The use of *Cicer reticulatum* L. for genetic improvement of cultivated chickpea

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ABSTRACT

The diverse gene pool of wild chickpea (*Cicer reticulatum* L.) has the potential for use to improve a range of agronomically important traits in cultivated chickpea. This study was conducted to evaluate the phenotypic and genetic variations of 486 lines derived from interspecific crosses between *C. arietinum* (CDC Leader) and 20 accessions of *C. reticulatum*. Field evaluations were done on the progeny at the F_4 and F_5 generations. The lines were grown at four locations in Saskatchewan over two consecutive years, 2017 (Saskatoon and Moose Jaw) and 2018 (Limerick and Lucky Lake). Significant variability was observed for different traits such as seed weight per plant, thousand seed weight, number of seeds per plant, and biomass. Correlation analysis showed significant positive correlation of seed yield with the yield components including seed weight per plant (r = 0.99), number of seeds per plant (r = 0.95), and biomass (r = 0.82), while negative correlation was obtained between thousand seed weight and number of seeds per plant (r = -0.16). The significant positive direct effects of the number of seeds per plant, thousand seed weight, and biomass on the seed weight was confirmed by path coefficient analysis. Cluster analysis based on the phenotypic data generated six clusters for potential identification of heterotic groups of the interspecific lines. Cluster I consisted of 67 lines potential for improvement of yield traits, while the lines in cluster VI showed improved resistance to ascochyta blight disease. Genotyping of the 381 interspecific lines and 20 parents using tGBS identified 14,591 SNPs ranging from 634 to 2244 per chromosome. Neighbour-joining cluster analysis based on the SNP data grouped the 401 germplasms into 20 clusters. Admixture analysis revealed 9 groups that had a substantial amount of intermixing. The marker-trait association analysis using the mixed linear model (MLM) identified 51 SNPs that had significant associations with different traits. The SNPs on chr 4 were significantly associated

with early flowering which were derived from the wild parents. Highest number of SNPs (13) were found to be associated with each of the trait such as thousand seed weight (g), and seed weight per plant (g).

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DEDICATION

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1. INTRODUCTION

The use of improved crop varieties can enhance crop production to achieve food and nutritional security for the growing global population. The role of chickpea in achieving nutritional security particularly in the developing countries is critical due to its high protein, vitamins and mineral content (Singh et al., 2008). Atmospheric nitrogen fixation capability in the root nodules of chickpea plays an important role to enhance soil fertility and increase yield of the succeeding crops in rotation (Jukanti et al., 2012). Chickpea is mainly cultivated in the arid and semiarid regions, but gradually the area of cultivation is expanding into other parts of the world including North America. Globally, the average yield of chickpea is 1.8 tonne/ha (Merga and Jema, 2019). At the same time, significant yield reduction also occurred due to adverse growing conditions like drought, disease, and cold temperature (Clarke and Siddique, 2004; Pande et al., 2005; Anbessa et al., 2006; Lobell, 2011). Despite the relatively low average global yield of chickpea, there is an opportunity to increase yield potential up to 5 tonnes/ha (Sudupak et al., 2002). Interspecific hybridization using wild species (*C. arietinum x C. reticulatum*) has a potential to increase the yield of chickpea (Jaiswal et al., 1986; Singh et al., 2015). Genetic improvement of chickpea is targeting traits including yield, abiotic and biotic stress resistances, above ground plant architecture (upright canopy), early flowering and maturing characteristics, nutritional, and processing qualities through different breeding strategies. Maintaining and increasing genetic diversity is important for crop adaptability in a changing environment. A population with high genetic diversity might allow the crop to adapt to substantially different environments. Generally, crop improvement is a continuous process of increasing crop adaptability, grain yield and nutritional content. To develop a new variety, breeders must explore the genetic diversity of cultivated chickpea. Low genetic diversity in the cultivated chickpea is due to frequent cultivation of limited varieties obtained from successive breeding (Robertson et al., 1997).

Farmers plant few varieties of the crop and those varieties have a high degree of genetic uniformity. This situation is quite different from the past practices in which farmers cultivated many locally adapted land races with potentially diverse genetic background. Overall, increased genetic variation is one of the important targets of successful crop improvement as it allows selection to increase or decrease the frequency of alleles in the population.

The genetic diversity of crops can be enhanced through introgression of desirable traits from their wild relatives. The wild species are valuable sources of genes for agronomic traits like early flowering, resistance to biotic and abiotic stresses, and yield potential that can be incorporated into cultivated genotypes (Harlan, 1976; McCouch, 2004; Dwivedi et al., 2008). Interspecific crosses have been implemented as a successful strategy for enhancing crop genetic diversity and yield by broadening the genetic base through transferring resistance genes and yield related alleles from the wild relatives to the cultivated species (Van Rheenen et al., 1993; Singh et al., 2015). *Cicer reticulatum* is considered to be part of the primary gene pool of chickpea, along with *Cicer arietinum*, and has the potential to increase genetic variability for seed yield (Jaiswal et al., 1986; Ahmad, 2005; Singh et al., 2015). *C. reticulatum* may possess useful genetic variation and have high cross-compatibility with *Cicer arietinum* and is being successfully used for introgression of desirable agronomic traits into the cultivated species (Collard et al., 2003; Sharma et al., 2013; Mason, 2016).

Genetic diversity is usually assessed by using different types of morphological and molecular markers (Sudupak et al., 2002; Singh et al., 2008; Aggarwal et al., 2011). Molecular markers have been widely used to determine the genetic variation and the relationship between cultivated crop species and their wild relatives (Gupta and Varshney, 2004). Recently Single Nucleotide Polymorphism (SNP) markers have been used for whole genome scans to reveal the natural

allelic diversity in chickpea (Varshney et al., 2013; Bajaj et al., 2015). The use of SNPs for Genome Wide Association Study (GWAS) is a promising approach for determining the population structure and genetic dissection of complex traits due its relatively low genotyping cost and high abundance in the plant genome (Jones et al., 2007). Association studies have been widely used to identify genomic regions associated with desirable traits and facilitate the discovery of candidate genes controlling the trait (Kujur et al., 2013). The overall objectives of this research were to evaluate the phenotypic and genetic variation of chickpea progeny derived from interspecific crosses between *C. arietinum* and *C. reticulatum,* and to establish the association between SNP markers and a series of important agronomic traits in chickpea.

1.1 Hypotheses

1. Lines with desirable agronomic traits (i.e., upright architecture, early flowering and commercially acceptable seed visual characteristics) will be identified from interspecific crosses between *C. arietinum* and *C. reticulatum*.
2. SNP markers associated with desirable agronomic traits (i.e., upright architecture, early flowering and commercially acceptable seed visual characteristics) will be identified through association analysis.

1.2 Objectives

1. To evaluate the phenotypic and genetic variations of chickpea lines derived from twenty interspecific crosses of *C. arietinum x C. reticulatum* for agronomic and morphological traits.
2. To assess the genome wide associations between agronomic traits with SNP markers.

2. REVIEW OF LITERATURE

Yield improvement is often considered a key breeding objective to ensure food security for the growing global population. The advancement of plant breeding and specific crop management activities has contributed to increase the productivity of major grain crops at a rate of about 0.8-1.2% per annum and it must continue to increase to fulfill future food demands (Li et al., 2018). However, some important issues such as crop adaptation to changing climate, disease and pest infestation, market acceptability, increased nutrient and water demand for high yielding crop varieties, and declining soil fertility need to be considered while developing a new crop variety. To address these underlying issues, more research attention should be given to develop varieties with novel agronomic traits that may improve yield.

On the contrary, several factors can restrict the yield improvement and breeding success. The low genetic diversity is one of them and might be limiting for the breeding opportunities of pulse crops (Abbo et al., 2003; von Wettberg et al., 2018). This situation can be overcome by reintroducing genetic diversity from related wild species. A much wider range of genetic diversity is carried by the wild relatives of crop species (Singh et al., 2008; Upadhyaya et al., 2008; Sharma et al., 2013). It is mainly due to their adaptability and existence in the natural environment without any human interference or without artificial selection for domestication. Wild species are a potential source of desirable traits including biotic and abiotic stress tolerance, improved nutritional quality and yield (Singh et al., 2008; Asif et al., 2013; Sharma et al., 2013; Man-Wah et al., 2016). Crop yield potential is determined by the genetic make up of the crop plant, therefore, genetic variation is a basic requirement in any crop improvement program. Plant breeders can depend on wild species to broaden the genetic bases of crop species.

2.1 Importance of chickpea

Chickpea plays an important role in the dietary requirement of people especially in the developing countries by ensuring alternate protein supply. Chickpea is a good and inexpensive source of protein, fiber and essential nutrients like iron (Jukanti et al., 2012; Merga and Jema, 2019). Food and nutritional security can be achieved with the optimum supply of affordable and nutritious food, which helps to eliminate hunger and all forms of malnutrition. As a legume, chickpea has a great influence on improving soil health by fixing atmospheric nitrogen. The use of chickpea as a rotational crop also helps to minimize disease and pest infestations.

2.2 Nutritional value of chickpea

Pulses are recommended for regular consumption to maintain good health. Usually, one cup of cooked chickpeas provides 268 calories, 14.5 g protein, 12.5 g fiber, and 0.2 mg of thiamin and vitamin B_6 (Wood and Grusak, 2007; Jukanti et al., 2012). As a plant-based protein source, chickpeas are beneficial to prevent chronic diseases like diabetes and cancer. They are consumed in many ways including as fresh immature seeds, fried, roasted or boiled whole seed, dal (split seeds without seed coat) and flour. The flour of chickpea is known as “besan” and is used for making gluten free cakes and other products. The green twigs and sprouted seeds are also consumed as a vegetable in the Asian region. Chickpea pod without seed and seed coats are also used as fodder for livestock (Ibrikci et al., 2003).

2.3 Beneficial effect on soil fertility improvement

Inadequate soil fertility has been identified as a major biophysical constraint for agricultural productivity. The indiscriminate use of chemical fertilizers is contributing to the decreasing inherent soil fertility (Austin et al., 2005). An integrated management approach is essential for conserving soil fertility. The use of grain legumes in cropping system is potential to increasing

nitrogen supply and microbial activity in soils. Chickpea improves soil quality through biological nitrogen fixation. The estimated amount of N fixed by well grown chickpea is about 60 kg/ha under cereal-chickpea cropping system in northern New South Wales (Unkovich and Pate, 2000). Research conducted in semiarid Canadian Prairies have reported to fix biological nitrogen by chickpea of about 106 kg N ha^{-1} (Hossain et al., 2017). By growing chickpea in crop rotation, the overall benefits are achieved from the reduced use of chemical nitrogenous fertilizers, which can minimize the adverse impacts on environment.

2.4 Origin, distribution and domestication of chickpea

In terms of area and production, chickpea (*Cicer arietinium* L.) is the worlds second most important pulse crops. It is an important source of essential nutrients that offers a range of health benefits. Chickpea is widely cultivated in about 57 countries including South and West Asia, China, North and South-East Africa, Australia, and North America (Croser et al., 2003; Warkentin et al., 2003; Knights et al., 2007; Merga and Jema, 2019). The productivity of chickpea is comparatively low in some developing countries, and the developed countries such as Australia, Canada, and Argentina are the major chickpea exporters around the world (Merga and Jema, 2019). Based on the observed diversity of chickpea and their wild relatives, the four regions including the Mediterranean, Central Asia, the Near East and India, and Ethiopia are considered as the centers of origin of chickpea (Vavilov, 1951). However, the historical evidence indicates that chickpea might have originated from southeastern Turkey (Ladizinsky and Adler, 1976; Roorkiwal et al., 2013). Later, chickpeas spread out to the distant part of the west including Mediterranean basin and south towards the Indian subcontinent via Silk Route (Singh, 1997). Increased production and consumption of chickpeas are mostly observed in tropical and subtropical areas (India, Middle East, and Mediterranean region).

Generally, two main types of chickpea i.e., *Kabuli* and *Desi* are used for commercial production globally (Malhotra et al., 1987; Singh et al., 2008; Sharma et al., 2013). *C. reticulatum* is the progenitor species of chickpea (Singh and Ocampo, 1997). The desi type seeds are mostly identical with *C. reticulatum*, and it easily explains the early domestication process (Mallikarjuna et al., 2007; Sharma et al., 2013; Singh et al., 2018). The desi type seeds are small, angular shaped, and dark in color with a rough surface. About 80-85% of the world's chickpea production area were desi type cultivation (Gaur et al., 2008). The desi type is mostly cultivated in the Indian subcontinent, Ethiopia, and Iran. It is usually processed by dehulling, consumed in split form (chana dal) or as flour (besan). In south Asia, the split form is used for curry preparation, while besan is used for baking purposes. The whole desi type chickpeas are also consumed as snacks in the Indian sub-continent. On contrary, the kabuli type is considered as a subsequently derived type of chickpea, which has a distinct genetic make-up than the wild progenitors (Pundir et al., 1985; Agarwal et al., 2012). The kabuli seeds are typically bigger than desi, round to ram-head shape with white or cream colour and have thin seed coat (Singh et al., 2008). The kabuli type is generally grown in the Mediterranean region, southern Europe, western Asia and northern Africa (Singh et al., 2008). In North America, most kabuli chickpea is consumed as salads, while in the Middle East it is consumed as hummus and falafels. Nowadays, hummus is popular in North America and it is available in most grocery stores. The kabuli type is marketed as canned whole seeds, dry seeds or as ground flour. Overall, there is a visible geographic boundary in domestication of each chickpea type, and production is largely controlled by local markets due to separate use, or consumption preferences.

2.5 Taxonomy and growth habit of chickpea

All the cultivated and wild species of chickpeas are taxonomically grouped under the genus *Cicer*, which belongs to the family Leguminosae, subfamily Papilionoideae, and tribe *Cicereae* (Kupicha, 1981; Van der Maesen et al., 2007). Chickpea is an herbaceous annual crop widely grown in subtropics or in the tropics during winter season. Plants are about 20 cm to 1 m tall and the stems are branched (Muehlbauer and Tullu, 1997). Different branches including primary, secondary and tertiary branching characteristics are related to the growth habit of chickpea. For example, a semi-erect variety produces fewer branches compared to semi-spreading type varieties (Paul and Ming, 2008). Chickpea plants exhibits different growth habits including erect, semi-erect, semi-spreading, spreading and flat or prostrate types growth. The root system of chickpea is comprised of a strong taproot system along with several rows of lateral roots. Therefore, chickpea is able to utilize nutrients and moisture from deeper soil zone. The lateral roots also produce symbiotic nodules with *Rhizobium* bacteria and fix atmospheric nitrogen for their growth and development. Usually chickpea leaves are glandular-pubescent and imparipinnate with serrated leaflets (Muehlbauer and Tullu, 1997). The entire plant including leaves, flowers and pods are covered with densely fine hairs known as trichomes. These glandular hairs can secrete malic acid and oxalic acids that helps to protect the plants from insect-pest infestation (Yoshida et al., 1997; Sharma et al., 2005; Narayanamma et al., 2007). The flowers of chickpea are pink, white, purplish or blue in colour (Taylor and Ford, 2007). Flowers are solitary and usually occur in an axillary raceme. Chickpea pods are oval shape and usually contain 1 to 2 seeds per pod. The seeds are rounded or angular in shape with rough or smooth seed surface. The seed characteristics are mostly related to the chickpea types.

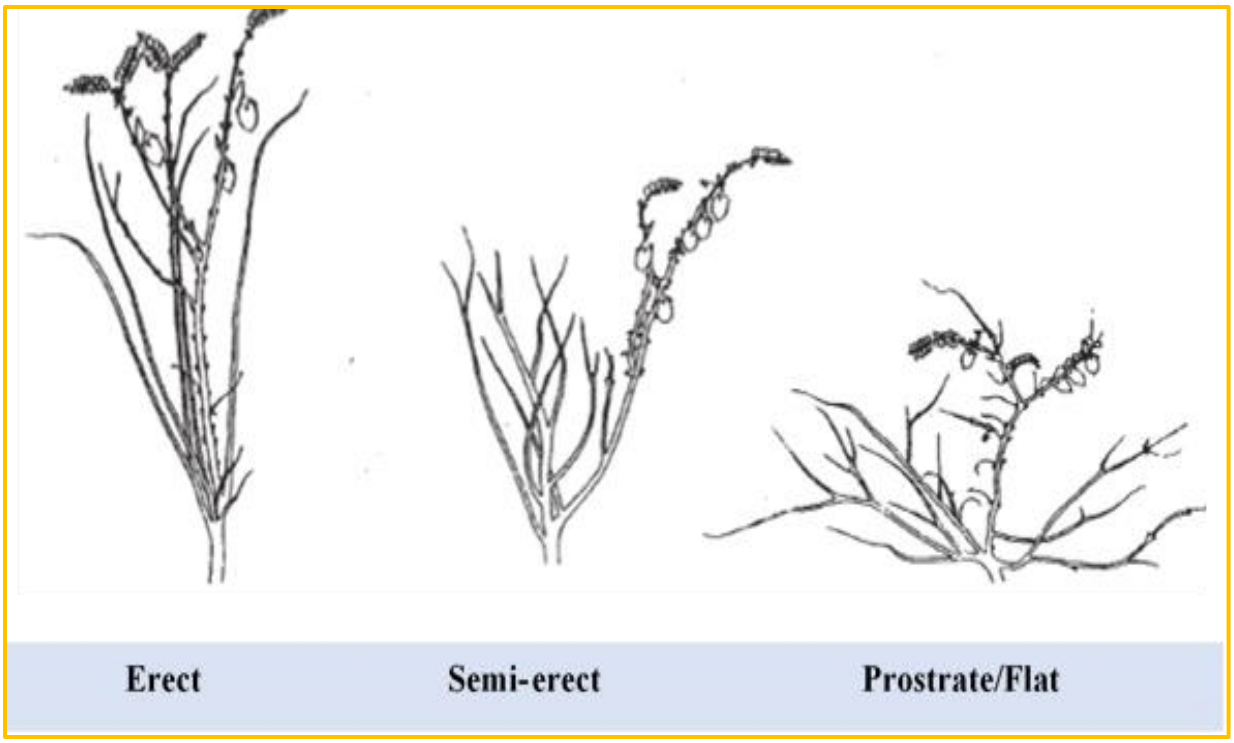


Figure 2.1. Growth habit of chickpea **(**Source: Guidelines for the conduct of test for Distinctiveness, Uniformity, and Stability on chickpea (*Cicer arietinum L.*), Protection of Plant Varieties and Farmer's Rights Authority, India).

2.6 Physiological requirements for chickpea cultivation

Chickpea is usually grown as a rainfed cool-weather crop or as a dry climate crop in semi-arid regions (Muehlbauer and Tullu, 1997). The physiological growth and development of chickpea is greatly affected by different abiotic stresses including salinity, water logging, and high or freezing temperature. Chickpea is fairly drought tolerant crop as the taproot system helps to extract water from deeper layers of soil. Relatively cooler climate and low rainfall in semi tropical regions is suitable for chickpea cultivation. The kabuli type chickpea is mostly grown in temperate regions, whereas the desi type is grown in the semi-arid tropics (Malhotra et al., 1987; Muehlbauer and Singh, 1987; Gaur et al., 2008; Singh et al., 2008). Chickpea can tolerate high temperatures during flowering stage, but chilling temperature or frost can cause severe damage

to bud and pod formation. The temperature between 5 and 15 ˚C is suitable for germination of chickpea. Both low (<15˚C) and high (<15˚C) temperature has been reported to cause substantial yield reduction of chickpea (Siddique et al., 1999; Rani et al., 2020). Similarly, the unexpected change in growing season temperature such as below 15˚C or above 35 ˚C may cause significant yield reduction due to the loss of pollen viability and possible flower abortion of field pea (Jiang et al., 2019). A recent study conducted at the University of Saskatchewan reported that there is significant temperature effect on flowering response of different photoperiod sensitive chickpea accessions (Daba et al., 2016b). Overall, the best performance of chickpea is observed with a daytime temperature of 18-25˚C and night temperatures of 17-21˚C (Duke, 1981; Muehlbauer and Singh, 1987; Anbessa and Bejiga, 2002). Other environmental factors like high annual rainfall may cause prolong vegetative growth along with increase disease susceptibility and stem lodging problems in chickpea. A well distributed annual rainfall ranging from 600-1000 mm is suitable for optimum yield and quality seed production (Duke, 1981; Anbessa and Bejiga, 2002).

2.7 Present status of chickpea production in Canada

About 95% of the world chickpea crop is produced and consumed in the tropical and sub-tropical continents (Kassie et al., 2009). In recent years, the chickpea production area has increased throughout the world including in the western Asia, Australia, and in the Northern Great Plains of North America (Merga and Jema, 2019). The majority of chickpea produced in Canada is for export.

The pulse production area in Canada has increased rapidly from 2.1 to 4.2 million hectares between 2011 to 2016 (Statistics Canada, 2017). The soil, climatic conditions of western Canada are favorable for pulse production. The pulse crops including chickpeas are a vital component of the cereal-based rotation practiced on the prairies. The Brown and Dark Brown soil zones are

favorable for chickpea production. These areas are comparatively drier than the northern regions of Canada, therefore, well suited for chickpea production. Long sunny days and well distributed rainfall throughout the growing season are favorable for chickpea production in Saskatchewan and Alberta. Among the Prairie provinces, Saskatchewan has the largest production area for growing different pulse crops. The total cropland in Saskatchewan is over 14.7 million ha (Statistics Canada, 2011). In 2016, the total pulse growing area of Saskatchewan was about 1.7 million ha (Statistics Canada, 2011). Further, the majority of chickpea production area of Canada (149,248 ha) is located in Saskatchewan (Government of Saskatchewan). Overall, the chickpea production trends are increasing in Saskatchewan. Several factors including susceptibility of chickpeas to ascochyta blight disease and low temperature in early fall are identified as limiting factors for chickpea production in Saskatchewan. Therefore, the successful production of chickpea in Saskatchewan requires improved varieties with the characteristics of early maturity and ascochyta blight resistance capabilities.

2.8 Factors affecting chickpea production

Around the world, chickpea is growing in a wide range of agroclimatic conditions. Depending on the variable climatic conditions several biotic and abiotic factors which are affecting the crop growth and development of chickpea are already identified (Chongo et al., 2003; Frimpong et al., 2009; Cobos et al., 2016; Tadesse et al., 2017). Some of the major factors are rainfall, soil and air temperature, disease, and insect infestation (Anbessa et al., 2006; Singh et al., 2008). Chickpea has many economic importance, but the productivity is very low. In most circumstances, the average yield of chickpea is about half their genetic yield potential. The low yield is mainly from the negative effect of abiotic and biotic stresses. For example, chickpea is grown as a post-rainy season crop in the tropics and subtropics where the terminal drought and heat stress are major abiotic factors responsible for yield reduction. Terminal drought can cause about 40-50% of the

average yield loss of chickpea (Ahmad et al., 2005; Varshney et al., 2010). Some of the negative impacts of abiotic and biotic stresses are discussed below.

2.8.1 Biotic stress

The most common biotic stress of chickpea is disease and pest infestations that result in significant yield reduction. Among the diseases, *Ascochyta* blight, *Rhizoctonia* root rot, *Macrophomina phaseolina* dry root rot, fusarium wilt, grey mold and bacterial blight are frequently observed in chickpea. However, *Ascochyta* blight is the most frequent and serious disease of chickpea commonly observed in West and central Asia, North Africa, Australia and North America (Gossen and Miller, 2004; Bhardwaj et al., 2010; Harveson et al., 2011). Depending on the weather conditions the pathogen can destroy chickpea crop and cause complete yield loss (Udupa and Baum, 2003; Chongo et al., 2003; Tadesse et al., 2017). It is a seedborne disease and very aggressive to chickpea compared to other pulse crops. Pathogen can survive on infested crop residue and cause brown spots on leaves, stems, pods and seeds of chickpea. These biotic constraints are often controlled by following proper crop rotation, using quality seed and disease resistant varieties (Spencer et al., 2005). Several accessions with improved resistance to *Ascochyta* blight have been identified for effective use in variety improvement (Pande et al., 2011). The presence of *Ascochyta* blight resistance gene in cultivated germplasm of chickpea is very limited. Successful efforts have been made earlier to identity the resource of *Ascochyta* blight resistance in chickpea (Rubiales and Fondevilla, 2012). For examples, the wild species *C. bijugum* and *C. pinnatifidum* have resistance to *Ascochyta* blight and some other diseases like fusarium wilt and cyst nematode (Singh et al., 1998; Ahmad et al., 2005). Although, the application of chemical fungicides could help in controlling the diseases, the frequent use of fungicides is harmful for the ecosystem and for human health. On contrary,

the disease resistant variety provides an opportunity to maintain a sustainable agricultural production system.

Further, it is also necessary to develop insect-resistant cultivars to reduce the dependence on pesticides and safeguarding crop productivity. Some common insects of chickpea are pod borer (*Helicoverpa armigera*), cutworms (*Agrotis* sp.), chickpea leaf miner (*Liriomyza cicerina*), and pea aphid (*Acyrthsosiphon pisum).* The most harmful pest of chickpea is pod borer (*Helicoverpa armigera*) which mainly feeds on young leaves, reproductive structures and developing seeds (Abbasi et al., 2007; Singh et al., 2008). Major economic losses are observed with the insect attack at flowering and pod formation stages. The severity of the disease and insect infestation are aggravated by favorable weather conditions. Apart from chemical and biological control strategies, the host plant resistance and insect resistance transgenic crops are used effectively to control insect infestation in chickpea. It is evident that the wild germplasm shows some levels of resistance to *Helicoverpa armigera* (Sharma et al., 2005). Some wild relatives of chickpea such as *C. bijugum, C. judaicum* and *C. reticulatum* are considered as highly resistance to multiple stresses (Gaur et al., 2008; Sharma et al., 2013). Therefore, plant breeders are trying to use wild species as the potential sources for the development of disease and pest resistant chickpea varieties.

2.8.2 Abiotic stress

The major abiotic stresses that adversely affect the growth and yield of chickpea are drought, cold and soil salinity (Mittler, 2006; Krishnamurthy et al., 2010). Among these abiotic stresses drought is the most severe problem in warm Mediterranean and low rainfall growing areas (Berger et al., 2005; Millan et al., 2006). On the Canadian Prairies, the extended cool winter, increasing summer temperature, and low rainfall during spring and summer are the common

abiotic stresses to successful crop production (Ahmad et al., 2012; Bueckert and Clarke, 2013; Pang et al., 2017). The range of yield loss of chickpea due to drought is high, which could reach 40 to 50% in some instances (Ahmad et al., 2012; Kaloki et al., 2019). More attention is needed for the development of early maturing and drought resistant chickpea varieties (Gaur et al., 2008). Improved varieties that are efficient in utilization of available water resources are in demand for rainfed agriculture. Conventional breeding efforts have successfully introgressed genes from wild relatives to the cultivated species for improvement of desired agronomic traits (Jaiswal et al., 1986; Singh et al., 2018). For example, a variety developed from the crosses between cultivated chickpea and wild species at ICRISAT is known to have wide adaptability and improved drought tolerance (Yadav et al., 2004).

2.9 Genetic improvement in chickpea

Globally due to the increase in population, climatic change, and rapid depletion of natural resources the need for foods, fuels and fibres are increasing. To provide food for the growing population there is a need for exploration of innovative ideas for crop production. In recent years, the combined use of conventional and advanced molecular approaches for genetic manipulation allowed improvement of agronomic traits to increase crop productivity (Azhaguvel et al., 2006; Abbo et al., 2014; Basu et al., 2018). The shifting in environmental patterns like uneven rainfall distribution throughout growing seasons, and several biotic and abiotic stresses are some of the major constraints to achieve higher crop productivity. The global productivity of grain legumes in general is not satisfactory, and there is a potential scope of yield improvement. Compared to other legumes, the average yield of chickpea is relatively low. Chickpeas are important for the developing countries where the rate of consumption is high (Jukanti et al.,

2012; Merga and Jema, 2019). Therefore, it is very important to increase the crop productivity to meet the requirement of future population.

The productivity of the existing crop varieties is usually affected by several factors including harsh climate, disease-pest infestation, and genetic factors (Lone et al., 2017; Kaloki et al., 2019). Improving crop varieties with desired agronomic traits is the primary goal of crop improvement program. A newly developed variety could be resistant to existing biotic and abiotic stresses along with increased yield potential. In recent years, the consideration of mechanized agriculture such as harvestability has become a major goal in chickpea breeding program. Several initiatives aimed at developing chickpea varieties with desired agronomic traits with some focus on regional problem (Qureshi et al., 2004; Ahmad et al., 2012; Singh et al., 2015; Pang et al., 2017). However, the yield improvement is still unsatisfactory. For example, the global productivity of chickpeas is about 1.8 tonnes/ha (Merga and Jema, 2019) whereas the estimated yield potential in some other countries is more than 2.0 tonnes/ha (Merga and Jema, 2019). Therefore, the major focus of chickpea improvement program includes desired agronomic traits along with increased yields.

Several breeding methods such as mass selection, pure line selection and wide hybridization were successfully used for the improvement of self-pollinated crops like chickpea. Chickpea production area has recently expanded in developed countries where the production system is fully mechanized. Some agronomic traits such as lodging resistance, height of pod setting, and upright growth habit are known to have greater influence on harvestability of chickpeas (Anbessa et al., 2007; Zohary et al., 2012). Although the harvesting loss of chickpea are not common, the plant architecture has a major influence on improving harvest efficiency. Harvestability is considered as a desired agronomic trait for the mechanized agriculture like in

Canada (Gan et al., 2003). The early maturity of chickpea is also desirable for Canadian farming to allow the crop to mature before the temperature drops in the early fall season. Other traits of interest include resistance to disease and pest, increased quality with high and stable yields.

2.10 Genetic diversity in chickpea

Variation in a trait can be identified by studying the genetic diversity within and between the crop species including their wild relatives (Singh et al., 2008; Sharma et al., 2013). Conventional tools and methodologies used in plant breeding have been very useful for improving crop varieties. Natural genetic variability within the crop species has been used in early plant breeding program for crop improvement. Nowadays, the availability of genomic tools and resources helps to study the genotypes and establish their relationships with the phenotypes (Mallikarjuna et al., 2007; Varshney et al., 2013). Genomic tools are being used to study the gene expression which provided useful biological information for plant breeding. Relationships among *Cicer* species have been assessed using morphological, biochemical, and molecular markers (Singh and Ocampo, 1993; Croser et al., 2003; Mallikarjuna et al., 2007; Sharma et al., 2013; Singh et al., 2018). Compared to different markers, evaluation of crop genotypes based on morphological traits are direct and inexpensive, but these traits may depend on environment. Molecular markers are useful tools to overcome the environmental dependency and provide precise measures of genetic diversity of crop species (Varshney et al., 2007; Varshney and Dubey, 2009; Glaszmann et al., 2010; Caruana et al., 2019).

However, the majority of chickpea improvement program are still using the conventional approaches like interspecific crossing between the cultivated and its wild species (Singh et al., 2008; Sharma et al., 2013). It is mainly due to the limited genetic diversity in the cultivated chickpea species (Abbo et al., 2003; von Wettberg et al., 2018). The responsible genes for some

of the desired agronomic traits like disease and pest resistance capability have been identified in wild germplasm of *Cicer reticulatum*. Several research (Singh et al., 1998; Ahmad et al., 2005; Govindaraj et al., 2015; Zhang et al., 2016) reported that *Cicer reticulatum* is tolerant to fusarium wilt and ascochyta blight disease. Thus, the use of wild species like *Cicer reticulatum* in chickpea hybridization is an effective approach. Moreover, considering the similarities of annual growth habit and genetic makeup of *Cicer reticulatum* and cultivated species, the conventional crossing has helped to increase the genetic diversity of chickpeas (Singh and Jana, 1993; Singh et al., 2008; Sharma et al., 2013). For development of improved variety, maintenance of genetic diversity of chickpeas is highly important.

In breeding programs, advanced molecular markers technology is being used by the geneticist for making efficient selection of genotypes (Singh et al., 2008; Lammerts et al., 2010; Varshney et al., 2013; Kantar et al., 2017). Molecular analysis combined with phenotypic assessment resulted in more accurate differentiation among the genotypes (Castro et al., 2011). The use molecular marker improves our understanding about a specific trait and helps to recognise the genes controlling the expression of these traits. The QTL analysis helps to identify the location of the genes or specific alleles in the genome**.** In addition, there is a possibility of using the marker assisted selection for introgression of identified genes into a desired cultivar (Singh et al., 2008). The commonly used genetic markers are Restriction Fragment Length Polymorphism (RFLPs), Amplified Fragment Length Polymorphism (AFLPs), Random Amplified Polymorphic DNAs (RAPDs), Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphism (SNP) (Upadhyaya et al., 2008; Aggarwal et al., 2011; Yu et al., 2011; Varshney et al., 2013; Bajaj et al., 2015; Kantar et al., 2017). For crop improvement program, the availability of the sequence

based genotyping methods can be used to establish marker-trait association, purity testing, genetic mapping and genomic selection (Varshney et al., 2014; Bajaj et al., 2015).

Breeders have made tremendous success in crop improvement through the combined use of conventional and molecular marker-assisted breeding. The recent advances in molecular genetics have opened the door for the plant breeder to speed up crop improvement (Varshney et al., 2007; Caruana et al., 2019). Molecular markers offer primary information for making genomic selection that is essential for successful breeding (Bajaj et al., 2015). It helps in proper selection of various yield contributing traits. The application of molecular marker mainly deals with the variations in DNA sequence for a specific trait and introduce new traits into the cultivated species (Singh et al., 2015; Long et al., 2019). Single nucleotide polymorphisms (SNPs) are highly preferred in plant genetic and genome analysis due to their abundance in the genome and amenability for various molecular genetic applications (Varshney et al., 2007; Caruana et al., 2019; Long et al., 2019).

Genome-wide SNP discovery is helpful to develop high density genetic and physical map and to study the genome-wide trait association (Barchi et al., 2011; Chutimanitsakun et al., 2011; Davey et al., 2011; Pfender et al., 2011; Yu et al., 2011). Recently, the high throughput SNP genotyping approaches using Illumina Golden Gate assay/Infinium (Bead Xpress array) and competitive allele specific PCR (KASPar) have been used in different chickpea accessions for whole-genome sequencing to identify the genomic regions and genes underlying plant stress responses (Varshney et al., 2013; Bajaj et al., 2015). The use of genetic markers increases the value and importance of genetic information for chickpea. About 3000 SNPs have been exploited to study the evolution and genetic diversity of chickpea (Gaur et al., 2012; Hiremath et al., 2012; Stephens et al., 2014).

2.11 Use of wild species in chickpea breeding

The wild species are a significant source of useful traits for contributing to variety improvement. The annual wild *Cicer* are potentially useful for breeders as they possess some desirable agronomic traits (Singh and Ocampo, 1997; Upadhyaya et al., 2008; Govindaraj et al., 2015; Zhang et al., 2016). Yield of cultivated chickpea has been improved through the introgression of desirable genes from *C. reticulatum* (Singh and Ocampo, 1997; Mallikarjuna et al., 2007; Zohary et al., 2012; Singh et al., 2018). The low seed yields of chickpea are attributed to low biomass production (Bidyarani et al., 2016). It is possible to increase the biomass production by crossing the cultivated species with the wild germplasm. The increase in biomass and plant vigour improvement were achieved by crossing of *C. reticulatum* and *C. echinospermum* with the *C. arietinum* (Zohary et al., 2012; Singh et al., 2018). The success of any breeding programme largely depends on the wealth of the genetic resources that can provide essential raw materials for crop improvement. The collection and use of wild annual and perennial species could be used to increase the genetic base of chickpeas.

Drought and frost are the major abiotic stresses for chickpea production in Canadian prairies. Early flowering genotypes are likely to extend the reproductive phase and can show early maturity characteristics. This could help to minimize the adverse effects of these stresses on yield of chickpea. The flowering time of chickpea is largely influenced by several factors including cropping season, temperature, and photoperiod (Anbessa et al., 2006; Craufurd and Wheeler, 2009). Some tremendous research efforts have been made by CDC, Saskatoon to manipulate flowering time of chickpea (Daba et al., 2016; Daba el al., 2016a; Ridge et al., 2017). In addition, the chickpea varieties released by CDC, Saskatoon are partially resistant to

ascochyta blight disease (Vandenberg et al., 2003; Warkentin et al., 2005; Tar'an et al., 2009), which is a major problem of chickpea production on the

Prairies. Early flowering in combination with upright growth habit, photoperiod insensitivity, ascochyta resistance and high yielding variety development are in priority of chickpea improvement program. By using the wild *Cicer* species in conventional breeding it is expected to develop new genotypes with desired agronomic traits (i.e., disease resistance, early maturity, upright growth habit and increased yield potential, and improve nutritional quality of chickpea).

2.12 Influence of genotype by environment interaction on the performance of chickpea

The phenotypic expression is a function of the genotype and influenced by environmental variation. Integrated analyses of genotype by environment interaction is mandatory during crop improvement to evaluate relative stability of new genotypes or varieties (Leon et al., 2016). Typically, yield performance evaluation trials are conducted over several locations and/or years with adequate replications. Similar research on evaluation of interspecific chickpea germplasms is known to identify the genetic basis of differential phenotypic expression under various environmental conditions (Singh et al., 2008; Sharma et al., 2013). It is well-known that the yield and yield contributing traits are controlled by numerous genes (Varshney et al., 2013). Therefore, a substantial amount of information obtained by genotype-environment interaction could be helpful in identifying the stable genotypes and potential genes controlling important agronomic traits. The recent advancement of molecular breeding provides an opportunity to identify the specific gene responsible for phenotypic variations in varying environmental conditions (Kashiwagi et al., 2008; Varshney et al., 2014). Overall, there is a need for meaningful assessment on genotype-environment interactions for successful breeding and further improvement of chickpea.

2.13 Implications of genotyping by sequencing in chickpea breeding

Genotyping-by-sequencing (GBS) has been used to perform studies ranging from SNP marker development to whole genome profiling in many crops including chickpea (Varshney et al., 2013; Verma et al., 2015). In breeding programs, the genetic variations tagged by DNA polymorphisms were used to improve the crop performance. The GBS is being used increasingly in crop genomic studies as it can identify the DNA sequence polymorphisms for a desirable trait (Poland and Rife, 2012; Long et al., 2019). Although phenotypic characterization is possible based on the visible or measurable traits, molecular markers facilitate genetic diversity assessment at the DNA level (Singh et al., 2008). In recent years, sequence-based molecular markers (SNPs) from GBS analysis have been used for genetic diversity, population structure, and genome-wide-association studies in chickpea (Varshney et al., 2013; Bajaj et al., 2015). Additionally, the advanced genotyping approach (tunable Genotyping By Sequencing or tGBS) is known to exhibits better performance due to its high SNPs calling accuracy compared to GBS (Ott et al., 2017). The tGBS method provides the opportunity for adjusting the number of targeted sites and uses single stranded oligos instead of double stranded adaptors used in GBS for amplification and sequencing of double digested DNA fragments. The oligos could be prepared and quantified simply as compared to the adaptors that enhances the reliability of tGBS library preparation. Therefore, application of tGBS in large-scale genotyping and validation of SNPs at genome wide level signifies its applicability in genomics-assisted breeding for chickpea improvement.

2.14 Accessing genetic diversity for chickpea improvement

The scarcity of genetically diverse germplasms with high yield potential and susceptibility to biotic and abiotic stresses are hindering the breeding progress of chickpea (Varshney et al., 2013;

Kantar et al., 2017). To improve the narrow genetic base of the cultivated chickpea, the utilization of wild *Cicer* species in breeding programs and introgression of economically important alleles are likely the ultimate options (Srivastava et al., 2016; Singh et al., 2018). Recently, chickpea breeding activities have made a considerable progress in accumulation of suitable alleles related to yield and quality parameters (Sharma et al., 2013; Belete et al., 2017; Singh et al., 2018). Overall, the enhancement of genetic diversity through interspecific hybridization between the wild and cultivated germplasms of chickpea could help to develop diverse high performing germplasms.

The collection and evaluation of wild annual chickpea indicates that the crossing between wild and cultivated accessions can results in potential variation for yield, agronomic, and quality traits (von Wettberg et al., 2018). These variations can be utilized to improve the adaptive strategies as well as widening the genetic diversity in cultivated chickpea for future variety development (Siddique et al., 2000; Varshney et al, 2013; Singh et al., 2015). Introducing the desired alleles from the wild accessions into the cultivated germplasms, and the use of genotyping by sequencing approach are likely to have a great potential for improving yield and adaptive quality traits.

3. MATERIALS AND METHODS

3.1 Sources of germplasm

The research was conducted at the University of Saskatchewan as a part of the chickpea improvement program in which a total of 20 accessions of *C. reticulatum* were used in crossing to develop the interspecific progeny of chickpea (Table 3.1). The wild species parents were part of the project of Chickpea Innovation Lab led by Dr. Doug Cook at the University of California, Davis, USA. The wild germplasms were collected from diverse geographical locations in Turkey (Figure 3.1), which varied significantly in terms of phenology, and resistance to biotic and abiotic stresses. It is well known that Turkey is the primary centre of origin of chickpea and a source of closely related annual wild species (van der Maesen, 1987). The diversity observed in the wild germplasm is related to the variation in soil texture, nutrient status, rainfall, temperature, and humidity that varied widely along the altitudinal gradients (von Wettberg et al., 2018). In 2013, about 60,000 km^2 area of Turkey were explored using a bioclimatic model that represented the locations of the diverse wild chickpea accessions (von Wettberg et al., 2018). A large variation in elevational gradient was found to be associated with the diversity of wild chickpea, and it was considered to select those locations for germplasm collection (Figure 3.1). The wild accessions of *C. reticulatum* has purple flower, and seeds with distinct shapes and colours such as Bari1-pink circle; Bari2-pink star; Bari-pink diamond; Beşev-black triangle; Derei-black diamond; Sarik-black square; Savur-black circle; Egill-red triangle; Kalka-red circle; Kayat-black star; Kesen-light blue star; Oyali-orange circle; Cudi A/B-royal blue circle; Şirnak-royal blue square (von Wettberg et al., 2018). Whereas, the cultivated variety CDC Leader used as a parent in this study is a kabuli type chickpea having high-yield potential, white flower colour, and seeds are light cream-beige color with typical ram-head shape. This variety can

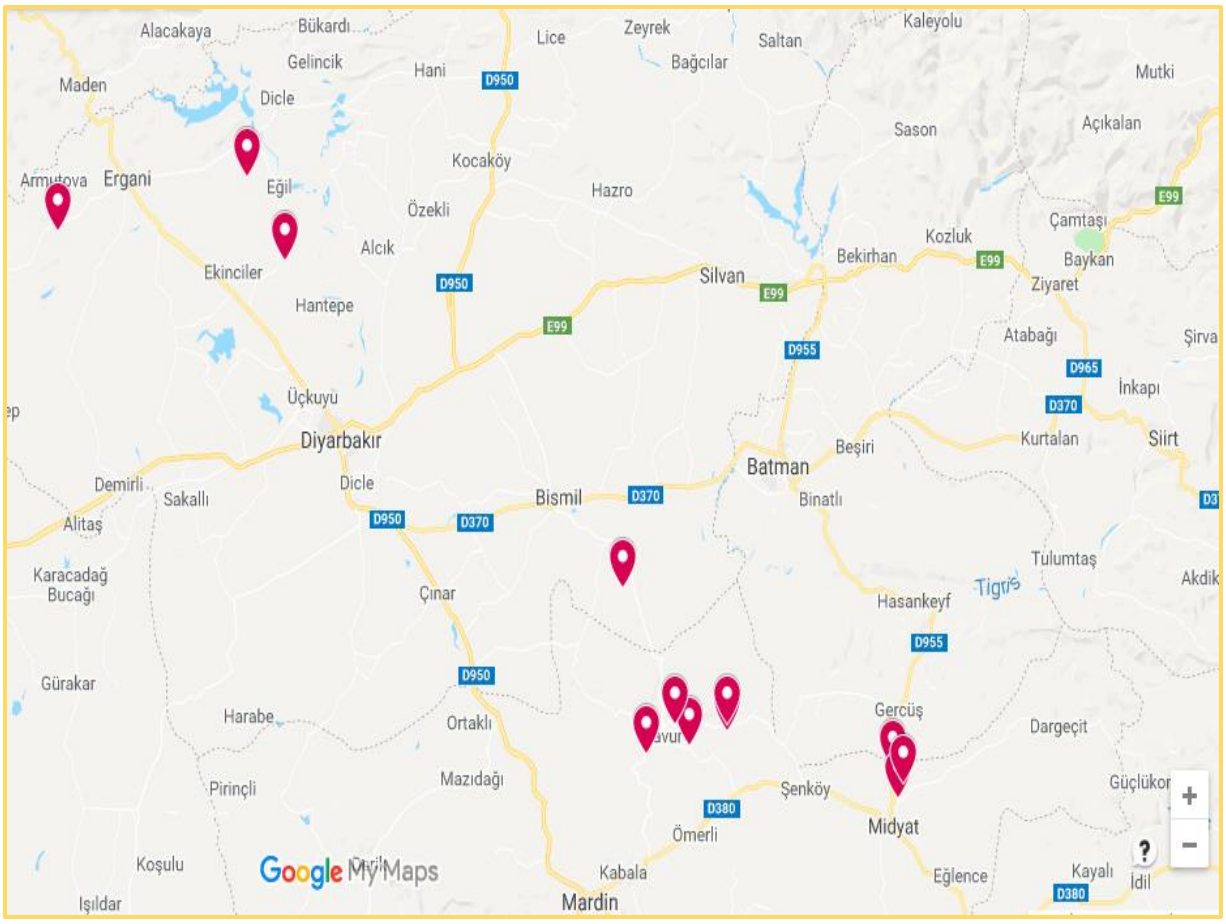


Figure 3.1. The centre of origin of chickpeas in Turkey. The wild parents (*C. reticulatum* L.) were collected from locations indicated in red after critical evaluation by the collaborative research group at UC-Davis, California. Multiple parents were collected from some of the locations with different elevational gradients.

attain 42 cm of plant height, has a medium maturity, and moderate resistance to ascochyta blight disease.

3.2 Development of chickpea lines from interspecific crosses of *C. arietinum x C. reticulatum*

In summer 2014, initial crosses between the adapted cultivar (CDC Leader) and 20 wild accessions of *C. reticulatum* (Table 3.1) were performed under greenhouse conditions by the chickpea breeding group led by Dr. Bunyamin Tar'an at the University of Saskatchewan. The F_1 seeds were grown and the plants were cloned by stem cuttings (Danehloueipour et al., 2006). The harvested F_2 seeds of approximately 400 seeds per population were screened for homozygous cultivated alleles at flowering time (FT). At this stage, screening was mainly performed to normalize the phenology of the breeding population for flowering time trait. The KASP (Kompetitive allele specific PCR) SNP marker and scratch cotyledon technique for DNA extraction developed in our lab (Pulse Crop Breeding Laboratory) were used in an assay to identify the homozygous alleles. The seeds with homozygous cultivated alleles at FT locus were selected and grown. After selection of lines for homozygous FT alleles, intrapopulation crosses were performed to increase the diversity. Plants with white flower and purple flower within each population were intercrossed to develop approximately 1,000 F_2 derived lines. All these crossing and population development were performed by Pulse Crop Breeding Group. The first generation from the intrapopulation crosses was designated as F_1. After that, phenotypic evaluation was performed in the F_3 generation (Figure 3.2).

Table 3.1. Selected parents of chickpea used for interspecies hybridization.

Genotypes	Locations	GPS coordinates	Altitude (m)	Climate (*Koppen Geiger class, AT and P)
Cicer arietinum (one cultivated parent from Canada)				
CDC Leader	Saskatoon			
Cicer reticulatum (twenty wild parents from Turkey)				
Bari1_092	Baristepe	37.49° N, 41.37° E	975.04	Csa, AT=16.6 °C, P= 648 mm
Bari2_072	Baristepe	37.45° N, 41.38° E	958.62	Csa, AT=16.6 °C, P= 648 mm
Bari3_072C	Baristepe	37.47° N, 41.39° E	962.92	Csa, AT=16.6 °C, P= 648 mm
Bari3_100	Baristepe	37.47° N, 41.39° E	962.92	Csa, AT=16.6 °C, P= 648 mm
Bari3_106D	Baristepe	37.47° N, 41.39° E	962.92	Csa, AT=16.6 °C, P= 648 mm
Besev_075	Besevler	37.51° N, 40.85° E	899.97	Dsa, AT=10.3 °C, P= 974 mm
Besev_079	Besevler	37.51° N, 40.85° E	899.97	Dsa, AT=10.3 °C, P= 974 mm
CudiA_152	Cudi	37.42° N, 42.50° E	1343.82	Csa, AT=18.7 °C, P= 724 mm
CudiB_022C	Cudi	37.43° N, 42.49° E	1286.82	Csa, AT=18.7 °C, P= 724 mm
Derei_070	Dereici	37.54° N, 41.02° E	993.81	Csa, AT=16.5 °C, P= 624 mm
Derei_072	Dereici	37.54° N, 41.02° E	993.81	Csa, AT=16.5 °C, P= 624 mm
Egill_073	Egil	38.27° N, 40.01° E	986.21	Csa, AT=16.6 °C, P= 648 mm
Egill_065	Egil	38.27° N, 40.01° E	986.21	Csa, AT=16.6 °C, P= 648 mm
Kalka_064	Kalkan	38.16° N, 40.09° E	840.48	Csa, AT=18.2 °C, P= 926 mm
Kayat_077	Kayatepe	37.52° N, 40.94° E	1083.17	Csa, AT=14.1°C, P= 695 mm
Kesen_075	Kesentas	38.20° N, 39.61° E	875.49	Csa, AT=15.3 °C, P= 626 mm
Oyali_084	Oyali	37.73° N, 40.80° E	914.02	Csa, AT=12.5 °C, P= 586 mm
Sarik_067	Sarikaya	37.55° N, 41.02° E	1005.10	Csa, AT=9.0 °C, P= 538 mm
Savur_063	Savur	37.55° N, 40.91° E	917.07	Csa, AT=16.5 °C, P= 624 mm
Sirna_060	Sirnak	37.54° N, 42.45° E	1658.92	Csa, AT=16.2 °C, P= 767 mm

Notes: *= Koppen Geiger climatic class, AT= Average Temperature, P= Precipitation, Csa= Hot-summer Mediterranean climate, Dsa= Mediterranean-influenced hot-summer humid continental climate.

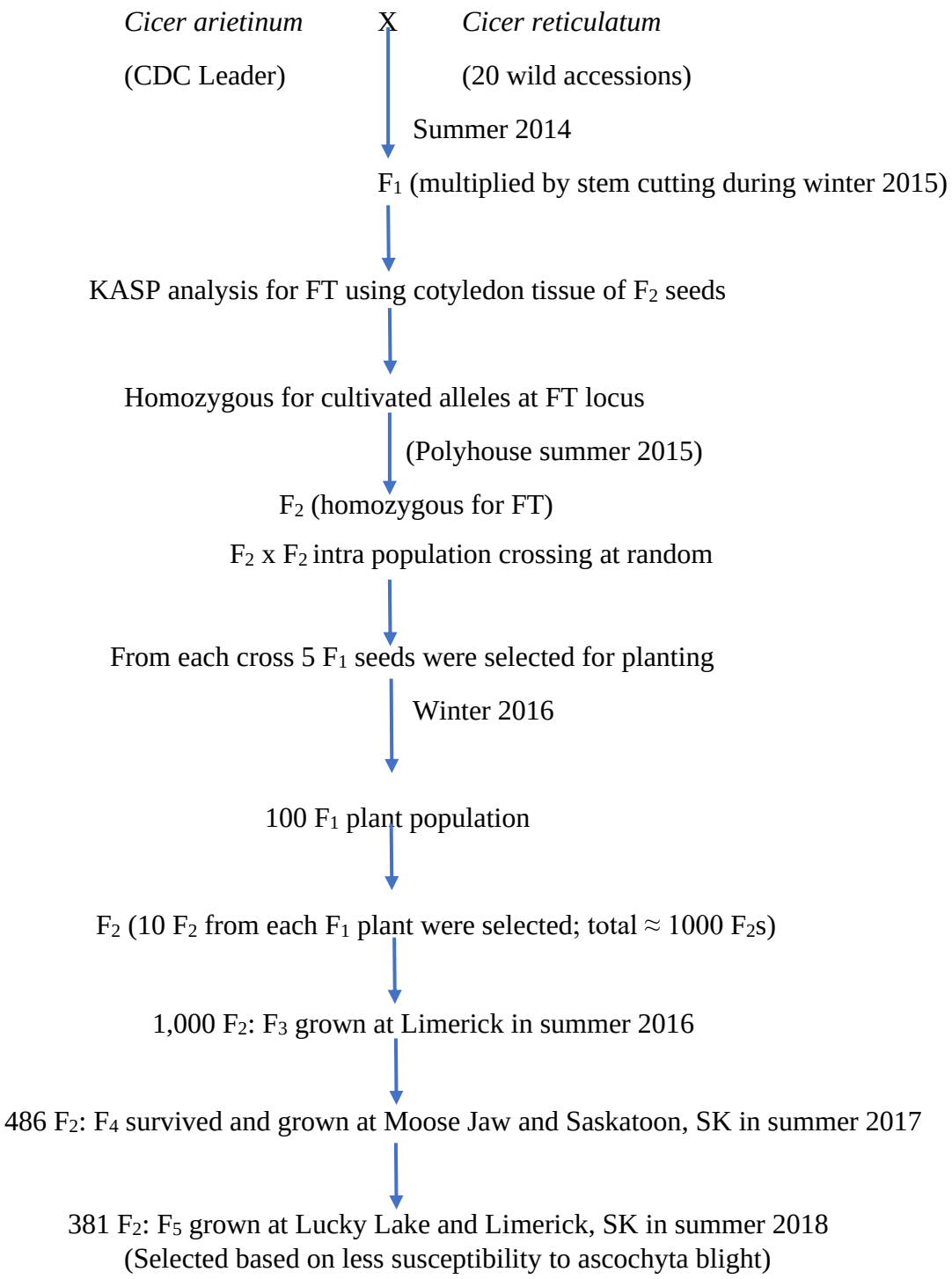


Figure 3.2. Flow diagram of the development of interspecific chickpea lines.

3.3 Experimental setup, data collection, and management

Field experiments to assess the variations of the F_4 and F_5 lines were conducted in 2017 and 2018 growing seasons, respectively, at two locations in Saskatchewan in each year. In 2017, the field sites were Saskatoon (52°07'27.2"N and 106°36'47.4"W) and Moose Jaw (50°01'16.0"N and 106°20'30.7"W). In 2018, the experimental field sites were Lucky Lake (51°3'57.94"N and 107°11'34.74"W) and Limerick (49°38'28.12"N and 106°29'15.91"W). The experimental field sites were located in the Brown (Lucky Lake and Limerick) and Dark Brown (Saskatoon and Moose Jaw) soil-climatic zones of Saskatchewan. The individual plot size was 1 x 1m. On average, 42 seeds were planted in three rows per plot. Prior to seeding, all seeds were treated with Insure® fungicide (Triticonazole, Metalaxyl, and Pyraclostrobin) as recommended for pulse cultivation.

In 2017, 486 F4 lines were evaluated in Moose Jaw with a single replication due to the limited amount of seeds. Multiple checks were used in the experiment. Simultaneously each of the 486 F4 lines and the checks were grown in two-gallon plastic pots in the yard of the Crop Science Field Lab in Saskatoon. These two experiments were laid out as Modified Augmented Design (MAD) where the checks were replicated three times (Federer, 1956). In this study, eight chickpea varieties released from the Crop Development Centre were used as the check variety such as CDC Leader, CDC Frontier, CDC Palmer, CDC Orion, CDC Alma, CDC Corrine, CDC Cory, and CDC Consul. At the Moose Jaw field site, selection was done based on the ascochyta blight disease infestation. Lines that had a very high ascochyta blight disease score (8 or higher on a 1-9 rating scale; Table 3.2) and produced no or limited number of seeds were eliminated for the next generation trial. In 2018, 381 selected F5 lines were evaluated at Lucky Lake and Limerick, SK. In these sites, the experimental design was randomized complete block design

(RCBD) with three replications. In 2017, seeds were sown on May 11th and May 23rd at the Moose Jaw and Saskatoon sites, respectively. In 2018, the seeding dates for Lucky Lake and Limerick field sites were May 3rd and May 7th, respectively. Only nitrogen fertilizer was applied as side band during seeding at all the field sites. No rhizobial inoculant was applied. Fungicide (Priaxor) and herbicide (spring burn-off Roundup, Clethodim, Amigo, and Axial) were applied for disease and weed control.

Data were collected for agronomic and yield traits including plant height, days to flowering, days to maturity, number of primary and secondary branches, ascochyta blight disease rating, growth habit, seed type, seed shattering, biomass per plant, number of seeds and seed weight per plant and 1000-seed weight. Randomly three plants (2018 field trial) and six plants (2017 field trial) per plot (1m x 1m) were harvested by hand to estimate the plant biomass and yield traits. The flowering, maturity, and growth habit data were recorded on individual plot basis. Plant height of each microplot at all the experimental sites were recorded at the maturity stage. Three plants were randomly selected from each microplot for plant height, which was measured from the ground level prior to harvesting. The branching pattern was used to categorize the plant architecture as erect, semi-erect and prostrate type of growth habit. The ascochyta disease score was done on the plot basis during pod formation period. The rating and scoring of ascochyta blight disease were performed by visual observation using 1 to 9 rating scale. The non-infected plants were scored as 1 and the disease infected dead plants received a highest score of 9 (Table 3.2; Reddy and Singh, 1984). Days to flowering was recorded as the number of days from sowing to the stage when 50% open flowered plants observed within a microplot. Similarly, days to maturity was calculated as the number of days required from sowing to the stage of 90% yellow colored plants in each plot. Prior to harvesting, the Reglone® (Diquat) was applied to

remove excess moisture and prepare the plants for harvesting. For 2017, six randomly selected plants were hand harvested for biomass and yield component measurements, whereas in 2018, three plants were randomly hand harvested from each microplot. Finally, individual plots were harvested within the two weeks after desiccation to ensure limited or no shattering loss. These plants were used to determine the total seed weight per plant (g), number of seeds per plant, and biomass per plant (g). All the harvested plants were dried with warm air circulation at 30 to 40 ^{o}C for 48 hours until a constant dry weight was achieved. Prior to threshing, the whole plant samples were weighed for total biomass, and then the samples were threshed using a rubber belt threshing machine. The harvested seeds were differentiated into three distinct categories such as kabuli, desi and pea type. The clean seeds obtained from hand harvested plants were used for grain yield measurements (g). Total number of seeds per plant were calculated by using an electronic seed counter (ESC-1; Agriculex Inc.).

Table 3.2. Scaling techniques for assessing ascochyta blight infection of chickpea based on the appearance of the disease symptoms in plants (adopted from Reddy and Singh, 1984).

Disease symptoms in chickpea	Score
Healthy plant, no disease	1
Lesions present, but small and inconspicuous	2
Lesions easily seen, but plant is mostly green	3
Severe lesions clearly visible	4
Lesions girdle stems, most leaves show lesions	5
Plant collapsing, tips die back	6
Plant dying, but at least three green leaves present	7
Nearly dead plant (virtually no green leaves left) but still with a green stem	8
Dead plant (almost no green parts visible)	9

The biomass and seed yield data were used to calculate seed yield and number of seeds per m^2 plot, and harvest index. The harvest index was calculated by the following formula:

$$Harvest\ index = (Total\ seed\ yield \div Total\ biomass\ yield) \times 100$$

3.4 Genotyping of chickpea populations and data analysis

The seeds of 381 lines at F_5 generation as well as 20 wild parents and the cultivated parent were grown in the greenhouse during the fall of 2017 to collect leaf tissue for DNA source and molecular analyses. The seedlings were grown up to four leaves stage to collect the required amount of leaf tissue for DNA analyses. Approximately 150 mg of fresh leaf tissues were carefully collected in microtubes. The collected fresh leaf tissues were then freeze-dried and stored at -80˚C; later the samples were sent to the genotyping service laboratory, Freedom Markers in Iowa, USA. Genotyping of the chickpea lines was conducted by a modified genotyping by sequencing (GBS) protocol called tunable Genotyping By Sequencing (tGBS) (Ott et al., 2017). The genomic DNA from the leaf tissue of 402 germplasms was extracted using the MagAttract 96 DNA Plant Core Kit (QIAGEN; Valencia, CA, USA) following the manufacturer protocol. The DNA samples were normalized using the Qubit dsDNA Broad Range Assay [Thermo Fisher (Waltham, MA, USA)]. In total 120 ng of DNA from each sample was used for tGBS library preparation according to the tGBS protocol (Ott et al., 2017). The tGBS libraries were then sequenced on Life Technologies' Ion Proton Systems following the Ion PI Hi-Q Sequencing 200 Kit User Guide. Clean reads were aligned to the CDC Frontier reference genome (V1.0) using GSNAP (Wu and Nacu, 2010) and SNPs were called. All these steps for genotyping of 402 chickpea germplasms were performed by the genotyping service laboratory, Freedom Markers in Iowa, USA.

3.5 Statistical analyses

Statistical analyses were carried out using the R package (3.4.0 version: an open source statistical software from the www.r-project.org). Prior to analyses, the data were tested for normality using Shapiro-Wilk's test, and homogeneity of variance was validated using Bartlett's test. The years and locations were used for descriptive analysis. Mean data from each location were used for calculating phenotypic correlation among the agronomic and yield traits. The SEM (Structural Equation Modelling) was used to calculate the direct and indirect effects of agronomic and yield components on the yield of chickpea in the R program (3.4.0 version). In SEM, the covariance and correlation estimate of the traits allowed a better estimate to determine the direct and indirect effects of the independent variables on yield (Figure 3.3). The mean values of nine phenotypic traits were used for cluster analysis. The genetic diversity was determined by Euclidean Ward's method and the data were standardized before analysis (Ward, 1963). Cluster visualization was done by heatmap using an online tool Clustvis (http://biit.cs.ut.ee/clustvis/).

Analysis of variance (ANOVA) for all agronomic and yield traits from 2018 field trials were performed using the mixed linear model (MLM). All the measured traits were considered as dependent variables. For ANOVA, the lines and locations were considered as fixed effects, while replications were considered as random. Levene's test was performed to test the equality of variance for each field site and combined locations. The variance components were calculated in R package and used to calculate the broad sense heritability (H^2). The H^2 for each trait was calculated using the following equation:

$$H^2 = \frac{\sigma^2 G}{\sigma^2 G + \sigma^2 er} \qquad and \qquad H^2 = \frac{\sigma^2 G}{\sigma^2 G + \sigma^2 GE + \sigma^2 er}$$

Where, $\sigma^2 G$, $\sigma^2 GE$, and $\sigma^2 er$ indicates the estimates of genotype, genotype-environment, and error variance respectively (Singh et al., 1993).

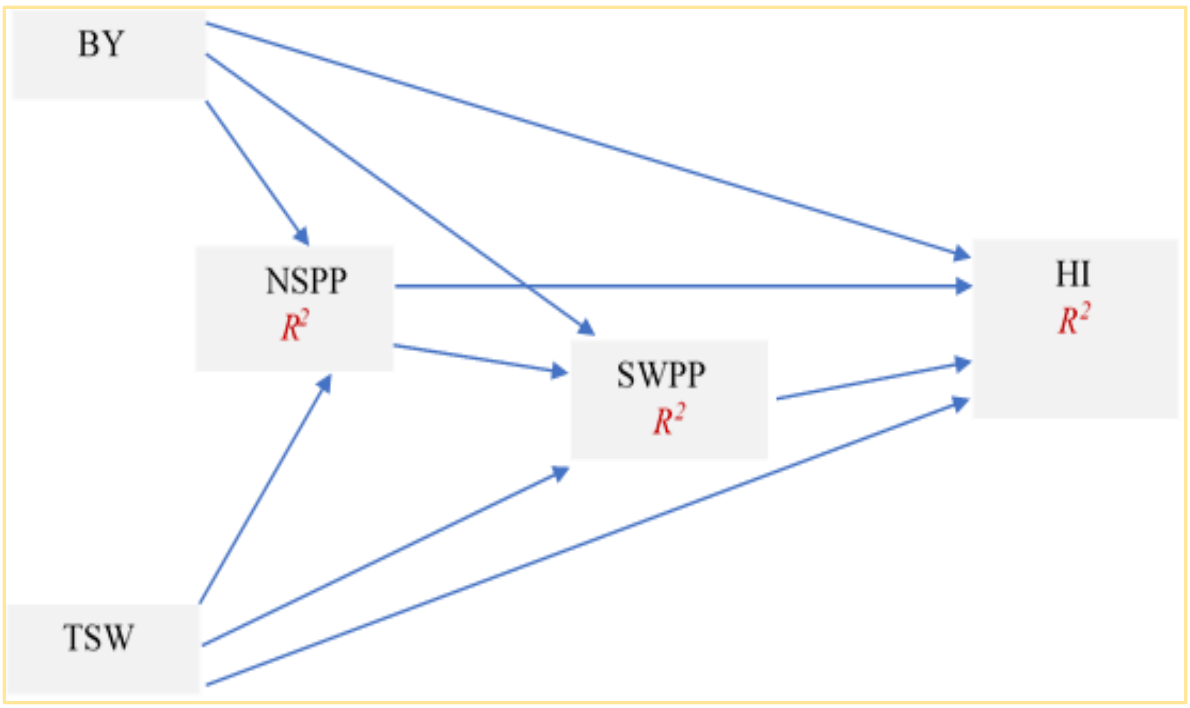


Figure 3.3. Predictive pathways model showing the cause-effect relationship among the yield components and their effect on harvest index of chickpea. Different traits are BY: biomass yield per plant (g); SWPP: seed weight per plant (g); NSPP: number of seeds per plant; TSW: thousand seed weight (g); HI: harvest index. The causal effects are indicated by single headed arrows. The variable at the tail affects the variable at the head. R^2 is the coefficient of determination and indicates the percentage of variance of dependent variable explained by the independent variable in the model.

3.6 Genetic diversity and population structure analyses

The SNPs used for genetic diversity and population structure analyses were obtained at minor allele frequency (MAF) ≥ 1% using TASSEL 5.2.13 software. To analyze the genetic diversity, similar software (TASSEL 5.2.13) was used for generating the phylogenetic relationship among the chickpea lines. The SNPs data were also used to determine the level of genetic diversity among the parental germplasms. A phylogenetic tree based on the genetic-distance of 381 F_5 lines plus 20 parents and one cultivated (total 401 genotypes) was generated by using the neighbor-joining method (Saitou and Nei, 1987). The MEGA 6 software was used to visualize the phylogenetic tree generated by neighbor-joining method (Tamura et al., 2013).

A total of 14,591 SNP markers with MAF ≥ 1% were used for population structure analysis based on the allele frequency by using the ADMIXTURE software 1.23 (Alexander et al., 2009; Alexander and Lange, 2011). To identify the number of *K* inferring the structure of the lines, the ADMIXTURE was set with a predefined *K* values (*K*= 2 to 10) which corresponds to the number of parental population clusters. Each population cluster was run for twenty times in order to find out the best *K* value. The optimum number of *K* was calculated using the STRUCTURE SELECTOR (an online visualizing program) by uploading the Q files generated from ADMIXTURE analysis (Li YL and Liu JX, 2018).

3.7 Genome-wide association study (GWAS)

The mean phenotypic data recorded during 2017 (Saskatoon and Moose Jaw) and 2018 (Limerick and Lucky Lake) were combined with the SNP markers information through genome-wide association analysis to identify significant markers associated with a particular trait. For association analysis, 381 F_5 lines obtained from twenty interspecific crosses were used. The presence of marker-trait association was calculated by using 5501 polymorphic SNPs with MAF ≥ 5%. Alleles in the F_5 lines were either inherited from the founder parents (19 wild parents) or the cultivated (reference) parent (CDC Leader). Therefore, the homozygous alleles of founder and reference parents were coded as 0 (zero) and 2, respectively, and the heterozygous allele was coded as 1 for GWAS. The association analysis was performed using the R statistical program using the NAM package (Xavier et al., 2015). This package was designed to carry out an association analysis suitable for populations grouped in multiple families. The NAM package was developed based on the mixed linear model (MLM) that consider SNPs and families as cofactors. The MLM calculated the *P*-values and the proportions of variance explained by all the SNPs for a particular trait that controls the genetic background and structure of the population. In

this MLM, the heterogeneity of the genetic background was separated as heterozygous alleles that reduces the chance of generating false positive association. After detecting large number of associations between markers and desired traits, the False Discovery Rate (FDR = 0.25) test was applied to declare the significant markers (Xavier et al., 2015). The FDR test reduced the number of markers associated with the individual trait. Candidate genes were identified on 100kb region on either side of the significant makers.

4. RESULTS

4.1 Variability of yield and selected yield contributing traits of chickpea

The descriptive statistics revealed a large variation in phenotypic expression of the chickpea interspecific lines, which could be associated with the genetic variation derived from the wild parents (Table 4.1 and 4.2). The maximum variance of the mean was observed for seed weight per plant, number of seeds per plant, and biomass yield per plant (i.e., 75%, 77% and 99%, respectively at Saskatoon site), which was irrespective of different years and sites. For days to flowering and days to maturity the variance was relatively lower than the other traits. The range and variance of days to flowering and days to maturity were relatively narrow. Ascochyta blight disease showed a high variability as some lines were identified as less susceptible to ascochyta with mean disease score of 4.0. The ascochyta disease infestation in 2018 was lower at Lucky Lake compared to the other site, which could be associated with the lower prevalence of this pathogen due to limited cultivation of chickpea, and drier conditions in this area. Interestingly, several lines produced flowers earlier than CDC Leader after 31 days of planting (Table 4.2), which could be suitable for the short growing season of the Canadian Prairies. The highest biomass yield per plant was obtained from some lines evaluated at Saskatoon (Table 4.1). Growing single plant per pot, irrigation, and less disease pressure helped to increase the biomass of chickpea. The variability in thousand seed weight was attributed to different seed size in the population. In general, the phenotypic variability for traits such as growth habit, seed type, and seed shattering were also present within the chickpea lines (Figure 4.1).

Table 4.1. Descriptive statistics of 486 F_4 lines derived from interspecific crosses of *C. arietinum* and *C. reticulatum* and CDC Leader for yield and selected yield contributing traits evaluated at two locations (Saskatoon and Moose Jaw) in Saskatchewan in 2017.

Traits	Saskatoon-2017				Moose Jaw-2017			
	F_4 lines		CDC Leader		F_4 lines		CDC Leader	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Days to emergence	7.00-18.0	9.00 (12.0)	-	-	-	-	-	-
Days to flowering	47.0-63.0	53.0 (5.00)	49.0-54.0	52.0 (2.00)	43.0-58.0	50.0 (5.00)	50.0-53.0	52.0 (2.00)
Days to maturity	72.0-99.0	89.0 (7.40)	87.0-93.0	90.0 (2.00)	70.0-99.0	88.0 (8.00)	89.0-92.0	91.0 (2.00)
Plant height (cm)	20.0-64.0	31.9 (19.0)	32.0-35.0	33.0 (1.73)	18.0-50.0	28.9 (15.0)	30.0-36.0	32.7 (3.06)
Ascochyta blight score	4.00-8.00	6.22 (1.40)	0.0	0.0	4.00-9.00	5.39 (2.30)	0.0	0.0
Biomass yield per plant (g)	10.5-346	53.1 (99.0)	10.7-20.9	14.6 (5.51)	1.30-98.8	13.5 (88.0)	9.28-15.6	13.1 (3.37)
Number primary branches per plant	3.00-30.0	10.0 (37.0)	3.00-5.00	4.00 (1.00)	-	-	-	-
Number of secondary branches per plant	3.00-62.0	19.0 (49.0)	6.00-15.0	10.0 (5.00)	-	-	-	-
Number of seeds per plant	1.00-180	43.0 (77.0)	21.0-42.0	33.0 (11.0)	1.00-53.0	18.0 (51.0)	14.0-49.0	27.0 (19.0)
Thousand seed weight (g)	101-695	361 (27.0)	211-250	227 (20.3)	121-453	246 (21.0)	239-268	256 (15.1)
Seed weight per plant (g)	0.10-96.0	10.7 (75.0)	4.44-8.74	7.00 (2.28)	0.10-14.2	4.37 (54.0)	8.86-11.7	10.1 (1.47)
Seed yield (kg/ha)	-	-	-	-	100-6000	1820 (55.0)	2392-2676	2563 (150)
Harvest index	0.02-0.84	0.31 (37.0)	0.48-0.56	0.50 (0.04)	0.01-0.69	0.40 (39.0)	0.35-0.56	0.40 (0.12)

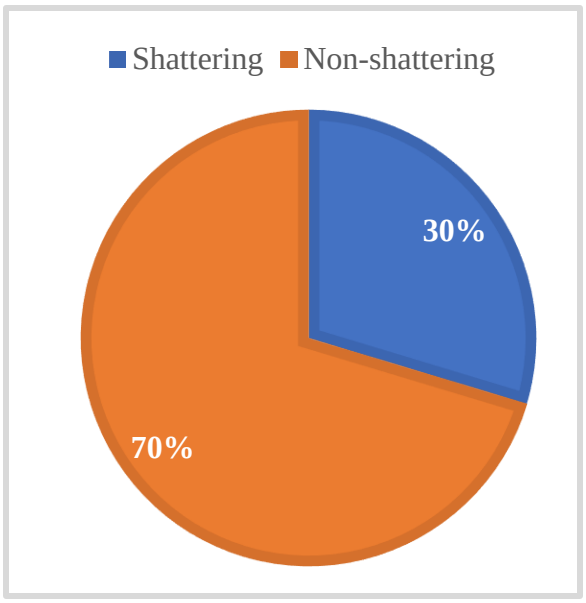
*Values in parentheses are the variance of the mean. Dash (-) indicates the traits were not measured for that site.

Table 4.2. Descriptive statistics of 381 lines (F_5 generation) derived from interspecific crosses of *C. arietinum* and *C. reticulatum* and CDC Leader for yield and selected yield contributing traits evaluated at two different locations (Limerick and Lucky Lake) of Saskatchewan in 2018.

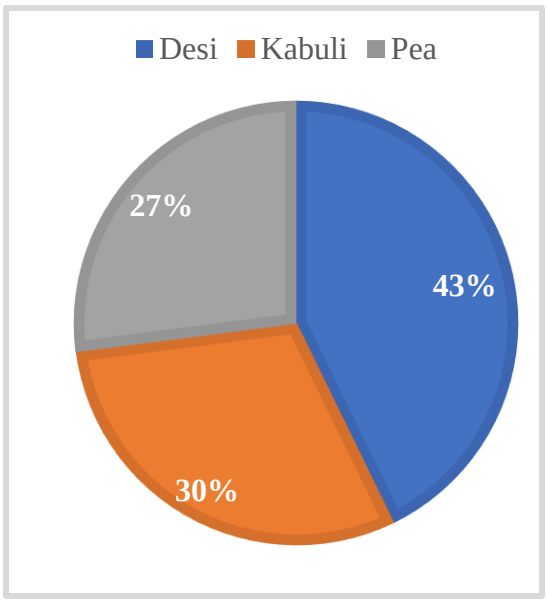
Traits	Limerick -2018				Lucky Lake -2018			
	F_5 lines		CDC Leader		F_5 lines		CDC Leader	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Percentage of germination	21.7-90.0	72.1 (18.0)	85.0-95.0	88.0 (5.88)	25.0-90.0	74.9 (17.0)	85.0-90.0	87.0 (3.00)
Days to flowering	42.0-57.0	49.0 (4.80)	55.0-57.0	56.0 (1.00)	31.0-53.0	45.0 (5.50)	50.0-54.0	52.0 (2.00)
Days to maturity	82.0-96.0	90.0 (2.70)	88.0-93.0	91.0 (3.00)	75.0-95.0	88.0 (2.90)	89.0-93.0	91.0 (2.00)
Plant height (cm)	22.0-45.0	32.0 (17.0)	34.0-38.0	36.0 (2.08)	16.7-35.0	27.4 (13.0)	28.0-32.0	30.0 (2.00)
Ascochyta blight score	4.00-9.00	6.90 (32.0)	0.0	0.0	0.0	0.0	0.0	0.0
Biomass yield per plant (g)	1.00-37.2	13.9 (19.0)	15.0-18.0	13.0 (3.66)	3.20-57.6	17.5 (17.0)	13.1-19.7	15.8 (3.50)
Number of seeds per plant	2.00-45.0	15.0 (16.0)	44.0-51.0	47.0 (4.00)	3.00-103	26.0 (16.0)	16.0-31.0	21.0 (8.00)
Thousand seed weight (g)	136-467	236 (15.0)	262-281	270 (9.00)	135-576	261 (11.0)	271-358	335 (57.0)
Seed weight per plant (g)	0.40-10.6	3.49 (32.0)	11.8-14.3	12.8 (1.34)	1.70-14.6	6.73 (30.0)	7.17-11.1	8.70 (2.01)
Seed yield (kg/ha)	20-4400	1460 (28.0)	2623-2807	2703 (95)	100-3900	1740 (28.0)	3302-3533	3395 (122)
Harvest index	0.02-0.71	0.27 (29.0)	0.77-0.80	0.78 (0.01)	0.13-0.66	0.38 (18.0)	0.54-0.56	0.50 (0.01)

*Values in parentheses are the variance of the mean. Dash (-) indicated that no ascochyta blight disease was observed for that site.

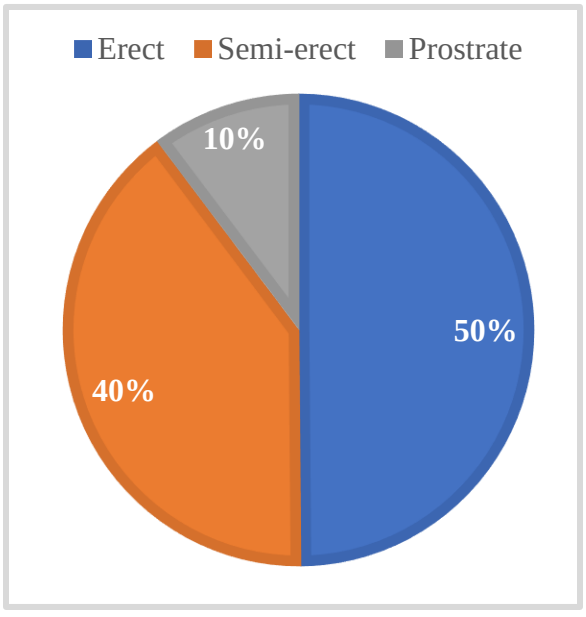
Figure 4.1. Variability for some qualitative traits among 381 chickpea lines of F_5 generation evaluated at two locations (Limerick and Lucky Lake) of Saskatchewan in 2018.



A. Seed shattering



B. Seed type



C. Plant growth habit

4.2 Correlation among the yield and yield contributing traits of chickpea

Correlation analysis was performed among the morphological and yield contributing traits of the chickpea lines evaluated in two-year field trials (2017 and 2018) at two locations in each year (Tables 4.3; 4.4; 4.5 and 4.6). Among the selected traits, the number of seeds per plant was found to show high significant positive relationship with seed weight per plant at 3 out of 4 locations (Tables 4.3; 4.4; 4.5 and 4.6). Biomass was positively correlated with thousand seed weight, number of seeds per plant at all locations. Plant height was positively correlated with the number of primary and secondary branches. Secondary branches per plant also showed a positive correlation with the yield and number of seeds per plant. Harvest index showed a significant positive correlation with number of seeds and seed weight per plant. However, the relationships of harvest index with days to flowering, days to maturity, plant height, and biomass yield were negative for all sites. Correlation of ascochyta disease scores with the harvest index was insignificant in all field trials. Plant height was found to be positively correlated with seed yield across different locations. The relationship of plant height and biomass was also positive. Thousand seed weight had a significant positive correlation with seed yield, whereas it had negative correlation with number seeds per plant. All the yield contributing traits had a negative correlation with the ascochyta blight disease scores. The relationship between days to flowering and ascochyta blight disease was also negative in the 2018 field trials.

Table 4.3. Pearson correlation coefficients among the yield and yield contributing traits of 486 F_4 lines derived from interspecific crosses of *C. arietinum* and *C. reticulatum* evaluated at Saskatoon, Saskatchewan in 2017.

Traits	DTM	PH	ABS	BY	NPB	NSB	NSPP	TSW	HI	SWPP
DTF	0.02^{ns}	0.04^{ns}	-0.04^{ns}	0.01^{**}	-0.05^{ns}	0.01^{ns}	-0.13^{*}	0.08^{ns}	-0.28^{***}	-0.11^{*}
DTM		-0.03^{ns}	0.07^{ns}	-0.07^{ns}	0.07^{ns}	-0.01^{ns}	-0.15^{**}	0.11^{*}	-0.25^{***}	-0.13^{*}
PH			-0.18^{***}	0.63^{***}	0.15^{***}	0.36^{***}	0.50^{***}	0.16^{***}	-0.18^{***}	0.51^{***}
ABS				-0.17^{***}	0.06^{ns}	0.03^{ns}	-0.22^{***}	0.01^{ns}	-0.05^{ns}	-0.24^{***}
BY					0.15^{**}	0.47^{***}	0.78^{***}	0.26^{***}	-0.20^{***}	0.82^{***}
NPB						0.32^{***}	0.07^{ns}	-0.04^{ns}	-0.16^{***}	0.04^{ns}
NSB							0.32^{***}	-0.07^{ns}	-0.30^{***}	0.25^{***}
NSPP								-0.05^{ns}	0.21^{***}	0.95^{***}
TSW									-0.24^{***}	0.09^{ns}
HI										0.22^{***}

Different evaluated traits are DTF: days to flowering; DTM: days to maturity; PH: plant height (cm); ABS: ascochyta blight disease score; BY: biomass yield per plant (g); NPB: number of primary branches per plant; NSB: number of secondary branches per plant; NSPP: number of seeds per plant (g); TSW: thousand seed weight (g); HI: harvest index; SWPP: seed weight per plant (g). ^{ns}, * ,** and ***: non-significant, significant at $p<0.05$, $p<0.01$ and $p<0.001$.

Table 4.4. Pearson correlation coefficients among the yield and yield contributing traits of 486 F_4 lines derived from interspecific crosses of *C. arietinum* and *C. reticulatum* evaluated at Moose Jaw, Saskatchewan in 2017.

Traits	DTM	PH	ABS	BY	SWPP	NSPP	TSW	HI	SY
DTF	0.19^{***}	0.21^{***}	0.03^{ns}	0.15^{***}	0.05^{ns}	0.01^{ns}	0.14^{**}	-0.18^{***}	0.08^{ns}
DTM		0.12*	0.09^{ns}	0.29^{***}	0.07^{ns}	0.07^{ns}	0.08^{ns}	-0.35^{***}	0.10^{*}
PH			-0.01^{ns}	0.33^{***}	0.29^{***}	0.23^{***}	0.16^{***}	-0.03^{ns}	0.28^{***}
ABS				0.07^{ns}	0.04^{ns}	0.04^{ns}	0.03^{ns}	-0.06^{ns}	0.02^{ns}
BY					0.80^{***}	0.75^{***}	0.22^{***}	-0.21^{***}	0.76^{***}
SWPP						0.90^{***}	0.37^{***}	0.35^{***}	0.99^{***}
NSPP							-0.04^{ns}	0.27^{***}	0.88^{***}
TSW								0.26^{***}	0.36^{***}
HI									0.33^{***}

Different evaluated traits are DTF: days to flowering; DTM: days to maturity; PH: plant height (cm); ABS: ascochyta blight disease score; BY: biomass yield per plant (g); SWPP: seed weight per plant (g); NSPP: number of seeds per plant; TSW: thousand seed weight (g); HI: harvest index; SY: seed yield (kg/ha). ^{ns}, *,** and ***: non-significant, significant at $p<0.05$, $p<0.01$ and $p<0.001$.

Table 4.5. Pearson correlation coefficients among the yield and yield contributing traits of 381 F_5 lines derived from interspecific crosses of *C. arietinum* and *C. reticulatum* evaluated at Limerick, Saskatchewan in 2018.

Traits	DTM	PH	ABS	BY	SWPP	NSPP	TSW	HI	SY
DTF	0.28^{***}	0.23^{***}	-0.16^{**}	0.22^{***}	0.01^{ns}	-0.01^{ns}	0.11^{**}	-0.15^{***}	0.00^{ns}
DTM		0.29^{***}	0.12^{**}	0.21^{***}	-0.07^{ns}	-0.06^{ns}	0.10^{*}	-0.15^{***}	-0.07^{ns}
PH			-0.03^{ns}	0.37^{***}	-0.05^{ns}	-0.05^{ns}	0.03^{ns}	-0.21^{***}	-0.04^{ns}
ABS				-0.33^{***}	-0.07^{ns}	-0.01^{ns}	-0.15^{***}	0.13^{*}	-0.06^{ns}
BY					0.07^{ns}	0.05^{ns}	0.11^{*}	-0.33^{***}	0.08^{ns}
SWPP						0.92^{***}	0.17^{***}	0.69^{***}	0.99^{***}
NSPP							-0.13^{**}	0.69^{***}	0.91^{***}
TSW								0.04^{ns}	0.18^{***}
HI									0.67^{***}

Different traits are DTF: days to flowering; DTM: days to maturity; PH: plant height (cm); ABS: ascochyta blight disease score; BY: biomass yield per plant (g); SWPP: seed weight per plant (g); NSPP: number of seeds per plant; TSW: thousand seed weight (g); HI: harvest index; SY: seed yield (kg/ha). ^{ns}, * ,** and ***: non-significant, significant at $p<0.05$, $p<0.01$ and $p<0.001$.

Table 4.6. Pearson correlation coefficients among the yield and yield contributing traits of 381 F_5 lines derived from interspecific crosses of *C. arietinum* and *C. reticulatum* evaluated at Lucky Lake, Saskatchewan in 2018.

Traits	DTM	PH	BY	SWPP	NSPP	TSW	HI	SY
DTF	0.53***	0.28***	0.25***	0.01ns	0.04 ns	0.17***	-0.14***	0.03 ns
DTM		0.31***	0.37***	-0.07 ns	0.14 ***	0.16***	-0.16***	0.05 ns
PH			0.18***	0.06 ns	0.02 ns	0.16***	-0.04 ns	0.13**
BY				0.03 ns	0.80***	0.17***	-0.20***	0.34 ns
SWPP					0.27***	0.16***	0.39***	0.54***
NSPP						-0.16***	0.48***	0.42***
TSW							0.30***	0.31***
HI								0.57***

Different traits are DTF: days to flowering; DTM: days to maturity; PH: plant height (cm); BY: biomass yield per plant (g); SWPP: seed weight per plant (g); NSPP: number of seeds per plant; TSW: thousand seed weight (g); HI: harvest index; SY: seed yield (kg/ha). ns, * ,** and ***: non-significant, significant at $p<0.05$, $p<0.01$ and $p<0.001$.

4.3 Path coefficient analysis

The Structural Equation Model (SEM) was constructed to determine the direct and indirect effects of the yield contributing traits on seed yield of chickpea (Table 4.7 and 4.8). This SEM statistical approach or path analysis was used to quantify the causal relationships among the selected intercorrelated traits. Results from the path analysis revealed that the number of seeds per plant had the highest direct positive effect on seed weight per plant, followed by thousand seed weight. These traits consistently showed positive effect on seed weight per plant in 3 out of 4 of the environmental conditions of different field sites. Therefore, these traits are potential objects for selection in the breeding program to increase chickpea yield. The direct effect of thousand seed weight on the number of seeds per plant was negative. The biomass also had a negative direct effect on harvest index. However, the indirect effect of biomass on the harvest index was positive in 1 out of 4 locations. Among all the traits, the number of seeds per plant, and seed weight per plant showed a positive direct effect on the harvest index. The direct effect of thousand seed weight on harvest index was positive in 3 out of 4 locations.

Table 4.7. Direct and indirect effects of yield contributing traits on seed yield of F_4 lines evaluated at Saskatoon and Moose Jaw in 2017.

Pathway	**----------Direct effect----------**			
	Saskatoon -2017		Moose Jaw -2017	
	Standardized estimates	Standard error	Standardized estimates	Standard error
BY ⟶ NSPP	0.85 ***	0.03	0.17 ***	0.00
BY ⟶ SWPP	0.11 **	0.01	0.01 ns	0.00
BY ⟶ HI	-0.98 ***	0.00	-0.63 ***	0.00
TSW ⟶ NSPP	-0.27 ***	0.00	-0.01 ns	0.01
TSW ⟶ SWPP	0.10 **	0.00	0.36***	0.00
TSW ⟶ HI	-0.06 ns	0.00	0.24***	0.00
NSPP ⟶ SWPP	0.87 ***	0.01	0.91***	0.00
NSPP ⟶ HI	0.01 ns	0.00	0.48***	0.00
SWPP ⟶ HI	0.98 ***	0.00	0.07 ns	0.01
	----------Indirect effect----------			
BY ⟶ HI (Through NSPP)	0.00	-	0.08	-
BY ⟶ HI (Through SWPP)	0.11	-	0.00	-
TSW ⟶ HI (Through NSPP)	-0.00	-	-0.00	-
TSW ⟶ HI (Through SWPP)	-0.01	-	0.03	-
NSPP ⟶ HI (Through SWPP)	0.00	-	0.43	-

Pathway of different traits are BY: biomass yield per plant (g); SWPP: seed weight per plant; NSPP: number of seeds per plant; TSW: thousand seed weight (g); HI: harvest index. The variable at the tail affects the variable at the head. ns, *,**, and ***: non-significant, significant at $p<0.05$, $p<0.01$, and $p<0.001$.

Table 4.8. Direct and indirect effects of yield contributing traits on seed yield of F_5 lines evaluated at Limerick and Lucky Lake in 2018.

Pathway	**----------Direct effect----------**			
	Limerick -2018		Lucky Lake -2018	
	Standardized estimates	Standard error	Standardized estimates	Standard error
BY ⟶ NSPP	0.06 ^{ns}	0.07	0.75 ***	0.06
BY ⟶ SWPP	-0.00 ^{ns}	0.00	0.01 ^{ns}	0.03
BY ⟶ HI	-0.38 ***	0.00	-0.59 ***	0.00
TSW ⟶ NSPP	-0.14 **	0.01	-0.22 ***	0.01
TSW ⟶ SWPP	0.30 ***	0.00	0.07 ^{ns}	0.00
TSW ⟶ HI	0.14 **	0.00	0.50 ***	0.00
NSPP ⟶ SWPP	0.96 ***	0.00	0.02 ^{ns}	0.02
NSPP ⟶ HI	0.62 ***	0.00	0.98 ***	0.00
SWPP ⟶ HI	0.13 *	0.01	0.06 ^{ns}	0.00
	----------Indirect effect----------			
BY ⟶ HI (Through NSPP)	0.04	-	0.74	-
BY ⟶ HI (Through SWPP)	-0.00	-	0.00	-
TSW ⟶ HI (Through NSPP)	-0.09	-	0.22	-
TSW ⟶ HI (Through SWPP)	0.04	-	0.00	-
NSPP ⟶ HI (Through SWPP)	0.12	-	0.00	-

Pathway of different traits are BY: biomass yield per plant (g); SWPP: seed weight per plant; NSPP: number of seeds per plant; TSW: thousand seed weight (g); HI: harvest index. The variable at the tail affects the variable at the head. ^{ns}, *,**, and ***: non-significant, significant at $p<0.05$, $p<0.01$, and $p<0.001$.

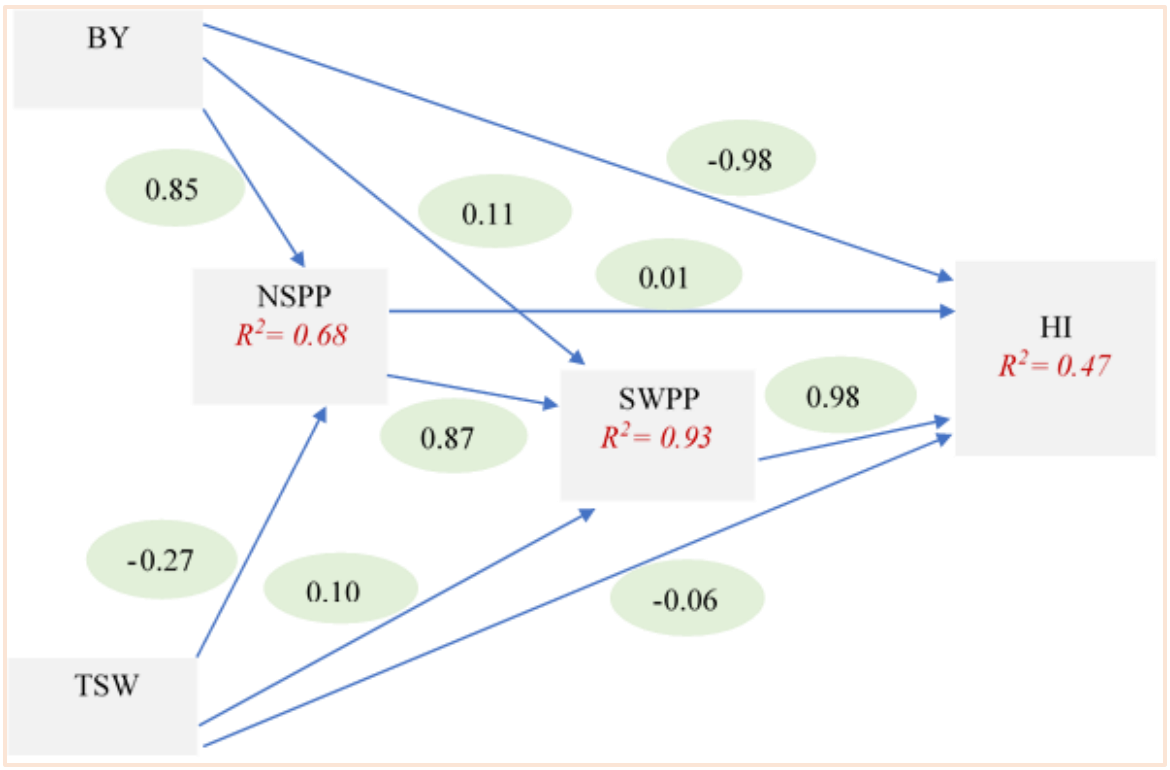


Figure 4.2. Path model showing direct effect of selected yield contributing traits on the yield of the chickpea lines (F_4 generation) evaluated at Saskatoon, SK in 2017. Different traits are BY: biomass yield per plant (g); SWPP: seed weight per plant (g); NSPP: number of seeds per plant; TSW: thousand seed weight (g); HI: harvest index. The causal effects are indicated by single headed arrows. The variable at the tail affects the variable at the head. Three residual effects are indicated by red color. R^2 is the coefficient of determination and indicates the percentage of variance of dependent variable explained by the independent variable in the model.

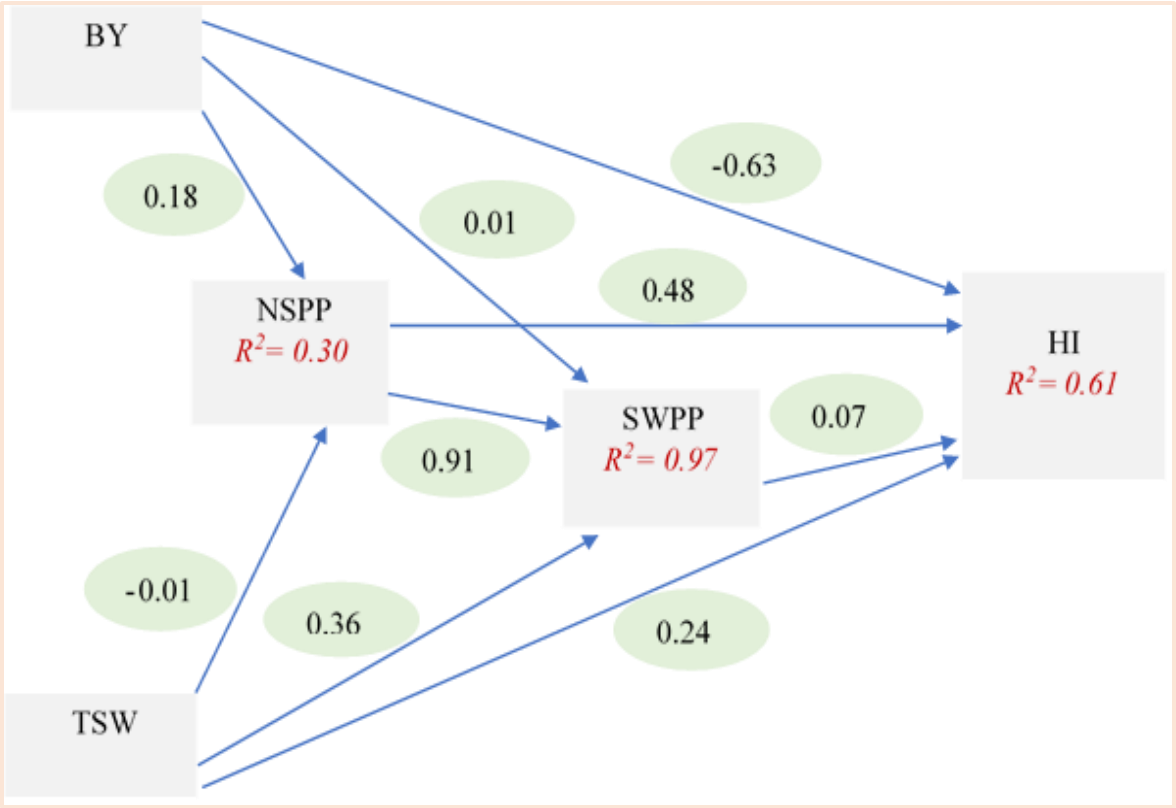


Figure 4.3. Path model showing the direct effects of selected yield contributing traits on the yield of chickpea lines (F_4 generation) evaluated at Moose Jaw, SK in 2017. Different traits are BY: biomass yield per plant (g); SWPP: seed weight per plant (g); NSPP: number of seeds per plant; TSW: thousand seed weight (g); HI: harvest index. The causal effects are indicated by single headed arrows. The variable at the tail affects the variable at the head. Three residual effects are indicated by red color. R^2 is the coefficient of determination and indicates the percentage of variance of dependent variable explained by the independent variable in the model.

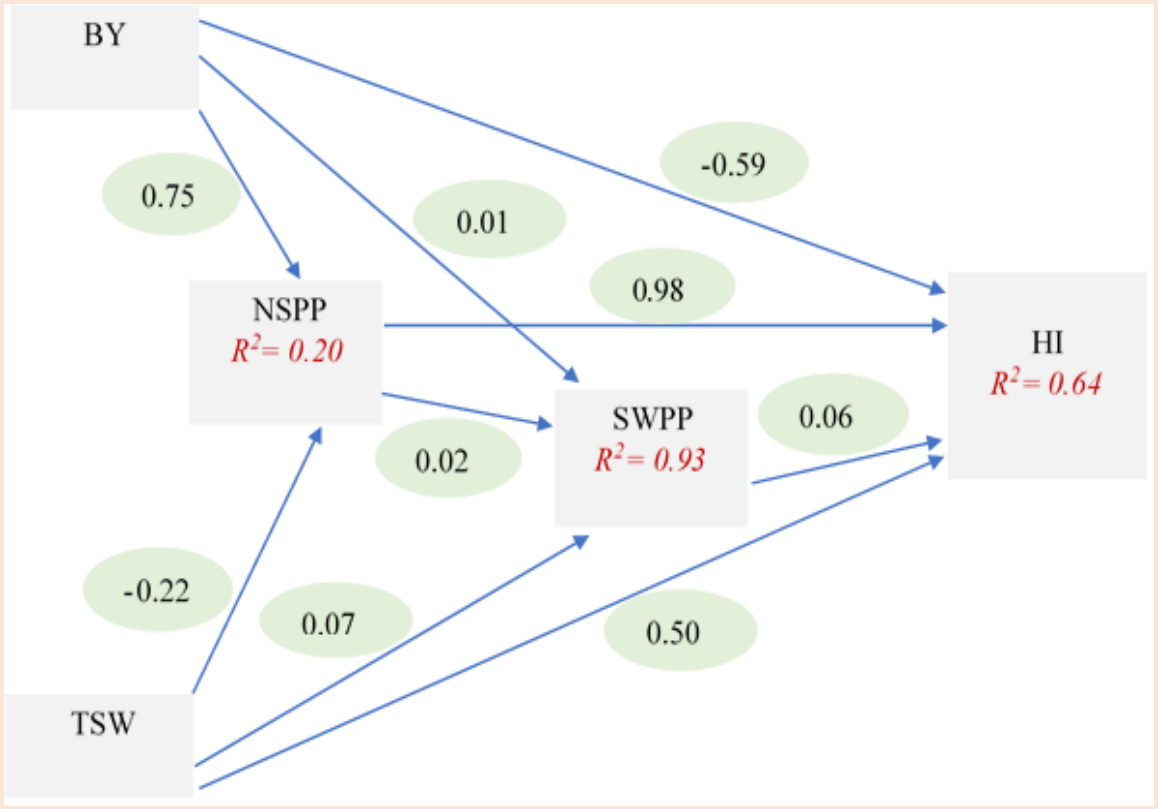


Figure 4.4. Path model showing the direct effects of selected yield contributing traits on the seed yield of chickpea lines (F_5 generation) evaluated at Limerick, SK in 2018. Different traits are BY: biomass yield per plant (g); SWPP: seed weight per plant (g); NSPP: number of seeds per plant; TSW: thousand seed weight (g); HI: harvest index. The causal effects are indicated by single headed arrows. The variable at the tail affects the variable at the head. Three residual effects are indicated by red color. R^2 is the coefficient of determination and indicates the percentage of variance of dependent variable explained by the independent variable in the model.

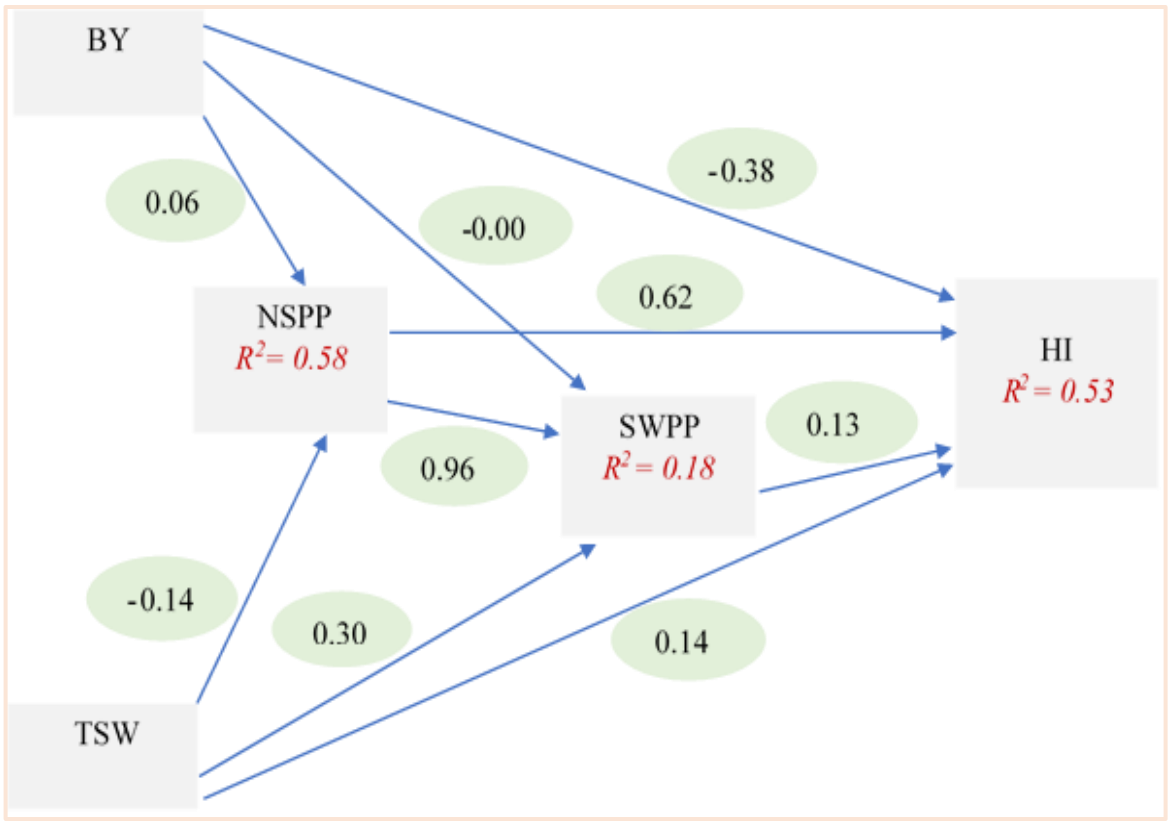


Figure 4.5. Path model showing the direct effects of selected yield contributing traits on the seed yield of chickpea lines (F_5 generation) evaluated at Lucky Lake, SK in 2018. Different traits are BY: biomass yield per plant (g); SWPP: seed weight per plant (g); NSPP: number of seeds per plant; TSW: thousand seed weight (g); HI: harvest index. The causal effects are indicated by single headed arrows. The variable at the tail affects the variable at the head. Three residual effects are indicated by red color. R^2 is the coefficient of determination and indicates the percentage of variance of dependent variable explained by the independent variable in the model.

4.4 Effects of genotype, environment and their interaction on seed yield and yield contributing traits of chickpea

Plant growth and seed yield of chickpea were greatly influenced by the genetic and environmental factors. The analysis of variance (ANOVA) using mixed linear model revealed significant effects of genotype (G) and environments (E) for all the traits (Table 4.9). The G×E interaction components were significant for days to maturity, number of seeds per plant, thousand seed weight, and seed yield, whereas their interaction effects on days to flowering, plant height, and biomass were not significant (Table 4.9). Broad-sense heritability estimates (H^2) showed low to medium heritability for all traits (Table 4.9). The maximum H^2 was observed for days to flowering (0.54) followed by seed weight per plant (0.45) and days to maturity (0.35). The yield contributing traits, such as number of seeds and biomass yield per plant had H^2 of 0.18 and 0.14, respectively. Thousand seed weight had the lowest H^2.

Table 4.9. Analysis of variance (ANOVA) and broad sense heritability estimates (H^2) of the chickpea lines (F_5 generation) for the yield and yield contributing traits evaluated at two locations (Limerick and Lucky Lake), Saskatchewan in 2018.

Traits	*F* values of the effects			H^2
	G	E	G X E	
Days to flowering	6.36 ***	919 ***	1.03 ns	0.54
Days to maturity	4.39 ***	262 ***	1.15 *	0.35
Plant height (cm)	2.06 ***	479 ***	0.93 ns	0.15
Biomass weight per plant (g)	1.93 ***	39.8 ***	1.03 ns	0.14
Number of seeds per plant	2.78 ***	681 ***	1.56 ***	0.18
Thousand seed weight (g)	6.47 ***	263 ***	6.16 ***	0.08
Seed weight per plant (g)	3.04 ***	710 ***	1.43 ***	0.45

G: Genotype; E: Environment; G x E: Genotype and Environment interaction. ns, * ,** and ***: non-significant, significant at p<0.05, p<0.01 and p<0.001.

4.5 Cluster analysis based on agronomic and yield traits

The standardized mean values of the nine agronomic traits from the 381 F_5 lines were used in cluster analysis (Figure 4.6). The means and standard deviations of six major clusters offered meaningful information regarding the genetic diversity of the lines. They provided an opportunity to identify the best line group (i.e., cluster I = 67 F_5 lines), which possessed high yield and a combination of desirable agronomic traits. The largest group belong to Cluster II (104 F_5 lines), while cluster VI had the lowest number of lines (28 F_5 lines). Cluster I produced the highest number of seeds per plant and seed weight per plant which were comparatively higher than the other clusters (Table 4.10). The cluster I lines could be recommended for future breeding program to improve the yield of chickpea. Moreover, lines in cluster VI contained several significant traits including early flowering and early maturity with the mean values of 45 and 86 days, respectively (Table 4.10). Cluster VI also showed the lowest ascochyta blight disease score indicating that these lines could be comparatively less susceptible to ascochyta disease. As such, cluster VI could be a potential source for further improvement of ascochyta blight resistance in the breeding program.

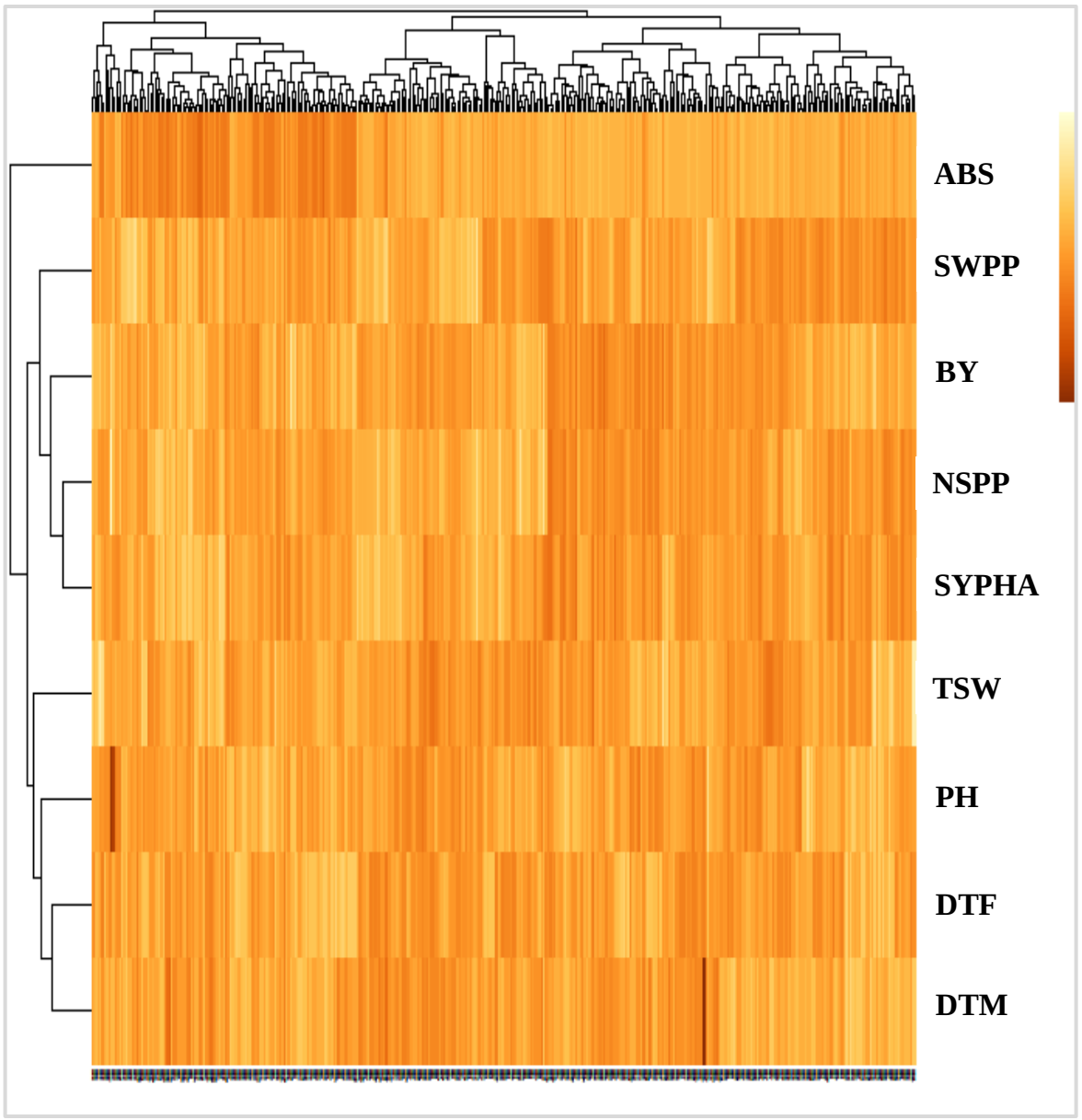


Figure 4.6. Heatmap based on the agronomic and yield components summarizing the differentiation among the 381 F_5 lines following the Euclidean Ward method. Different traits are DTF: days to flowering; DTM: days to maturity; PH: plant height (cm); TSW: thousand seed weight (g); SYPHA: seed yield per hectare (kg/ha); NSPP: number of seeds per plant; BY: biomass yield per plant (g); SWPP: seed weight per plant (g); ABS: ascochyta blight disease score.

Table 4.10. Means and standard deviation of 6 clusters for yield and yield contributing traits toward genetic divergence in 381 chickpea lines at the F_5 generation.

Traits	Cluster-I (67)	Cluster-II (104)	Cluster-III (68)	Cluster-IV (72)	Cluster-V (42)	Cluster-VI (28)
Days to flowering	47.0± 2.0	47.0± 3.0	46.0± 2.0	50.0± 3.0	51.0± 2.0	45.0± 2.0
Days to maturity	88.0± 2.0	90.0± 2.0	87.0± 2.0	91.0± 2.0	89.0± 2.0	87.0± 1.0
Plant height (cm)	29.2± 3.6	28.8± 2.4	28.2± 2.7	33.3± 2.4	28.9± 2.1	29.0± 2.4
Ascochyta blight score	6.64± 1.6	7.81± 0.7	8.00± 0.7	6.83± 1.4	5.48± 1.5	4.65± 0.9
Biomass yield per plant (g)	17.6± 4.0	14.7± 3.3	10.8± 5.5	20.5± 4.3	16.4± 3.8	13.6± 4.3
Number of seeds per plant	28.0± 7.0	19.0± 6.0	17.0± 6.0	22.0± 6.0	21.0± 5.0	18.0± 4.0
Thousand seed weight (g)	253± 28	238± 31	234± 34	268± 43	245± 31	260± 45
Seed weight per plant (g)	6.87± 1.4	4.20± 1.1	5.32± 1.5	4.90± 1.4	4.65± 1.1	4.96± 1.4
Seed yield (kg/ha)	2310± 394	1332± 384	1360± 432	1680± 394	1598± 300	1650± 528

*Values in parentheses are the number of lines in each cluster. "± values" indicates the standard deviations.

4.6 Genetic diversity and population structure analyses

The genetic diversity of 381 chickpea F_5 lines and the 19 wild parents and one cultivated parent (CDC Leader) were evaluated by NJ tree clustering using MEGA programs. Genotyping of the population by tGBS (a modified genotyping by sequencing method) identified a total of 15,186 SNP markers. These markers were filtered with MAF ≥ 1% in order to consider the effects of minor alleles; therefore, the number of SNPs were reduced to 14,591 and used to calculate the genetic diversity of the chickpea lines. The distribution of SNP markers on the chromosomes indicated the highest number of SNPs on chromosome 4 (Figure 4.7). This implies that chromosome 4 might have a greater contribution towards the diversity of the chickpea population. The NJ cluster analysis divided the 381 F_5 lines into 20 distinct groups according to their respective cultivated and wild parents from which they were developed (Figure 4.8.a). It was expected that some useful genetic information in the respective wild parents were transferred to the progeny lines. Further, the clustering patterns of the 381 F_5 lines were consistent with their wild parents (Figure 4.8.c). The diversity analysis of the cultivated and wild parents produced 16 different clusters (Figure 4.8.b).

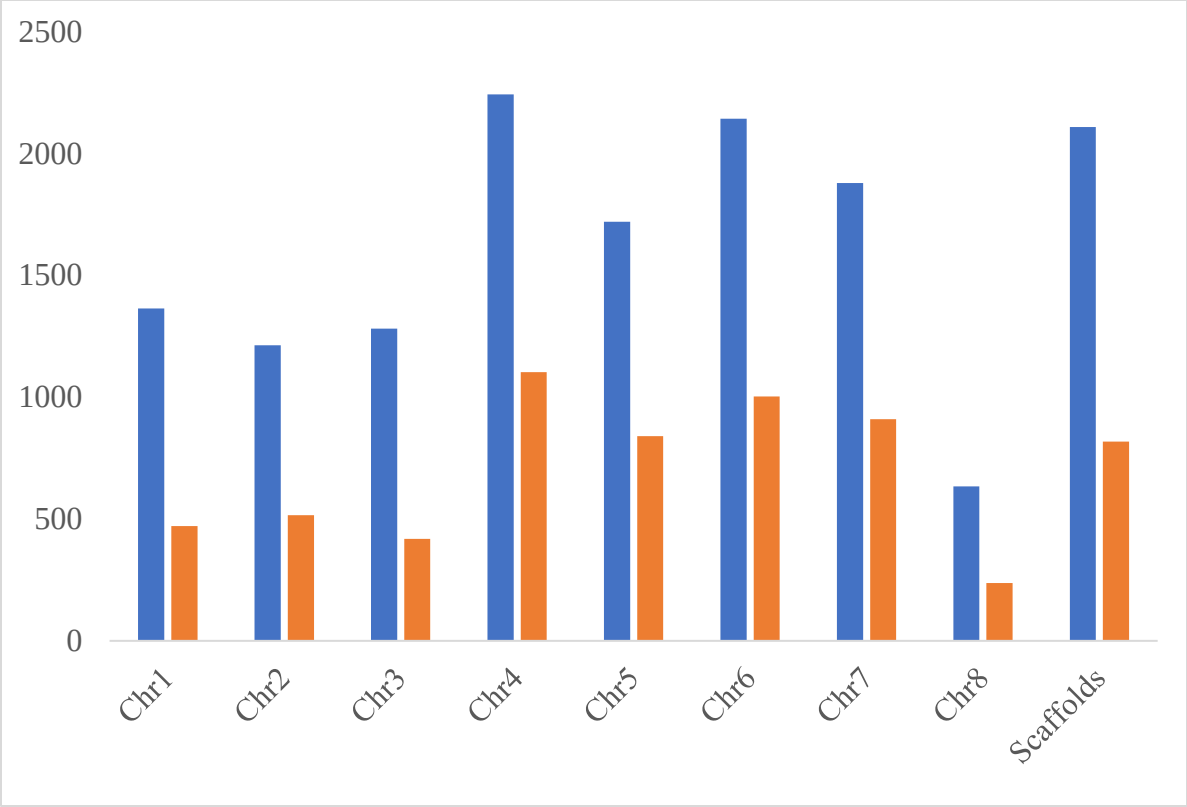


Figure 4.7. Distribution of SNPs in the chickpea genome of 381 F_5 lines arising from interspecific crosses of *C. arietinum* and *C. reticulatum*. About 14,591 and 6,319 SNPs were identified at MAF ≥ 1% (blue bars) and MAF ≥ 5% (brown bars), respectively using tGBS approach.

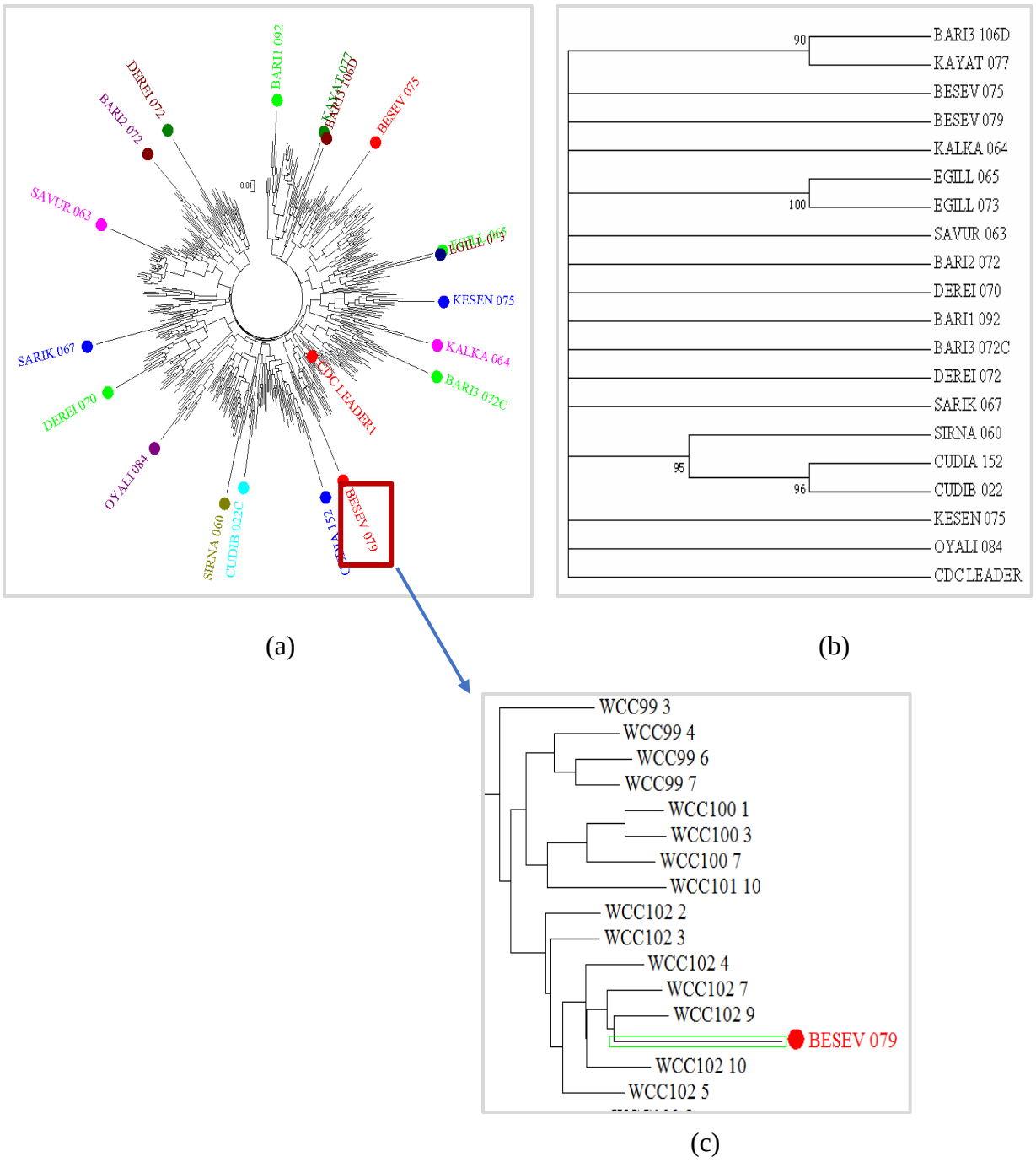
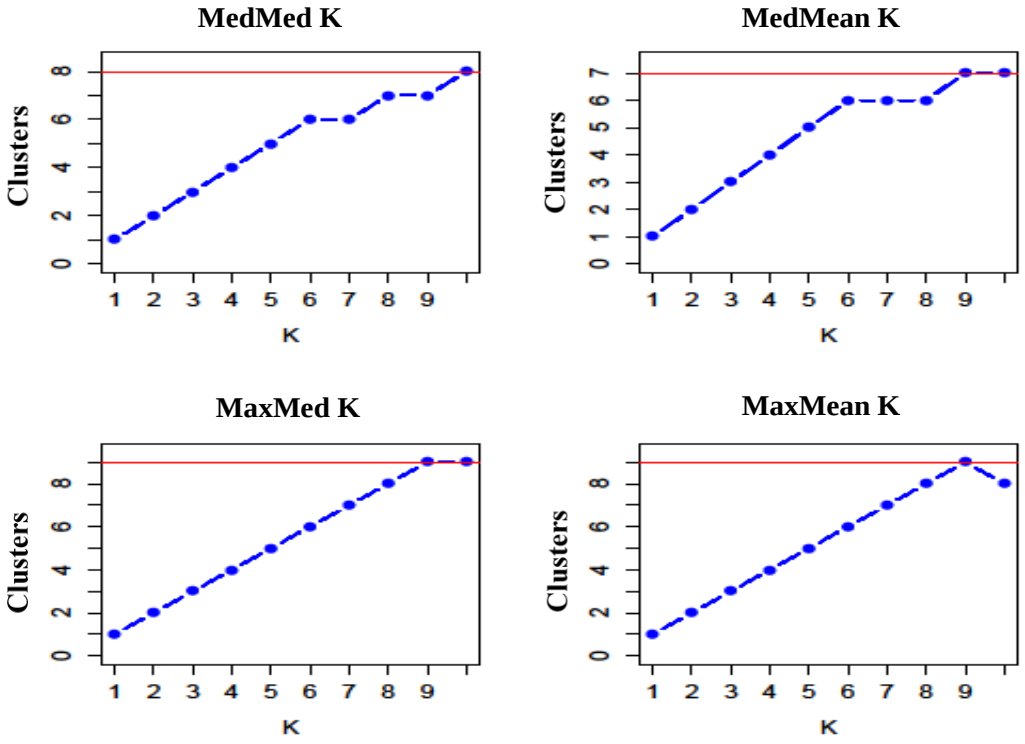


Figure 4.8. (a) Neighbor-joining (NJ) clustering revealed the genetic relationships of 381 chickpea lines including 19 wild and one cultivated parent using 14,591 SNPs markers with MAF ≥ 1%. The color dots indicate different parents that were crossed with the cultivated parent (CDC leader) to develop the chickpea lines (b) Phylogenetic tree and bootstrap values of 19-wild, and one cultivated parent (CDC Leader) based on the SNP markers. (c) Visualization of the formation of clusters of the chickpea lines with their respective parent.

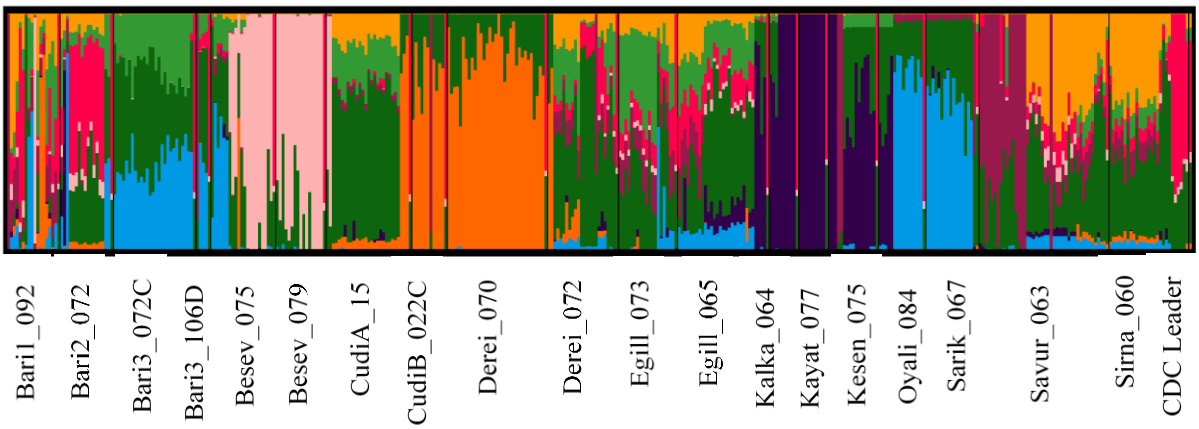
To provide further insights into the genetic diversity, the population structure was determined using the ADMIXTURE analysis. In this analysis, the 14,591 SNPs with MAF ≥ 1%, and the number *K* from 2 to 10 (repeating each analysis 20 times) were used to find the best *K* peak. The highest peak value was observed at $K = 9$, which indicated the possibilities of the presence of 9 clusters within the 381 lines (Figure 4.9.a). As the curve became plateaued or starts to decline at $K = 9$ (Figure 4.9.a), it provided a strong support to form 9 clusters from the lines. Furthermore, the ADMIXTURE analysis revealed some degrees of intermixing of the lines in each cluster. Thus, the sample lines could be considered as weakly differentiated. However, the population structure as shown in Figure 4.9.(b) indicated that the lines developed from Besev_075 and Besev_079 as well as Egill_073 and Egill_065 were clustered together, and these two clusters were clearly distinct from other groups. Formation of two different line groups derived from these four parents could be associated with the geographical variations from which they were collected.

4.7 Association analysis and potential candidate genes

The association between the SNPs and the variation in phenotypic traits were calculated using 5,501 SNPs that have MAF ≥ 5% and the mean values of the agronomic and yield contributing traits obtained from the field evaluation of 381 F_5 lines (Figures 4.10, 4.12-4.18). After filtering with MAF ≥ 5%, the scaffolds were removed in order to exclude the potential redundant markers (Figure 4.8). There were 51 SNPs identified on different chromosomes which showed significant association with five traits such as days to flowering, biomass yield (g), thousand seed weight (g), number of seeds per plant, and seed weight per plant (g) (Table 4.11; 4.12).



(a)



(b)

Figure 4.9. Admixture analysis of the 381 chickpea F_5 lines with their 19 wild parents and one cultivated parent (CDC Leader) was performed with K = 2 to 10 based on the polymorphic markers. Individual line was represented by a thin vertical line and the colour-coded admixture proportions indicate the genetic contributions of the parents. (a) Identification of the number of clusters of 401 chickpea lines. (b) Visualization of the chickpea population clusters revealed by ADMIXTURE analysis. When K = 9, the population was classified into nine groups.

A SNP locus was identified on chr4 that showed significant association with days to flowering (Figure 4.10). The SNP marker (*P* value = 10.4) detected within the 13.0 Mbp region on chr4 (Ca4_V1_P-13022400) was mainly responsible for early flowering trait. Further analysis confirmed that the alleles from the wild parents were associated with the early flowering (Figure 4.11). The highly significant SNP marker (Ca4_V1_P-13022400) was identified using the combined data of 2018 field sites which was associated with days to flowering and explained 0.3 to 5.0% phenotypic variance (R^2) for this trait. There was no SNP significantly associated with plant height. The number of SNPs significantly associated with different traits were: one SNP for days to maturity (R^2=12%), three SNPs for biomass yield (R^2=1.0 to 6.0%), 13 SNPs for number of seeds per plant (R^2=0.4 to 1.1%), 12 SNPs for thousand seed weight (R^2=0.2 to 3.0%), and 13 SNPs for seed weight per plant (R^2=0.1 to 32%). The highest mean difference between wild and cultivated alleles for these traits were 3.0 days for days to maturity, 8.5 g for biomass, 4.0 for number of seeds per plant, 16 g thousand seed weight, and 4.3 g seed weight per plant, respectively (Table 4.11; 4.12).

Potential candidate gene identification was performed within 100 Kb region on either side of the significant markers via LIS (Legume Information System). Seven candidate genes were found in the regions, three of these genes are related to flowering and four candidate genes are related to the growth, development, and yield (Table 4.13).

Table 4.11. List of significant SNPs from genome-wide association analysis for the traits evaluated at Saskatoon and Moose Jaw during 2017.

Traits	Field sites	Chromosome	Most significant SNP	Number of SNPs	-log$_{10}$ *P* value
Days to flowering	Combined	VI	Ca6_V1_P-46744160	1	5.24
Biomass	Combined	III	Ca3_V1_P-31624927	1	6.26
Number of seeds per plant	Saskatoon	II	Ca2_V1_P-33400910	2	10.2
		IV	Ca4_V1_P-27008886	2	6.63
		V	Ca5_V1_P-28287194	1	6.04
	Moose Jaw	VI	Ca6_V1_P-22032893	2	5.09
Thousand seed weight (g)	Moose Jaw	I	Ca1_V1_P -710760	1	5.30
		IV	Ca4_V1_P-9707182	5	6.60
Seed weight per plant (g)	Saskatoon	I	Ca1_V1_P-25733193	1	5.01
		II	Ca2_V1_P-30049933	2	6.32
		IV	Ca4_V1_P-40127929	1	5.12
		V	Ca5_V1_P-41263716	3	6.94
		VI	Ca6_V1_P-41147990	3	9.32
		VII	Ca7_V1_P-45085937	3	5.19

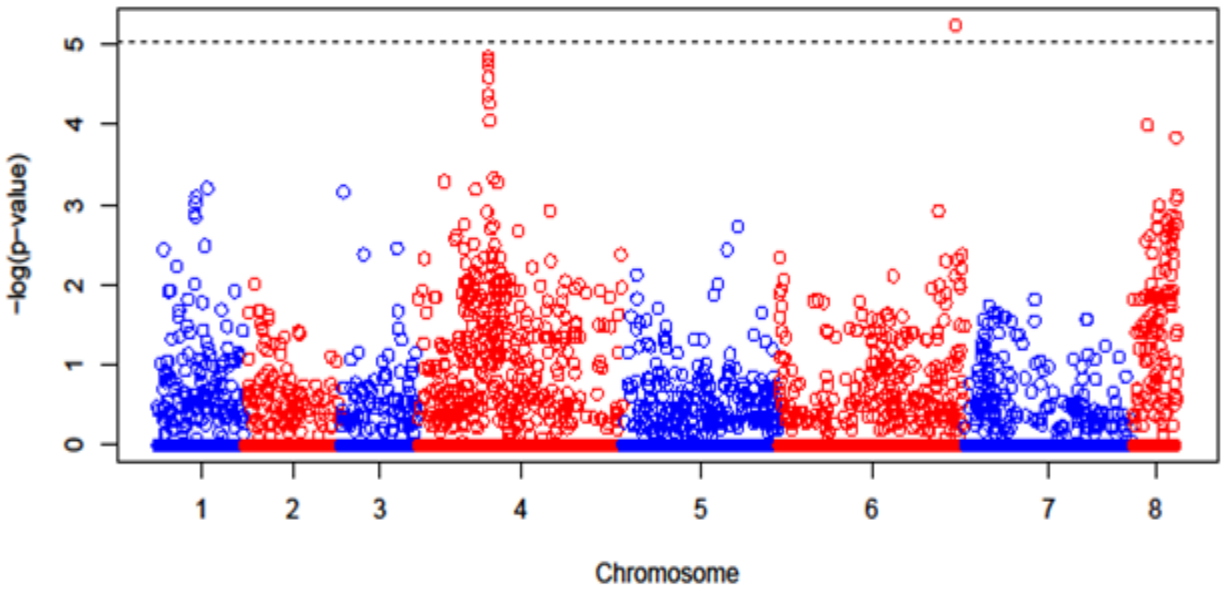
*Ca = *Cicer arietinum*; V1 = Version 1; P = Position on chromosome in base pairs.

Table 4.12. List of significant SNPs from genome-wide association analysis of the traits evaluated at Limerick and Lucky Lake in 2018.

Traits	Field sites	Chromosome	Most significant SNP	Number of SNPs	-log$_{10}$ *P* value
Days to flowering	Combined	IV	Ca4_V1_P-13022400	9	10.4
Days to maturity	Lucky Lake	VIII	Ca8_V1_P-957257	1	5.08
Biomass	Combined	VII	Ca7_V1_P-34285390	2	12.1
Number of seeds per plant	Lucky Lake	II	Ca2_V1_P-15088105	1	5.02
		III	Ca3_V1_P-14460088	1	5.14
		VII	Ca7_V1_P-34285390	2	6.79
		VIII	Ca8_V1_P-310610	1	8.95
Thousand seed weight (g)	Limerick	I	Ca1_V1_P-14313744	1	6.54
		II	Ca2_V1_P-17609263	2	6.28
		V	Ca5_V1_P-32629686	1	5.32
	Lucky Lake	V	Ca5_V1_P-29349635	1	5.23
		VI	Ca6_V1_P-16130634	1	11.2

*Ca = *Cicer arietinum*; V1 = Version 1; P = Position on chromosome in base pairs.

(a) Manhattan plot of days to flowering (Combined-2017)



(b) Manhattan plot of days to flowering (Combined-2018)

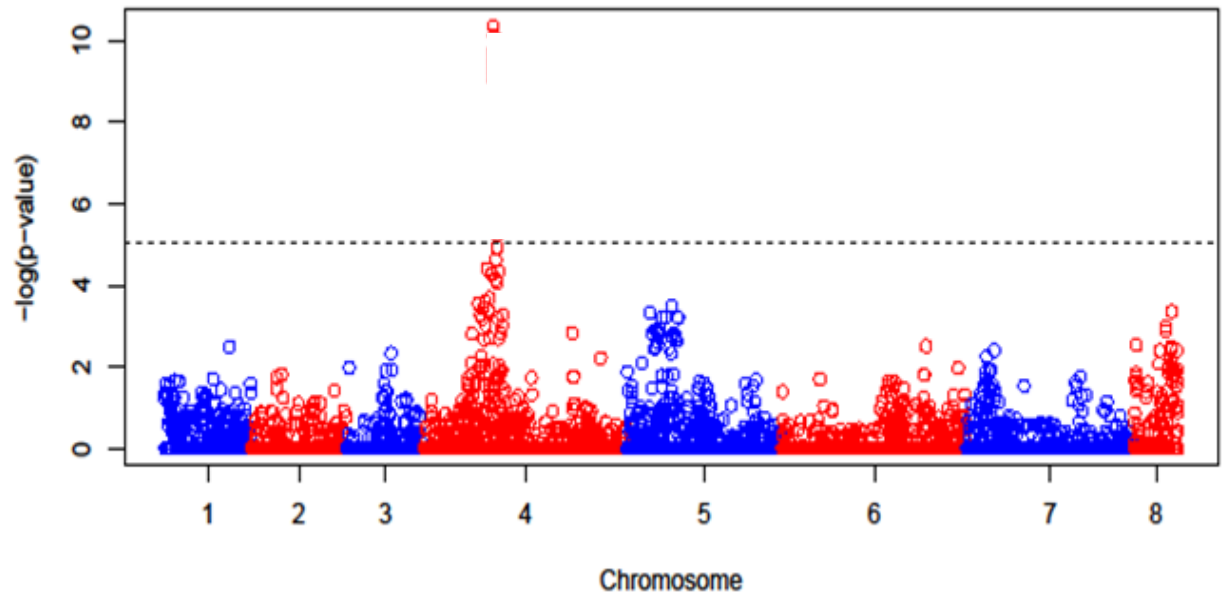
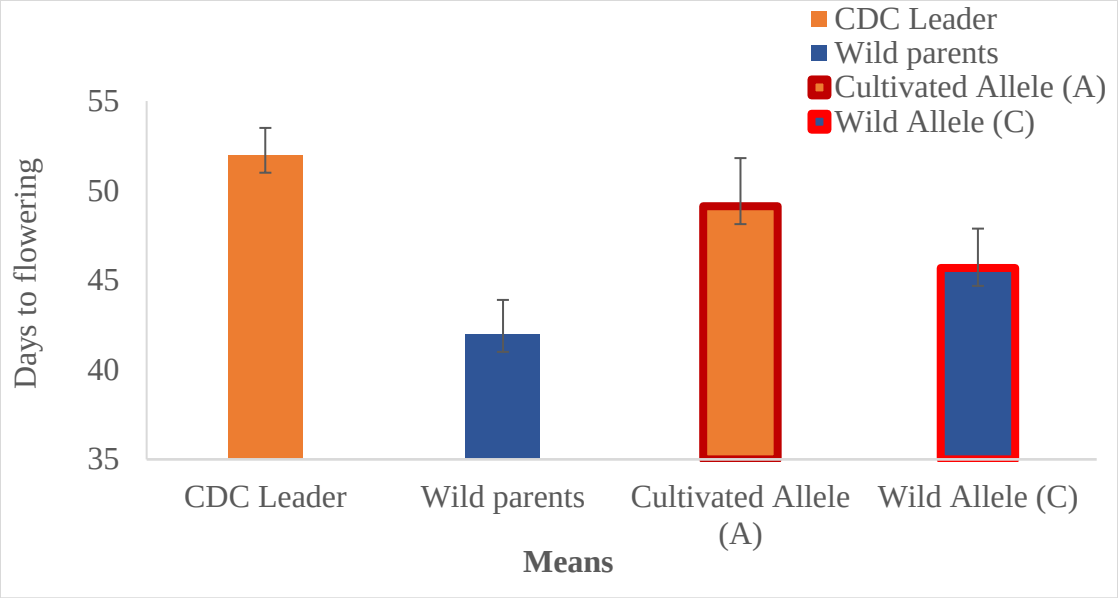


Figure 4.10. Manhattan plots from genome-wide association analysis for days to flowering using the combined phenotypic data of two locations in each year of (a) 2017 and (b) 2018. Horizontal axis indicates the position of the SNPs on chromosomes. Vertical axis shows -$\log_{10}$ *P* value of the SNP loci.



(A) Ca4_V1_P-13022400

Figure 4.11. Comparison of the frequency of cultivated parent (CDC Leader) with the average of wild parents (n = 19) for days to flowering and the relative contribution of cultivated alleles (187 lines) and wild alleles (167 lines) explained by the most significant SNP ($-\log_{10} P$ value = 10.35) for early flowering of the 381 F_5 lines.

Manhattan plot of days to maturity (Lucky Lake-2018)

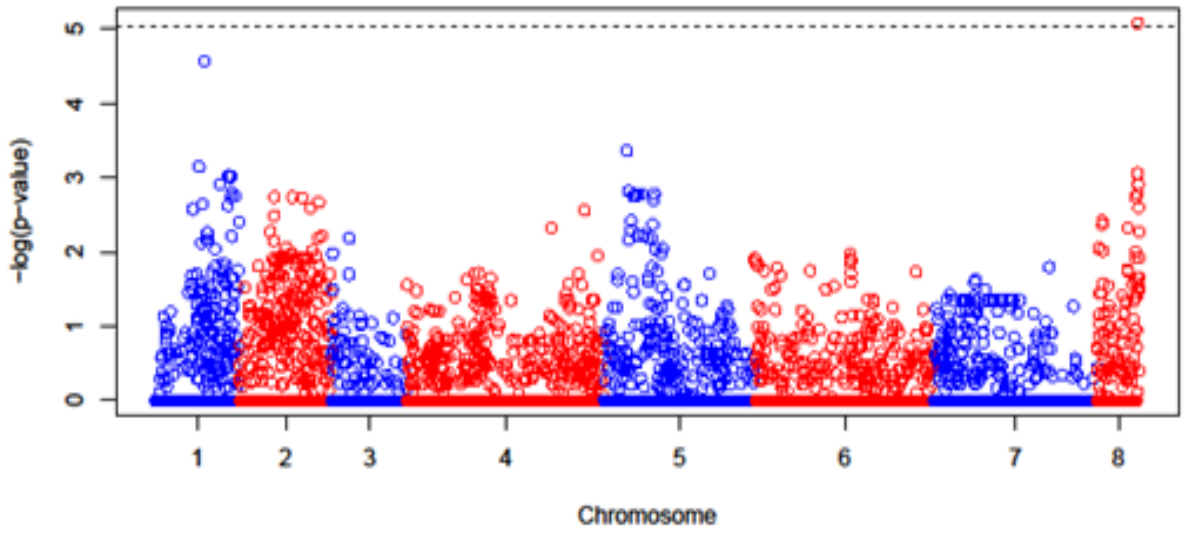
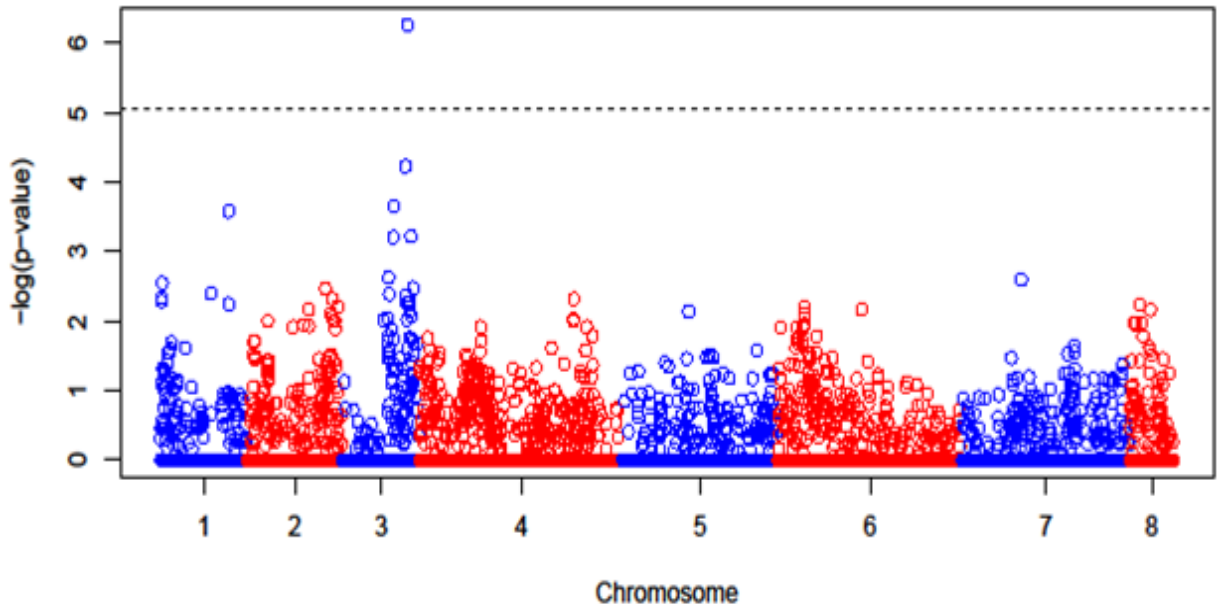


Figure 4.12. Manhattan plots from genome-wide association analysis for days to maturity using the phenotypic data of Lucky Lake-2018. Horizontal axis indicates the position of the SNPs on chromosomes. Vertical axis shows $-\log_{10} P$ value of the SNP loci.

(a) Manhattan plot of biomass yield (Combined-2017)



(b) Manhattan plot of biomass yield (Combined-2018)

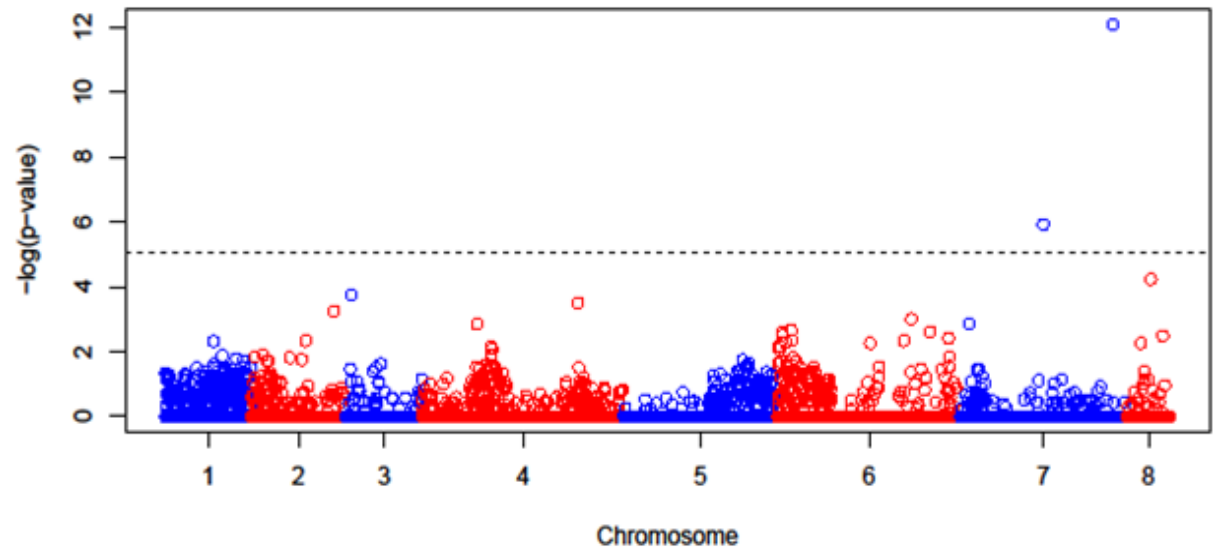
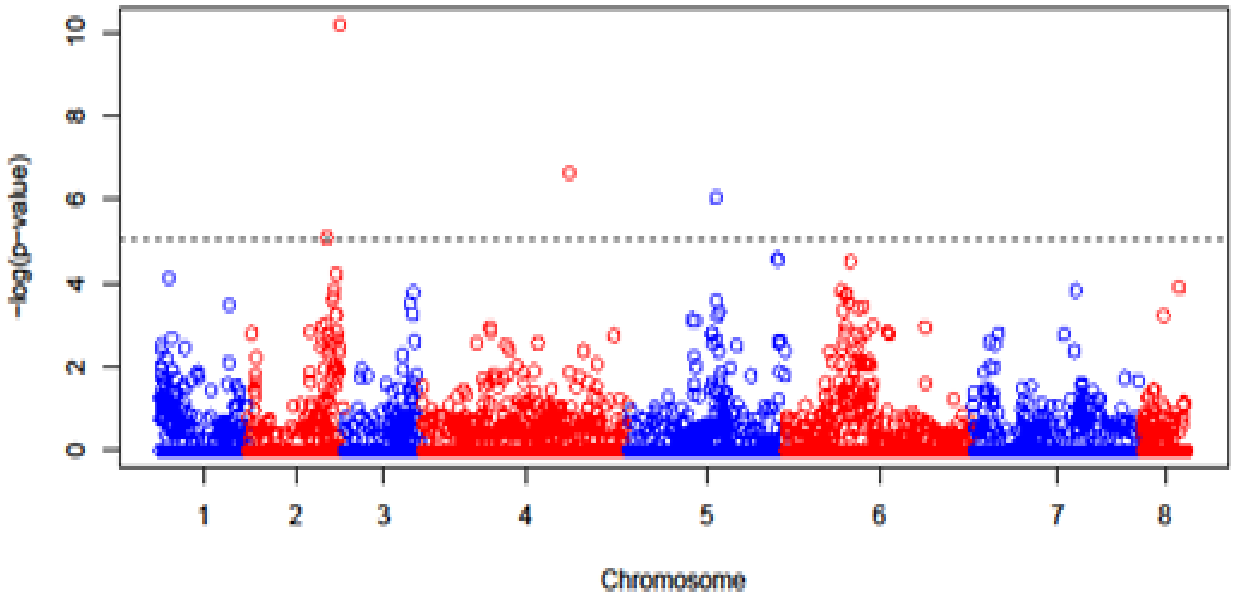


Figure 4.13. Manhattan plots from genome-wide association analysis for biomass yield using the combined phenotypic data of two locations in each year of (a) 2017 and (b) 2018. Horizontal axis indicates the position of the SNPs on chromosomes. Vertical axis shows -$\log_{10}$ *P* value of the SNP loci.

(a) Manhattan plot of number of seeds per plant (Saskatoon-2017)



(b) Manhattan plot of number of seeds per plant (Moose Jaw-2017)

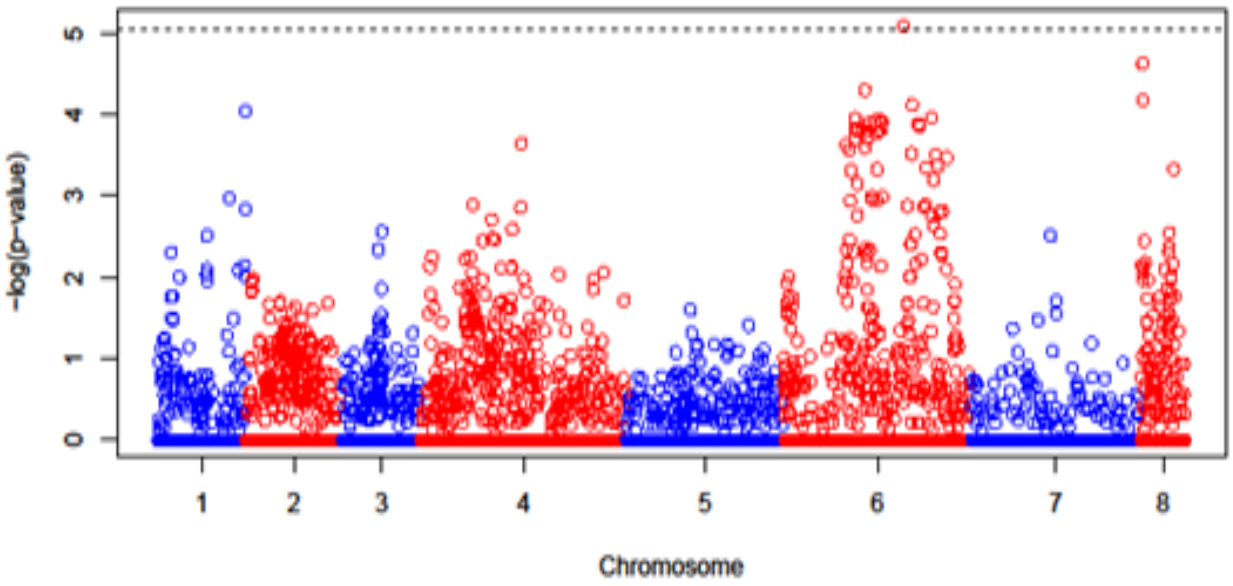


Figure 4.14. Manhattan plots from genome-wide association analysis for number of seeds per plant using the phenotypic data of 2017 (a) Saskatoon and (b) Moose Jaw. Horizontal axis indicates the position of the SNPs on chromosomes. Vertical axis shows -$\log_{10}$ *P* value of the SNP loci.

Manhattan plot of number of seeds per plant (Lucky Lake-2018)

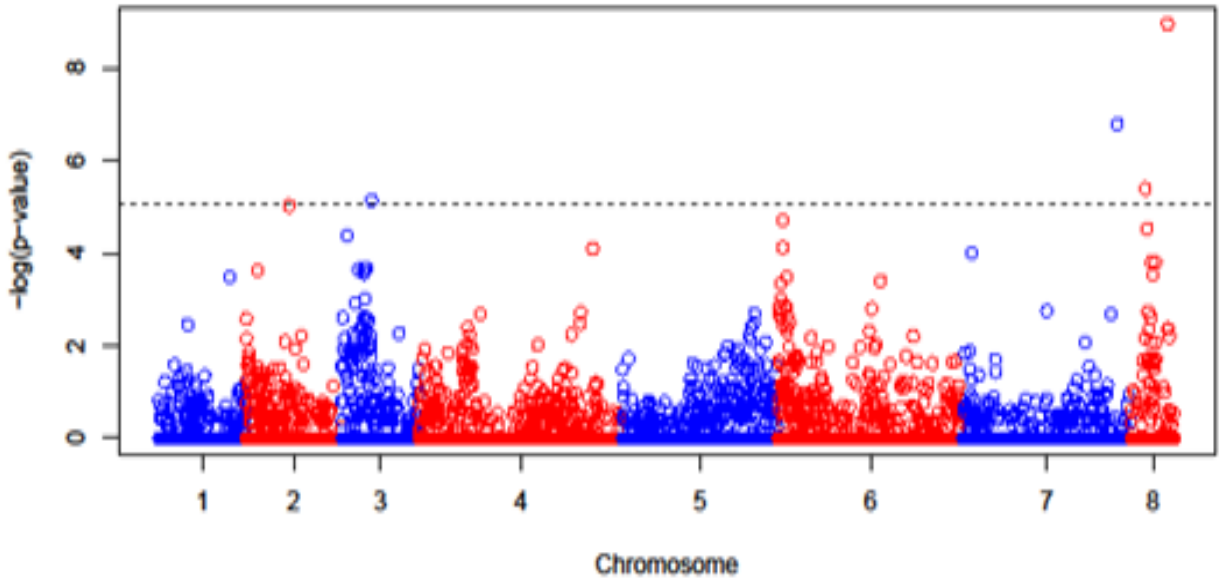


Figure 4.15. Manhattan plots from genome-wide association analysis for number of seeds per plant using the phenotypic data of Lucky Lake-2018. Horizontal axis indicates the position of the SNPs on chromosomes. Vertical axis shows -$\log_{10}$ *P* value of the SNP loci.

Manhattan plot of thousand seed weight (Moose Jaw-2017)

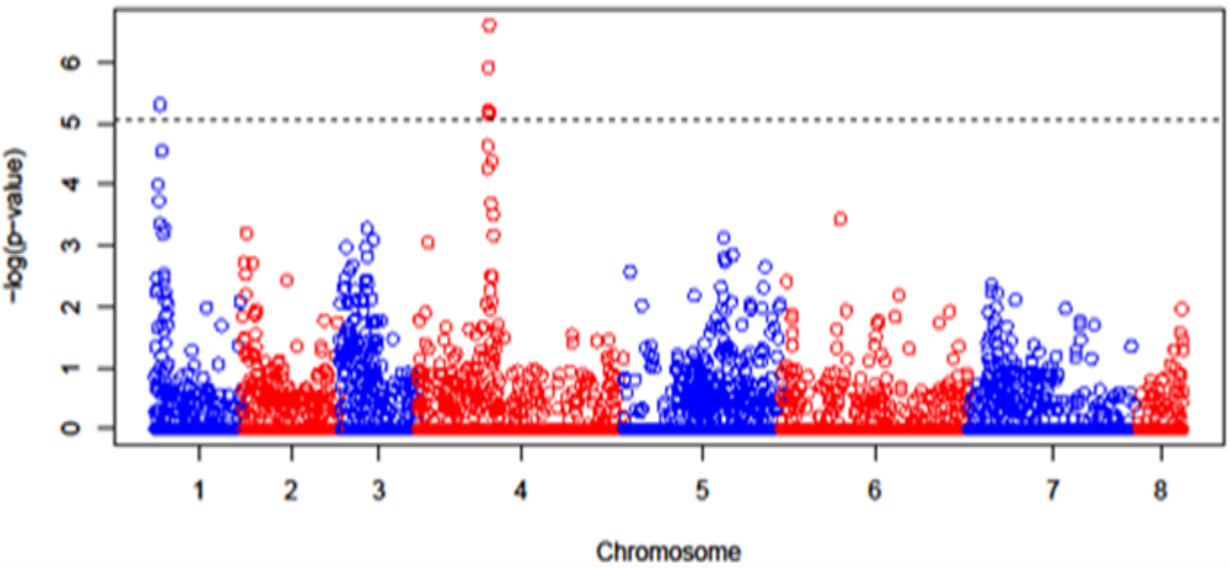
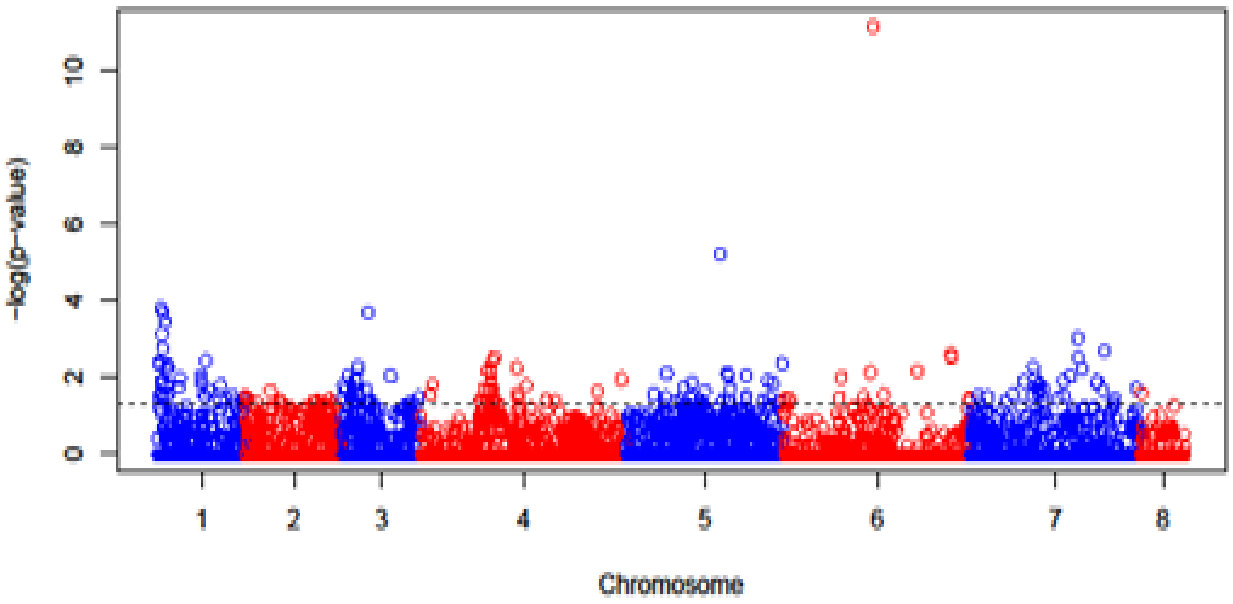


Figure 4.16. Manhattan plots from genome-wide association analysis for thousand seed weight (g) using the phenotypic data of Moose Jaw-2017. Horizontal axis indicates the position of the SNPs on chromosomes. Vertical axis shows -$\log_{10}$ *P* value of the SNP loci.

(a) Manhattan plot of thousand seed weight (Limerick-2018)



(b) Manhattan plot of thousand seed weight (Lucky Lake-2018)

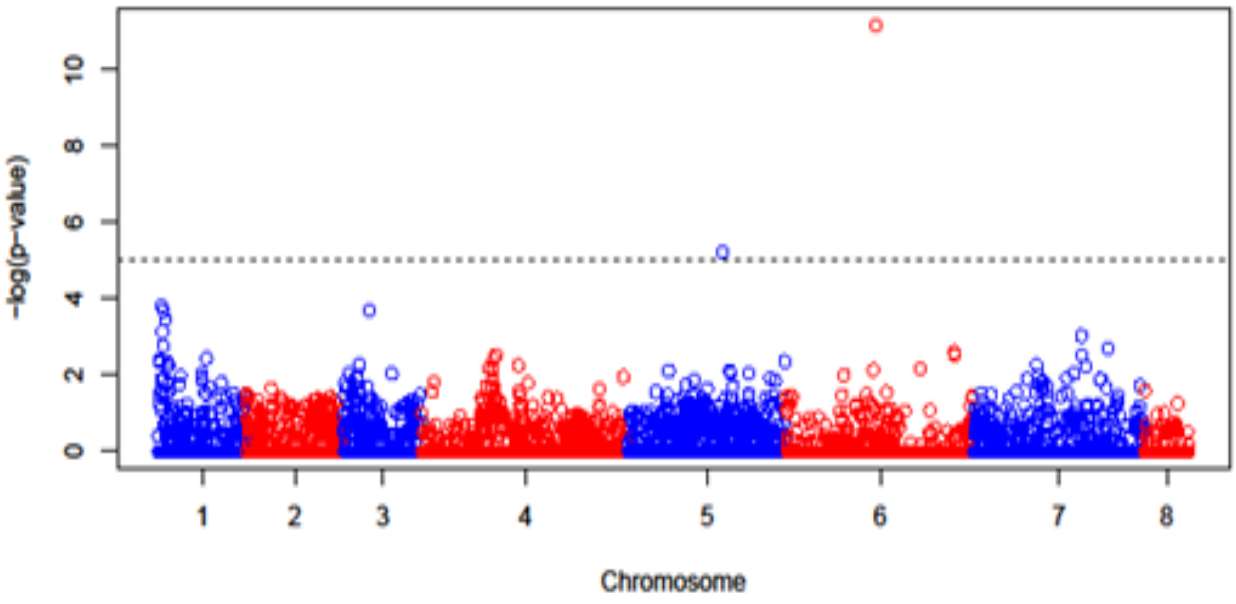


Figure 4.17. Manhattan plots from genome-wide association analysis for thousand seed weight using the phenotypic data of 2018 (a) Limerick and (b) Lucky Lake. Horizontal axis indicates the position of the SNPs on chromosomes. Vertical axis shows -$\log_{10}$ *P* value of the SNP loci.

Manhattan plot of seed weight per plant (Saskatoon-2017)

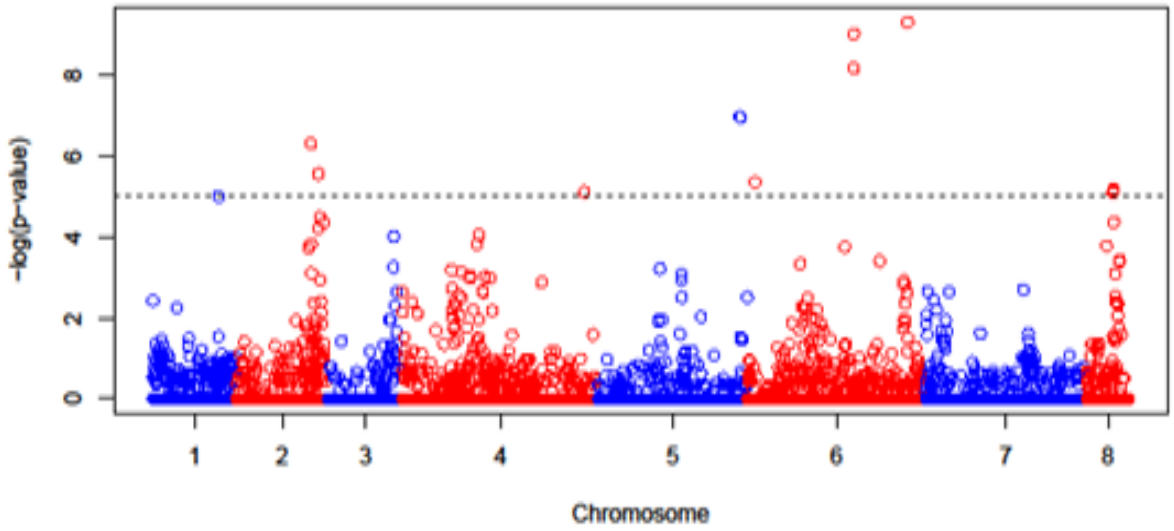


Figure 4.18. Manhattan plots from genome-wide association analysis for seed weight per plant using the phenotypic data of Saskatoon-2017. Horizontal axis indicates the position of the SNPs on chromosomes. Vertical axis shows -$\log_{10}$ *P* value of the SNP loci.

Table 4.13. Candidate gene annotations for the studied traits and their position on the chickpea genome.

Gene Id	Chromosome	Start	End	Description	Gene Function	Reference
Related to flowering						
Ca_TIC	IV	13836536	13844034	protein (tic)	Early flowering	Hall et al., 2003
Ca_GA 20OX2	IV	13002067	13004480	gibberellin 20 oxidase 2	Associated with flowering time	Rieu et al., 2008
Ca_PC L1	VI	54242622	54245220	transcriptio n factor PCL1-like	Associated with flowering time	Onai et al., 2005
Related to yield						
Ca_102 65	II	32585905	32594820	Protein kinase	Regulates photophosp -horylation activity	Peng et al., 2008
Ca_102 21	II	33011956	33016412	Protein kinase	Regulates photophosp -horylation activity	Peng et al., 2008
Ca_102 04	II	33172919	33174836	Plastocyani n-like	Involved in electrons to photosyste m I	Weigel et al., 2003
Ca_149 21	IV	39853369	39854663	Photosyste m I PsaG/PsaK protein	Involved in photosynth esis	Friso et al., 2004

5. DISCUSSION

The narrow genetic base of cultivated chickpea germplasm is restricting the opportunities of genetic advancement for higher yield, quality, and desired agronomic traits. Several studies (Siddique et al., 2000; Varshney et al., 2013; Kantar et al., 2017) reported that the valuable genes that were lost through the domestication and recurrent selection process could have a significant contribution in the development of new varieties with higher yield, quality, and increased tolerance to biotic and abiotic stresses. Conversely, the wild relatives of chickpea are considered as the most significant sources of genetic variability and have promising potential for variety improvement (Singh et al., 2008; Sharma et al., 2013). *C. reticulatum* is considered as one of the most important wild species closely related to cultivated *C. arietinum* and exhibits a high cross-compatibility (Singh and Ocampo 1993; Mallikarjuna et al., 2007; Sharma et al., 2013; Singh et al., 2018). This ultimately provides an opportunity to successfully utilize the potential advantage of cross-compatibility between *C. reticulatum* with *C. arietinum* for developing interspecific hybrids of chickpea (Mallikarjuna et al., 2007; Singh et al., 2015).

5.1 Variability and performance of the interspecific lines

The segregating populations used in this research were developed from the interspecific crossing between twenty wild (*C. reticulatum*) and one cultivated (CDC Leader) parents. Two successive generations (F_4 and F_5) of chickpea lines derived from the interspecific crosses were evaluated at four locations in Saskatchewan. The populations were completely fertile and capable of producing fertile progenies. The population revealed a considerable variation for seed yield and agronomic traits (Table 4.1; 4.2 and Figure 4.1). Our results were in line with the findings of Singh et al. (2018).

The wild accessions used in this research were known to have high genetic variation (von Wettberg et al., 2018) and contributed in improving the productivity as well as resistance to biotic and abiotic stresses under the environmental conditions of California, USA (von Wettberg et al., 2018). The initial anticipation of genetic variability in these wild germplasms is based on their diverse geographical distribution and adaptability to varying environmental conditions. The variability in studied lines is described by using the mean, range and variance of the means of a specific trait (Table 4.1; 4.2 and Figure 4.1). The results showed considerable variations among the studied lines for all the yield contributing and agronomic traits. Different yield contributing characteristics such as seed weight per plant, number of seeds per plant, and biomass yield per plant showed the maximum variability. The large variation in yield in the progeny lines suggested that the favourable genes have been transferred by interspecific crossing. In many instances, the segregating lines developed from the interspecific crosses showed high genetic variability for different traits including the number of branches per plant and harvest index (Gaur et al., 2007; Singh et al., 2015; Singh et al., 2018). Both genetic and environmental factors, as well as the interactions between genes and environmental factors, might have contributed to this type of observed variation. A recent study of chickpea grown at eight different locations in Australia showed a significant influence of the environment on the genetic variation for yield (Kaloki et al., 2019). The large yield variation is typically due to the introgression of genes from the wild *Cicer* species (Srivastava et al., 2016; Singh et al., 2018). Similar findings on the increased phenotypic variability in the cultivated chickpea that was derived from interspecific crossing between wild and cultivated variety were reported in India (Jaiswal et al., 1986; Singh et al., 2018). The variation observed for the growth habit such as erect and semi-erect type of plants can facilitate a potential advantage for mechanical harvesting. Thus, proportions of lines

with preferred qualitative traits listed in figure 4.1 can offer to identify some erect or semi-erect genotypes suitable for mechanized agriculture of Canadian Prairies. Moreover, the variation in seed types such as kabuli, desi and round or pea type could be utilized for further development of varieties to satisfy consumer's demand. Overall, the genotypes with erect and semi-erect characteristics that exhibited less susceptibility to diseases, better harvest index, and high seed yield could be utilized for the development of commercially acceptable chickpea varieties for growers of western Canada.

5.2 Interrelationship among the yield and yield contributing traits for efficient selection

Extensive knowledge of genetic variability and relationship among the yield contributing traits can easily justify the success and effectiveness of breeding strategies. Usually, seed yield is considered as a complex trait and it is profoundly influenced by all agronomic and yield contributing characteristics. Correlation analysis is one of the most common approaches to evaluate the relationships among traits and to identify the most important ones contributing to seed yield (Kozak et al., 2012). The relationships among various traits is useful for selecting genotypes with higher productivity based on groups of desired traits that significantly contribute to the increased yield of chickpea.

The use of correlation statistics is well documented for genotype selection in variety improvement programs (Patane, 2006; Kozak et al., 2012; Hu et al., 2019). However, the selection strategies could be effective only when the traits exhibit a significant and positive correlation with seed yield. For instance, the high yielding chickpea genotype selections were performed in a study conducted in Pakistan depending on the traits that showed a significant and positive correlation with yield (Qureshi et al., 2004). The positive correlation was between the number of seeds per plant and seed yield observed in this study, suggesting that the increase of

number of seeds per plant could be an effective way to increase the chickpea yield under the western Canadian soil-climatic condition. Similar, positive correlation of total seed yield with biomass yield and harvest index was also observed in earlier studies (Ahmad et al., 2012; Belete et al., 2017). These traits were used for further breeding to increase chickpea seed yield (Ahmad et al., 2012). The biomass yield showed a significantly positive correlation with the number of seeds per plant in 3 out of 4 locations over two years. Path analysis also confirmed this finding and indicating that the biomass yield has a significant direct effect on the number of seeds per plant. Therefore, the selection of genotypes with higher biomass yield could be a potential option to improve the seed yield of chickpea. Path coefficient analysis also confirmed the significant and high positive direct effects of other yield components such as thousand seed weight on the total seed weight per plant in 3 out of 4 locations over two years.

The thousand seed weight was negatively correlated with the number of seeds in 2018 field trials, which agreed with the findings of Belete et al. (2017). Apart from plant height, the other traits such as the number of days to flowering and ascochyta blight disease infestation showed negative relationships with seed yield at 1 out of 4 locations and the relationship between days to maturity and seed yield was inconsistent. The research conducted by Jha et al. (2012) under tropical weather condition revealed that the days to flowering and days to maturity had a negative correlation with seed yield. Severe yield loss of chickpea with the increase of ascochyta blight disease was also observed (Chongo et al., 2003; Tadesse et al., 2017). Conversely, Frimpong et al. (2009) reported that chickpea yield is mostly affected by the environment, and the yield performance of a variety was inconsistent under different environmental conditions of the Canadian prairies.

5.3 Genotype by environment interaction, and broad sense heritability

Evaluation of the chickpea lines under two different environmental conditions provided an opportunity to select stable genotypes that could be useful for further development of breeding populations with higher adaptability in the changing environment (Crossa, 2012; Kaloki et al., 2019). Generally, the seed yield is a complex trait, controlled by multiple genes, and strongly influenced by the interaction between the environmental factors and yield contributing traits (Kashiwagi et al., 2008; Varshney et al., 2014). The most significant genotype by environment interaction effects were observed for days to maturity, the number of seeds per plant, thousand seed weight, and seed yield. Therefore, better documentation on the genomic approach for the perception and processing of environmental signals need to be developed. Several researchers (Toker, 2004; Yadav et al., 2014; Abdi, 2018; Kaloki et al., 2019) have evaluated the influence of environment on the yield components of different legumes and selected the identical genotypes with improved yield under varying environmental conditions. However, chickpea yields are highly influenced by genotype and environment interactions and exhibit poor heritability under marginal and unfavourable environments (Kashiwagi et al., 2008; Varshney et al., 2014). Overall, the interaction between genotypes and the environment for economically important traits deserve further attention.

The broad-sense heritability estimated from the variance components resulted in low to moderate heritability for the traits in the current study. The highest heritability was observed for days to flowering which indicated that this trait is favourable for the selection of better-performed chickpea lines under the Canadian prairie conditions. A similar finding on high heritability for days to flowering was reported in research conducted with 47 chickpea genotypes in Pakistan (Khan and Farhatullah, 2011). Using the knowledge of the heritability for the selection of the

best progenies is crucial for better transmissibility of traits in variety improvement programmes (Mba et al., 2012; Addisu and Shumet, 2015; Yirgu, 2017). The low to medium H^2 expressed by the traits in the current study was associated with the significant effects of the environments on the phenotypic expression. The low heritability for the yield components observed in this study agreed with the previous research findings of Zali et al. (2011) and Jha et al. (2012). In general, the heritability of a specific trait changed over time due to variation of temperature and environmental conditions (Kashiwagi et al., 2008; Varshney et al., 2014).

5.4 Clustering of the chickpea lines based on the phenotypic traits

Cluster analysis using the phenotypic traits can be used to separate the genotypes into distinct groups based on a particular trait of interest (Nath et al., 2014). In this study, the Euclidean distance following Ward's method was used to identify six clusters. The result also indicated the morphological diversity of the interspecific lines. Grouping of 381 chickpea lines into those clusters was based on similarity matrix, therefore, it was considered a completely random process as no relationship between pedigree and genetic diversity was observed. Furthermore, a given cluster included some diverse lines that were developed from different parents. Among the six groups, the cluster I comprised 67 lines with important yield traits such as the seed weight per plant, the maximum number of seeds per plant, and the highest total seed yield. The cluster IV consisted of 72 lines which have a high thousand seed weight. The lowest number of lines (28) were found in cluster VI, which was categorized with early flowering, early maturity, and reduced susceptibility to ascochyta blight disease. These observations suggested the possibility of yield improvement by combining high seed yield with increased seed weight through effective selection and hybridization between the genotypes of cluster I and cluster IV. Previous research suggested the possibility of attaining hybrid vigour from crossing between genotypes of distant

clusters (Sharifi et al., 2018). Furthermore, the hybridization between genotypes of cluster I and cluster VI will facilitate the opportunity to incorporate the commercially demanding traits of prairies (such as early flowering, early maturity, and reduced ascochyta susceptibility) in high yielding genotypes.

Many researchers reported the clustering of chickpea genotypes through a similarity matrix to evaluate the phenotypic diversity for desirable traits and successfully identified the most diverse genotypic groups (Admas and Abeje, 2017; Sachdeva et al., 2018). However, the clustering pattern of the chickpea lines was irrespective of their parental germplasms. Additionally, the lines derived from crossing between parents were found to group into different clusters. A similar clustering pattern was used to evaluate the genetic diversity of chickpea genotypes based on the highest performance in desired agronomic and yield traits (Admas and Abeje, 2017; Singh et al., 2018).

5.5 Genetic diversity in the chickpea lines using SNP genotyping

Effective utilization of plant genetic resources for variety improvement largely depends on the available genetic diversity of the breeding population. Singh et al. (2008) reported that a better understanding of genetic diversity in chickpea germplasms can contribute to select and adopt the novel breeding strategies for superior variety development. Usually, the natural allelic variation in the wild *Cicer* is much higher than the cultivated species (Upadhyaya et al., 2008). Therefore, the wild *Cicer* species were considered as a potential source of desirable genes for commercially valuable traits (Singh et al., 2008; Sharma, 2017). The introgression of desirable alleles from wild species through interspecific hybridization is considered the best approach for improving the genetic variation in cultivated chickpea (Adak et al., 2017; Sharma, 2017). Some studies (such as Verma et al., 1990 and Singh et al., 2015) showed successful breeding with wild *Cicer*

species to increase the genetic diversity of chickpea. The genetic diversity and population structure results of this study also confirmed a substantial amount of genetic variation in the developed breeding lines.

Recent improvements in genotyping-by-sequencing has led to generate a large number of cost-effective genome-wide molecular markers such as SNPs (Varshney et al., 2007; Yang et al., 2011; Caruana et al., 2019). Additionally, the molecular marker technology is being used widely in chickpea breeding programs to investigate the diversity, genetic relationship and marker-trait association due to their tight linkage with important agronomic and adaptive traits (Cobos et al., 2005; Singh et al., 2008; Tadesse et al., 2017). Several researchers (Gupta and Varshney, 2004; Huang and Han, 2014; Caruana et al., 2019; Long et al., 2019) reported abundant of SNP markers throughout the genome and their effective association with genes controlling a specific trait. Therefore, SNPs are used for estimating the genetic diversity, population structure, and marker-trait associations which are essential for evaluating the genetic potential of the experimental germplasms.

Different approaches (i.e., Neighbour-joining and Admixture; Skotte et al., 2013; Farahani et al., 2019) were used in this study which were known to give better indications of genetic diversity and structure of the studied chickpea lines. Also, the studied lines were genotyped by a modified genotyping-by-sequencing method called tGBS using two restriction enzymes (NspI and BfuCI) as described by Ott et al. (2017). This method used single-stranded oligos instead of double-stranded adaptors that simplified the tGBS library preparation (Ott et al., 2017). Moreover, it is well suited for genotyping germplasms with available reference genome and showed high SNPs calling accuracy and generation of less missing data per site. In this study, the SNPs identification was performed using the reference genome of CDC Frontier (Version 1.0;

Varshney et al., 2013). The SNP markers generated by tGBS method were used to determine the genetic relationship among the 401 chickpea genotypes by the neighbour-joining method. This method was used extensively to explain the evolutionary relationships among the diverse crop genotypes (Zhao et al., 2011; Saxena et al., 2014). Increased diversity in the chickpea germplasms is evident from the grouping of breeding lines with their respective wild parents used in this crossing program. The information derived from diversity analysis could be utilized for developing cultivars with desirable agronomic traits through crossing between genotypes from a different cluster. The SNPs were found capable to explain the reason for the clustering of some lines and irrespective parents as greater similarities with other progenies were identified. Additionally, the genetic relationship analysis in the parents indicated the formation of 16 groups out of nineteen wild and one cultivated parent used in crossing. However, the lack of distinct differentiation in wild and cultivated accessions were also reported in some recent studies (such as Saxena et al., 2014; Nguyen et al., 2018) and likely to be associated with the low genome coverage sequencing (Basu et al., 2018). These specific or isolated groups could be associated with the adaptability, growth pattern of the wild parents, and the environmental conditions of the areas from which they were collected. The wild parents were collected from different elevation gradients. These accessions may possess useful genetic variation for adaptability and seed quality. For example, the Sirna_060 parent was collected from the highest elevation (1658.92 m) which had distinct environmental condition such as low temperature in winter and high annual rainfall (von Wettberg et al., 2018). The results agreed with similar studies conducted by Roorkiwal et al. (2013) and von Wettberg et al. (2018), who reported the presence of large diversity in the wild accessions collected from the similar regions of Fertile Crescent and successfully utilized those wild parents for improving the genetic diversity in chickpea.

Admixture analysis using SNPs is essential for determining the genetic structure of introgressed lines with important agronomic and yield contributing traits (Basu et al., 2018; Farahani et al., 2019). These results indicated that the SNP markers categorized the lines into nine groups (K = 9) along with a little intermixing of lines in them. However, it is not unusual to exhibit admixed ancestry traces in the developed breeding lines as also have been reported in different studies (Winkler et al., 2016; Fu et al., 2017; Basu et al., 2018). Furthermore, the phylogenetic relationship analysis also supports the formation of groups with admixed chickpea lines derived from different parental crosses. Typically, the presence of admixed ancestry in the populations was likely related to the wild parental accessions that inherited similar gene pool (Saxena et al., 2014; Farahani et al., 2019). In a recent study by Basu et al. (2018), it was confirmed that admixture analysis was capable to identify the relationships between the breeding population with their ancestry that could be utilized for marker-assisted breeding programs of chickpea. Finally, the genetic diversity and population structure revealed in this study could be used in future breeding efforts to improve chickpea.

5.6 Association mapping of the studied traits

The use of association mapping is considered as a powerful tool to identify the SNP markers associated with important agronomic traits (Zhao et al., 2011). In the past, several studies were known to use the SNP markers to depict marker-trait associations in segregating population evaluation for new variety development of chickpea (Azhaguvel et al., 2006; Abbo et al., 2005; Thudi et al., 2014; Basu et al., 2018). The identification of the molecular markers showed that the genes governing yield and agronomic traits are widely distributed throughout the genomic region of chickpea (Varshney et al., 2013). Therefore, the identification of markers associated with the candidate genes that govern novel agronomic traits is vital for variety improvement.

Our association analysis integrated the phenotypic data of 381 chickpea lines with the genotypic information to identify the SNPs associated with the commercially acceptable traits. All phenotypic data obtained from the field study have been used for association mapping, however, four traits have shown significant variation under two different environmental conditions. Based on the physical position of the SNP markers several genes associated with flowering and yield-related traits were identified. The presence of candidate genes on different chromosomes (documented by Ridge et al., 2017; and Basu et al., 2018) are closely matched with the locations of our significant makers. The significant SNPs were found on chromosomes 4 and 6 and showed a relationship with the flowering time of chickpea. These results agreed with earlier studies that reported the presence of markers on similar chromosomes (i.e., 4 and 6), and significantly associated with the flowering time of chickpea (Varshney et al., 2014; Upadhyaya et al., 2015; Daba et al., 2016; Ridge et al., 2017). Additionally, the markers identified for yield and yield contributing traits such days to maturity, biomass (g), number of seeds per plant, thousand seed weight (g), and seed weight per plant (g) of this study are distributed widely on different chromosomes. Similar results were reported by Srivastava et al. (2016) and Basu et al. (2018) who identified that the SNP loci associated with seed yield are widely distributed throughout the genomic regions of chickpea. The genetic basis of the protein produced by the flowering related genes have been well characterized in *Arabidopsis thaliana* (Hall et al., 2003; Onai et al., 2005; Rieu et al., 2008). The identified genes involved in encoding kinase protein, plastocyanin, and PsaG/PsaK protein are known to be associated with seed yield trait in chickpea as it controls the molecular pathways of underlying growth, development and yield traits (Weigel et al., 2003; Friso et al., 2004; Peng et al., 2008). Overall, seed yield is considered as a complex quantitative trait and the continuous marker-assisted breeding research has identified numerous

genomic regions that can govern crop yield (Xu et al., 2011; Varshney et al., 2014; Kujur et al., 2015; Srivastava et al., 2016).

6. CONCLUSION

The lines derived from interspecific crosses between a cultivated chickpea variety and wild accessions of *Cicer reticulatum* were highly variable for the agronomic and yield traits. The valuable alleles derived from the wild accessions were confirmed by phenotypic and genotypic evaluations. The correlation and path coefficient analyses revealed that seed weight per plant, thousand seed weight, number of seeds per plant, and biomass yield were the most significant yield contributing traits to enhance the seed yield potential of cultivated chickpea. Cluster analysis based on the agronomic and yield contributing traits categorized the lines into six distinct clusters, which provides the potential for future improvement by crossing the lines among the clusters for yield improvement and resistance to ascochyta blight disease. The heritability estimate showed a range of moderate to high values indicating that selection could be made for the traits for further gain in genetic improvement. The SNP markers employed for genetic diversity and population structure analyses confirmed the high genetic diversity of the progeny lines. The results of the SNP based genetic diversity are highly correlated with their pedigree. Association analysis identified SNPs that are significantly associated with early flowering and yield per plant. Overall, our study results revealed the successful development of breeding lines from interspecific crosses between cultivated and *C. reticulatum* which had a greater genetic diversity as well as a significant marker-trait association for important traits.

7. FUTURE RESEARCH

This research findings indicates that the wild *Cicer reticulatum* has considerable potential for widening the genetic base of cultivated chickpea and to serve as a source of valuable alleles for future variety improvement. The findings could be used for selecting the genotypes of chickpea with economically important traits. However, the following research areas are identified for further investigation.

i) The seeds of the breeding lines should be further evaluated for their nutritional qualities such as vitamins and micronutrients content.

ii)To identify the stress resistance qualities, the lines could be tested under temperature and moisture stress conditions.

iii) The variable genotype-environment interactions observed in this study needs further investigation in a wide range of environmental conditions to identify the most stable genotypes. The selected genotypes with early flowering and high yielding characteristics could be evaluated following the multi-years and multi-sites experimental approach.

iv) Whole genome resequencing could be used for SNP validation and association study which will improve the possibility to identify markers that are tightly linked with the desired gene pools.

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9. APPENDICES

APPENDIX A. List of progeny lines and their pedigree with improved agronomic traits.

Traits	Selected populations	Pedigree
Days to flowering (early)	WCC68-5	CDC Leader/Bari1-092//CDC Leader/Bari1-092
	WCC69-2	CDC Leader/Bari1-092//CDC Leader/Bari1-092
	WCC71-1	CDC Leader/Bari1-092//CDC Leader/Bari1-092
	WCC71-2	CDC Leader/Bari1-092//CDC Leader/Bari1-092
	WCC71-4	CDC Leader/Bari1-092//CDC Leader/Bari1-092
	WCC118-5	CDC Leader/ Derei_072//CDC Leader/ Derei_072
	WCC118-8	CDC Leader/ Derei_072//CDC Leader/ Derei_072
	WCC122-10	CDC Leader/ Derei_072//CDC Leader/ Derei_072
	WCC136-8	CDC Leader/Kalka_064//CDC Leader/Kalka_064
	WCC159-1-1	CDC Leader/Savur_063//CDC Leader/Savur_063
Days to maturity (early)	WCC73-5	CDC Leader/Bari2_072//CDC Leader/Bari2_072
	WCC78-10	CDC Leader/Bari3_072C//CDC Leader/Bari3_072C
	WCC80-3	CDC Leader/Bari3_072C//CDC Leader/Bari3_072C
	WCC103-3	CDC Leader/CudiA_152//CDC Leader/CudiA_152
	WCC118-8	CDC Leader/ Derei_072//CDC Leader/ Derei_072
	WCC118-9	CDC Leader/ Derei_072//CDC Leader/ Derei_072
	WCC159-2-1	CDC Leader/Savur_063//CDC Leader/Savur_063
	WCC159-2-2	CDC Leader/Savur_063//CDC Leader/Savur_063
	WCC159-3-1	CDC Leader/Savur_063//CDC Leader/Savur_063
	WCC162-9	CDC Leader/Sirna_060//CDC Leader/Sirna_060
Plant height (cm)	WCC97-1	CDC Leader/Besev_075//CDC Leader/Besev_075
	WCC97-6	CDC Leader/Besev_075//CDC Leader/Besev_075
	WCC102-4	CDC Leader/ Besev_079//CDC Leader/ Besev_079
	WCC104-10	CDC Leader/CudiA_152//CDC Leader/CudiA_152
	WCC106-1	CDC Leader/CudiA_152//CDC Leader/CudiA_152
	WCC143-1	CDC Leader/Kesen_075//CDC Leader/Kesen_075
	WCC145-7	CDC Leader/Kesen_075//CDC Leader/Kesen_075
	WCC148-2	CDC Leader/Oyali_084//CDC Leader/Oyali_084
	WCC150-5	CDC Leader/Oyali_084//CDC Leader/Oyali_084
	WCC165-1	CDC Leader/Sirna_060//CDC Leader/Sirna_060

APPENDIX A. Continued.

Traits	Selected populations	Pedigree
Ascochyta blight score (4-5)	WCC73-7	CDC Leader/Bari2_072//CDC Leader/Bari2_072
	WCC76-5	CDC Leader/Bari2_072//CDC Leader/Bari2_072
	WCC76-10	CDC Leader/Bari2_072//CDC Leader/Bari2_072
	WCC114-5	CDC Leader/Derei_070//CDC Leader/Derei_070
	WCC118-4	CDC Leader/ Derei_072//CDC Leader/ Derei_072
	WCC118-9	CDC Leader/ Derei_072//CDC Leader/ Derei_072
	WCC118-10	CDC Leader/ Derei_072//CDC Leader/ Derei_072
	WCC120-8	CDC Leader/ Derei_072//CDC Leader/ Derei_072
	WCC127-4	CDC Leader/Egill_073//CDC Leader/Egill_073
	WCC131-5	CDC Leader/Egill_065//CDC Leader/Egill_065
Biomass yield per plant (g)	WCC82-3	CDC Leader/Bari3_072C//CDC Leader/Bari3_072C
	WCC104-1	CDC Leader/CudiA_152//CDC Leader/CudiA_152
	WCC106-1	CDC Leader/CudiA_152//CDC Leader/CudiA_152
	WCC118-3	CDC Leader/ Derei_072//CDC Leader/ Derei_072
	WCC140-10	CDC Leader/Kayat_077//CDC Leader/Kayat_077
	WCC143-10	CDC Leader/Kesen_075//CDC Leader/Kesen_075
	WCC154-1	CDC Leader/Sarik_067//CDC Leader/Sarik_067
	WCC160-9-1	CDC Leader/Savur_063//CDC Leader/Savur_063
	WCC164-8	CDC Leader/Sirna_060//CDC Leader/Sirna_060
	WCC165-1	CDC Leader/Sirna_060//CDC Leader/Sirna_060
Number of seeds per plant	WCC73-5	CDC Leader/Bari2_072//CDC Leader/Bari2_072
	WCC93-2	CDC Leader/Besev_075//CDC Leader/Besev_075
	WCC107-7	CDC Leader/CudiA_152//CDC Leader/CudiA_152
	WCC115-4	CDC Leader/Derei_070//CDC Leader/Derei_070
	WCC118-7	CDC Leader/ Derei_072//CDC Leader/ Derei_072
	WCC143-10	CDC Leader/Kesen_075//CDC Leader/Kesen_075
	WCC154-1	CDC Leader/Sarik_067//CDC Leader/Sarik_067
	WCC154-4	CDC Leader/Sarik_067//CDC Leader/Sarik_067
	WCC154-7	CDC Leader/Sarik_067//CDC Leader/Sarik_067
	WCC159-2-1	CDC Leader/Savur_063//CDC Leader/Savur_063

APPENDIX A. Continued.

Traits	Selected populations	Pedigree
Thousand seed weight (g)	WCC105-10	CDC Leader/CudiA_152//CDC Leader/CudiA_152
	WCC118-4	CDC Leader/ Derei_072//CDC Leader/ Derei_072
	WCC125-7	CDC Leader/Egill_073//CDC Leader/Egill_073
	WCC127-3	CDC Leader/Egill_073//CDC Leader/Egill_073
	WCC141-9	CDC Leader/Kayat_077//CDC Leader/Kayat_077
	WCC145-10	CDC Leader/Kesen_075//CDC Leader/Kesen_075
	WCC150-1	CDC Leader/Oyali_084//CDC Leader/Oyali_084
	WCC160-4-1	CDC Leader/Savur_063//CDC Leader/Savur_063
	WCC163-3	CDC Leader/Sirna_060//CDC Leader/Sirna_060
	WCC164-10	CDC Leader/Sirna_060//CDC Leader/Sirna_060
Seed weight per plant (g)	WCC73-5	CDC Leader/Bari2_072//CDC Leader/Bari2_072
	WCC94-9	CDC Leader/Besev_075//CDC Leader/Besev_075
	WCC105-7	CDC Leader/CudiA_152//CDC Leader/CudiA_152
	WCC111-2	CDC Leader/CudiB_022C//CDC Leader/CudiB_022C
	WCC115-2	CDC Leader/Derei_070//CDC Leader/Derei_070
	WCC115-9	CDC Leader/Derei_070//CDC Leader/Derei_070
	WCC118-7	CDC Leader/ Derei_072//CDC Leader/ Derei_072
	WCC121-10	CDC Leader/ Derei_072//CDC Leader/ Derei_072
	WCC125-2	CDC Leader/Egill_073//CDC Leader/Egill_073
	WCC125-10	CDC Leader/Egill_073//CDC Leader/Egill_073
Seed yield (kg/ha)	WCC68-4	CDC Leader/Bari1-092//CDC Leader/Bari1-092
	WCC93-2	CDC Leader/Besev_075//CDC Leader/Besev_075
	WCC102-7	CDC Leader/ Besev_079//CDC Leader/ Besev_079
	WCC107-7	CDC Leader/CudiA_152//CDC Leader/CudiA_152
	WCC110-3	CDC Leader/CudiB_022C//CDC Leader/CudiB_022C
	WCC111-2	CDC Leader/CudiB_022C//CDC Leader/CudiB_022C
	WCC115-4	CDC Leader/Derei_070//CDC Leader/Derei_070
	WCC118-7	CDC Leader/ Derei_072//CDC Leader/ Derei_072
	WCC159-8-2	CDC Leader/Savur_063//CDC Leader/Savur_063
	WCC165-5	CDC Leader/Sirna_060//CDC Leader/Sirna_060

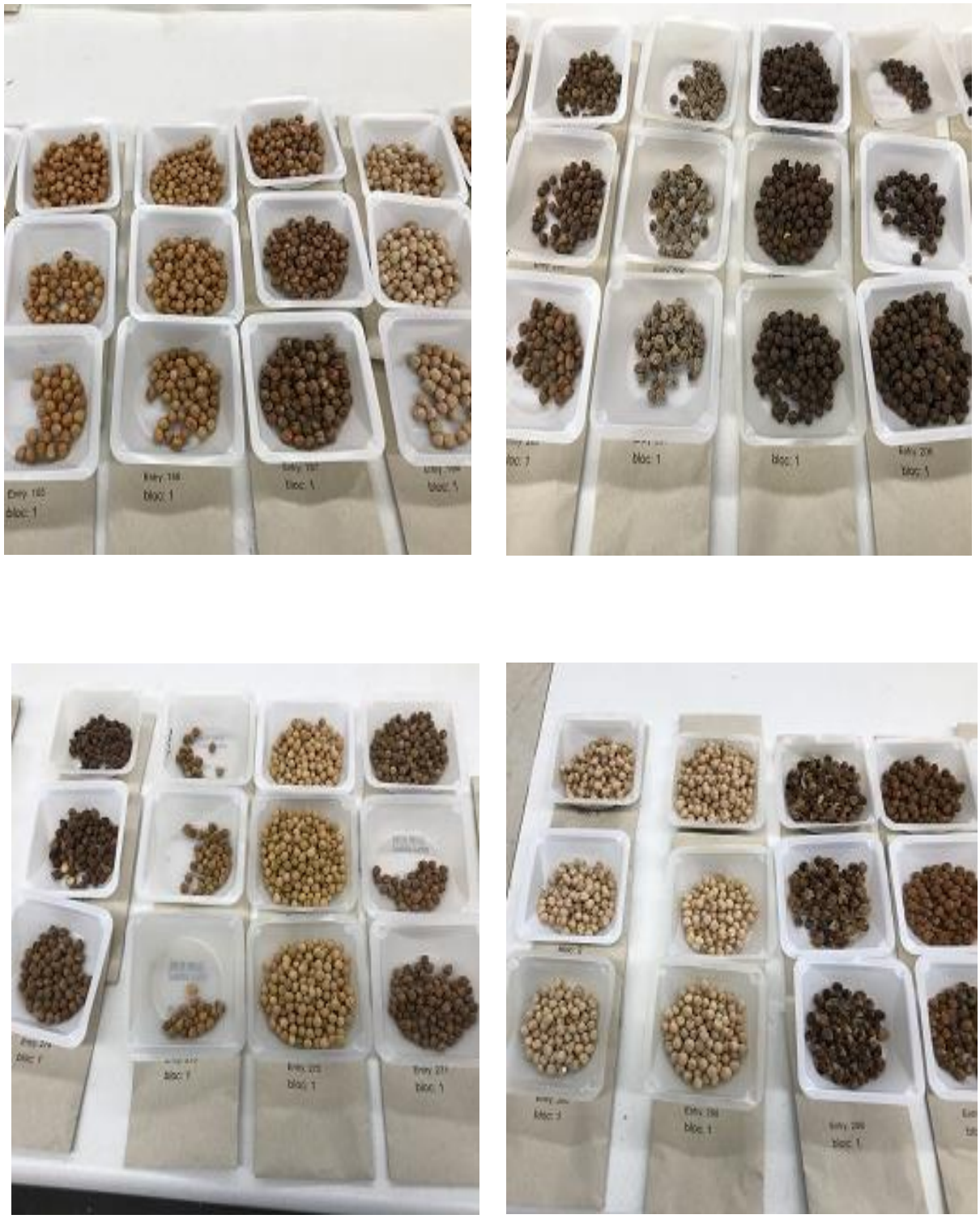
APPENDIX B. Saskatoon field site (2017).

APPENDIX C. Moose Jaw field site (2017).


APPENDIX D. Limerick field site (2018).


APPENDIX E. Lucky Lake field site (2018).

APPENDIX F. Leaf tissue sampling for genotyping.



APPENDIX G. Some portion of the seeds displayed for selection.