikelet number per spike may offer an avenue for increasing kernel number and harvest index in wheat (Gaju, 2009).

The number of grains is itself a function of the number of fertile ears per area and the number of grains per spike. To count the number of grains per spike, it has been reported that samples are taken randomly from 10 spikes per repetition The e number of eventual grains per spike (ear) varies from season to season and is affected by a number of factors including: disease control during flowering, weather conditions during flowering and temperature and day length before terminal spikele, (Castro, N.,2011).

2.3.4 Harvest index (HI)

Harvest index indicates the efficiency of a crop in converting photosynthetic products or assimilates produced before and afteranthesis into final grain yield. Most often it is expressed as the ratio of grain yield to above-ground dry matter. Although harvest index was not used as a selection criterion in wheat yield improvement in the past (e.g., during the Green Revolution), the achieved yield progress was actually due to an increase in the number of kernels and a genetic shift towards greater harvest index (Blum, 2005; Zhang, 2012). The harvest index improvement in wheat has been mostly due to introduction of dwarfing gene alleles, Rht-D1b and Rht-B1b, into the background of modern cultivars. These genes reduced overall plant height and improved availability of assimilates which increased survival of growing florets to increase potential kernel number (Rebetzke, 2012). The harvest index of spring wheat is lower than that of winter wheat, and it rarely exceeds 45% for the former (Zhang , 2012).

2.3.5 Phenological traits: Days to heading (DTH) and Days to Maturity

Heading time is an important trait for adaptation of wheat to its target environments including moisture stress areas (Lin, 2008). It is one of the traits effectively used in classical plant breeding programs as a mechanism of escaping terminal moisture stress and freezing injury in early spring. Furthermore, heading date is a highly heritable trait in wheat and hence selection is usually effective. Heading date is regulated by three well-characterized groups of loci namely, 1) vernalization requirement (VRN), 2) photoperiod response (Ppd) and 3) earliness per se (Eps). Physiological maturity is when the grain reaches the maximum dry weight and the grain becomes viable. It is most easily determined in field when 50% of the peduncles are ripe (i.e., yellow), and at this point the glumes (which are frequently the last part of the plant to senesce) will also be losing their color (M. Reynolds, 2012).

2.3.6 Morphological and drought related traits: Plant height

Optimum plant height is required for better yield in wheat, as tall plants are susceptible to lodging and excessively short plants are often associated with a yield penalty in resource limited areas (e.g., moisture stress environment) (Griffiths, 2012). Dwarfing genes *Rht-D1b* and *Rht-B1b*, which are insensitive to gibberellic acid, have increased grain yield in most resource rich environments through reducing lodging susceptibility and increased grain number (Rebetzke, 2012). Since the *Rht-D1b* and *Rht-B1b* alleles are also associated with reduced coleoptile length and poor seedling vigor, there is an interest in introducing alternative gibberellic acid responsive dwarfing alleles with a potential for reducing plant height without affecting coleoptile length. The *Rht8* gene on chromosome 2DS is a potential candidate in the development of semi-dwarf wheat varieties with long-coleoptiles (Rebetzke, 2012; Griffiths, 2012).

2.4 Wheat Diseases

Cool and wet weather is favorable for the development of fungal and bacterial diseases. Wheat is susceptible to more diseases than most grains (Gramene, 2006). Wheat diseases caused by fungal pathogens are more threatening for crop yields and grain quality than those caused by bacteria and viruses (Goyal AK, 2015). The fungal pathogens are very adaptable and can rapidly evolve into new strains that can infect earlier disease resistant plants. Infection of wheat fungal

diseases are influenced by various factors, nature of pathogen, susceptibility of host, diversity of virulence, density of inoculums and temperature (Rajaram and Van Ginkel, 1996). The most important fungal diseases in wheat include different types of rust as powdery mildew and Karnal bunt. Some of the small grain diseases that thrive in warm, humid environments are fusarium head blight (*Fusarium graminearum*), stripe rust (*Puccinia striiformis*), leaf rust (*Puccinia triticina*), stem rust (*Puccinia graminis*), Septoria glume blotch (*Stagnospora nodorum*), Septoria leaf blotch (*Septoria tritici*), tan spot (*Pryenophora tritici-repentis*), barley yellow dwarf virus (*Luteoviridae luteovirus*) and bacterial steak (*Xanthomonas campestris*) (Schafer, 1987).

2.4.1 Wheat rust Pathogens

Wheat rust pathogens belong to genus *Puccinia*, family *Pucciniaceae*, order *Uredinalesand* class *Basidiomycetes*. The rust diseases of wheat such as leaf rust, stem rust, and stripe rust have historically been among the major bioticcon straints in the world (Saari and Prescott, 1985). The rusts of wheat is caused by fungal pathogens that can be disseminated thousands of kilometers by windandare capable of causing considerable economic loss throughout the world (Kolmer, 2005; Goyal and Prasad, 2010).

2.4.2 Taxonomy and life cycle of the rust fungi

The rust fungi called *Puccinia* species have complex life cycles with many different spore-producing stages. These rust fungi are obligate parasites which need live plant tissues for survival. The fungus has infectious structures with limited secretory activity. They also have carbohydrate and protein rich layers which secrete the fungal and host plasma membranes (Bolton, 2008).

The three economically important rust fungi of wheat include leaf rust (brown rust); yellow rust (stripe rust) and stem rust (black rust). Optimal temperature and light requirements for germination and infection differ for stem, leaf and yellow rust resulting in each rust fungus having distinct geographical distributions (Table 2).

Table 2. Optimal environmental conditions required for development of wheat rust (Roelfs,1992).

Stage	Temperature °C			Light intensity		
	Stem	Leaf	Yellow	Stem	Leaf	Yellow
Germination	15-24	20	9-13	Low	Low	Low
Germ tube formation	20	15-20	10-15	Low	Low	Low
Appresorium development	16-27	15-20	Not formed	None	None	None
Stomatal penetration	29	20	9-13	High	No effect	Low
Hyphal growth	30	25	12-15	High	High	High
Sporulation	30	25	12-15	High	High	High

The rusts are heteroecious, requiring two phylogenetically distinct or unrelated host plants to complete their life cycle (Schumann and Leornard, 2000). The heteroecious life cycle involving two different unrelated hosts, a microcyclic asexual stage on a gramineous host and a sexual stage which occurs on an alternative host. The sexual stage of the life cycle initiates late in the cereal growing season with mature telia producing teliospores. These teliospores remain dormant over winter, but following the onset of warm weather in the spring they develop into basidia which produce basidiospores. These basidiospores infect the alternative host plant forming after 3 – 4 days spermangonium that rupture the surface of the leaf revealing receptive hyphae and spermatia. Following fertilisation, aesciospores are produced that are wind dispersed to infect the primary cereal host. The life cycle of *P. striiformis* was believed to be autoecious, having only a gramineous host upon which its reproduction was exclusively asexual (Figure 1). However, teliospores are produced by *P. striiformis* on cereals, indicating a possible sexual cycle (Roelfs, 1992).

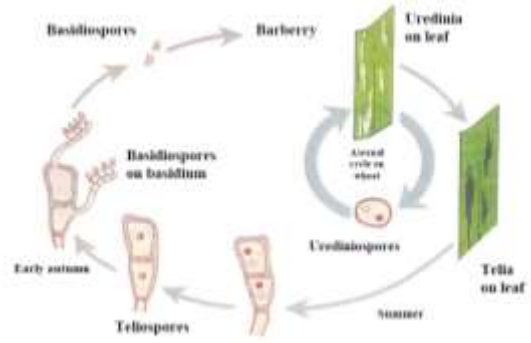

Figure 1. The autoecious life cycle of *Puccinia striiformis* (figure modified from Roelfs, 1992).

2.4.3 Stripe Rust (*Puccinia striiformis*)

The causal agent for Stripe rust is *Puccinia striiformis. sp. Tritici.* The symptoms and signs of the disease include chlorotic or necrotic flecks, and formation of uredia, a pustule- like structure enclosing abundant amounts of yellow to orange urediniospores (Chen, 2005). The uredinium form stripes along the leaves of adult plants as the pathogen development follows the elongation of leaf vascular system (Roelfs, 1992). Pustules are light yellow and occur on leaves in distinct straight sided stripes about 1/16 inches wide and of regular length. Some of the effects that this disease cause is to reduced dry matter production, root growth, plant height, size and number of flowering spikes, and the size and number of grains are the parameters affected by infection. These effects were more pronounced with infection beginning at the seedling stage, although infections initiated at anthesis were also associated with reduced root weight and grain yield (Wellings, 2011). The distribution of stripe rust is usually limited to the locations of northern or southern latitudes at high elevations with temperate climates, and higher precipitation levels (Roelfs, 1992). Cool and wet weather is favorable for the development of yellow rust.

2.4.4 Control of Stripe Rust

A major objective of plant breeding, agronomists and the agricultural chemical industry is the control of disease epidemics (Martin, 2003). In many parts of the developed world plant diseases have been controlled through the use of agrochemicals as well as disease resistant varieties. In contrast to agrochemicals, breeding for disease resistance is an economical and environmentally safe measure to reduce crop losses. The most effective and environmentally sustainable method of controlling wheat rust is through the transfer of resistance genes into modern cultivars (Fetch, 2011). A goal in breeding programs should be to screen germplasm for durable resistance genes, and then attempt to combine them in a cultivar for long-term durable resistance (McCallum, 2007). Gene pyramiding, or combining several resistance genes into one genotype, is one strategy for developing durable resistance that the pathogen may not be able to overcome. For this reason, a constant search for new genes for resistance is required, and wild relatives of wheat may be a rich resource for identifying novel resistance genes for stripe rust (Knott, 1987, Jiang, 1994). Gene pyramiding incorporates many desirable genes into elite

genotypes (Ayliffe, 2008). The pyramids used have involved major genes, minor genes, effective genes, race or race non specific genes which confer resistance (Pedersen and Leath, 1988).

2.4.5 Race specific resistance and Race non-specific resistance

Van der Plank established in 1963 the theoretical bases and concepts of two types of plant resistance, which call vertical and horizontal, where the first is a single genotype effective pathogen while horizontal by definition is effective against all genotypes of the pathogen.

The specific resistance also called vertical resistance or seedling confers complete protection against the pathogen, is based on a single major gene or a combination of major genes. The major genes are vulnerable to pathogenic variation, and longevity is short (Singh, 2001).
Seedling resistance can be expressed in all stages of plant development, conferring a completely resistant phenotype which is easy to select for in breeding programs. This type of resistance is however normally race-specific, the resistance gene conforming to the gene-for-gene hypothesis (Flor, 1955, 1971). The gene-for-gene hypothesis refers to the specific plant-pathogen interactions between the host resistance gene (R-gene) and a corresponding avirulence (avr) gene in the pathogen, resulting in an incompatible interaction (McDowell and Woffenden, 2003, Dangl and Jones, 2001). Usually a hypersensitive defence response is triggered by this incompatible interaction. Genotypes possessing only race specific resistance in most cases lose their resistance over time due to the occurrence of more virulent stripe rust races. These races evolve due to high selection pressure on the pathogen, which is caused by growing resistant wheat cultivars on large numbers of acres (Line and Qayoum, 1992).

Horizontal resistance also called durable, polygenic, minor gene resistance or adult plant resistance (APR) (Watson, 1970) is characterized by a slow development of the disease; it is based on interactions of genes with small and medium effects. The term APR describes resistance that is expressed at post seedling growth stages (Bariana, 2001). Cultivars that contain only APR show a susceptible phenotype at seedling growth stages. One small partial resistance gene cause moderate reduction in the progress of the disease, but the combination of genes additive effect causes high resistance. Genetic analysis of cultivars with APR in general finds the resistance to be conferred by the additive effects of several minor genes (Singh, 2005;

Navabi, 2004). The expression of the resistance is reduced when more resistance genes are present and partial together, that is, the stability of resistance increases with the presence of more genes (Singh, 2003). This resistance level is considered durable type and specified. Individually APR genes express a partially resistant phenotype, while stacking multiple APR genes can achieve a complete resistant phenotype (Singh, 2005).

2.4.6 Resistance genes used for stripe rust

According to Milus (2009) resistant varieties are the most cost-effective means of managing stripe rust, but new races of the pathogens can overcome some types of resistance. Little is known about the genes for stripe rust. Some of the genes that have conferred general but not race specific resistance are Yr17, Yr18, and Yr29 (Suenaga, 2003). There are also a number of major, specific genes that provide race specific resistance. Durable resistance can be obtained through a combination or pyramiding of major resistance genes and non-specific genes.

Currently there are 65 designated stripe rust resistance genes (Yr1-Yr65) and approximately 40 temporarily designated genes (Cheng, 2014). Some of the permanently designated genes that confer adult plant resistance are Yr11, Yr12, Yr13, Yr14, Yr16, Yr18, Yr29, Yr30, Yr34, Yr36, Yr39, Yr46, Yr48 and Yr52 (Xu, 2013). These genes are not race specific so they can provide partial protection against several races of stripe rust and are more durable. However most resistance genes are considered all stage or seedling stage resistance. A small number of slow rusting genes are also available for breeders to incorporate into their material and complement with race specific genes. Slow rust genes provide horizontal or partial resistance to a number of races and work by increasing latent period and by decreasing uredinial size, infection frequency and spore production. Two of the commonly used slow rust genes for stripe rust are Yr18 and Yr29 (Rosewarne, 2006). The gene Yr9 is considered one of the most widely used resistance sources for stripe rust in wheat breeding history (Hao, 2011). Yr9 is commonly found in soft red winter wheat varieties.

The Yr17 gene is derived from *Aegilops ventricosa* and is found on chromosome 2A, specifically in the short arm. It is part of the gene cluster Lr37/Yr17/Sr38 and is commonly found in varieties in Europe, Australia and the US. This cluster has been widely used in breeding programs in the

US and has provided durable resistance to stripe rust for years (Hao, 2011). Because of the evolution of new virulent races of stripe rust, deployed resistance genes are often overcome by the pathogen; therefore, new genes for resistance are needed to develop new improved varieties with more durable resistance.

Figure 2. The Yellow pustules indicative of stripe rust on a wheat leaf. (www.hcga.co.uk)

2.5 Plant Breeding

The fundamental basis of plant breeding is the selection of specific plants with desirable traits. Selection typically involves evaluating a breeding population for one or more traits in field or glasshouse trials, among these we can find agronomic traits, disease resistance or stress tolerance, or with chemical tests as grain quality. The goal of plant breeding is to assemble more desirable combinations of genes in new varieties (Bertrand C.Y, 2008).

Despite optimism about continued yield improvement from conventional breeding, new technologies such as biotechnology will be needed to maximize the probability of success (Huang, 2002). One area of biotechnology, DNA marker technology, derived from research in molecular genetics and genomics, offers great promise for plant breeding. Owing to genetic linkage, DNA markers can be used to detect the presence of allelic variation in the genes underlying these traits. By using DNA markers to assist in plant breeding, efficiency and precision could be greatly increased.

Código de campo cambiado

2.6 Marker assisted selection (MAS)

The development of comprehensive genetic maps was not possible until the introduction of DNA based molecular marker technologies. DNA markers are relatively simple tools that are completely independent of plant environmental conditions and can be detected at any stage of development. The use of DNA markers in plant breeding is called marker-assisted selection (MAS) and is a component of the new discipline of 'molecular breeding' (Bertrand C.Y, 2008).

The advantages of MAS can be exploited by breeders to accelerate the breeding process (Ribaut & Hoisington 1998; Morris, 2003). Target genotypes can be more effectively selected, which may enable certain traits to be 'fast-tracked', resulting in quicker line development and variety release. Other is that the total number of lines that need to be tested can be reduced since many lines can be discarded after MAS early in a breeding scheme, this permits more efficient use of glasshouse and/or field space which is often limited (Bertrand C.Y, 2008). Molecular markers have been used to tag important resistance genes thus improving selection of genotypes which contain combinations of non-race specific genes providing durable resistance (Todorovska, 2009).

2.7 Types of molecular markers

Genetic marker is any heritable character showing variation or polymorphism in individuals studied. There are different types of genetic markers, morphological, protein and which are based on DNA polymorphism (Phillips, 1995). DNA markers used in this selection can be either dominant or co-dominant. Dominant markers detect only one allele per locus, while co-dominant markers generally have the potential to reveal all alleles of a locus (Phillips, 1995).

There are five main considerations for the use of DNA markers in MAS: reliability, markers should be tightly linked to target loci, preferably less than 5 cM genetic distance; DNA quantity and quality of DNA required, some marker techniques require large amounts and high quality of DNA, which may sometimes be difficult to obtain in practice, and this adds to the cost of the procedures; technical procedure for marker assay, high-throughput simple and quick methods are highly desirable; level of polymorphism the marker should be highly polymorphic in breeding material; and cost (Mackill & Ni 2000; Mohler & Singrun 2004, Bertrand C.Y, 2008)

2.7.1 Simple sequence repeats (SSR) markers

Simple sequence repeats (SSRs), also known as microsatellites, are molecular markers that have become available to researchers for general use (Quarrie, 2005). SSR markers are small regions based on 2 to 10 bp, which are tandemly repeated. A different "allele" occurs at a micro satellite locus as a result of changes in the number of times the core element is repeated, altering the length of the repeated region. These PCR based SSR markers are highly polymorphic, co-dominant and chromosome or locus specific enabling differentiation of homozygotes and heterozygotes in the early generations (Babiker, 2009; Narasimhamoorthy, 2006), SSRs continue to be the main marker type for QTL studies in wheat, either alone or in combination with other types of markers.

2.7.2 Single nucleotide polymorphism

Single nucleotide polymorphism (SNP) consists of a single base difference within a given segment of DNA between two individuals. SNP's contribute about 90% of the genetic variation in living organisms and provide high-density markers. These markers are high throughput, abundant and uniformly distributed throughout the genome (Gupta, 2008). SNP output is also of binary type enabling easy data interpretation. Moreover, there is no need for electrophoresis and the SNP locus is a qualitative character; the allele is defined by the identity of a particular base in the sequence (Koebner and Summers, 2003).

2.8 Genetic mapping or linkage mapping

A genetic or linkage map is like a road map of an organism's chromosome using molecular markers. These maps indicate the position and genetic distance between the markers in the different chromosomes (Patterson and Tanksley, 1991). There are two main variants of maps: genetic, defined by recombination frequency units, and physical, where the distances between loci are expressed in units of distance in nucleotides (Collard, 2005). The linkage maps are based on the principle that (markers or loci) genes segregate by recombination of chromosomes during meiosis. The linkage maps allow a full analysis of the genotypes, breaking down complex characteristics in Mendelian components, locate genomic regions that control traits of agronomic importance, in addition to quantify the effect of these regions (Ferreira y Grattapaplia, 1998).

2.8.1 Construction of linkage maps

Since we know the location of the markers, we can determine the location of the gene of interest by using the tightly linked marker. Markers that are different in the parents (polymorphic) are used to construct the linkage map. The genotypic information obtained from scoring these markers is compared to the phenotypic information obtained in field trials. Once these calculations are completed a test of likelihood is used to determine the linkage between a location on the linkage map and the QTL. For the construction of a linkage map is important to take into account some characteristics:

- Recombination frequency

The construction of a genetic map involves estimating the genetic distance or distance between two loci, this distance is defined as the value of recombination between two genes, that is, the rate at which new associations between two gene pairs are formed with respect the total number of associations. The distance between markers is a relative value for each crossing, it depends on the markers that are considered, the minimum value is zero and the maximum 0.5, and the last value represents the genetic independence of loci (Kearsey and Pooni, 1996; Nuez, 2000; De Vienne, 2003).

- Linkage

The linkage is determined by calculating an odds ratio (odds of linkage versus no linkage). This ratio is expressed as a logarithm of ratio and is known as the logarithm of odds (LOD) value. The threshold LOD value to consider a QTL as linked to a location on a map is typically around 3. A LOD value of 3 states that linkage is 1,000 times more likely to occur tan no linkage (Collard, 2005).

- Genetic distances and mapping functions

For the construction of a linkage map it is necessary to develop a population and polymorphic markers for this population. Thus, from the recombination frequency between markers can infer their relative position and distance in centiMorgan (cM) that is the unit of genetic distance between two loci and amounts to a recombination frequency of 1% of individuals recombinants (Semagn, 2006). It must be noted that there are different factors such as maternal, nutritional or genotypic that can distort recombination frequencies and therefore the distances obtained.

Three mapping functions are commonly used:

a) Kosambi: when considering a degree of interference.
b) Haldane: when not considered interference.
c) Morgan: complete interference and small distances is considered (Semagn, 2006).

2.9 Quantitative trait loci (QTL) analysis

QTL maps are a common method used to discover the location of genes for complex traits. "A QTL is a region of any genome that is responsible for variation in the quantitative trait of interest" (Doerge, 2002). The QTL are regions within the genome with genes associated with a quantitative trait in a segregating population (Collard, 2005). The position of a QTL can be determined with respect to the highest LOD value and the location of the flanking markers. It is very important in the identification of genes with major and minor effects. Its value also lies in establishing the number and the chromosomal location of genes involved in the inheritance of important traits (Haile, 2012).

Analysis QTLs is based on the detection of an association between the phenotype and genotype of a population, it should be noted that for the realization of a map QTL a segregating population is needed as F2 or backcross besides biometrics showing whether marker associated with the phenotypic trait and detect parameters detected QTL (Ferreira and Grattaplagia, 1998). For efficiency in QTL mapping, the phenotyping should be accurate; repeated over locations and in years; involve appropriate genetic analyses; presence of a wide genetic background and use of advanced generations (Young, 1999).

3. MATERIALS AND METHODS

3.1 Phenotypic data

3.1.1 Plant material

Two hundred sixty F6 recombinant inbred lines (RILs), *Triticum aestivum*, were used in this study. Plant derived F6 was by single seed descent (SSD), the germplasm was developed from the cross of synthetic line (*Botno/ Ae. Squarrosa 666*) with elite line Kachu.

Synthetic hexaploid wheat constitutes an effective genetic bridge for transferring agronomically important genes from *Aegilops tauschii* to common wheat. Scientists have replicated the pathway of natural origin of hexaploid wheat to create synthetic hexaploid wheat, which is produced by the hybridization of *Triticum turgidum L.* with *Aegilops tauschii* (Talbot, 2011). Kachu is an elite germplasm and it has desirable agronomic characteristics. To introgress desired trait, breeders use the backcross. Backcrossing is a well-known and long established breeding scheme where a characteristic is introgressed from a donor parent into the genomic background of a recurrent parent. The characteristic could be a trait, a gene or even an anonymous locus or chromosome segment (F. Hospital, 2005). These two wheat lines were developed by CIMMYT and are good genetic combiners.

Kachu was crossed to Botno/ Ae. Squarrosa (666) to produce F_1 seeds and detailed is showing in Figure 3. Plants from these seeds were self-pollinated and from each plant was harvested just a F_2 seed in Batán, 2013. F_3 was sowing in CENEB, Obregón on 2013-2014 season. F_4 individual plant was harvested as bulk in Batán 2014. From this F4 generation leaf tissue from each plant were sampled for DNA extraction and the 347 lines were sent for sequencing using genotyping by sequencing (GBS) technology to SAGA ("Servicio de Análisis Genético para la Agricultura") in Spanish. F_5 generation was planted and harvested as bulk in Obregon and Batán.

Parents and F_6 Recombinant Inbreed Lines (RILs) were planted in three different environments and phenotypic evaluation was record for each trial (Obregón, Sonora 2014; Batán, México 2015 and Toluca, México 2015.)

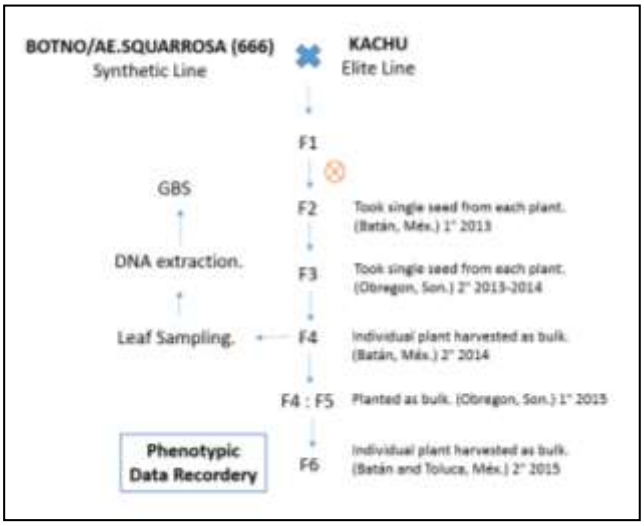

Figure 3. F_6 Recombinant inbred lines (RILs) scheme of *Botno/ Ae. Squarrosa 666* population.

3.1.2 Experimental Design

Strategies for efficient plans for data collection, which lead to proper estimates of parameters relevant to the researcher's objective, is known as experimental design. In agriculture, the main objective of the experimental design is to estimate the average of the differences between varieties or treatments even if the response varies from one environment to another or between years. In this research, the experimental design was "Control of local variability – by blocking" following an alpha lattice design. Blocking is the arrangement of experimental units into groups (blocks) that are similar to one another (Reynolds, 2012).

347 blocks corresponding to the lines that form this population were formed, each block covered an area of 50 x 50 cm, and the distance between plot and plot was 50 cm. The 347 entries (lines) were distributed in 17 rows consecutively (Figure 4). The level of fertility may introduce a level of variability within the experiment; therefore, blocking is required to remove the effect of low and high fertility. Blocking is used to reduce or eliminate the contribution of nuisance factors to experimental error (Reynolds, 2012). To improve the significance of an experimental result, the repetition of the experiment was performed. Replication reduces variability in experimental results, increasing their significance and the confidence level, also permit control of error variance (Reynolds, 2012).

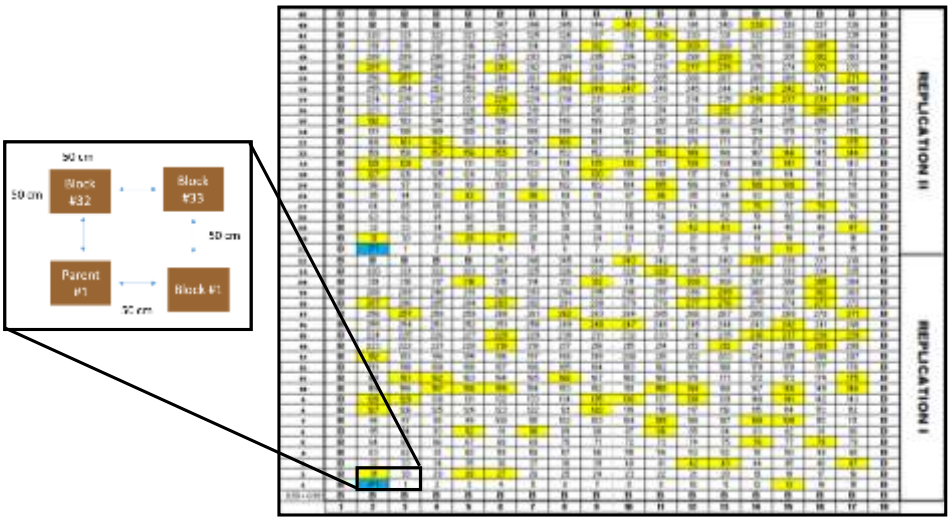

Figure 4. Map field experimental design used in Batán and Toluca, Estado de México.

3.1.3 Crop planting

For sowing plant materials, 347 seed envelopes with 5 grams of each line were prepared. Sowing was done by hand in wet conditions on a soil clay type; it was performed following the order described in the distribution map, spreading and covering the seeds on the surface corresponding to each block. Sowing dates were recorded: June 26, 2016 in Batán and July 9, 2016 in Toluca.

3.1.4 Environments evaluated

Lines were tested in different experimental sites. Phenotypic data for yellow rust was evaluated in two different environments. The first environment is Batán-Mexico situated at an elevation of 2250 m above sea level with monthly average temperature of 16.6 °C, dew point 11.8 °C and humidity of 75%. The second is Toluca- Mexico with slightly different characteristics. It is located at an elevation of 2660 m above sea level, monthly average temperature of 14.8 °C , dew point around 9.0 °C and a humidity of 74%. These data were obtained through the Personal Weather Station Info (PWS) for Batán, CIMMYT (IESTADOD2) and Toluca, Mexico; using Weather Underground web site, wich provides local and long range Weather Forecast. For the construction of the graph was used the monthly average daily data for the trial period from planting to harvest.

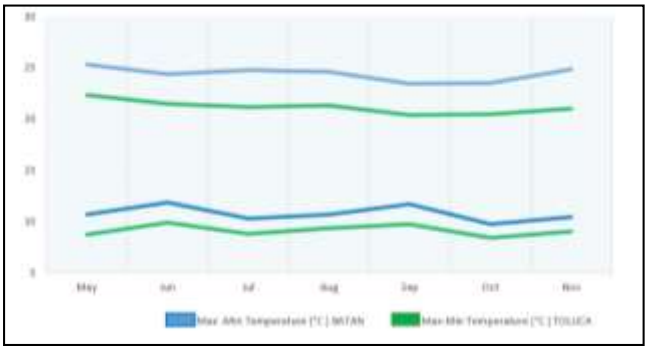

Figure 5. Weather history graph for two different environments. (Batán and Toluca)
May 1st to November 1 st, 2015.

Phenotypic data of traits associated with grain yield in wheat were taken from replication trial in Batán; data for thousand-kernel weight (TKW) was also taken in CENEB, Obregon 2015. Data collection for height and Days to Heading were taken in Batán, 2015 replication trial and in Obregon was performed only one measurement.

3.1.5 Field evaluation for different quantitative traits

- *Vegetative damage caused by Stripe rust.*

Vegetative damage may affect physiological processes there by reducing growth, biomass and ultimately yield, with effects to the spike typically causing the largest reduction in yield (Reynolds, 2012-b). For this evaluation, the first step was to identify those plots (blocks) that showed resistance and susceptibility to yellow rust observing the presence or absence of the fungus. The average percentage of leaf area of adult plants covered by stripe rust was visually estimated according to the scale described by Cobb, 1948. Two ratings were done (Day 1 and Day 2), the latest usually shortly before leaf senescence when the upper leaves were still green and scorable. The proportion of damage was also estimated in relation to the total area of each plot using a scale of 0% to 100%

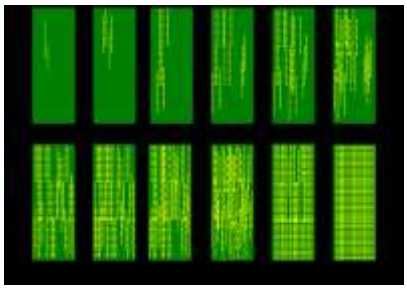

Figure 6. Scale of rust severity (percent of leaf area infected). Estimates of severity were measured according to Modified Cobb Scale (Paterson, 1948), which is used to determine the percentage of possible tissue rusted and was evaluated from 1% to 100%.

- *Plant height (PH)*

Plant height is controlled by Rht genes (height reducing genes), and highly heritable. It was measured as the height of the tallest culm from the soil surface avoiding any mounds or cracks in the soil, to the tip of the spike, awns excluded (Reynolds, 2012-b). This measurement was performed manually for the 347 lines in both replicates using a large wooden ruler.

- *Heading (spike emergence)*

This measurement was taken when 50% of the spike is emerged; however, often it is recorded as when the base of 50% of the spikes have emerged from the flag leaf sheath. Measurement is visual assessment of each plot basis (Reynolds, 2012-b).

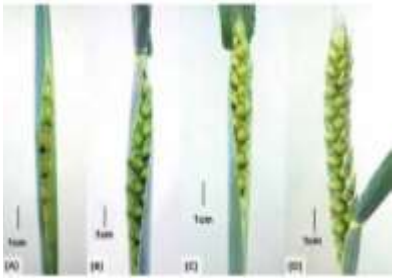

Figure 7. The sequence of spike emergence: the end of booting (A-B), the start of spike heading (C), spike have emerged (D). (Credit: wheatbp.net).

- *Number of grains per spike*

To collect data for components associated with grain yield in wheat, 15 spikes randomly were harvest per each plot (block). The goal of good harvesting is to maximize grain yield, and to minimize grain losses (Reynolds, 2012-b); for that reason, the collection of spikes made carefully and each spike kept in a different glassine bag to control the loos of grain. After that, the grain count were

performed manually by going threshing each spike. For data analysis was taken the average number of grains from the 15 spikes.

- *Thousand Kernel Weight (TGW)*

The 1,000-kernel weight is the weight in grams of 1,000 seeds of a grain sample. Test weight of wheat is considered the most common and easiest way to quantify wheat. Reduction in TKW may be caused by weather or biological stress during grain-filling or in-field effects due to the plasticity of yield components. The basic factors that affect the test weight of wheat are kernel size and shape, kernel density, maturity of wheat and actual wheat variety (Reynolds, 2012-b). To perform the measurement a random sample was taken of whole grains, it was clean carefully to remove all broken and aborted grains and chaff, but was not discard small grains (Reynolds, 2012-b). 200 grains were counted by hand for each line. To obtain the final weight of 1000 grains, the 200 grains weight multiplied 5 times.

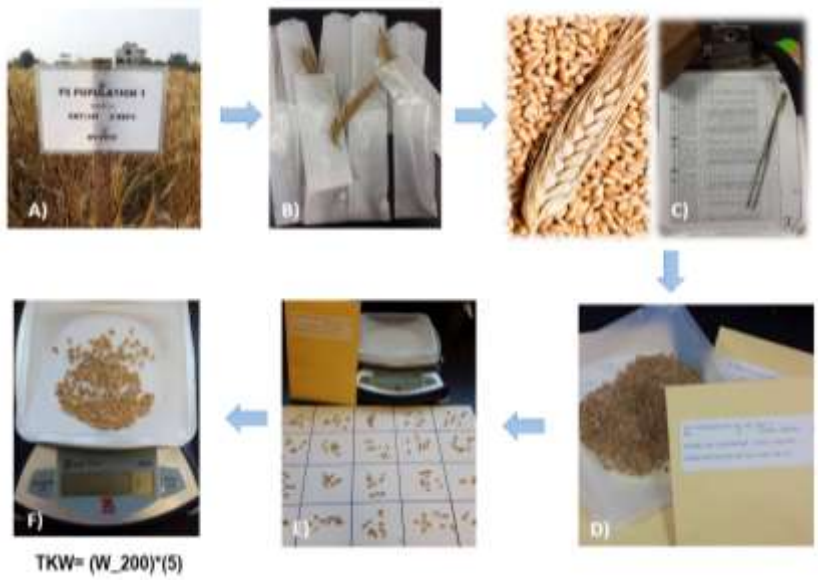

Figure 8. Sequence of activities for data collection of the two yield components: Number of grains per spike and thousand kernel weight. (A) Harvested plots, (B) harvested randomly spikes, (C) Hand-threshing sample, (D) Grain collected - 15 spikes, (E) 200 grains count and (F) 200 grains weight.

3.2 Genotypic data

3.2.1 DNA extraction

CTAB method was used (CIMMYT, 2005). Tissue samples were collected and placed at 80°C for 12 hours. Then, samples are placed in to a lyophilizer for 3 days. After that, 60 mg of tissue are added into a 2 ml Eppendorf tube. Samples are grinded using the tissuelyzer for 3 min, then CTAB solution (1 mL) is added and the tubes, which are then closed and shaken for 1.5 h at 65°C. Then 1 mL of a mix chloroform: octanol (24:1) is added and shaken for 15 min at room temperature. The tubes are centrifuged at 3750 rpm for 30 min. The supernatant is transferred into new tube, RNAase 1 mg/ml is added, the tubes are put in oven at 37°C for 30 min, and then cool isopropanol is added, the tubes are softly shaken and placed into -20 °C for 1 h. Then, the tubes are centrifuged at 3750 rpm for 30-40 min, the supernatant is discarded and ethanol (70%) is added, the tubes are centrifuged for 15 min. The supernatant is discarded and the pellet is dried at room temperature. The DNA is suspended in 200 ml of water, the DNA quantitation is made in Nanodrop-8000 TM. The dilutions are adjusted to 50 ng/ul. These dilutions were used to send for sequencing (GBS) and to evaluated SSR markers.

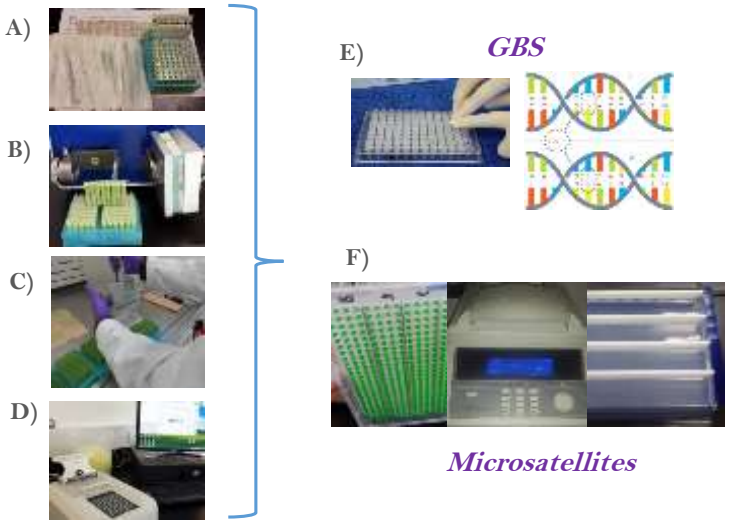

Figure 9. Sequence of activities for genotypic data. (A) Tissue collection, (B) Grinding tissue, (C) DNA extraction, (D) DNA quantitation, (E) Sample preparation for genotyping by sequence and (F) PCR and gels for SSR markers.

3.2.2 Polymerase chain reaction (PCR)

The conditions for PCR - SSR markers were set using the follow reagents and volumes from GoTaq® DNA polymerase (Promega Corporation). Buffer 5X (1.55µL), $MgCl_2$ 25mM (0.75µL), dNTP´s 100mM (0.6µL), Primer 1µM (2µL) and Taq enzyme (0.4µL). The annealing temperature of each SSR was obtained from Grain Genes 2.0 Web page. The PCR program used for SSR markers was the next: 30 cycles (94°C- 5 min // 94°C- 1 min/ **60°C- 2 min**/ 72°C-1 min// 72°C -7 min/ 10°C- ∞); only alignment temperature (highlighted in bold) change for each marker. The PCR product was loaded on agarose gels (3%) and ran for 2 h at 150 volts. The banding pattern in the population is scored according to each parent, genotype parent 1 and genotype parent 2, a mix of two genotypes is heterozygous genotype.

3.2.3 Genetic and statistical analysis

Genotyping of the population was made through 2694 SNPs markers and were used 11 SSR markers to identify and validate the linkage maps.

Software IciMapping 4.0 was employed for construction of a genetic linkage map (Want, 2015). Map distances between markers were calculated using the Kosambi (1944) mapping function. The positions of detected QTLs were determined by inclusive composite interval mapping (ICIM) (Zeng 1993, 1994). A logarithm of odds (LOD) of 3.0 was set to declare significance of QTLs. QTL effects were estimated as the proportion of phenotypic variance explained (PVE) by the QTL. The success of QTL (marker)-assisted selection in breeding depends on adequate prediction models and sufficient number of markers for trait phenotypes (Cooper, 2009).

To identified fragment containing stripe rust resistance gene Yr17 translocated between the short arm of *Triticum ventricosum* 2NS in chromosome 2AS, two PCR primers URIC, VENTRIUP, LN2 were used. These markers were used to construct genetic linkage maps that were generated using IciMapping QTL 4.1 (Meng, 2015) software. The markers were assigned to linkage group with a minimum LOD of 5.0; the map distances were calculated based on Kosambi mapping function and detection of QTL analysis was done with 1,000 permutations.

Statistical analysis was performed using SAS 9.4 software (SAS Institute, Cary, NC). In this study, instead of the Best Linear Unbiased Estimators (BLUEs), the Best Linear Unbiased Predictors (BLUPs) were calculated, using Restricted Maximum Likelihood (REML) in a Mixed Model Framework. The corresponding linear models were implemented in PROC Mixed of SAS using REML to estimate the variance components.

For analyses of individual locations using a lattice design the model was [Eq 1].

$$Y_{ijk} = \mu + Rep_i + Block_j(Rep_i) + Gen_k + \varepsilon_{ijk} \quad \text{[Eq 1]}$$

where Y is the trait of interest, μ is the mean effect, Rep_i is the effect of the ith replicate, $Block_j(Rep_i)$ is the effect of the jth incomplete block within the ith replicate, Gen_k is the effect of the kth genotype, and ε_{ijk} is the error associated with the ith replication, jth incomplete block, and kth genotype, which is assumed to be normally and independently distributed with mean zero and homoscedastic variance σ^2.

For the analyses combined across locations, new terms are added to the above model. For a lattice design, the model is [Eq. 2]

$$Y_{ijkl} = \mu + Loc_i + Rep_j(Loc_i) + Block_k(Loc_i Rep_j) + Gen_l + (Loc_i \times Gen_l) + \varepsilon_{ijkl} \quad \text{[Eq. 2]}$$

where the new terms Loc_i and $(Loc_i \times Gen_l)$ are the effects of the ith location and the location × genotype interaction, respectively.

Analysis of variance (ANOVA) test were performed, using the general linear model, for disease rating obtained from each location and for estimating genetic and environmental effects of lines. The variance components were carried out in the field trials in which two replications in Batan were evaluated and one replication for Toluca.

4. RESULTS

Result section has beed presented in to four sub-headings:

4.1 Phenotypic evaluation of recombinant inbred line population for yellow rust and other traits

The recombinant inbred lines of Botno/A.e squearosa // Kachu population, were screened for yellow rust in two different locations in the season 2015. 'Kachu', the resitant parent showed 10% of disease severity at the adult plant stage whereas, for synthetic parent it ranged between 35-40%. The yellow rust mean ranged between 31.62% to 43.94% for RILs in the two environments (Table 3).

Table 3. Summary of the disease severities (%) for Botno/Ae.squarrosa (666) × KACHU population evaluated for yellow rust resistance in two environments in Mexico.

	Yellow rust severity, % (Batán, México)		**Yellow rust severity, % (Toluca, México)**
Parent, Parameter	Day 1[a]	Day 2[b]	Day 1[c]
Kachu	10	10	10
Botno/Ae.squarrosa (666)	35	40	35
Population F_6 grand mean	31.62	43.94	33.86
Population range: low	10	10	5
Population range: high	70	90	85

[a] Percent yellow rust severity at El Batán, Day 1.
[b] Percent yellow rust severity at El Batán, Day 2.
[c] Percent yellow rust severity at Toluca, Day 1.

Analysis of variance (ANOVA) test were performed, using the general linear model (GLM), for disease rating obtained from each location and for estimating genetic and environmental effects of lines. The variance components were carried out in the field trials in which two replications in Batan were evaluated and one replication for Toluca (two replications were planted but due to hail storm and flooding only one replication data was recorded). The genotypie variance (σ^2_g) was 235.40 for day 1, 417.56 for day 2 in Batan and 665.03 for Toluca. The residual variance (σ^2_e) was 70.80 for day 1, 76.98 for day 2 in Batan and 114.04 for Toluca. The ANOVA results were used to estimate the heritability (h^2) calculated as $h^2 = \sigma^2_g / (\sigma^2_g + \sigma^2_e / \text{nreps})$. The heritability between reps for Batan experiment has been mentioned in Table 4.

Table 4. Summary of heritability, variance components, least significant difference and coefficient of variation for individual analysis of the measurements made in Batán and Toluca.

	Yellow rust (Batán, México)		Yellow rust (Toluca, México)
Parent, Parameter	Day 1[a]	Day 2[b]	Day 1[c]
Replications	2	2	1
Genotype variance (σ^2_g)	235.40	417.56	665.03
Residual variance (σ^2_e)	70.80	76.98	114.04
Heritability (h^2)	0.87	0.92	0.92
LSD	15.21	16.73	20.29
CV	26.61	19.97	31.54

[a] Percent yellow rust severity at El Batán, Day 1.
[b] Percent yellow rust severity at El Batán, Day 2.
[c] Percent yellow rust severity at Toluca, Day 1.

For the combined analyses, the heritability is calculated as $h^2 = \sigma^2_g / (\sigma^2_g + \sigma^2_{ge} / \text{nlocs}) + (\sigma^2_g / \text{nlocs X nreps})$ where the new term σ^2_{ge} is now the genotype × environment interaction variance component and "nlocs" is the number of locations in the analysis. The variance components shows for combine analysis both days in Batan and across both locations, Batan and Toluca (Table 5). The heritability is 0.81 and 0.87 respectively.

Table 5. Summary of heritability, variance components, least significant difference and coefficient of variation for combined analysis in Batán and combined across both locations in Mexico.

Parameter	Combined analysis (Day1 and Day 2 Batan)	Combined analysis across both locations. (Batán and Toluca)
Population F_6 grand mean	37.77	36.52
Genotype variance (σ^2_g)	248.78	333.33
Location x genotype variance	76.13	106.01
Residual variance (σ^2_e)	73.71	86.92
Heritability (h^2)	0.81	0.77
LSD	18.78	18.30
CV	22.73	25.53

The frequency distribution for disease severity was determined and plotted for both environments. The frequency distribution of RILs for yellow rust severity was continuous in the two field experiments, Batan and Toluca (Figure 10).

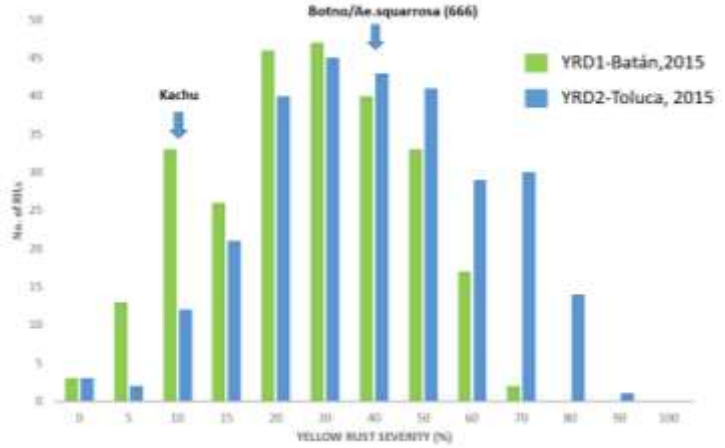

Figure 10. Phenotypic frequency distributions for disease severity in Botno/Ae.squarrosa (666) x Kachu F_6 recombinant inbred lines (RILs) for leaf rust severity in both field trials in Mexico.

The recombinant inbred lines of Botno/A.e squearosa // Kachu population, were screened for physiology and grain yield related traits in two locations in Obregon, 2014 and Batan, 2015. Four characters were evaluated, days to heading, heigth, thousand kernel weight and grains per spike. Values shown in the following table.

Table 6. Summary of four traits related to physiology and grain yield: days to heading (DTH), height (HT), thousand kernel weight (TKW) and grains per spike (GS) in Botno/Ae.squarrosa (666) x KACHU wheat population in Obregon 2014 and Batán 2015.

Parent, Parameter	DTH (days)		HT (cm)		TKW (g)		GS
	Batán (Rep I-II)	Obregon (Rep I)	Batán (Rep I-II)	Obregon (Rep I)	Batán (Rep I-II)	Obregon (Rep I)	Batán (Rep I-II)
Kachu	58	-	89	-	35	-	-
Botno/Ae.squarrosa (666)	64	-	73	-	15.5	-	22
Population F_6 mean	57	73	96.9	103.1	30.4	40.1	30
Population range: low	45	60	60	55	11	14	13
Population range: high	66	101	120	130	47	55	50

Analysis of variance (ANOVA) test were performed. The variance components were carried out in the field trials in which two replications in Batan were evaluated. The genotype variance (σ^2_g) shows for individual analysis were the variance components of DTH, HT, TKW and GS has been presented in the table 5. The genotypie variance (σ^2_g) was 29.69, 114.33, 33.96 and 57.38 respectively. The residual variance (σ^2_e) was 1.31, 8.07, 29.55 and 8.61 for the four characters. The ANOVA results

were used to estimate the heritability (h^2) calculated as $h^2 = \sigma^2_g/(\sigma^2_g + \sigma^2_e/\text{nreps})$. The heritability was 0.98 for days to heading, 0.97 for height, 0.69 for thousand kernel weight and 0.93 for grains per spike.

Table 7. Summary of heritability, variance components, least significant difference and coefficient of variation for individual analysis of the measurements made in Batán.

Parameter	DTH	HT	TKW	GS
Population F6 grand mean	56.54	96.9	30.4	29.9
Genotype variance (σ2g)	29.69	114.33	33.96	57.38
Residual variance (σ2e)	1.31	8.07	29.55	8.61
Heritability (h2)	0.98	0.97	0.69	0.93
LSD	2.32	5.47	9.30	5.97
CV	2.03	2.93	18.12	9.72

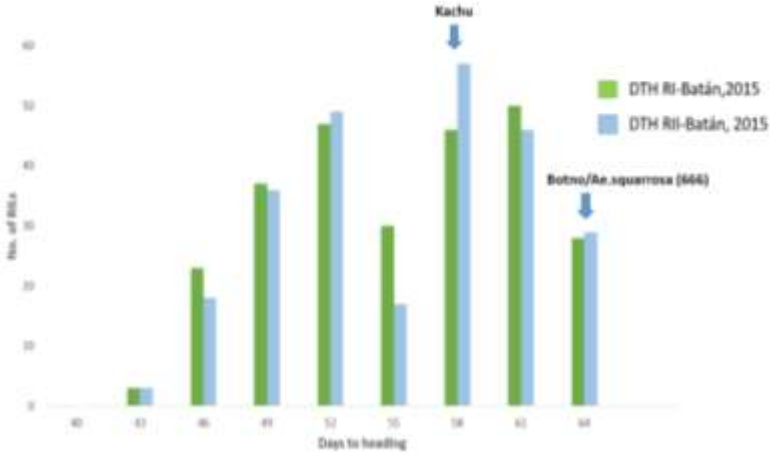

Figure 11. Phenotypic frequency distributions for Days to Heading in Botno/Ae.squarrosa (666) x Kachu F_6 recombinant inbred lines (RILs) in both field repetition trials in Batán and Obregón.

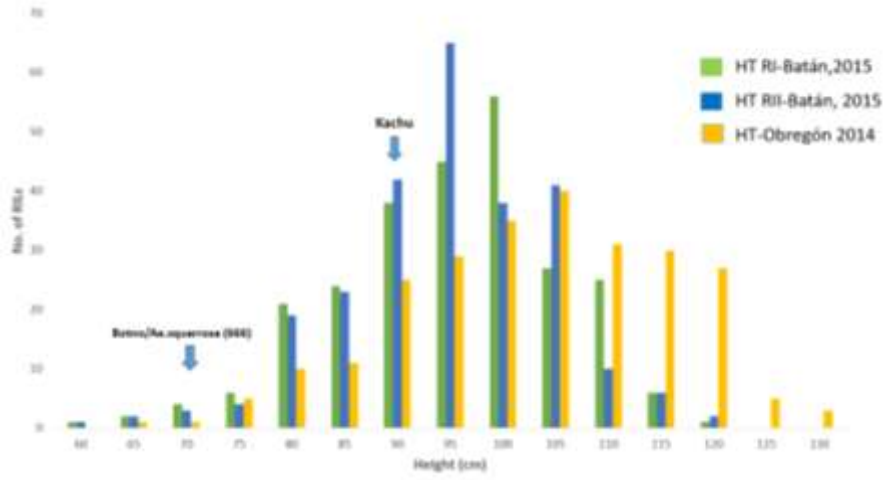

Figure 12. Frequency distributions for height in Botno/Ae.squarrosa (666) x Kachu F_6 recombinant inbred lines (RILs) in field trials at Batán and Obregón.

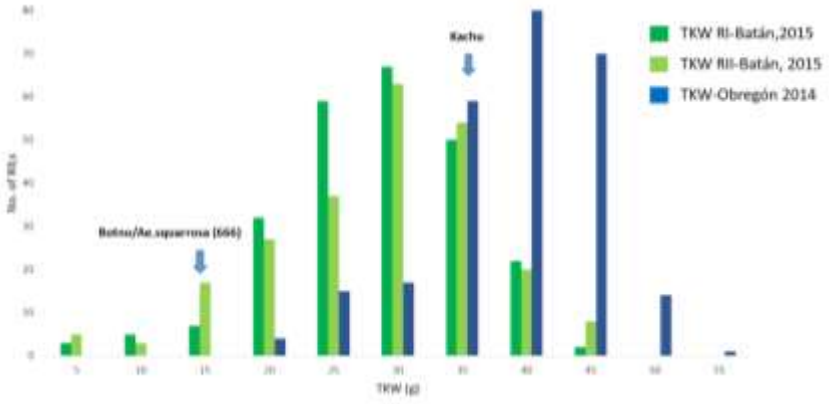

Figure 13. Frequency distributions for Thousand Kernel Weight in Botno/Ae.squarrosa (666) x Kachu F_6 recombinant inbred lines (RILs) in field trials in Batán.

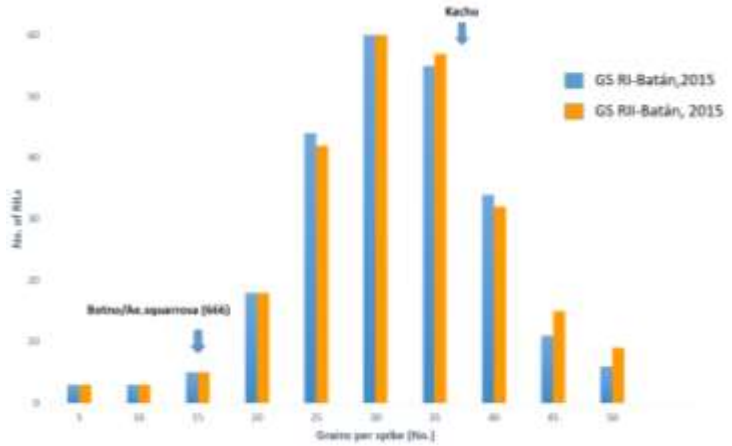

Figure 14. Phenotypic frequency distributions for Grains per Spike in Botno/Ae.squarrosa (666) x Kachu F_6 recombinant inbred lines (RILs) in both field repetition trials in Batán.

4.2 Linkage map construction

The genetic linkage map was constructed for RIL population of Botno/Ae. Squarrosa// Kachu using ICIM software. To define the significance of genetic distance, LOD of 3.0 was considered as threshold. The number of linkage groups, markers in each group and length is presented in Table 8.

Table 8. Details of linkage map of *Botno/Ae. Squarrosa//* Kachu RIL population.

Chromosome	Number of markers	Chromosome length
1A	125	187.09
1B	186	241.68
1D	122	170.04
2A	308	318.63
2B	340	423.02
2D	-	-
3A	126	191.84
3B	51	71.39
3D	117	169.06
4A	79	171.48
4B	118	127.11
4D	91	154.36
5A	194	260.74
5B	112	252.35
5D	90	176.86
6A	109	126.76

6B	120	124.21
6D	83	182.79
7A	192	239.75
7B	-	-
7D	98	227.41

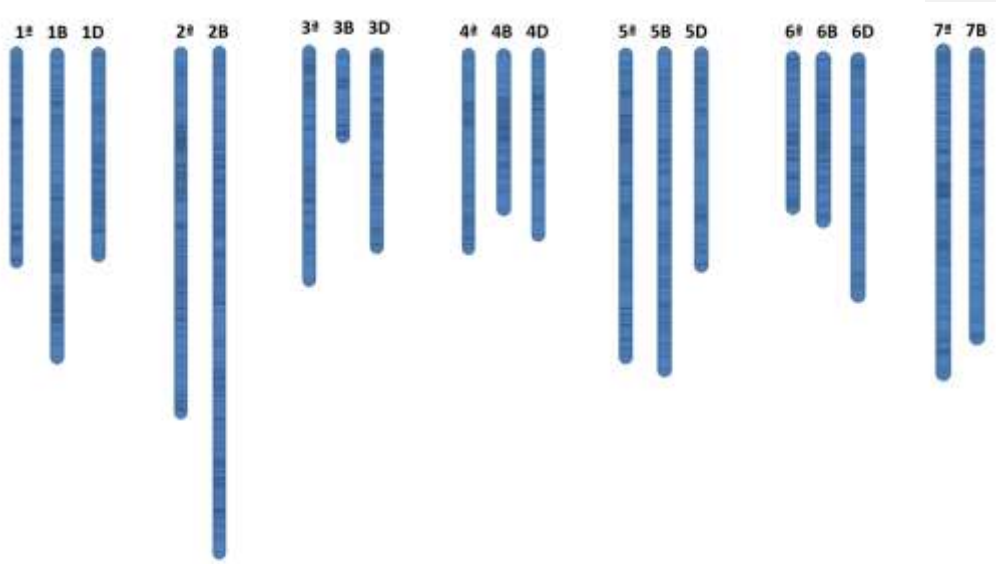

Figure 15: Linkage groups of *Botno/Ae. Squarrosa//* Kachu RIL population. Chromosome detailed information (name and position of each marker) is in supplementary material.

The chromosome position was defined based on the BLAST of cloneID sequences with wheat reference genome (Kent, 2002). Number of markers on chromosomes 1A, 1B, 1D, 2A, 2B, 3A, 3B, 3D, 4A, 4B, 4D, 5A, 5B, 5D, 6A, 6B, 6D, 7A, 7D, were 125, 186, 122, 309, 340, 126, 51, 117, 79, 118, 91, 194, 112, 90, 109, 120, 83, 192, 98 respectively and length of chromosomes were 187.09, 241.68, 170.04, 318.63, 423.02, 191.84, 71.39, 169.06, 171.48, 127.11, 154.36, 260.74, 252.35, 176.86, 126.76, 124.21, 182.79, 239.75, 227.41 respectively. The total length of 21 chromosomes was 3816.57.

4.3 QTL analysis

4.3.1 QTL mapping for Yellow rust resistance

Composite interval mapping (CIM) revealed quantitative trait loci on chromosome 2AS significantly associated with the yellow rust resistance in *Botno/Ae. Squarrosa* x Kachu RIL population. This QTL explained phenotypic variance up to 48.8 %. Additive effect up to 18.2 % was observed. LOD score in mapping ranged 18.6 – 34.7 in different trials. The positive QTL allele was contributed from elite parent of the population, 'Kachu'. The QTL was located on proximal end of chromosome 2A short arm as shown in Figure 16 and 17. Markers linked with this QTL were BARC122, 1028859|F|0--50:G>C and VENTRIUP-LN2. Genetic linkage map showed that marker intervals 3.3 cM by BARC122-1028859|F|0--50:G>C- VENTRIUP-LN2 in different experiments. Results were consistent across two environments. Summary of QTL mapping results in different experiments have been presented in table 9.

Table 9. Summary of QTL for stripe rust in the Botno/Ae.squarrosa (666) × Kachu F6 RIL population (Batán and Toluca, México), position and effect of quantitative trait loci using composite interval mapping.

Trait	Chr	Pos	Left Marker	Right Marker	LOD	PVE (%)	Add
M-YRBatan-D1	2A	11	BARC122	1028859|F|0--50:G>C	34.7265	48.8416	-11.6629
M-YRBatan -D2	2A	12	1028859|F|0--50:G>C	VENTRIUP-LN2	18.6075	22.1807	-10.1747
M-YRToluca-D1	2A	11	BARC122	1028859|F|0--50:G>C	31.58	46.0896	-18.2789

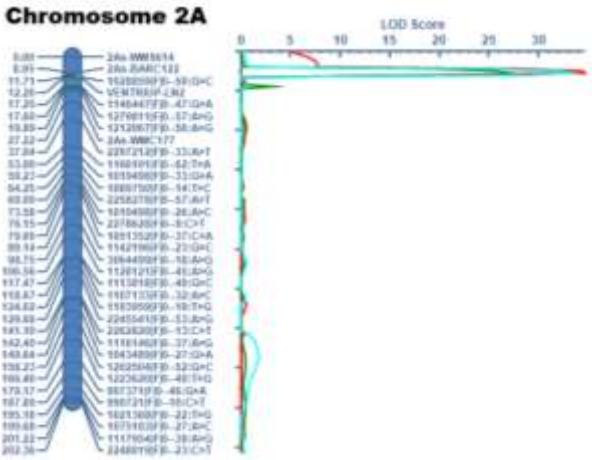

Figure 16. Genetic linkage map chromosome 2AS and QTL identified for stripe rust showing map distances on the left side (cM) and name of molecular markers on the right.

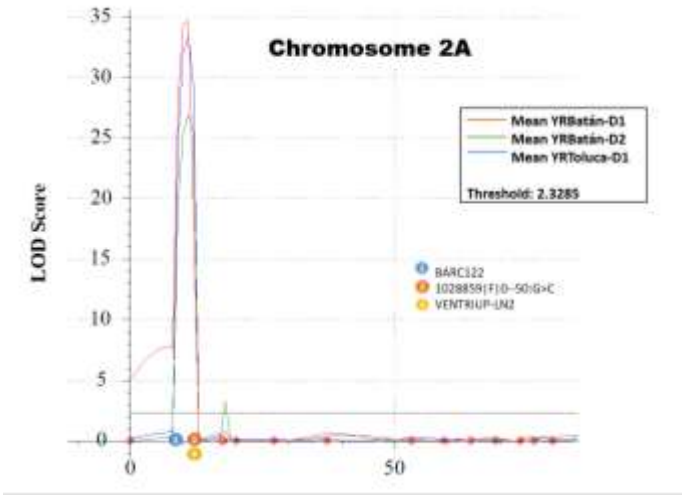

Figure 17. The QTL for stripe rust on chromosome 2AS, detected in two environments. Likelihood plot of the quantitative trait loci (QTL) for yellow rust (Yr) on chromosome 2A, identified by IciMapping 4.0 program in F6 RIL population Botno/Ae.squarrosa (666) x KACHU. The significant logarithm of odds (LOD) thresholds was detected based on 1,000 permutations. D1 = Day 1, D2 = Day 2.

4.3.2 QTL mapping for grain yield and related traits

Genetic analysis using ICIM in Botno/Ae. Squarrosa × Kachu RIL population, identify a genomic regions on chromosome 5A, 2A and 4B significantly associated with DTH, HT, TKW and GS.

Two QTLs for **days to heading (DTH)** were identified on chromosomes 5A and 2A. These QTLs explained phenotypic variance up to 8.06% and 20.60% respectively. Additive effect up to 6.61 % was observed. LOD score in mapping ranged 5.43 – 15.43 in different trials (Batán and Obregón). The positive allele for QTL on chromosome 5A and 2A were contributed from synthetic and elite parents respectively. Markers associated with DTH on chromosome 5A were 1089190|F|0--41:G>T and 1106489|F|0--46:C>G. On the chromosome 2A linked markers were 1202504|F|0--52:G>C and 2252376|F|0--60:G>A. Results have been presented in table 10, figure 18 and 19.

Two QTLs for **height (HT)** were identified on chromosomes 2A and 4B. Theses QTLs explained phenotypic variance up to 11.69% and 25.28% respectively. Additive effect up to 2.7 % was observed. LOD score in mapping ranged 5.20 – 13.92 in different trials (Batán and Obregón). The positive QTLs alleles found in 2A and 4B chromosomes were contributed from elite parent. Markers

linked with QTL-1 were 1667741|F|0--13:G>C and 1146447|F|0--47:G>A in Chromosome 2A and with QTL-2 were 1089520|F|0--43:G>A and 996008|F|0--11:T>G in Chromosome 4B (table 10, figure 20).

For **thousand kernel weight (TKW)** one QTL were identified on chromosome 2A. This QTL explained phenotypic variance up to 16.49%. Additive effect up to 3.80 % was observed for QTL-1 and 7.34% for QTL-2. LOD ranged 7.20 – 8.66 in different trials in Batán (table 10, figure 18 and 21). The positive allele for QTL was contributed from elite parent. Markers linked were 1667741|F|0--13:G>C and 1146447|F|0--47:G>A.

Two QTLs were identified associated with **grains per spike (GS)** on chromosomes 2A and 4B. The first QTL explained phenotypic variance up to 24.0%, the second up to 19.05%, third up to 6.41% and fourth 13.98%. Additive effect up to 4.05%, 3.21%, 2.09% and 2.77% was observed respectively (table 10, figure 22). LOD ranged 5.12 – 13.79 in different trials in Batán. The positive alleles for QTLs on chromosome 2A and 4B were contributed from elite parent.

Markers associated with GS on chromosome 2A were 1667741|F|0--13:G>C and 1146447|F|0--47:G>A for first QTL and 2As-WMS 614 and 1028859|F|0--50:G>C for second QTL. Two QTLs were founded in chromosome 4B. Markers linked were 2278417|F|0--27:T>A and 1090296|F|0--61:A>G for first QTL and 2279884|F|0--17:C>T and 3222514|F|0--5:T>C for second QTL.

QTL results reveal the consistent effects across two environments. Summary of QTL mapping results in different experiments have been presented in table 10.

Table 10. Summary of QTLs for different traits related to grain yield in the Botno/Ae.squarrosa (666) x Kachu F6 RIL population (Batán 2015 and Obregón 2014, México), position and effect of quantitative trait loci using composite interval mapping (ICIM-ADD) by IciMapping 4.0.

Trait	QTL		Chr	Pos	LeftMarker	RightMarker	LOD	PVE (%)	Add
Days to Heading	QTL 1	DTH-B-RI	5A	180	1089190|F|0--41:G>T	1106489|F|0--46:C>G	5.431	6.832	1.512
		DTH-B-RII	5A	180	1089190|F|0--41:G>T	1106489|F|0--46:C>G	6.775	8.057	1.623
	QTL 2	DTH-B-RI	2A	273	1202504|F|0--52:G>C	2252376|F|0--60:G>A	13.605	20.600	-2.585
		DTH-B-RII	2A	273	1202504|F|0--52:G>C	2252376|F|0--60:G>A	11.379	16.102	-2.258
		DTH-O	2A	272	1202504|F|0--52:G>C	2252376|F|0--60:G>A	16.438	25.420	-6.618
Height	QTL 1	HT-B-RI	2A	23	1667741|F|0--13:G>C	1146447|F|0--47:G>A	5.208	10.956	-3.646
		HT-B-RII	2A	23	1667741|F|0--13:G>C	1146447|F|0--47:G>A	5.657	11.691	-3.800
	QTL 2	HT-B-RI	4B	35	1089520|F|0--43:G>A	996008|F|0--11:T>G	12.306	17.743	-4.643
		HT-B-RII	4B	32	1089520|F|0--43:G>A	996008|F|0--11:T>G	11.747	16.787	-4.555
		HT-RI-O	4B	32	1089520|F|0--43:G>A	996008|F|0--11:T>G	13.924	25.283	-7.349
Thousand Kernel Weight	QTL 1	TKW-B-RI	2A	23	1667741|F|0--13:G>C	1146447|F|0--47:G>A	7.200	16.494	-2.640
		TKW-B-RII	2A	23	1667741|F|0--13:G>C	1146447|F|0--47:G>A	8.665	14.026	-2.786
Grains per spike	QTL 1	GS-B RI	2A	23	1667741|F|0--13:G>C	1146447|F|0--47:G>A	12.512	24.006	-4.056
	QTL 2	GS-B RII	2A	5	2As-WMS 614	1028859|F|0--50:G>C	13.792	19.055	3.215
	QTL 3	GS-B RI	4B	39	2278417|F|0--27:T>A	1090296|F|0--61:A>G	5.122	6.417	-2.094
	QTL 4	GS-B RII	4B	52	2279884|F|0--17:C>T	3222514|F|0--5:T>C	9.598	13.985	-2.771

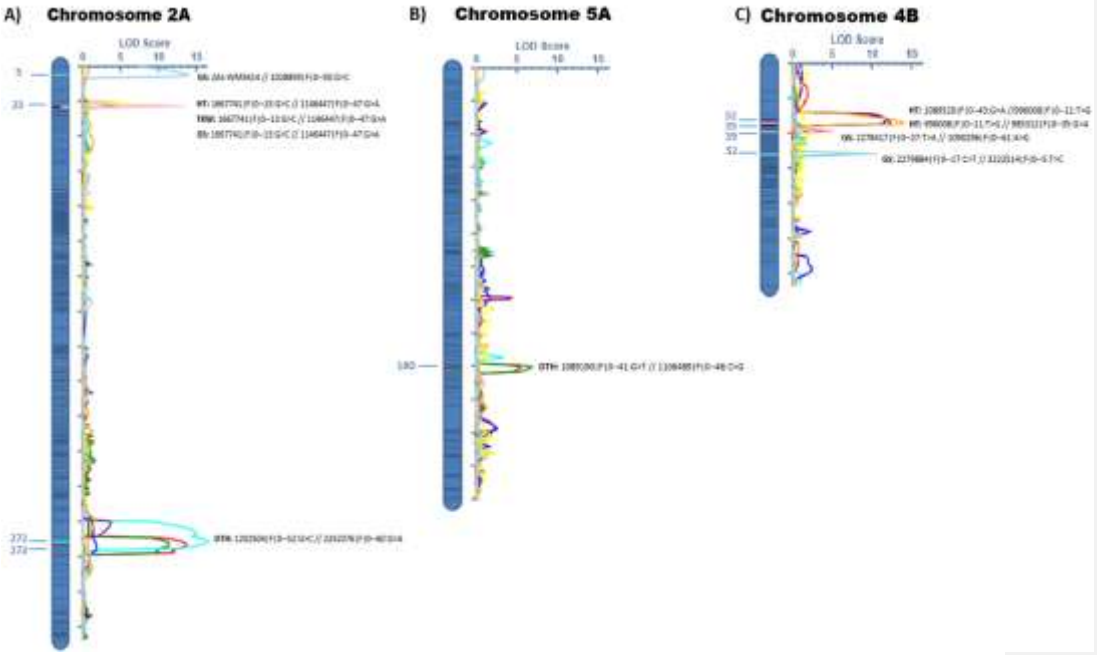

Figure 18. Summary of Genetic linkage map of the three chromosomes where QTLs related to grain yield were identified. Mapped and validated position of each QTL.

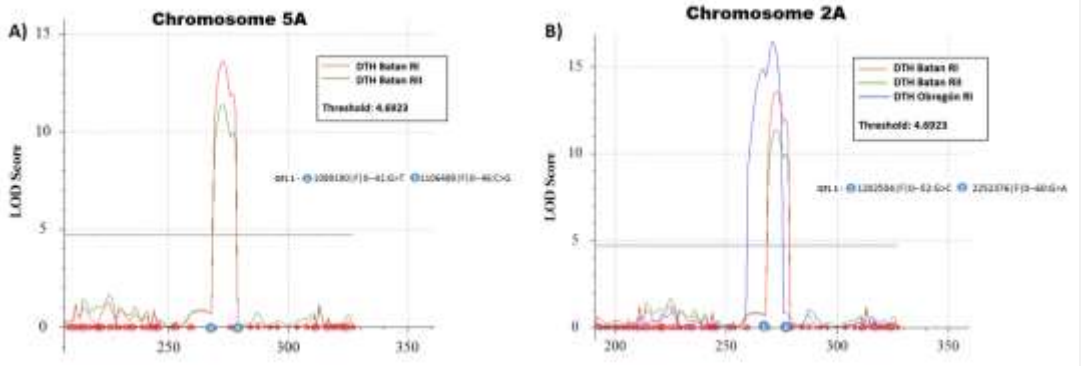

Figure 19. LOD plots of each significant QTL region for Days to Heading on chromosome 5A and 2A, with the threshold included in each scale, detected in two environments.

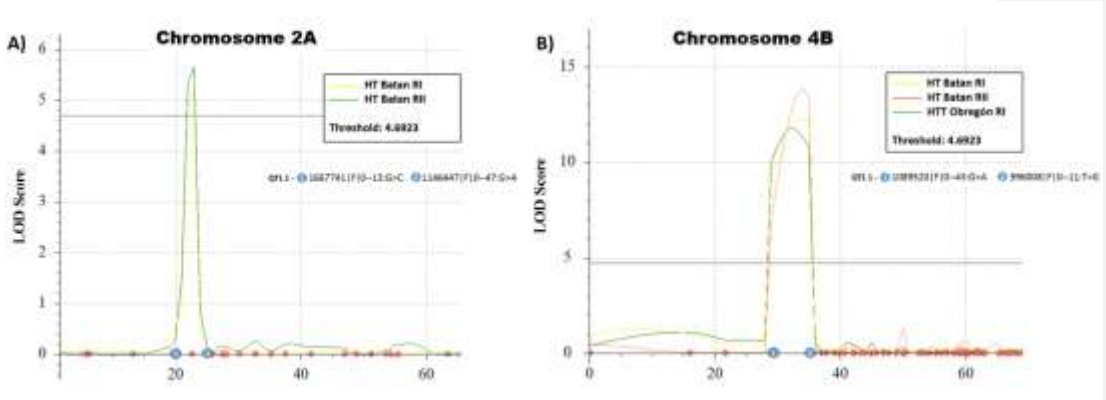

Figure 20. LOD plots of each significant QTL region for Plant height on chromosome 2A and 4B, with the threshold included in each scale, detected in two environments

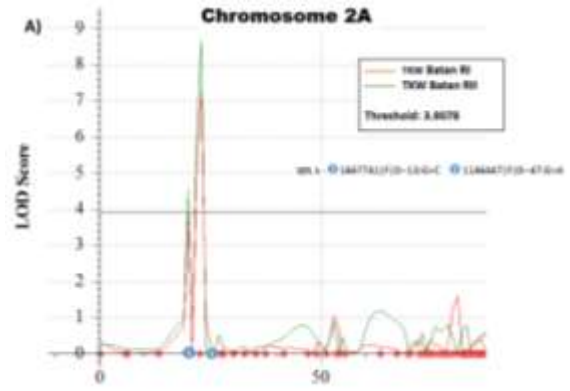

Figure 21. LOD plot significant QTL region for Thousand Kernel Weight Height on chromosome 2A, with the threshold included in each scale, detected in Batán, Mexico.

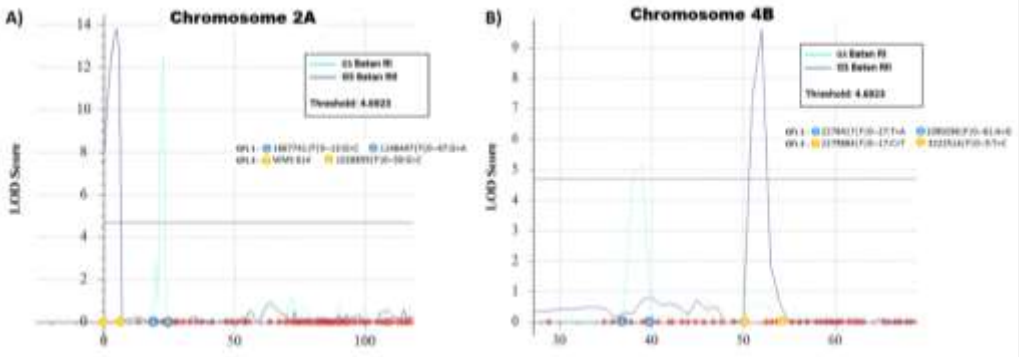

Figure 22. LOD plots of each significant QTL region for Grains per spike on chromosome 2A and 4B, with the threshold included in each scale, detected in Batán, Mexico.

4.4 Yellow Rust QTL characterization

Characterization of the QTL on chromosome 2A for yellow rust resistance was carried out in way to understand the origin of resistance gene(s) associated with the QTL. An *Aegilops venticrossa* specific marker was amplified from population lines (Figure 16) to validate whether this genomic region is ultimately contributed by *Aegilops venticrossa*. It is important to note that this wild relative is one of the parents of QTL contributing line, 'Kachu'. Results clearly indicated towards effect of *Aegilops venticrossa* allele to be responsible for the effect of QTL.

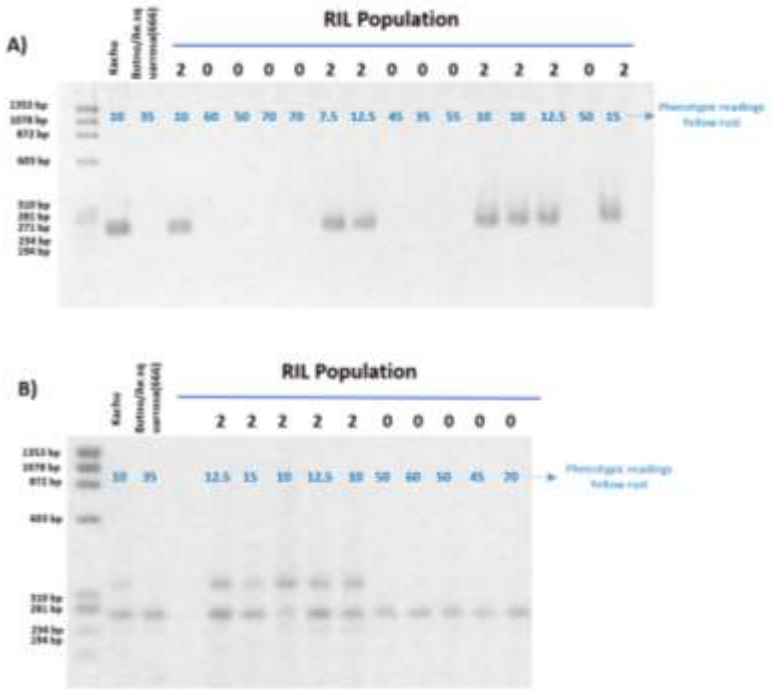

Figure 23. PCR amplification with 2NS-specific marker VENTRIUP/LN2 for YR17 and SSR Barc-122.

5. DISCUSSION

Understanding the genetic control of quantitative traits helps plant breeders to improve crop yield, disease resistance, end-use quality, and other important characteristics. Quantitative trait locus (QTL) analysis identifies the association of molecular markers withphenotypic traits to ultimately locate genes governing traits. The main goal of this research was to identified genomic regions associated with phenology and grain yield related traits in wheat mapping population derived from *Botno/Ae. Squarrosa (666)//* Kachu cross.

5.1 Phenotypic evaluation of recombinant inbred line population for yellow rust and other traits

An accurate phenotyping is the most important pre requisite for QTL analysis. In this study we evaluated a synthetic × elite bi-parental RIL population (Botno/A.e squearosa // Kachu) for important adaptive traits in wheat i.e. grain yield related parameters along with yellow rust disease.

The grain yield related traits measured in our study showed a normal distribution in the population. Graph showing distribution of genotypes for HT, TKW and GS are normal, however, for days to heading it was not little bit deviated from the normal (Figure 11, 12, 13 and 14). This kind of distribution of genotypes in the population clearly suggests towards presence of sufficient recombinants. Deviation from normal for DTH should be due to presence of major genes in the population. Though the graph is not perfectly biomodal, possiblity of presence of single major gene controlling DTH in population is ruled out (Figure 11).

For the yellow rust disease severity, again normal distribution was observed in the population (Figure 10) rendering the population to be a fit one for QTL analysis study.

Study of phenotypic and genotypic coefficient of variation, heritability and genetic variance for yellow rust and quantitive yield traits are shown in table 4 and 7. Heritabilities of the traits observed in this study were at higher score providing another line of evidence. Greater magnitude of heritability (0.98) were observed for days to heading in two replication trails in Batan under irrigated conditions. Similar heritability estimates for heading were also reported from research work by

Ikramullah, 2011. In the previous reports the heritability score of plant height were medium to low compared as our result (0.97). Height heredability (0.85) have been reported by MA Rahman, 2016 in bread wheat.

Heredability score reports of TKW and GS showed low to moderate ranged if we compered with our results (0.69 and 0.93). MA Rahman, 2016; reported a higher value of heritability for TKW (0.72) but low value for GS (0.54) in bread wheat compared with our resutls. Ikramullah, 2011, reported under irrigated environment a lower heritability value related to TKW compared with our result.

The phenotypic variance explained by RIL population (Botno/A.e squearosa // Kachu) subjected to the QTL analysis study also indicated towards presence of large number of recombination events which is the most important and pre requisite for a breeding/molecular breeding program. This population can be analyzed for a number of adaptive traits to identify suitable trait donors as well as useful alleles governing adaptive traits. The in depth genetic characterization of above mentioned RIL poulation through using molecular markers is discussed in subsequent sections.

5.2 Genotypic analysis and linkage map construction

Phenotypic analysis if the RIL population analyzed in this study indicates the presence of large number of recombinants. Therefore in order to capture maximum number of recombination events genetic characterization was carried out using high density genotyping platform coupled with sequencing of short reads of ~65 bp called as genotyping-by-sequencing. The challenge after achieving GBS tags is to anchor them to a reference chromosome sequence whiich can not be done in wheat efficiently as compared to other model crop species (rice and maize). Therefore construction of a quality linkage map in wheat is of prime importance while using GBS for genetic characterization. Linkage groups were established by considering all estimates of recombination frequencies.

Linkage map construction was done using polymorphic marker scores in the population. Marker polymorphism in wheat is reportedly lower than in other major crops such as corn and soybean. Studies have shown that SSR marker polymorphism in corn and soybean is over 50% (Sa, 2012; Singh, 2010), whereas it is around or even below 40% in wheat (37.5%) (Cregan, 2012). Common hexaploid wheat also has extremely low levels of polymorphism at DNA marker loci compared to its

parent species, especially *Aegilops. squarrosa* (Nishikawa, 1980). Polymorphism also depends on the parents. In the present study two contrasting parents, synthetic wheat and an elite variety were crossed so as to obtain large number of polymorphic markers. The polymorphic markers which did not show significant segregation distortion were taken in to account for map construction. After filtering the data set based on missing genotype score, call rate, average lead length and segregation distortion a total of 2707 markers were used for map construction. These marker set covered a genetic distance of 3816.57 cM among total of 21 chromosomes. This linkage map was used for QTL analysis for traits observed in our study.

5.3 QTL mapping for Yellow rust resistance

QTL analysis detected one major QTLs for yellow rust showing a consistent effect across in two different locations. This QTL was located on chromosome 2A and showed a high significance as revealed by LOD scores in different trials (Table 9). This genomic region was identified using GBS marker data. Later on based on the pedigree of elite parent 'Kachu' we hypothesized that positive allele might be contributed from the wild ancestor (*Aegilops ventricosa*). SSR markers, Barc-122 and VENTRIUP-LN2 were amplified in the population lines to find their association with yellow rust resistance. It is important to note here that 'VENTRIUP-LN2' is a SSR marker designed from the sequence of *Aegilops ventricosa*. This marker corresponds to the chromosome 2N of *Triticum ventricosum* (*Tausch*), also known as *Aegilops ventricosa*. This chromosome region is known to harbor resistance gene cluster (Lr37-Sr38-Yr17) conferring resistance against leaf rust, stem rust and stripe rust (Dyck and Lukow 1988; McIntosh, 1995; Robert, 1999; Seah, 2000). The marker used in our study (VENTRIUP-LN2) is used for detecting the presence of chromosome 2N of *Triticum ventricosum* (*Tausch*). This 2NS chromosome segment was translocated to the short arm of bread wheat chromosome 2A (Helguera, 2003).

Characterization of this chromosome segment by SSR marker confirms that the effect of yellow rust resistance QTL in RIL population is contributed 2NS translocation in Kachu from *Triticum ventricosum* (*Tausch*). These results are quite important for current and future genomic studies related to rust resistance. Ample number of elite wheat genotypes used in breeding or varietal pipelines are harboring segments from one or the other wild accessions. Genetic understanding of such germplasms should provide better understanding of genetic basis of resistance genes in wheat.

The approach we followed in this study for yellow rust can be successfully applied for other traits such as grain quality, heat, drought or other traits.

5.4 QTL mapping for grain yield and related traits

Grain yield improvement is the ultimate goal for a wheat breeding program. Grain yield has complex inheritance and is difficult to manipulate through a per se approach. However, selection based on certain component traits may lead to considerable improvement in grain yield. In the era of yield stagnation in staple cereals (maize, wheat, rice) it is important to follow different approaches toward achieving the overarching goal of yield potential enhancement. One approach could be accumulate genes associated with different yield component traits. Envisioning this, we identified QTLs for DTH, HT, TKW and GS in the RIL population subjected to analysis.

Wheat cultivars with appropriate heading time to the target environment and life cycle duration will help maximize yield potential in any environment. Two QTLs were identified on chromosomes 5A and 2A associated with Days to Heading (DTH). These QTLs explained phenotypic variance up to 8.06% and 20.60% respectively. Additive effect up to 6.61 % was observed. LOD score in mapping ranged 5.431 – 15.43 in different trials (Batán and Obregón). Heading time in wheat is governed by three major genetic factors: vernalization requirement, photoperiod sensitivity and narrow-sense earliness. Differences in the vernalization genes (*Vrn*) determine spring and winter wheat habits. The photoperiod genes (*Ppd*) play a major role in determining the flowering time and the sensitivity to photoperiodism.

Christine Zanke, 2014; reported in a suty of genome-wide association study (GWAS) that found for the vernalization gene Vrn-A2 is locating on chromosome 5A, as well as for the photoperiodism genes Ppd-A1and Ppd-B1 onchromosomes 2A and 2B were detected. Further investigation would reveal the uniqueness or repeatebility of gene on chromosome 5A. Another QTL on chromosome 2A is a novel region identified in this study for DTH. Gel based markers need to be developed to use these informations in plant breeding as we performed for yellow rust.

The reduction of crop height has therefore been an important breeding goal for many decades (Griffiths S., 2012). Plant height (PH) is one of the most studied phenotypes in wheat due to its involvement in plant architecture and ultimately in grain yield, also plant height in small grain cereals is an important agronomic trait affecting crop performance, particularly lodging and consequently

grain yield and grain quality. QTLs controlling variation for HT were identified on chromosomes 2A and 4B (Table 10). Chromosme 4B is well known to harbor green revolution gene, rht1. In hexaploid wheat, dwarfing has been achieved mainly through the introduction of the Rht alleles Rht-B1b and Rht-D1b, now found in the majority of varieties grown worldwide (Evans, 1996). It was found that the wheat Rht genes encode growth repressors that are normally suppressed by gibberellin (GA) (Peng, 1999). Six additional alleles at Rht-B1 and Rht-D1 have been identified by genetic mapping, four on chromosome 4B and two on chromosome 4D, which produce dwarfs with a broad range of plant height (Gale and Youssefian, 1985; Borner, 1996). For Chromosome 2A there are no reports of QTLs associated with height.

TKW is another target trait for plant breeders and is considered as one of the main grain yield components in wheat . TGW is usually stably inherited and can be divided into individual components including physical parameters (grain length, width, area) and grain filling characteristics, which are also under independent genetic control (James Simmonds, 2014). In our present investigation we focused to identify QTLs associated with TKW as the first step. Similar to DTH and HT one QTL was identified on chromosme 2A for TKW explaining phenotypic variance up to 16.49%. In addition to TKW, two QTLs were identified for GS on chromosome 2A and 4B.

QTL mapping results may be very helpful for QTL introgression. We also found that beneficial alleles existed in wild species and synthetic wheat for some of the traits which will be uselful for wheat improvement. QTL results reveal the consistent effects across two environments. Summary of QTL mapping results in different experiments have been presented in table 10.
Our study provided an initial basis for presence of QTL(s) or gene(s) that can be pursued successfully in marker assisted breeding for enhancing grain yield of wheat in Mexican environments.

6. CONCLUSIONS

1. Wheat *(Triticum aestivum L.)* is the most widely grown staple food crop and is important for global food security. One fifth of the total calories consumed by the world population comes from wheat. Yellow Rust, caused by *Puccinia striiformis*, is one of the most devastating diseases in world's staple food crop, wheat, causing significant lost to the grain yield globally. Genetic modification of varieties is a prefer alternative among different management options. Genomics assisted approaches offer promise of fast track breeding in wheat.
2. A synthetic by elite population (RIL population; synthetic parent: Botno/ *Ae. Squarrosa* (666) and elite parent: Kachu) was evaluated for yellow rust disease in two different environments in Mexico, CIMMYT experimental fields El-Batán and Toluca. This population was subjected to Genotyping by sequencing (GBS) for an in-depth genetic characterization.
3. A major effect rust resistance QTL explaining up to 45% phenotypic variance was found to be contributed from "Kachu". Further analysis revealed this QTL to be contributed from a segment of *Triticum ventricosum* on chromosome 2NS translocated at short arm of bread wheat chromosome 2AS in "Kachu". QTL position was confirmed using *T. ventricosum* specific primer VENTRIUP-LN2.
4. Two QTLs were identified associated with days to heading (**DTH)** on chromosomes 5A and 2A explaining phenotypic variances up to 8.05% and 20.60% respectively. Two QTLs were identified associated with height **(HT)** on chromosomes 2A and 4B explaining phenotypic variance up to 11.69% and 25.28 respectively. For thousand kernel weight **(TKW)** one QTL were identified in chromosome 2A which explained phenotypic variance up to 16.49%. Two QTLs were identified associated with grain per spike **(GS)** on chromosomes 2A and 4B. The first QTL explained phenotypic variance up to 24.0%, the second up to 19.05%.
5. Identified genomic regions are being introgressed in to the popular but susceptible wheat varieties through marker assisted breeding for enhancing yellow rust resistance and thereby grain yield.

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