MARKER ASSISTED BACKCROSS SELECTION FOR VIRUS RESISTANCE IN PEA

(*PISUM SATIVUM* L.)

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Viruses are destructive plant pathogens, which cause significant yield loss and reduced grain quality. *Pea Seed-borne Mosaic Virus* (PSbMV) is an economically important viral disease in pea (*Pisum sativum* L.) and recently detected in the Northern Great Plains (NGP) in 2012. PSbMV is aphid-transmitted from plant to plant and can be seed-borne. It causes malformed leaves, discolored or split seed, and reduced size and number of seeds. Host resistance to PSbMV-P4 is conferred by a recessive gene, *sbm-1*. Marker assisted backcross breeding using the 4Egenomic primers previously developed assisted in transferring the single resistance allele located on LG VI from 'Lifter' into locally adapted breeding lines. After two backcrosses and allowing plants to self-pollinate to the B_2F_2, individuals were inoculated with PSbMV-P4 isolate to validate resistance. The BC_2F_3 populations were tested in a field evaluation trial for disease resistance against the PSbMV-P4 strain in the NGP and for agronomic adaptation.

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CHAPTER I. GENERAL INTRODUCTION

Introduction and justification

Grain legumes serve important roles in livestock feed, human consumption, and crop rotations. Grain legumes include chickpea (*Cicer arietinum* L.), faba bean (*Vicia faba* L.), lentil (*Lens culinaris* Medik.), common bean (*Phaseolus vulgaris*), lima bean (*Phaseolus lunatus* L.), and dry pea (*Pisum sativum* L.). Legumes benefit cropping systems through symbiotic relationship with Rhizobium bacteria to fix nitrogen and offer nitrogen credits to subsequent crops such as small grains. Leguminous crops leave nitrogen in the soil creating a more economic and sustainable production system, reducing the use of inorganic fertilizers (Crews and Peoples, 2005). Dry pea and other legumes included in rotations breaks disease cycles, and reduces insect and weed pressure.

Pea, along with many significant crops, was domesticated in the Near East (Zohary and Hopf, 1973) and belongs to the Fabaceae family. Domestication dates back 9,000 to 8,000 years ago making it one of the oldest grown crops, which was cultivated with wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) (Zohary and Hopf, 1973). The multiple agronomic benefits of having pea in crop rotations has led to increased pea production. Today, dry pea is grown around the world in various climatic regions, but is most suited to the temperate regions. From 1980 to 2016, the annual average rate of pea production increased 8.5% in the United States, with the exception of 2011 (Janzen et al., 2014; USDA/NASS, 2016). In 2011, adverse weather conditions during planting decreased production area by nearly one-half. In the United States, the primary pea producing states include North Dakota, Montana, Washington, Idaho, and Oregon. In 2015, North Dakota ranked number one in dry pea production, and between North Dakota and Montana, approximately 400,000 hectares were planted, which

accounts for 85% of the total U.S. dry pea production (USDA/NASS, 2015). Both spring and winter pea types are produced, but in North Dakota spring-sown pea is most common due to the harsh winters experienced in the Northern Great Plains (NGP) region. Winter pea is primarily grown in the Palouse region of the Pacific Northwest where there are mild winter conditions with more snow cover to protect the plants.

Pea is used as a broad term for various pea market classes including smooth seeded green or yellow dry pea, marrowfat, edible podded types, freezer, canner, and Austrian winter peas (Muehlbauer and McPhee, 1997). The edible podded, freezer, and canner types have seeds that remain within the pod or are shelled and consumed as an immature seed, while yellow and green dry pea, marrowfat, and Austrian winter types are harvested and consumed as mature seed. Marrowfat pea is a smaller market class mainly sold for the seasoned snack pea market. The largest market class is dry edible pea often sold whole or as split peas commonly found in soup, or ground pea flour for ingredients in pasta, cereal, or cookies for gluten-free alternatives.

Dry pea is a nutritional food source for human and livestock consumption containing approximately 25% protein and 60% carbohydrates (Muehlbauer and Summerfield, 1989). Peas are high in protein and the essential amino acids, lysine and methionine, which are important for several metabolic functions (Akibode and Maredia, 2011). The high protein content in pea makes them a desirable replacement when other high protein products are in short supply or are less cost effective. Each market class is part of the export markets making it an economically important crop for the United States. More than 70% of dry pea production is exported for food processing or livestock feed (Janzen et al., 2014). Pea exported from the U.S. must maintain grading standards of size, color, and uniformity with absence of defects from insects, disease, or damage due to harvest to maintain quality assurance of U.S. products.

Pea production is limited by abiotic and biotic stress agents that impede growth, resulting in reduced crop yield and quality losses. Biotic stress agents that pea is susceptible to are fungal, bacterial, and viral pathogens. PSbMV recently has been detected in the NGP region in 2012 (Pasche, personal communication, 2017). It is unknown when it first appeared, but was confirmed in 2012. It is a *Potyvirus* belonging to the *Potyviridae* family. PSbMV first appeared in the Pacific Northwest of the United States in 1968, and was reported under many different names by scientists from Oregon (Hampton, 1969), Washington (Mink et al., 1969), and Wisconsin (Stevenson and Hagedorn, 1969). During this time, the virus was also present in Japan (Inouye, 1967) and Czechoslovakia (Musil, 1970). After further investigation, the universal name known today as *Pea Seed-borne Mosaic Virus* was proposed (Mink et al., 1974). The most common arrival of the virus into new uninfected regions is the dissemination of infected seed sources. PSbMV can be spread within a field by several vectors, the most common vector is *Acyrthosiphon pisum* (pea aphid) and through mechanical sap inoculation.

Aphid vectors can acquire the virus from alternative host such as alfalfa (*Medicago sativa* L.) or weed species (Wunsch et al., 2014). They transmit in a non-persistent manner with an external borne stylet by piercing leaf tissue. PSbMV is known to have varying symptomology based on the pathotype. Some pathotypes may affect the plant more aggressively resulting in more prominent symptom expression, but commonly the plant overall is stunted, has shortened internodes near the top of the plant, budding at the internodes delaying senescence, leaves are malformed and discolored with swollen leaf veins (reviewed by Khetarpal and Maury, 1987). In addition, the flowers or seed may be aborted. The seed that is developed sometimes has discoloration, scarring, split seed coat, or water-soaked lesions.

During early recognition of the virus in the U.S., host resistance was not readily accessible by growers, but scientists have since identified resistant sources (Hagedorn and Gritton, 1973). Northern India serves as an important reservoir of PSbMV resistant germplasm (Hampton and Braverman, 1979). The gene conferring resistance is recessively inherited, which can hinder breeders' efforts to release resistant germplasm by adding cost and time to the breeding process. When the virus was first observed in the U.S., host resistance was not being implemented into breeding programs, but rather destroying entire infected seed stocks (Hampton et al., 1976). Since those efforts, Muehlbauer (1983) has developed germplasm lines with the USDA-ARS and Washington Agricultural Research Center, Pullman, WA, and later McPhee and Muehlbauer (2002) released a resistant cultivar, 'Lifter', which possesses the *sbm-1* resistance gene, but lacks the desirable semi-leafless leaf type.

The reported resistant germplasm has allowed scientists to conduct genetic studies to identify the gene location. It was proposed by Hagedorn and Gritton (1973) that the single recessive resistance gene be designated as *sbm*. There have been many PSbMV resistance genes located on LG VI in a resistance gene cluster including *sbm-1*, *sbm-1*^{1}, *sbm-3*, and *sbm-4*; however, there is a second resistance gene cluster located on LG II containing *sbm-2* (Gritton and Hagedorn, 1975; Provvidenti and Alconero, 1988a; Provvidenti and Alconero, 1988b; Gao et al., 2004b). PSbMV has been detected around the world and there have been three reported pathotypes (P1, P4, and P2/L1) identified in the United States (Alconero et al., 1986). PSbMV-P1 and PSbMV-P4 are specific to pea, but only PSbMV-P4 has been detected in the NGP region (Pasche, personal communication, 2017). The first linkage map for *sbm-1* was developed by Timmerman et al. (1993) using restriction fragment length polymorphism (RFLP) and random amplification of polymorphic DNA (RAPD) markers. From the study creating the first linkage

map, to what is currently known today, many molecular markers have been identified, which are either linked to *sbm-1* or are located within the gene (Timmerman et al., 1993; Frew et al., 2002; Gao et al., 2004a; Smýkal et al., 2010).

Developments in molecular marker technology have increased scientific discoveries and simplified breeding efforts with the use of marker assisted selection. This process assists in selecting a desirable phenotype based on a marker that is closely linked to or is located within the gene of interest. During backcross population development of introgressing a recessive gene, there are constraints when only using phenotypic selection. Transferring a recessive gene requires an additional cycle of self-pollination to identify homozygous recessive individuals from progeny tests (Acquaah, 2012). However, marker assisted backcross breeding has been implemented to improve efficiency and reduce the additional cycle after each backcross. To deliver a reliable cultivar to producers, reduce time, cost, and optimize their breeding efforts, many researchers have used marker assisted backcrossing (Neeraja et al., 2007; Yi et al., 2009; Chu et al., 2011). The 4Egenomoic primer pair was developed by Gao et al. (2004a) to identify the recessive resistance gene (*sbm-1*), to aid in developing resistant germplasm. This work was later expanded by Smýkal et al. (2010), with the complete sequence of the eukaryotic translation initiation factor gene (*eIF4E)*, and the generation of more markers.

Objective

The objective of this project was to transfer the recessive resistant gene (*sbm-1*) from cv. Lifter via marker assisted backcross selection. The development of backcross populations have resistance to *Pea Seed-borne Mosaic Virus* P4 pathotype present in the NGP region. It is also expected that progeny with resistance and good agronomic performance will be released as germplasm for the scientific community and may be considered for release as new varieties.

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CHAPTER II. LITERATURE REVIEW

Origin and domestication of field pea

Domestication of various plant species significant to modern civilization originated in the Near East or Fertile Crescent region, which includes Jordan, Syria, Lebanon, and Israel. Pea was domesticated around 7,000 to 6,000 B.C. in Neolithic farm villages and is one of the oldest grown legumes often cultivated with wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) (Zohary and Hopf, 1973). This region is known as the primary center of origin, but there is a secondary center of diversity located in the highlands of the Asiatic region or the Hindukusch near the southern slopes of the Himalayan mountain range (Ambrose, 2008). Smýkal et al. (2011) discusses the challenges from human activity and climate changes that have hindered identifying the exact location of origin. Historical artifacts have allowed scientists to identify where and when wild and cultivated species were found based on phenotypic characteristics. The unique seed coat of carbonized pea distinguishes wild species from cultivated pea. Wild pea has a rough or granular seed coat, while cultivated varieties have a smooth surface (Zohary and Hopf, 1973). Helbaek (1964) excavated carbonized pea seed with a smooth seed coat in Catal Hukyuk, Turkey, from the sixth millennium along with naked, six-row, compact spike barley, which provides evidence that both pea and barley were being cultivated during this time. In further studies conducted in Cayonu, Turkey, researchers looked at seed from the seventh millennium. Many seeds found were missing their seed coat; however, those with a seed coat contained a rough seed coat suggesting they were wild progenitors (van Zeist, 1972). Throughout the 1900s many expeditions to the primary and secondary centers of origin were made to fill in knowledge gaps, and still much information is unknown. Today, the literature remains constant

in stating that pea has a long history dating back to 7,000 to 6,000 B.C. era where cultivation, adaptability, and popularity began and is present in the cultivars grown today.

Pea cultivation, along with other crops including barley, wheat, and lentil, continued to thrive in the Near East and spread throughout the world (Zohary and Hopf, 1973; Smartt, 1990). It spread north to Russia, west to Europe, and east to India and China. Discovery of the New World by Columbus expanded cultivation of pea into North America, primarily grown in Canada and northern parts of the United States (Wade, 1937). In the U.S., the Palouse region in the Pacific Northwest and in recent years, cultivation of pea has expanded into the Northern Great Plains (NGP), specifically North Dakota and Montana. Pea grown throughout the world today is from a single species, but there are various species included in the *Pisum* genus.

The relationship between wild and cultivated species was further analyzed to gain a better understanding of archeological digs during the early 1970s. Ben-Ze'ev and Zohary (1973) discussed their results on the *Pisum* genus indicating three wild type species, *P. elatius*, *P. humile*, and *P. fulvum*. Although the *Pisum* gene pool is quite diverse, it is categorized into three distinct groups, *P. abyssinicum*, *P. fulvum*, and a single-species complex of *P. elatius*, *P. humile*, and *P. sativum* (Vershinin et al., 2003; Ben-Ze'ev and Zohary, 1973). Within the *Pisum sativum* species, there are sub-species *Pisum sativum* ssp. *sativum*, *Pisum sativum* ssp. *elatius* and *humile*, which are categorized in the primary gene pool and are readily intercrossed. The progeny from crosses within the primary gene pool will also be completely or almost completely fertile (Muehlbauer et al., 1994). *P. fulvum* is classified as a *Pisum* member; however, is not closely related. Therefore, it is part of the secondary gene pool and hybridization is more difficult, in addition, the progeny may be sterile due to translocations among chromosomes. To increase successful hybridization among individuals of the primary gene pool and *P. fulvum* species, it is

noted that *P. fulvum* should be used as the pollen parent. This concept is important in the development of modern *P. abyssinicum* cultivars grown because literature indicates *P. fulvum* was used as a parent.

Distribution and production

There is a relationship between domestication, cultivation, and worldwide distribution. Jing et al. (2010) used retrotransposon-based insertion polymorphism (RBIP) and tagged microarray markers (TAM) to analyze genetic diversity among field pea. Their conclusions support the research conducted by Vershinin et al. (2003) and other researchers. One conclusion they drew, which also supports Vershinin et al. (2003), is that modern *P. abyssinicum* species grown in Ethiopia were derived from a cross between *P. elatius* and *P. fulvum*. This hybrid event is thought to have occurred in the western half of the Fertile Crescent, but through human activity was translocated to areas of northern Africa where it continued to develop into what is known as *P. abyssinicum*. While *P. sativum* lineage remains unclear, Jing et al. (2010) believe that domesticated *P. sativum* was derived from wild selections of *P. elatius* made by early farmers in the Fertile Crescent. From this region, the Old World crop continued to spread throughout the world; the exact number of countries that produce pea today is unknown, but there are about 100 known countries (FAOSTAT/FAO, 2017).

Total world production of dry pea in 2014 was approximately 11 million MT (FAOSTAT/FAO, 2017). In the past 30 years, the peak production occurred in 1990 with 16.6 million MT. However, there has been a gradual declined until 2007 with the lowest production rate at 9.3 million MT, but since then production continued to increase. Countries of origin in the Fertile Crescent are not among the top producers, but since 1985 the top five average producing counties include USSR, Canada, France, Russian Federation, and China, respectively. Like many

other commodity trends and production demands shift, in the last 10 years of available data, Canada ranks as the top dry pea producer worldwide with an average production rate of 3.2 million MT per year, while Russia ranks second with 1.3 million MT followed by China, France, and India. Production increased in Canada to 3.4 million MT in 2014. The most recent data for 2014 alone shows Canada, Russia, China, United States, and India ranked as the top dry pea producers in the world.

The United States ranked fourth in dry pea production, produced approximately 900,000 MT of dry edible pea (USDA/NASS, 2016). In 2015, North Dakota ranked number one as the dry edible pea producer with 410,000 MT followed by Montana, Washington, Idaho, and Oregon producing 401,000, 76,000, 35,000, and 6,000 MT, respectively. North Dakota and Montana plant approximately 400,000 hectares, which is approximately 85% of the total U.S. dry pea production (USDA/NASS, 2015). The United States also produces a limited amount of wrinkled pea primarily grown in Idaho and Washington, fresh and processing pea in Wisconsin, Minnesota, Illinois, New York, Oregon, Washington, Delaware, and Maryland (UDSA/NASS, 2017) and Austrian winter pea grown in Idaho, Washington, Montana, and Oregon.

Market classification

There are different market classes to distinguish pea characteristics and uses. The different types explained by Muehlbauer and McPhee (1997) are dry, marrowfat, edible podded types, freezer, canner, and Austrian winter pea. The different types of pea are based on varying harvest times in regard to the development stage of the crop. Pea can be harvested as immature or mature seed. Immature harvested seed is succulent and fleshy, which is a fresh pea type, and includes edible-podded types also known as snap pea, freezer pea, and canner pea. Conversely, mature harvested seed is allowed to reach physiological maturity and is dried on the plant prior

to harvest. Mature seed types include dry, marrowfat, and Austrian winter pea. The duration in which the plant is at the immature seed stage for the fresh market is relatively short. In this case a single variety is grown in successional plantings or multiple varieties are selected for varying flowering times to extend harvestability (Ambrose, 2008). The seed fills with vitamins, protein, and sugar, but as the plant reaches physiological maturity, the sugar levels decrease and the seed begins to dry as it accumulates starch and protein. Both forms are important for human and livestock consumption markets today.

Each pea type is utilized for various purposes other than human and livestock consumption, additional uses include forage, green manure, and cover crop production. Dry pea is used as a commercial crop for human consumption when sold as whole, split, or ground products, which are common in soup or as pea flour in pasta, cookies, and many other products. Dry pea can have green or yellow colored cotyledons that are rich in protein and starch (Muehlbauer and McPhee, 1997). Marrowfat pea is used for commercial pea production as mushy peas when canned, which are commonly found in the United Kingdom. Marrowfat pea is a dimpled green pea with a larger seed, leaves, and robust stem structure, but relatively short plant stature with many branches. In Southeastern Asian countries, marrowfat pea is dried, seasoned, and sold as a snack pea, referred to as wasabi pea. Austrian winter pea has multiple purposes such as livestock feed, cover crop, and a source of green manure. They are sometimes referred to as *Pisum sativum* ssp. *arvense*, but are still cross compatible with *P. sativum* (Muehlbauer and McPhee, 1997). They have pigmented seed, stem, and flowers compared to other pea types. In the United States, Austrian winter pea is usually sown in the fall in the Pacific Northwest, and has the ability to survive most winters. The increasing demand of products from diverse markets contribute to the rise in U.S. production.

Each market class is economically important to the United States and contributes to foreign market exports. In the United States, USA Dry Pea and Lentil Council provides grading standards for whole dry pea to maintain set standards for quality assurance of U.S. products. The six classes used include: smooth green dry pea, smooth yellow dry pea, wrinkled dry pea, mottled dry pea (Austrian winter pea), miscellaneous dry pea, and mixed dry pea (USADPLC, 2009). In 2003, the U.S. exported nearly 2.5 million MT of dry pea, while about 140 countries imported them (USADPLC, 2010). The U.S. exports more than 70% of dry pea produced to Canada, India, China, and Spain. India and China primarily utilize pea for food processing, while Belgium, Italy, Spain, and Germany import for livestock feed (Janzen et al., 2014). Pea for human consumption, must be good in size, appealing color, and uniform, while maintaining quality with absence of seed defects from insects, disease, or harvest damage.

Agronomic characteristics

Pea is a cool season broadleaf annual crop in the legume family (Fabaceae). It is sensitive to climate extremes, and often found in temperate climates, higher altitudes, or in warm regions during the cool growing season (Elzebroek and Wind, 2008). Pea can be sown in the fall and spring like other crops including wheat. Pea produces optimal yields with adequate moisture, temperatures of 13 to 18 ℃, and adequate amounts of the essential nutrients (Muehlbauer and McPhee, 1997). Germination and plant growth is best suited for well-drained soil with good texture. Dry pea cultivars are indeterminate, but due to the environment, growth is stopped making it appear determinant. However, the fresh pea market is seeing a shift towards release of determinacy cultivars due to the uniformity of seed at the proper developmental stage necessary for processing. There are still indeterminate types for home garden peas to extend harvest period. The eight distinct leaf morphologies include normal, semi-leafless, tendrilless, leafless effects of

‘afila’ with reduced stipule, reduced stipule, ‘afila’ with tendrilless, reduced stipule and tendrilless, and ‘afila’ with reduced stipule and tendrilless (Muehlbauer and McPhee, 1997). The differences in leaf morphology are due to three genes, *af*, *st*, and *tl*. The *af* gene controls leaf types with tendrils, *st* gene is responsible for smaller stipules, and *tl* gene converts tendrils to leaflets. Goldenberg (1965) observed a mutation where the leaflets were now tendrils, and called it the ‘afila’ type, but is commonly known as semi-leafless. The transition from a normal leaf having all the dominant genes (*AfAfStStTlTl*) to a semi-leafless (*afafStStTlTl*) is due to change in the single *af* gene where the tendrill or semi-leafless morphology is recessive. A plant with ‘afila’, reduced stipules, and tendrilless has all three of the recessive genes (*afafststtltl*). Semi-leafless cultivars are preferred over normal leaf types because of their resistance to lodging making harvest easier (McPhee, 2003). The most commonly grown varieties in North Dakota and Montana have either normal or semi-leafless morphology. The other leaf types have not been studied to the extent that normal, semi-leafless, and leafless types have been.

A mature seed contains an embryo and two cotyledons, which are surround by the testa (Muehlbauer and McPhee, 1997). The composition of pea is about 10% water, 25% protein, 60% carbohydrate, 1% fat, and 3-3.5% minerals (Muehlbauer and Summerfield, 1989). Their high protein content makes them desirable for replacement of other high protein substances in animal feed or cereal for human diets when there is a shortage or utilizing pea is more cost effective. The seed has a scar, also referred to as the hilum, on the pea seed from the attachment point where the seed was connected to the inter wall of the pea pod (Muehlbauer and McPhee, 1997). The hilum; the micropyle, opening in the testa to allow sperm cells to enter egg during fertilization; and raphe, a small ridge connecting the two halves created by the radicle, are

located along the separation line between the cotyledons. This separation region is where the radicle protrudes from during germination.

During the germination process, the seed goes through the imbibition and activation phases before emergence (Sutcliffe and Bryant, 1977). The first phase is imbibition, the seed begins to uptake moisture after planting; this phase is about twenty hours (Sutcliffe and Bryant, 1977). As the seed swells and doubles in size, water intake begins to slow as it reaches the end of the first phase, then the seed enters the activation phase. The second phase activates metabolic processes, and can last for several days. During this time, the seed undergoes change causing the components in the cotyledons to degrade thus providing nutrients to the growing seedling before root development has occurred. The third phase is about five days after imbibition has begun resulting in a decrease of oxygen uptake and the cotyledons start to senesce. As the cotyledons change, the embryo experiences similar phases. The first phase corresponds to the imbibition phase and water enters the cells, followed by an increase in metabolic processes in the second phase, and in the third phase oxygen enters into the seed allows respiration to occur resulting in synthesis of DNA and RNA (Muehlbauer and McPhee, 1997). The radical emerges between the two cotyledons during the second phase, which allows oxygen to enter into the cells as the root grows during the third phase.

The initial growth of the seedling begins when the radicle emerges, and grows downward. Establishment of the radicle supplies nutrients to the epicotyl above ground as it grows and emerges through the soil surface (Muehlbauer and McPhee, 1997). Pea, unlike other legumes such as common bean (*Phaseolus vulgaris*) that have epigeal germination, is characterized by hypogeal emergence where the cotyledons remain below the soil surface. The taproot system with lateral roots can reach depths of 1.5 meters or more (Muehlbauer and

Summerfield, 1989). To support the growing seedling, secondary or lateral roots branch off the radicle to provide additional resources as the plant continues to go through a vegetative stage.

Before the plant is able to develop and optimize efficiency with extended rooting zone to develop nodes, leaves, and branches, it must utilize resources from the cotyledons. After the root has been developed, it can mine nutrients from the soil. Throughout the growing season, plants require adequate moisture and nutrients, and can grow in areas where rainfall is 400 mm annually (Muehlbauer and McPhee, 1997). However, in areas with limited rainfall, the soil must be capable of retaining moisture to support crop growth and development. In North Dakota, the average precipitation is approximately 450 mm; however, there is a precipitation gradient across the state (Current Results Publishing Ltd., 2016). Regions receive approximately 480-570 mm, 440-500 mm, and 360-460 mm on average for the eastern, central, and western part of the state, respectively. Soil type also varies across the state with different water holding capacities. Soil in the Red River Valley (RRV) on the eastern region is comprised of clay, clay loam, to loam texture, while soils in Williams County, located in western North Dakota, contain silt, sand, and gravel (Freers, 1970).

Soils with fine particles such as clay have a reduced infiltrate rate compared to coarse soils with sand or gravel (Brouwer et al., 1985). Pea can grow in various soil types from light sand to heavy clay, but proper drainage is necessary when planted in clay soils. In poorly drained soils, pea is not tolerant to waterlogging conditions, which can result in severe injury. Studies show that during waterlogged conditions, the plant experiences physiological changes and there is a reduction in transpiration (Jackson, 1985; Belford et al., 1980). The plant has decreased stem growth, leaves retain water, but under prolonged conditions the leaves will begin to lose water and start to desiccate (Jackson, 1979). Pea is not a deep-rooted plant, and is prone to various root

diseases partially due to their inability to tolerate waterlogged environments. The amount of moisture and soil texture will also influence the available nutrients for pea during the growing season.

Pea requires adequate nutrients for proper growth and development to produce reasonable yield. Growth can be limited in leached soils with a lack of the 14 essential plant nutrients in the root zone. All nutrients are important, but some deficient nutrients can influence the growth of the crop more by affecting the seed quality, reduced pod formation, root nodules fix less nitrogen, and a reduction in yield (Muehlbauer and McPhee, 1997). Inoculants placed on the seed during planting will assist in nitrogen fixation, but in soils where nitrogen is lacking, a starter dose of nitrogen fertilizer may be necessary. Nitrogen in excess can reduce nodulation and N_2 fixation. Phosphorus is another important nutrient, but it is important to note that seedlings are salt-sensitive (Muehlbauer and Summerfield, 1989). It is important to avoid placement in high concentration next to the seedling, and is best to broadcast in low quantities and then incorporated or applied in bands under the seed. The source, rate, and placement can ensure proper uptake. Nutrient deficiency is detectable through soil testing, plant tissue analysis, or visual symptoms on the foliage. However, visual symptoms of deficiencies can be mistaken for other disorders such as moisture deficiencies associated with the plant, and are not always distinct unless under severe conditions.

Moisture and temperature influence pea growth during the growing season. Germination occurs after the testa has been softened or is broken allowing imbibition. During this time, the seed is susceptible to pathogens present in the soil possibly causing the seed to deteriorate if it is not treated. Pea seed soaking in water at low temperatures causes seed to die or become damaged (Perry and Harrison, 1970). Another study in chickpea by Auld et al. (1988) supports the

findings of Perry and Harrison (1970). Auld et al. (1988) reported in cold soils, germination is significantly reduced at lower temperatures. This often occurs when planting too early in the season in addition to high soil moisture. Gan et al. (2002) found soil temperatures under 9-10 ℃ caused poor germination. However, in years with warm temperatures and lower soil moisture, planting early in the growing season resulted in consistently higher yields compared to a later planting date. On the contrary, when planted later, plants may experience drought stress early during vegetative stages and can cause early flowering, improper pod formation, and reduced yields.

Proper planting date, soil texture, moisture, and adequate nutrients result in a healthy dark green plant. During the vegetative stage a white to yellow plumule hook emerges through the soil surface. The plumule hook exposed to light will initiate chlorophyll synthesis and begin to straighten allowing the first leaves to become visible. The first two nodes remain under the soil and may contain small stipules. The pea stem develops 20-25 nodes, which is comprised of a compound leaf, stipule, and a petiole. A normal leaf type has a petiole containing 2-3 leaflets and the terminal end has 3-5 tendrils, but a semi-leafless or 'afila' type only has tendrils on the petiole (Muehlbauer and McPhee, 1997). The change in leaf morphology allows for lodging resistance and decreases foliar diseases because air is able to move freely throughout the canopy. A less dense canopy and upright plant structure allows more light to penetrate through to the lower leaves and increase the photosynthetic process resulting in increased yields according to a study conducted by Kielpinski and Blixt (1982). The vegetative growth is influenced by both genetics and environmental factors. Kielpinski and Blixt (1982) saw that under normal field conditions where lodging is present in normal leaf type cultivars, pea cultivars with the *af* allele do yield higher. However, when allowing cultivars with a normal leaf type to grow on a wire

trellis, they observed a higher yield compared to *afila* peas. Despite the increased yield potential of normal leaf type cultivars under a lodging resistant environment, they concluded that pea with *afila* allele is observed to have higher yield because of its improved plant stature and light penetration into the canopy under normal field conditions. The plant continues to extend in length and develop more leaves, but the vegetative stage transitions into the reproductive cycle.

Pea is an autogamous crop, and during reproduction flowering begins from the lower part of the stem and continues to flower to the top of the plant. Several stages occur during floral development prior to the flower fully opening exposing internal components of the developing pod after fertilization. Pea is a complete and prefect flower that is self-pollinated. Pollination occurs between 24 and 36 hours before the flower opens, and then the fertilized embryo, now a zygote, starts to divide (Cooper, 1938). During division, the pod starts developing and increases in length and width (Pate and Flinn, 1977). Eventually the pod creates a hollow cavity, and the plant will translocate nutrients into filling grain. The seed has a high moisture content at the beginning of development, but as the plant approaches maturity, moisture content will decline. Once the moisture content has reached approximately 14%, the crop is ready to harvest and be stored properly without issues of seed quality deterioration (Muehlbauer and Summerfield, 1989). A lower moisture content increases split seed and seed coat cracking during harvest.

Agronomic benefits

Pea and other legume crops contribute many benefits to a cropping system. Pea is often included in rotation with cereals to assist with weed control, pest and disease control, and fixation of nitrogen (Muehlbauer and Summerfield, 1989). As a broadleaf crop, it is easier to eliminate grassy weed species that may be difficult to terminate in a cereal crop, thus reducing the viable seed that can return to the soil seed bank for the following growing season. In

addition, it can reduce inoculum build up in wheat stubble with the inclusion of a non-host crop such as pulse crops, which do not support the pathogen life cycle (Krupinsky et al., 2002). In a study pertaining to tillage and a crop rotation system, the average disease rating for leaf spot in wheat was reduced when wheat followed pea or summer fallow compared to following another cereal (Bailey et al., 1992). Pea reduces fertilizer cost because it supplies nitrogen credits to subsequent nonlegume crops in rotation due to the symbiotic relationship it has with nitrogen-fixing soil bacteria. Soil moisture availability impacts the amount of nitrogen fixation, and under dry conditions N_2 fixation rates are reduced to nearly half (Bremer et al., 1988). Pea not only provides residual N, but also promotes yield as seen by Wright (1990) and Evans et al. (1991) in wheat and barley, respectively. They reported a seed yield increase of at least 20% or more when following pea, and Stevenson and van Kessel (1996) reported a greater rotational benefit of pea followed by wheat with a 43% increase in yield. The roots provide necessary nutrition for subsequent crops, but the seed is rich in protein, which enhances food security for both human and livestock.

The use of cover crops in rotation is becoming a common practice in the United States. A cover crop survey conducted by the Sustainable Agriculture Research and Education/ Conservation Technology Information Center (NCSARE/CTIC) (NCSARE, CTIC, and ASTA, 2016) reports an increase in cover crop acreage each year according to survey respondents. Winter pea was the second most popular legume used. Austrian winter pea serves to protect against soil erosion during harsh winters with little snow cover when planted in the fall (Holderbaum et al., 1990). Winter pea used as green manure adds organic matter to the soil, as well as reduces disease severity of soil borne pathogens that attack cereal crops (Mahler and

Auld, 1989). Despite the multiple benefits of incorporating pea in rotation and for consumption, both abiotic and biotic stress agents can limit pea production.

Viral establishment

The Northern Great Plains, particularly Montana and North Dakota, are important pea producing states to the U.S. economy. However, environmental and pathogen related limitations result in significant yield and grain quality losses. Among these limitations are several viral diseases including bean yellow mosaic (BYMV), pea enation mosaic (PEMV), red clover vein mosaic virus (RCVMV), and pea streak virus (PeSV) (Pavek, 2012), and recently *Pea Seed-borne Mosaic Virus* (PSbMV). There are three distinct PSbMV pathotypes that have been detected and are reported as PSbMV-P1, P2/L1, and P4 (Alconero et al., 1986). PSbMV-P1 and P4 are specific to pea, whereas PSbMV-P2/L1 is a lentil strain. The genes conferring resistance to these pathotypes include *sbm-1*, *sbm-1^{1}*, *sbm-2*, *sbm-3*, and *sbm-4*. The *sbm-1* gene confers resistance to PSbMV-P1 and PSbMV-P4 pathotypes (Gao et al., 2004b). The *sbm-1^{1}* is an allele of *sbm-1*, and it confers resistance to the P1 and P2/L1 PSbMV pathotypes. In addition, the *sbm-2* and *sbm-3* genes prevent susceptibility to the PSbMV-P2/L1 pathotype (Congdon et al., 2016; Makkouk et al., 2014; Gao et al., 2004b; Provvidenti and Alconero, 1988a). Lastly, the *sbm-4* gene confers resistance to the PSbMV-P4 pathotype (Provvidenti and Alconero, 1988b). The isolate found in the NGP region has high sequence homology to the PSbMV-P4 pathotype (Pasche, personal communication, 2017). It is unknown when it first appeared in North Dakota, but the first confirmation occurred in 2012.

The virus was referenced by several names as scientists made their discoveries (reviewed by Khetarpal and Maury, 1987). PSbMV appeared in the Pacific Northwest in 1968 (Hampton, 1969). In 1969, United States scientists alone reported several names for this virus, Hampton

(1969) of Oregon State University reported, “pea fizzle top virus”, Mink et al. (1969) from Washington reported, “seed-borne virus of pea”, and Stevenson and Hagedorn (1969) of Wisconsin stated, “a new seed-borne virus of pea”. Each reported case found in the United States resembled those in Japan (Inouye, 1967) and Czechoslovakia (Musil, 1970), which led to the universal name of *Pea Seed-borne Mosaic Virus* (Mink et al., 1974).

Taxonomy and transmission

Pea Seed-borne Mosaic Virus is a member of the *Potyviridae* family belonging to the genus *Potyvirus*. It is a single stand positive-sense RNA virus with a flexuous rod-shaped particle that is approximately 770 nm in length and 12 nm in diameter (Hampton et al., 1974). The viral RNA has been completely sequenced and is 9,924 nucleotides in length (Johansen et al., 1991).

The newly discovered virus found in 1968 and later reported in 1969 began to interest research groups across the United States. The virus is transmissible through seed, vector, and mechanical sap inoculation. Scientists had a growing concern over the impact because of the seed borne nature of the disease, and at the time, Stevenson and Hagedorn (1970) were unsure of the vector, but thought PSbMV was vectored by aphid species. Gonzalez and Hagedorn (1971) confirmed the aphid vector in their study by obtaining information on transmission potential of three aphid species. Of the three species included in the study, *Macrosiphum euphorbiae* (potato aphid) were more efficient vectors than *Acyrthosiphon pisum* (pea aphid) and *Myzus persicae* (green peach aphid). The vector may also feed on alternative hosts of PSbMV such as alfalfa (*Medicago sativa* L.) or weed species to acquire the virus, and then enter pea fields and begin infecting healthy plants (Wunsch et al., 2014). However, seed trade is the most common method of virus spread into uninfected regions.

Infected seed can act as the initial source of inoculum, and from there the virus can be subsequently vectored by a total of 21 aphid species, including the pea aphid (*Acyrthosiphon pisum*) (reviewed by Khetarpal and Maury, 1987). The rate of seed transmission varies across studies when using different cultivars. Some cultivars are more prone to infection and seed transmission. One study found that cultivar Dark Skin Perfection had a higher rate of seed transmission than other cultivars (Stevenson and Hagedorn, 1973). Seed transmission rates identified in some studies were as high as 100%, but most commonly were only 30%.

Aphids transmit PSbMV in a non-persistent manner using an external borne stylet that pierces the leaf tissue to extract the sap. Due to the aphid-virus interaction, the 'non-persistence' is the inability to infect after a few minutes or hours (Watson and Roberts, 1939). Gonzalez and Hagedorn (1971) studied three aphid species, *Acyrthosiphon pisum* (pea aphid), *Myzus persicae* (green peach aphid), and *Macrosiphum euphorbiae* (potato aphid), and concluded that the pea and green peach aphids can transmit the virus after 10 to 90 seconds of feeding, and do not require a latent period before transmitting the virus to a healthy plant. Cool growing conditions are favorable for aphid population development.

Symptomatology and detection

Virus infection in pea results in deformities in plant physiology and seed. The interaction between cultivar, pathotype, and environmental conditions cause varying symptoms. The plant appears stunted with shortened internodes that occur near the top of the plant. Budding at the internodes results in delayed senescence of infected plants causing uneven maturation. The leaves are malformed and the apical meristem can have a rosette appearance (Wunsch et al., 2014). The leaf veins may appear swollen with downward curling of leaves (reviewed by Khetarpal and Maury, 1987). Overall, the leaves may display mottling, mosaic, or chlorotic

appearance and become necrotic. Due to virus infection, flowers may be aborted or if the ovary becomes fertilized, the virus can be transferred into the developing seed. Pods will also have similar discoloration and appear abnormal. In addition, pods may appear flat, shrunken, curved, or have a 'figure-eight' appearance from a lack of seed development. The seed is reduced in size and mature infected seed may show symptoms of scarring, split seed coat, and water-soaked lesions. Some may also refer to it as "tennis ball" lesions on the seed.

Virus detection is accomplished through laboratory analysis. Some of the methods used include immunodiffusion in gels, enzyme-linked immunosorbent assay (ELISA), serologically specific electron microscopy (SSEM) (Hamilton and Nichols, 1978), nucleic-acid based techniques (reverse transcriptase PCR (RT-PCR), and tissue blot immunoassay (TBIA) (Wunsch et al., 2014).

Economic importance

According to Hampton et al. (1993), PSbMV is distributed worldwide through the dissemination of infected pea germplasm or seed trade. The devastation of the virus led seed companies to destroy entire seed lots in the U.S. from 1969 to 1974 to prevent further spread of the virus through seed transmission (Hampton et al., 1976). Unfortunately, many of the current commercial pea cultivars are PSbMV susceptible leading to extreme economic losses in yield and reduction in grain quality. Yield trial analysis in Australia detected seed yield reductions up to 25% (Coutts, 2016). Lower yield and unmarketable seed caused by the virus resulted in producers experiencing reduced profits. Reduced seed size and the distorted seed coat has a lower test weight contributing to reduced yield. PSbMV also causes additional legumes such as faba bean, lentil, and chickpea to suffer from crop yield loss (Coutts et al., 2008). The economic losses may be minimized by eliminating virus reserves, controlling the vector, and host

resistance. Due to the recessive nature of host resistance, breeding for resistance was difficult with a lack of molecular marker technology to assist, which has simplified PSbMV breeding efforts today. During early recognition of the virus in the U. S., the virus could have gone unnoticed making it difficult to introgress a recessive resistance gene by including progeny tests after each backcross generation. Markers linked to gene were first identified in the 1990s with the first linkage map for *sbm-1* (Timmerman et al., 1993).

Sources of resistance

Genetic studies have been conducted to reduce the economic impacts of the virus. The resistance gene conferring PSbMV is recessively inherited (*sbm*), whereas susceptibility is the result of having the dominant allele (*Sbm*) (Hagedorn and Gritton, 1973). There are PSbMV resistant pea genotypes available as reported by Hagedorn and Gritton (1973), Muehlbauer (1983), and McPhee and Muehlbauer (2002). Cultivar Lifter is one source of virus resistance and is a green dry pea released for its multiple disease resistant package (McPhee and Muehlbauer, 2002). Lifter is a semi-dwarf, normal leaf type developed by the USDA-ARS with the Washington Agricultural Research Center, Pullman, WA, and Idaho Agricultural Experiment Station, Moscow, ID, and released in 2001 (McPhee and Muehlbauer, 2002). It is resistant to race 1 of Fusarium wilt caused by *Fusarium oxysporum*, pea enation mosaic virus, pea seed-borne mosaic virus (*sbm-1*), and powdery mildew caused by *Erysiphe polygoni*, which are all diseases limiting pea production in the Palouse region, North Dakota, and Montana. Lifter acquires resistance to PSbMV from cultivar 'Alaska 81' developed by USDA-ARS and Washington State University (Muehlbauer, 1987). Lifter is known to have one of the resistant genes, *sbm-1*, and was confirmed by Smýkal et al. (2010). There are additional genes conferring resistance, which include *sbm-1*, *sbm-1*^{1}, *sbm-2*, *sbm-3*, and *sbm-4* (Gritton and Hagedorn, 1975;

Provvidenti and Alconero, 1988a; Provvidenti and Alconero, 1988b; Gao et al., 2004b). The locus conferring resistance to PSbMV-P4 is *sbm-1* and *sbm-4* (Gao et al., 2004b; Provvidenti and Alconero, 1988b).

Host genetic resistance research

Resistance to PSbMV was first identified in USDA *Pisum* plant identification accessions (Stevenson and Hagedorn, 1971). Further studies conducted by Hagedorn and Gritton (1973) identified two additional lines and proposed that the trait controlling resistance is a single recessive resistance gene designated as *sbm*. Additional PI lines were later identified as resistant (Hampton and Braverman, 1979). Further testing of PI lines suggests that pea plants grown at the Northeastern Regional Plant Introduction Station in Geneva, NY, could have been exposed to PSbMV carrying aphids from 1961 to 1969 without recognizing disease presence. Lines from India, Yugoslavia, and Peru tested positive for PSbMV and likely served as inoculum sources for the *Pisum* PI collection. Although Indian lines contributed to the introduction of PSbMV into the U.S., it is noted that northern India is also an important reservoir of PSbMV resistant germplasm. Resistant germplasm was not yet implemented into breeding programs during this time. Many seed companies responded to the outbreak by destroying virus-infected seed stocks to prevent further spread of the disease (Hampton et al., 1976). PSbMV was not detected in the United States after elimination efforts until 1974 when seed was sent to Brawley, CA for winter increase along with pea breeding lines sent from Canada in turn infecting USDA lines (Hampton et al., 1976). It was soon learned that the virus might exist for several years going unnoticed by breeders or commercial companies.

There have been various reports of resistance, which have led scientists to conduct genetic studies to determine where the *sbm* gene is located. Gritton and Hagedorn (1975) found

that *sbm-1*, which confers resistance to pathotype 1 and 4 is closely linked to gene *wlo*, gene controlling wax on pea leaflets on LG VI, as well as gene *p*, which has been associated with the type and amount of pod membrane. Hampton and Marx (1981) confirmed the *wlo-p-sbm-1* relationship, but further study led Skarzynska (1988) to propose that *sbm-1* is closer to *Pl*, a gene controlling black hilum (Skarzynska, 1988). The results of Skarzynska (1988) were later confirmed and *Prx-3* was also demonstrated to be in close proximity to *sbm-1* (Weeden et al., 1991). Provvidenti and Alconero (1988a) first identified *sbm-2* and *sbm-3*, which confer resistance to PSbMV-P2/L1 on LG II and LG VI, respectively (Congdon et al., 2016; Gao et al., 2004b; Makkouk et al., 2014). Gene *sbm-2* is closely linked to gene *mo*, which confers resistance to bean yellow mosaic virus, and additional genes that confer resistance to other potyviruses. The two genes (*sbm-2* and *sbm*-3) do confer resistance to the same pathotype, but are inherited independently (Provvidenti and Alconero, 1988a). *sbm-4* has been suggested that it is closely linked to *sbm-1* and *sbm-3* on LG VI and confers resistance to PSbMV-P4 (Alconero et al., 1986). Timmerman et al. (1993) proposed the first linkage map for *sbm-1* by conducting linkage studies using restriction fragment length polymorphism (RFLP) and random amplification of polymorphic DNA (RAPD) markers.

The use of molecular markers is a powerful tool that allows breeding programs to identify individuals that contain the marker for the gene conferring resistance, and can be especially useful for recessive genes. An RFLP marker, GS185 (Timmerman et al., 1993), and a STS marker, sG05_2537 (Frew et al., 2002), were located approximately 8 cM and 4 cM from the gene, respectively. Dhillon et al. (1995) mapped *sbm-4*, and identified the same RFLP marker GS185 to be closely linked to select for virus resistance. Gao et al. (2004a) used a candidate gene approach and *Medicago truncatula* (barrel medic) genome to identify markers linked to *sbm-1*.

After cloning fragments of the pea eukaryotic translation initiation factor (*eIF4E*) gene, they found linkage between *eIF4E* and *eIF(iso)4E* to *sbm-1* and *mo*, respectively. Size differences were detected in intron 2 of the *eIF4E* gene sequence between the genomic DNA of JI1405 and JI2009. Polymorphism from a RFLP marker observed between the resistant (JI1405) and susceptible (JI2009) lines was used to develop a perfect PCR polymorphic marker using 4Egenomic3' and 4Egenomic5' primers, which provide a co-dominant assay for screening segregating populations. The work of Gao et al. (2004a) was expanded by Smýkal et al. (2010) with the complete sequence of *eIF4E* with an additional intron and comparing it among resistant and susceptible accessions. Whereas Gao et al. (2004b) only characterized the partial gene sequence, and missed the first intron that Smýkal et al. (2010) identified.

Recent research has focused on elucidating the relationship between *sbm-1* and PSbMV. It is known that there is an interaction between the virus genome-linked protein (VPg) and translation factor eIF4E; however, it is not understood how their role impacts one another. The VPg is required for virus infection to occur. The current speculation is that there are amino acid differences between *Sbm-1* and *sbm-1*, which inhibits VPg interaction with *sbm-1*; therefore, the viral protein is not translated and the virus does not replicate in the plant cell (Ashby et al., 2011). If the dominant gene is present, the virus is able to replicate and cause a systemic infection (Gao et al., 2004b). Currently, there is ongoing research to further understand the mechanism or role of the VPg.

Pea genome

Pea is a diploid species containing seven chromosome pairs (2n=2x=14). Various scientists dating back to the "Father of Genetics", Gregor Mendel, have long studied pea genetics. Genetic studies suggest that pea has a relatively large haploid genome estimated to be

4.45 Gbp in size (Doležel et al., 1998; Doležel and Greilhuber, 2010), but to date, there is no complete pea genome sequence. The consensus maps use reference populations to map traits of interest including, but not limited to disease resistance, seed color, and leaf type. The first map proposed by Wellensiek 1925 contained six linkage groups, but further study led to the addition of a seventh (Rozov et al., 1999). Lamprecht (1948) presented seven linkage groups that correspond to the seven pea chromosomes. Although this work has been revised, much of the literature today reports seven pea linkage groups (Murfet, 1990; Ellis et al., 1992; Laucou et al., 1998; Loridon et al., 2005; Duarte et al., 2014; Tayeh et al., 2015). Random amplification of polymorphic DNA and RFLP markers were used to develop early consensus maps, and through advancements in technology and knowledge, scientists use sequence tagged sited (STS) markers and microsatellite markers or simple sequence repeats (SSR). The most recent consensus map was created with the use of single nucleotide polymorphism (SNP), which provides a high-density composite map with even marker distribution along the genome. Tayeh et al. (2015) published the latest consensus map consisting of 15,079 SNP markers resulting in 794.4 cM in length. Consensus maps are powerful tools for marker assisted selection to improve breeding efforts with more precision and a shorter breeding cycle.

Two legume species, *Medicago truncatula* and *Lotus japonica*, have been selected as model species to understand and study legume genetics (Stougaard, 2001). Both have a syntenic relationship to the pea genome, but have a relatively small genome compared to pea. The pea genome constitutes many repetitive sequences contributing to its large genome size (Neumann et al., 2001). The use of model species and other species such as alfalfa, chickpea, and lentil with syntenic relationships to pea have aided in understanding the pea genome by transferring knowledge between them (Table 1) (Weeden et al., 1992; Kaló et al., 2004; Aubert et al., 2006;

Leonforte et al., 2013; Sindhu et al., 2014). Additional synteny with pea include pigeon pea (*Cajanus cajan* L.) and soybean. Using fluorescent in situ hybridization (FISH), Fuchs et al. (1998) were able to assign each of the seven linkage groups to the seven pea chromosomes. The results were later confirmed by Ellis and Poyser (2002); they denote the chromosome numbers and linkage group numbers as Arabic and roman numerals, respectively, 1: VI, 2: I, 3: V, 4: IV, 5: III, 6: II, and 7: VII.

There are two recessive disease resistant gene clusters located on linkage groups II and VI (Provvidenti and Hampton, 1991). Using a candidate gene approach based on the *Medicago truncatula* genome, scientists were able to identify two homologous genes, *eIF4E* and *eIF(iso)4E*, which confer resistance to PSbMV at the *sbm-1* and *sbm-2* loci (Gao et al., 2004a; Bruun-Rasmussen et al., 2007). Understanding this region of the genome provides valuable information that is currently being implemented with the use of marker assisted selection (MAS) to develop resistant germplasm or screen existing lines within the program. However, there are still many unanswered questions about the pea genome, but an association of scientists part of the International Consortium for Pea Genome Sequencing (PGS) committee are working to provide a complete genome sequence (CSFL, 2017).

Table 1. Syntenic relationship between pea linkage groups and *M. truncatula*, *L. japonica*, alfalfa, chickpea, and lentil linkage groups (Compiled from: Weeden et al., 1992; Kaló et al., 2004; Aubert et al., 2006; Leonforte et al., 2013; Sindhu et al., 2014).

Pea	*M. truncatula*	*L. japonica*	Alfalfa	Chickpea	Lentil
I	5	1, 2	5	2, 8	5
II	1	5	1	4	1, 5
III	2, 3	1, 3, 6	2, 3	7	3
IV	4, 8	2, 3, 4	8	7	7
V	7	1, 3	7	3	6
VI	2, 6	1, 2, 4, 6	2, 6	1, 2, 8	2
VII	4, 8	3, 4, 6	4	6	2

Developments in technology enhance breeding efforts

Breeders seek to enhance and improve plants by manipulating their composition as they cross elite lines, landraces, or even wild species depending on the breeding objective. Throughout history, the breeding objective has changed as breeding goals are achieved. In the past, breeders made selections based on phenotypic characteristics solely; however, in recent years new advancements in technology have allowed them to make genotypic selections. This is the art and science of plant breeding; breeders have a trained eye and rely on intuition, skill, and judgment, but also are using scientific methods and technology to assist in selecting (Acquaah, 2012b). Marker assisted selection is a process that allows breeders to make selection of desirable phenotypes by selecting a marker closely linked or within the desirable gene expressing the phenotype of interest. Advancements in molecular technology have simplified genetic studies by improving the efficiency, less labor intensive, and more cost effective. Three steps of MAS include the construction of a genome linkage map, identify markers located near the trait of interest, and select PCR markers linked to the QTL of interest (Sleper and Poehlman, 2006). The use of marker assisted technology can be a significant tool in breeding programs by allowing plant breeders to gain an understanding of the germplasm available, introgress traits and reduce linkage drag, and test and select traits of interest in earlier generations of the breeding process (Acquaah, 2012b). Another useful process is marker assisted backcross selection (MABS), which can speed up the backcrossing process while maintaining a recessive allele (reviewed by Xu and Crouch, 2008). Often MABS is used for simple inherited traits such as disease or pest resistance. These processes assist in gene pyramiding by developing cultivars with multiple resistant genes.

Molecular markers have greatly contributed to the knowledge and understanding that is available today. Many improvements have been made since the beginning of molecular marker

development, and many different types exist. Restriction fragment length polymorphism markers and RAPDs were some of the first markers developed during the 1980s to conduct mapping studies (Acquaah, 2012b; Sleper and Poehlman, 2006; Williams et al., 1990). However, RFLP are rarely used due to safety of radioactive materials. Random amplified polymorphic DNA markers are a PCR-based molecular marker, but more commonly used markers today include SSR and SNP markers. Both SSRs and SNPs can be co-dominant allowing easy identification of heterozygous individuals, which can easily be visualized on an agarose gel. SNPs, however, are not always co-dominant, they can also have a dominate nature allowing for only present/absent banding patterns on gels (Sleper and Poehlman, 2006). Simple sequence repeat markers are short repetitive DNA sequences that are versatile and are the most exploited PCR-based marker that are distributed throughout the genome. These markers have an advantage of being tightly linked or sometimes located within the gene providing more accurate results when making selections compared to RFLPs and RAPDs (reviewed by Xu and Crouch, 2008; Acquaah, 2012b).

Backcrossing population development using marker assisted selection

Backcross breeding was introduced by Harlan and Pope in 1922 to improve current cultivars by replacing the undesirable allele with an alternative, while maintaining the quality of the cultivar lacking a particular trait. The two parental lines used in hybridization are referred to as the recurrent parent, the desirable parent lacking the trait of interest, and donor parent, the source of the desirable gene. The inbreeding process is modified by crossing the F_1 to the recurrent parent to recover the characteristics and quality traits that were lost in the initial cross. This modification is the first backcross (BC_1) and is typically followed with additional cycles of backcrossing the new BC_nF_1 to the recurrent parent until a desired level of homozygosity for each recurrent parent has been recovered, while still maintaining the desirable trait.

Each backcross will be preceded with selection to ensure crosses are made to individuals with the trait of interest. Phenotypic selection can be utilized, but in the case of a recessive gene, the individuals must be self-pollinated to identify the recessive homozygotes using progeny tests (Acquaah, 2012a). Transferring a recessive resistant gene adds cost and time to breeding. Gao et al. (2004a) and Smýkal et al. (2010) have developed primers to assist in selection for PSbMV resistance to improve efficiency during cultivar development. To minimize the time and cost, while providing a reliable product to producers, many researchers have used marker assisted backcross selection to optimize their breeding efforts (Neeraja et al., 2007; Yi et al., 2009; Chu et al., 2011).

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CHAPTER III. VIRUS RESISTANCE IN PEA (*PISUM SATIVUM* L.)

Introduction

Pea (*Pisum sativum* L.) serves important roles as a grain legume commonly found in livestock feed, human consumption, and crop rotations. For human and livestock consumption, dry pea is a nutritional food source containing approximately 25% protein and 60% carbohydrates (Muehlbauer and Summerfield, 1989). Their high protein content and essential amino acid support metabolic functions, which makes it a desirable protein replacement. More importantly they serve as a beneficial role in rotational systems by fixing nitrogen through a symbiotic relationship with Rhizobium bacteria, break disease cycles, and reduce insect and weed pressure. However, pea production is constrained by several abiotic and biotic stress agents.

Pea *Seed-borne Mosaic Virus* (PSbMV) is found worldwide with the first observations in the United States occurring in the Pacific Northwest region and Wisconsin in 1968 to 1969 (Hampton, 1969; Mink et al., 1969; Stevenson and Hagedorn, 1969). As well as in Japan (Inouye, 1967) and Czechoslovakia (Musil, 1970) during the same time. It is unknown when it arrived in North Dakota, but first conformation was in 2012 (Pasche, personal communication, 2017). PSbMV impedes growth, resulting in reduced crop yield and quality losses. Currently, growers in Montana and North Dakota are experiencing repercussions from PSbMV. It is a *Potyvirus* belonging to the *Potyviridae* family. The primary spread of the virus is through dissemination of infected seed, but alternative methods include several vector species with the most common vector being *Acyrthosiphon pisum* (pea aphid) and mechanical sap inoculation. PSbMV causes varying symptomology depending on the pathotype. Commonly, the plant is stunted containing shortened internodes near the top of the plant, budding at the internodes,

delayed senescence, leaves are malformed and discolored with swollen leaf veins (reviewed by Khetarpal and Maury, 1987). The flowers may be aborted or pods fill improperly with missing seed. The seed that is developed sometimes has discoloration, scarring, split seed coat, or water-soaked lesions.

The Palouse region of the Pacific Northwest is known for pea production. Resistant germplasm has been developed for this region and McPhee and Muehlbauer (2002) released cultivar 'Lifter', which possesses the *sbm-1* gene. However, Lifter is not adapted to North Dakota, it has the dominant allele (*Af*) for a normal leaf type morphology and has a delayed flowering period that matures later than cultivars grown in the NGP region. The gene conferring resistance (*sbm-1*) is recessively inherited and is located on linkage group (LG) VI. Lifter was reported to have the resistant gene (*sbm-1*) (USDA-ARS, 2001) and confirmed by Smýkal et al. (2010). The *sbm-1* gene confers resistance to pathotypes PSbMV-P1 and PSbMV-P4 (Gao et al., 2004b). It has been identified and renamed as the eukaryotic translation initiation factor, *eIF4E*, by Gao et al. (2004b). The mechanism of the recessive gene prevents transcription of the virus and inhibits virus movement from cell to cell. There are additional PSbMV resistant genes located on LG VI including *sbm-1*^{1}, *sbm-3*, and *sbm-4*, and *sbm-2* is also a resistance gene to PSbMV, but located in a second resistance gene cluster on LG II (Gao et al., 2004b; Provvidenti and Alconero, 1988a; Provvidenti and Alconero, 1988b). The *sbm-1*^{1} is an allele that confers resistance to PSbMV-P1 and PSbMV-P2/L1 pathotypes; however, is susceptible to PSbMV-P4 (Gao et al., 2004b). PSbMV-P2/L1 pathotype is also conferred by *sbm-2* and *sbm-3*, which are independently inherited (Congdon et al., 2016; Gao et al., 2004b; Provvidenti and Alconero, 1988a). Lastly, *sbm-4* only confers resistance to PSbMV-P4 pathotype (Provvidenti and Alconero, 1988b).

Therefore, by transferring the *sbm-1* gene from Lifter which confers resistance to pathotypes PSbMV-P1 and PSbMV-P4 (Gao et al., 2004b), we are transferring resistance to the PSbMV-P4 pathotype present in the NGP region. The 4Egenomic primer pair previously developed by Gao et al. (2004a) is a 'perfect marker' to select the *sbm-1* gene to assist in developing resistant germplasm. Marker assisted backcross breeding was used in this study to follow inheritance of the recessive resistance gene (*sbm-1*) to minimize time, cost, and other resources. The developing populations were validated for resistance using manual inoculation of the PSbMV-P4 isolate to confirm results from DNA analysis. Agronomic performance of resistant backcross lines was evaluated in an early generation trial during the 2017 field season.

Materials and methods

Backcross population development

Backcross populations were developed using 10 parental breeding lines adapted to North Dakota, which were previously developed by the NDSU Pulse Crops Breeding Program (Table 2). Each adapted breeding line was crossed with Lifter as the donor parent for its multiple disease resistance characteristics especially to P*ea Seed-borne Mosaic Virus*. Lifter has a normal leaf type and green cotyledons, but it is not adapted to North Dakota and lacks lodging resistance. The selected recurrent parent breeding lines consist of both green and yellow dry pea types.

Table 2. Susceptible adapted breeding lines from the NDSU Pulse Breeding Program used as recurrent parents in a marker assisted backcrossing scheme to develop PSbMV resistant germplasm.

Breeding line	Leaf type	Cotyledon color	Market class	Trait selected
NDP080173	semi-leafless	green	marrowfat	marrowfat seed
NDP121166	semi-leafless	green	smooth green	high yield
NDP121221	semi-leafless	yellow	smooth yellow	high yield
NDP121322	semi-leafless	yellow	smooth yellow	high yield
NDP121334	semi-leafless	yellow	smooth yellow	high yield
NDP121361	semi-leafless	yellow	smooth yellow	high yield
NDP121443	semi-leafless	yellow	smooth yellow	high yield
NDP121548	semi-leafless	green	smooth green	high yield
NDP121613	semi-leafless	yellow	smooth yellow	high yield
PS07ND0190	semi-leafless	green	smooth green	high yield

Greenhouse: Fall 2015

The recurrent parents were sown on 3 September 2015 in the Agricultural Experiment Station (AES) greenhouse, Fargo, North Dakota. Each parent was planted 2 cm deep in a six inch round pot (BFG Supply, Burton, OH) filled with Pro-Mix Flex potting soil (Premier Horticulture Inc. Quakertown, PA) and fertilized with Osmocote Plus 15-9-12, 3-4 month formula (Everris NA Inc., Dublin, Ohio). Three pots of each parental type were planted to ensure viable flowers throughout crossing. In addition, a second crossing block was planted two weeks later, 21 September 2015, to ensure proper timing of anthesis. The plants were grown on greenhouse benches allowing them to grow up nylon twine attached as a trellis. Each pot was watered daily as needed under constant temperatures of 25 and 20 °C day and night, respectively, and a 16:8hr day:night photoperiod.

Crossing began as the plants flowered with the pollinating objective in the fall of 2015 to make a minimum of three attempts per cross combination. Each of the ten selected lines were crossed with cultivar Lifter, the resistant donor parent, to develop F_1 populations (McPhee and Muehlbauer, 2002) (Figure 1). Artificial hand pollinations were made in the morning when the pollen was viable. The crosses were made on the recurrent parents as the female plant, with an

exception when flowers were not adequate and the donor parent was used as the female parent. After crossing the two individuals, a #5 1 1/8 x 1 ¾ inch merchant tag (Uline Pleasant Prairie, WI) was attached to the female flower to identify the parents of the cross, date, and individual whom performed the cross. The successful F_1 pods contain between 1 to 7 seeds were harvested in December as they matured coin envelope.

Greenhouse: Spring 2016

The harvested F_1 seeds were planted on 11 January 2016, in the AES greenhouse under the same growing conditions along with the recurrent parents. Each F_1 plant was backcrossed to their respective recurrent parent (Figure 1). The F_1 individuals were used as the female parent and the recurrent parent was used as the pollen parent typically, but reciprocal crosses with the F_1 parent as the pollen donor were made occasionally. A goal of three attempts per cross combination was attempted. The crosses performed in the spring were the first backcross (BC_1) populations and each BC_1F_1 cross was harvested individually with its corresponding crossing tag. After the BC_1F_1 crosses were harvested, the individual F_1 plants from each population were hand harvested as well in order to recover the F_2 seed. The F_1 seeds were not confirmed to be derived from a successful cross until after the first backcross was made, and then DNA analysis was performed on BC_1F_1 populations identified the successful F_1 and BC_1F_1 individuals.

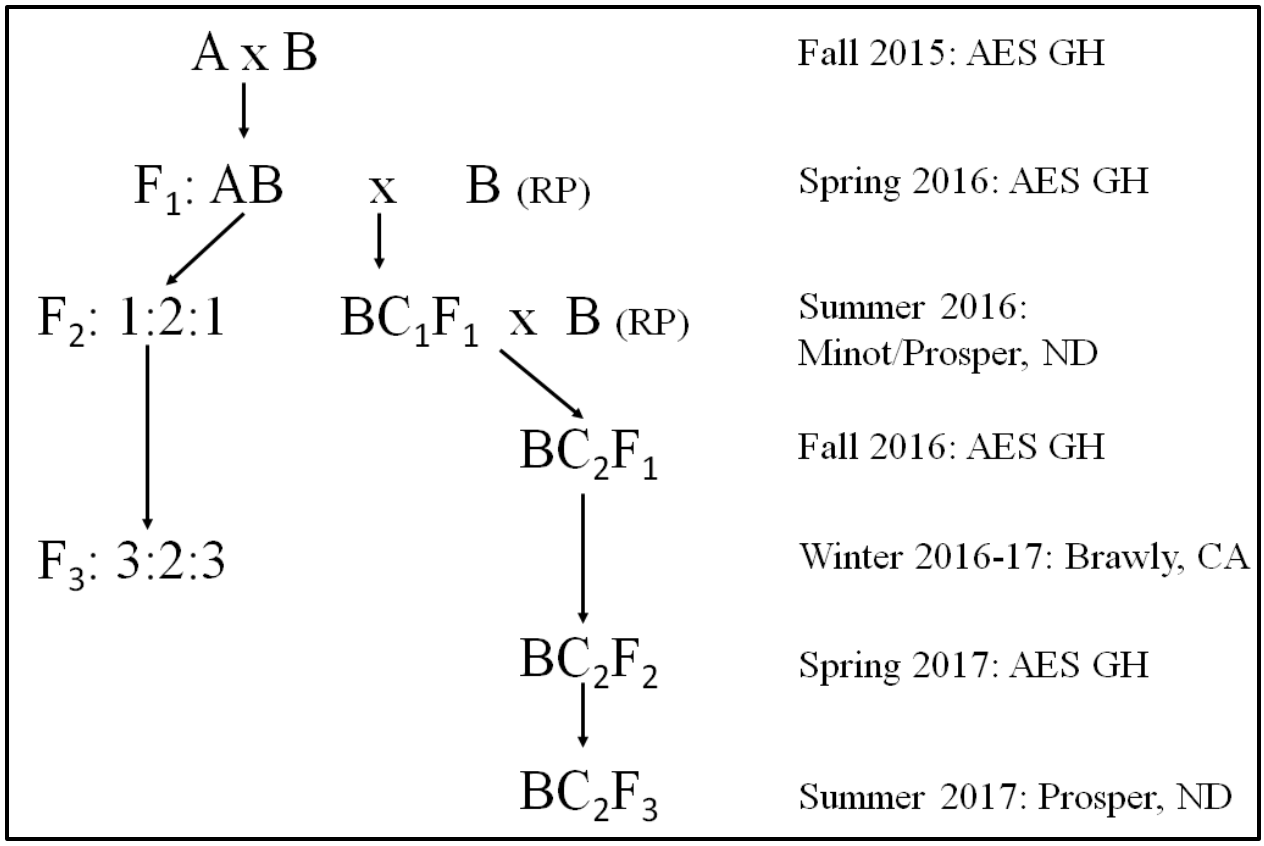

Figure 1. Flow diagram for a marker assisted backcrossing approach for PSbMV resistance in pea. 'A' represents Lifter as the resistant parent and 'B' represents the respective recurrent parent.

Field: Summer 2016

The BC_1F_1 populations were sown in the field using a 75 horsepower tractor (New Holland Agriculture, New Holland, PA) with a three point Wintersteiger XL 8-row planter (Wintersteiger Ag, Ried, Austria). Each plot was sown on 13 May 2016 as a single 2.4 m row near Prosper, North Dakota, (47° N, 97° W). The amount of seed per row/plot was variable, one to seven seeds, depending on the number of developed seeds from each successful backcross. A field stake label indicated each plot, and within each plot, individual BC_1F_1 plants were labeled to distinguish each plant for DNA analysis and crossing purposes. The co-dominant nature of the SSR marker selected was able to detect a true cross pollination versus a self-pollination based on the results from DNA analysis. The results from DNA analysis identified heterozygous

individuals possessing the *sbm-1* resistance allele, which were crossed to the respective recurrent parent to develop BC_2F_1 populations in the 2016 field growing season. Each cross BC_2F_1 cross was hand harvested first, and then followed by individual BC_1F_1 plants harvested and threshed with an Almaco Small Bundle Thresher (Almaco, Nevada, IA).

The self pollinated F_2 seeds were advanced in the breeding program and were sown in a similar manner as the BC_1F_1 individuals in the field at the North Central Research Extension Center (NCREC) in Minot, North Dakota. Approximately 40 seeds were planted per single row. The F_2 plants were allowed to self-pollinate and flowering dates were collected. At the end of the season, plots were hand harvested and threshed with an Almaco Large Plot Thresher (Almaco, Nevada, IA). The F_3 populations along with parental lines were sent to Brawley, California for winter increase and generation advancement for the NDSU Pulse Crops breeding program. The focus of this study is directed to the development of the backcross populations.

Greenhouse: Fall 2016

BC_2F_1 progeny were planted in the AES greenhouse on 31 August 2016 in four inch square pots (BFG Supply, Burton, OH) due to space constrains in the greenhouse. Each plant was self-pollinated, and DNA analysis was preformed to select heterozygotes possessing the recessive allele. The heterozygous individuals were retained to allow them to segregate into the three genotypic classes and be identified in next generation of DNA testing. The BC_2F_2 seed from each heterozygous plant was hand harvested and placed in separate envelopes to be planted in the spring of 2017 to identify homozygous recessive individuals following the second backcross.

Greenhouse: Spring 2017

The identified heterozygotes were grown in the AES greenhouse as BC_2F_2. Due to the large number of individuals, there were two planting dates to support half of the populations in each cycle. The first set was planted on 4 January 2017 and the second set on 20 January 2017 in six inch round pots and four inch square pots, respectively, due to limited greenhouse bench space. The first set had difficulty germinating properly due to a hard seed coat. Seeds that did not germinate after two weeks were dug up to scarify the seed and encourage germination. To ensure even germination, all seed in the second planting went through the scarification process by nicking all seeds with a scalpel. Tissue samples were collected two weeks after planting for DNA extraction. Concluding DNA analysis, only homozygous recessive individuals conferring resistance to pea seed-borne mosaic virus were kept, a few heterozygotes were retained for their potential to segregate for desired phenotypes, and all homozygous dominant individuals were discarded. To validate resistant individuals, each plant was inoculated with PSbMV-P4 isolate and tested with DAS-ELISA. All homozygous recessive BC_2F_2 individuals were hand harvested.

Field: Summer 2017

Experimental homozygous recessive *sbm-1* individuals from eight BC_2F_3 populations were planted near Prosper, North Dakota, (47° N, 97° W). The experimental lines were evaluated in the field for agronomic performance and validation of virus resistance. The experiment was created as a partially replicated incomplete block design with five blocks, similar to an augmented design where test entries are non-replicated. Due to a scarcity of seed in early generation testing, the test entries were not replicated throughout each block. The eight susceptible recurrent parental lines, two check cultivars (‘Agassiz’, green dry pea, and ‘CDC Striker’, yellow dry pea), and one resistant parental check (Lifter), were replicated one, five, and

four times per block, respectively. The three check cultivars were systematically placed in a diagonal fashion with the non-replicated test lines randomly arranged in the remaining plots according to designs discussed by Clarke and Stefanova (2011).

The three check cultivars were selected based on the current cultivars commonly grown in North Dakota (Agassiz and CDC Striker) and the last (Lifter) because it is the resistant parent used in each of the eight developing families. An estimate of experimental error based on repeated checks and parental lines were used to compare the populations. The non-replicated BC_2F_3 populations and checks were planted as 2.1 m, single rows spaced 0.53 m apart. There were 25 seeds planted per plot. The entire experiment was designed in Agrobase containing 360 entries with 72 entries per block; within each block there were 50 non-replicated test entries, 8 parental lines, and 14 checks. There was a pre-plant herbicide applied, Treflan, to control grasses and broadleaf weeds, and on 8 June 2017 a labeled rate of Raptor and Basagran 5L with a non-ionic surfactant was applied to control broadleaf weeds specifically volunteer canola. It must be applied before flower initiation.

Agronomic trait measurements were collected for number of days to first flower, bloom period, and physiological maturity, and near physical maturity, two plants per row were used to measure vine length, number of nodes to first reproductive node, number of reproductive nodes, and average number of pods per peduncle. Flowering data was recorded when 10% of the plot began to flower, and then again when only 10% of flowers remained was recorded as the last flowering date. The two dates were used to calculate the bloom period. Before the plants reached physical maturity, a bulk tissue sample of 4 to 6 leaves was collected from all non-replicated, parental, and check plots for ELISA testing to ensure there was no virus present in non-replicated test entries. Physical maturity was based on all of the pods being brown or yellow with a few of

the top pods on the plants being almost yellow or had a leathery pod membrane. Vine length was measured from the soil surface to the top of the plant. Stand counts were collected before harvest. Each single row plot was bulk harvested and threshed with an Almaco Large Plot Thresher. The plot weight in grams, 1000 seed weight in grams, and seed color were recorded post-harvest. Plot weight was measured in grams and converted to kilograms per hectare.

DNA analysis

Each of the BC_1F_1, BC_2F_1, and BC_2F_2 populations were tested for the presence of the virus resistant gene, *sbm-1*, using a perfect PCR marker developed by Gao et al. (2004a). QIAGEN DNeasy Plant Mini Extraction Kit (QIAGEN Inc., Valencia, CA) was used for BC_1F_1 populations, and QIAGEN DNeasy 96 Plant Extraction Kit (QIAGEN Inc., Valencia, CA) was used to extract DNA from BC_2F_1 and BC_2F_2 populations due to the number of samples being processed. Leaf tissue samples of approximately 300 mg were collected from individual plants into two milliliter VWR Microcentrifuge Tubes (VWR International, Radnor, PA) for BC_1F_1, and a one milliliter 96-well deep well polypropylene plate (USA Scientific, Ocala, FL) for BC_2F_1 and BC_2F_2. The samples were dried with a lyophilizer (SP Scientific, Gardiner, NY) and ground using a Geno/Grinder 2000 (SPEX CentiPrep, INC., Metuchen, NJ). A stainless steel 3.2 mm bead (BioSpec Products, Inc., Bartlesville, OK) was placed in each sample tube or well after samples were dried to assist with grinding samples. After DNA extraction, all samples were placed in a -20 °C freezer.

BC_1F_1 extraction

The quick start protocol provided in the DNeasy Plant Mini Kit was followed as directed using a Heraeus Biofuge Pico Microlitre Centrifuge (DJB Labcare Limited, Buckinghamshire, England) to centrifuge the samples. DNA samples were quantified using a Nanodrop 2000

Spectrometer (Thermo Scientific, Wilmington, DE) and adjusted to an average concentration of 50 ng/ul.

BC_2F_1 and BC_2F_2 extraction

The Quick-Start Protocol provided in the DNeasy 96 Plant Kit was modified to follow similar protocol in the DNeasy Plant Mini Kit. The protocol indicates that it is for fresh plant tissue, but the samples were dried, ground, and then 400 ul of working lysis solution was added. After the lysis solution was added, the plates were incubated for ten minutes in a hot water bath at 65 °C. After this step, the remaining steps were followed as directed using a Beckman Coulter Allegra X-12R Centrifuge (Beckman Coulter, Brea, CA) to centrifuge the samples. The samples were not quantified due to the large quantity of samples.

Polymerase chain reaction

Each PCR plate included BC_nF_n progeny, respective recurrent parent of the progeny being tested, and Lifter. A PCR master mix composed of Promega Go Taq® Flexi DNA Polymerase kit (Promega Corporation Madison, WI), 2 mM final concentration from a 100 mM deoxynucleotide (dNTP) solution set (BioLabs Inc. New England), and forward and reverse 4Egenomic primers (Eurofins Genomics Louisville, KY) was used to amplify the target sequence. The 20 ul PCR reactions included 18.5 ul master mix, 1.5 ul of DNA, and were run in an Applied Biosystems Thermal Cycler (Applied Biosystems Corporation Foster City, CA). The thermal cycler is programed for three stages. The first stage heats the sample to 94 ℃ for one minute before entering stage two. Stage two has three steps for denaturing, annealing, and extension, which run for one minute at 94 ℃, another minute at 61℃, and two minutes at 72 ℃, respectively. As it begins the third stage, a constant temperature of 72 ℃ is maintained for seven minutes before allowing the sample to remain at 4 ℃ until sample is removed from the machine.

Gel electrophoresis

A 10 ul sample of each PCR reaction was run on a 3% TAE agarose gel containing ethidium bromide (EtBr) and 5 ul of a DNA ladder, 1kb Plus (Affmetrix, Inc. Cleveland, Ohio) in the outer wells of the gel. Lifter and the recurrent parent were included as positive checks to observe banding patterns for resistant individuals. An AlphaImager HP (Protein Simple San Jose, CA) aided in capturing gel images of amplified products for each individual plant.

Chi-square test

To determine if the observed values deviate significantly from the expected ratio, a Chi-Square Test (Goodness of Fit Test) was used for each generation of DNA analysis. The chi-square value (X^2) was calculated using the following formula,

$$X^2 = \sum \frac{(O-E)^2}{E}$$

where 'O' is the observed values and 'E' is the expected values. The calculated X^2 value was compared to the critical value of 6.635 for probability level of 0.01 with one degree of freedom (df) presented in a Chi-Square Distribution table was used for BC_1F_1 and BC_2F_1 populations. The expected ratio changes when individuals are allowed to self, so for BC_2F_2 populations the segregation ratio is 1:2:1 with two degrees of freedom. The critical value of 9.210 for $P<0.01$ and 2 df will be used to determine if the population fits as expects.

Validation of resistance

In the AES greenhouse in the spring of 2017, the BC_2F_2 plants that were homozygous recessive were mechanically inoculated with PSbMV-P4 and confirmed resistant individuals with DAS-ELISA. Two sets of inoculations were conducted for the two planting dates. To prepare for inoculations, cultivar Ginny was used as the maintainer line that was inoculated one to two weeks after emergence. The maintainer lines were tested with ELISA two to three weeks

after inoculation to determine infected individuals. The PSbMV inoculum was obtained from the top leaves of an infected pea plant at six weeks maturity. It is crucial to have young new tissue when preforming inoculations because that is where virus titers are the highest. The individuals with a high virus titer were used to inoculate BC_2F_2 homozygous recessive and a few homozygous dominant plants to increase probability that inoculations were effective. Leaf tissue was ground using a mortar and pestle and homogenized in a 0.01 M Phosphate buffer with a pH of 7.1 at a 1:10 ratio of tissue to buffer. Silicon carbide, also known as Carborundum 320 grit, was added to the slurry (crude sap) of crushed leaf tissue and Phosphate buffer to serve as an abrasive to create leaf surface injury. Approximately 0.1 grams of silicon carbide to five milliliters of crude sap was added. The virus is active for a short time, and a new set of inoculum was prepared every five to ten minutes. The plants were inoculated prior to flowering at six to seven weeks after planting. The top six leaves or the top three nodes were brushed with infective solution by applying slight pressure to damage the leaf surface using a Q-tip. Enough pressure was applied, but not too much that the leaf tears or appears water soaked when visualizing the leaf from the bottom of the inoculated leaf. Ten minutes after inoculation, inoculated leaves were rinsed with water to remove silicon carbide. A second inoculation was conducted about a week after the first inoculation to increase infection frequency. The same procedure was used to inoculate the second planting date.

Three to four weeks after inoculation, the plants completed flowering and pods were formed or beginning to form at the top of the plant, tissue samples were collected from the top of the plant in Bioreba extraction bags (Bioreba, Reinach, Switzerland). Samples were screened with a Double Antibody Sandwich-Enzyme Immunosorbent Assays (DAS-ELISA) (AC Diagnostic, Catalog #V036-K1, Fayetteville, AR) following the protocol as directed. The plates

were read with an Epoch Microplate Spectrophotometer controlled by the Gen5 Software interface (BioTek, Winooski, VT). To identify individuals as positive or negative, a standard of two time the background of the negative control was used. Everything above two times the negative control was considered positive. Plates were washed and kept in a -20 °C freezer.

To ensure resistance in the field during the summer of 2017, a bulk sample of 4 to 6 leaves was collected from each BC_2F_3 plot including the checks and parents following the same ELISA protocol. Aphids were first observed on the west side of the field during the first week in July, and in a week they slowly migrated to the east across the field before insecticide was sprayed. A labeled rate of Warrior II was applied on 13 July 2017. Only one insecticide application was used since the aphids were not observed later in the season.

Statistical analysis

Data gathered on BC_2F_3 populations from 2017 field growing season were analyzed using SAS version 9.4 (SAS Institute, Cary, NC). In SAS using a generalized linear model (GLM) procedure, where the experiment and genotypes were considered fixed effects in a non-spatial augmented block analysis (Figure A1). The GLM procedure generated an analysis of variation (ANOVA) for ten agronomic traits collected during the growing season and post-harvest to evaluate significance among genotypes. The analysis included a type III sum of squares (SS) ANOVA table containing the genotype and experiment, which is the model term containing two sources of variation, the genotype and experiment. The type III SS was used to interpret the significance ($P<0.05$, $P<0.01$, and $P<0.001$) among the genotypes and the differences among the blocks within the experiment for each trait. The use of a type III SS corrects for any missing data points or block effects that impact the genotype by assessing differences and adjusting by computing the least square (LS) means for each genotype. The LS means were used to calculate

trait averages, minimum and maximum values, and ranges to identify superior lines. In addition, the same GLM procedure was used, but was altered to conduct a probability of differences analysis which is a t-test to analyze each recurrent parent to their respective populations/families derived from that recurrent parent. (Figure A1).

Results

Backcross population development

Greenhouse: Fall 2015

There were 10 unique cross combinations resulting in twenty-four F_1 crosses that were harvested in the fall of 2015 representing each of the susceptible parents (Table 3). The progeny were advanced as F_1 plants used to develop the first backcross generation. The F_1 individual plants were not confirmed to be a true cross until after the BC_1F_1 populations were developed and confirmed with DNA tested. Since Lifter is a normal leaf type carrying the dominant gene, and if it was used as the male parent, then the leaf type will be an indicator if the F_1 progeny was a true cross pollination.

Table 3. Number of F_1 seeds derived from crosses of 10 susceptible parents and cv. Lifter in the fall 2015 crossing block.

Recurrent parent	Seed harvested
NDP080173	7
NDP121166	9
NDP121221	1
NDP121322	10
NDP121334	4
NDP121361	8
NDP121443	5
NDP121548	10
NDP121613	13
PS07ND0190	6
Total	73

Greenhouse: Spring 2016

Thirty-one BC_1F_1 crosses were generated and 91 seeds were harvested. Progeny representing all recurrent parents were generated and used to develop the BC_2 populations (Table 4). DNA analysis of the BC_1F_1 progeny was conducted before BC_2 crosses were attempted to determine which individuals possessed the recessive resistant allele (Table 4).

Table 4. Number of successful BC_1F_1s from spring 2016 crossing block and those confirmed based on DNA analysis to have the resistant allele.

Recurrent parent	Crosses	Confirmed
NDP080173	1	0
NDP121166	3	3
NDP121221	3	1
NDP121322	4	2
NDP121334	3	2
NDP121361	6	5
NDP121443	1	1
NDP121548	1	1
NDP121613	3	2
PS07ND0190	6	4
Total	31	21

DNA analysis

Field: Summer 2016

Twenty-one crosses were used to develop the second backcross populations. The 21 representative crosses consisted of 73 plants, of which 25 were homozygous dominant and 48 were heterozygous (Table A1). The parents were tested to indicate the banding pattern and confirmed the band pattern for the progeny (Figure 2-3). Each parent was represented in the field except for NDP080173, which resulted in a self-pollination when developing the first backcross. Chi-square analysis showed that the BC_1F_1 populations did not fit the expected 1:1 ratio (X^2=7.246, p=0.01) (Table 5). The tabulated X^2 value was 6.635, which is less than the calculated value indicating that it does not fit the expected segregation pattern.

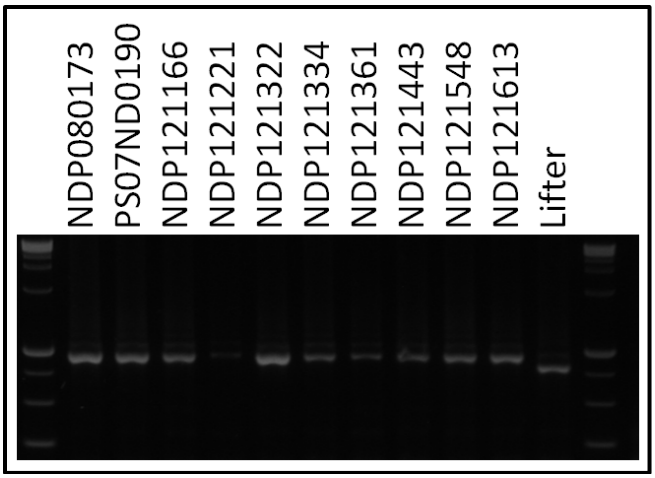

Figure 2. Segregation of a co-dominant PCR marker among resistant and susceptible parents.

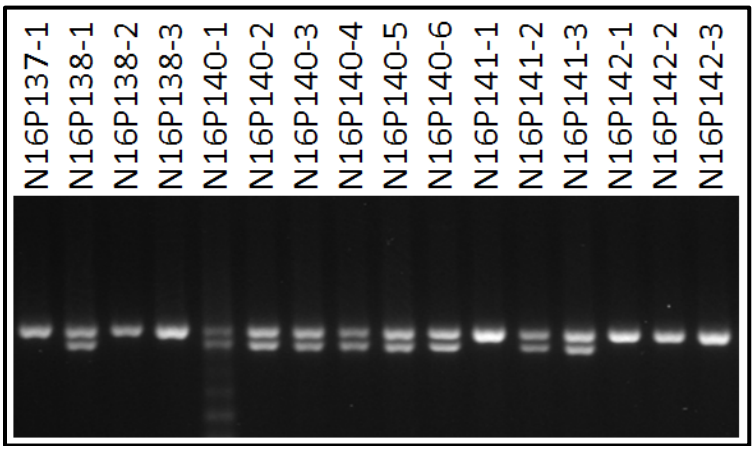

Figure 3. BC_1F_1 DNA analysis showing segregation of homozygous dominant and heterozygous individuals in the 2016 field study.

Table 5. Chi-square test for the expected 1:1 ratio for BC_1F_1 generation.

Genotype	(O)†	(E)‡	(O-E)	$(O-E)^2/E$
HO§ dominant	25	36.5	-11.5	3.623
Heterozygous	48	36.5	11.5	3.623
Total	73	73	0	7.246

† observed value
‡ expected value
§ homozygous

One hundred thirty-one successful BC_2F_1 crosses were harvested and individual seeds planted in the AES greenhouse for DNA analysis and generation advancement by self-pollination (Table 6).

Table 6. Number of BC_2F_1 crosses produced during the 2016 field season and the number of crosses based on DNA analysis.

Recurrent parent	Crosses	Confirmed
NDP121166	21	19
NDP121221	7	7
NDP121322	7	6
NDP121334	14	13
NDP121361	24	24
NDP121443	1	1
NDP121548	15	14
NDP121613	16	16
PS07ND0190	26	24
Total	131	124

Greenhouse: Fall 2016

There were 124 crosses confirmed by DNA analysis from the original 131 BC_2F_1 crosses that were developed in the field (Table 6). Among the 377 individuals tested for the presence of the resistant allele, 190 were homozygous dominant and 187 were heterozygous progeny (Table 7) (Figure 4). Of the 187 heterozygous progeny confirmed, only 160 were advanced to the next generation since some were lost in the AES greenhouse. Segregation of the *sbm-1* locus in the BC_2F_1 had a X^2 value of 0.022, indicating the observed values fit the expected 1:1 ratio since it was lower than the tabular value from the chi-square table of 6.635 (p=0.01) (Table 8). Only one cross with one seed from NDP121443 was successful which happen to be homozygous dominant, therefore this population was lost.

Table 7. Results from DNA analysis of 377 BC_2F_1 populations and the number of populations advanced to the BC_2F_2.

Recurrent parent	Homozygous dominant	Heterozygous	Advanced heterozygotes
NDP121166	29	26	24
NDP121221	15	15	13
NDP121322	9	5	4
NDP121334	21	15	12
NDP121361	36	38	36
NDP121443	1	0	0
NDP121548	16	23	22
NDP121613	27	20	16
PS07ND0190	36	45	33
Total	190	187	160

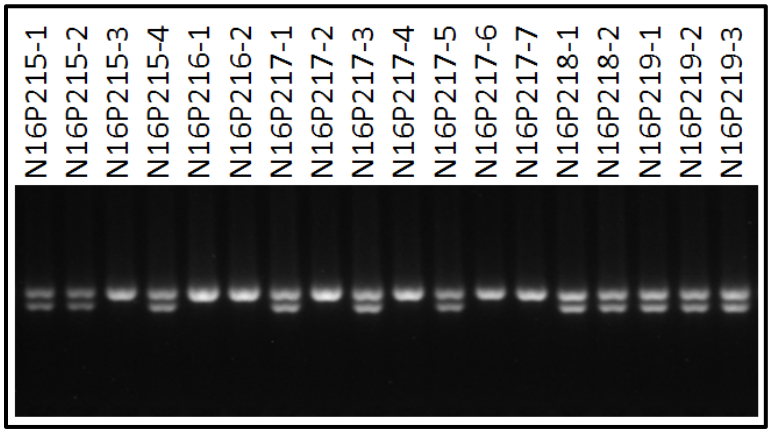

Figure 4. BC_2F_1 DNA analysis showing segregation of homozygous dominant and heterozygous individuals in the fall 2016 AES greenhouse study.

Table 8. Chi-square test for the expected 1:1 ratio for the BC_2F_1.

Genotype	(O)†	(E)‡	(O-E)	$(O-E)^2/E$
HO§ dominant	190	188.5	1.5	0.011
Heterozygous	187	188.5	-1.5	0.011
Total	377	377	0	0.022

† observed value
‡ expected value
§ homozygous

Greenhouse: Spring 2017

Among the 2080 BC_2F_2 plants tested to identify those possessing the *sbm-1* allele, 569 were homozygous dominant, 1031 were heterozygous, and 480 were homozygous recessive (Table 9). Chi-square analysis showed that the segregation frequency does fit the expected 1:2:1

ratio (X^2=7.772, p=0.01) (Table 10) (Figure 5). The tabulated X^2 value was 9.210, which is higher than the calculated value indicating that the segregation pattern observed in the BC_2F_2 population does fit the expected segregation frequency.

Table 9. Frequency of individuals in each segregating class for the *sbm-1* allele for each of the 8 families tested.

Recurrent parent	Homozygous dominant	Heterozygous	Homozygous recessive	Total
NDP121166	71	142	68	281
NDP121221	39	64	29	132
NDP121322	5	12	5	22
NDP121334	72	143	61	276
NDP121361	185	310	148	643
NDP121548	67	126	57	250
NDP121613	77	153	65	295
PS07ND0190	53	81	47	181
Total	569	1031	480	2080

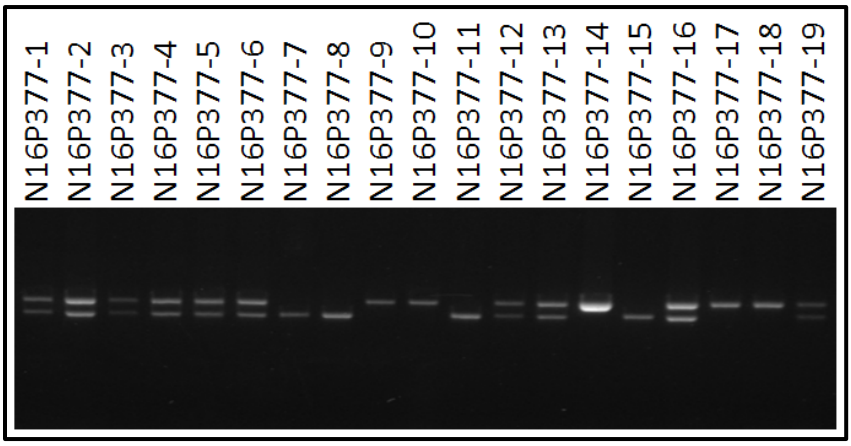

Figure 5. BC_2F_2 DNA analysis showing segregation of resistant, homozygous recessive, and susceptible, homozygous dominant and heterozygous individuals in the spring 2017 AES greenhouse study.

Table 10. Chi-square test for the expected 1:2:1 ratio for alleles at the *sbm-1* locus in the BC_2F_2.

Genotype	(O)†	(E)‡	(O-E)	$(O-E)^2/E$
HO§ dominant	569	520	49	4.617
Heterozygous	1031	1040	-9	0.078
HO recessive	480	520	-40	3.077
Total	2080	2080	0	7.772

† observed value
‡ expected value
§ homozygous

Validation of resistance

Greenhouse: Spring 2017

Following DNA analysis, all homozygous recessive individuals were inoculated with the PSbMV-P4 pathotype to validate the results from DNA testing. Four hundred sixty-nine BC_2F_2 plants were tested and 466 lines tested negative for the virus (Table 11; Figure A3). Individuals testing negative were grown in the field to evaluate agronomic performance and virus resistance under environmental growing conditions. Two individuals tested positive for the virus in families NDP121322 and NDP121361, and a third progeny was excluded from family NDP121361 because it did not show a clear negative or positive reaction when confirmed with DAS-ELISA.

Table 11. Evaluation of BC_2F_2 lines for *Pea Seed-borne Mosaic Virus* resistance in the greenhouse using mechanical inoculation.

Recurrent parent	Number tested	Tested negative for virus
NDP121166	68	68
NDP121221	29	29
NDP121322	5	4
NDP121334	59	59
NDP121361	145	144
NDP121548	55	55
NDP121613	63	62
PS07ND0190	45	45
Total	469	466

Field: Summer 2017

All test entries were virus free based on ELISA, but some of the susceptible checks and parents throughout the experiment tested positive for presence of PSbMV (Figure A4). From the bulk samples collected, 10 plots were considered to have PSbMV. CDC Striker, NDP121166, NDP121334, and NDP121613 each had one plot test positive, and Agassiz and NDP121221 had two plots test positive based on ELISA.

Statistical analysis

Field: Summer 2017

A partially replicated incomplete block design with diagonal checks for spatial effects was used to assess eight early-generation BC_2F_3 families with the aim to appropriately estimate the means of 246 lines. ANOVA using GLM procedure detected significant variation due to genotype for flower date, bloom period, physical maturity, vine length, number of nodes to first flower, seed yield, and 1000 seed weight at $P<0.001$, and pods per peduncle and stand count at $P<0.05$ (Table 12). The coefficient of determination (R^2) ranged from 0.73 for number of reproductive nodes to 0.96 for vine length (Table 13). R^2 value ranges from 0 to 1 indicating a level of variability of one factor is influenced by another factor. A coefficient of determination is the square of the correlation coefficient (R), which displays a linear correlation between two variables. A R^2 value of '1' states that the dependent variable can be predicted from the independent variable without error. Yield, for example, had an R-square value of 0.86 meaning that 86% of the variation observed in yield is predicted from the independent variable, and not all of the variability in the model could be explained (Table 13).

The coefficient of variation (CV) measures the dispersion of observed data points in reference to the experimental mean of the trait being analyzed. The lowest was 2.47 for days to first flower indicating low variation observed in the test entries. There was a range of 47 to 59 days to first flower with an average of 53 days (Table 14). However, yield had the highest CV of 20.74 (Table 13) indicating a high level of variation because the observed data points where further from the experimental mean of 2452 kg ha^{-1} with a range of 33 to 4390 kg ha^{-1} (Table 14). The higher CV and lower R^2 values could be a result of small plot size with a limited

number of individuals represented in each plot. The CV is calculated by dividing the root mean square error (MSE) by the overall mean for each trait observed.

Table 12. Combined ANOVA for agronomic data for early generation testing in 2017 field season.

Source	DF	Type III SS	Mean Square	F value	Pr>F
FlwrDt†					
Genotype	255	1142.06	4.48	2.63	<0.0001***
Block	4	4.97	1.24	0.73	0.5730NS
Bloom period					
Genotype	255	4748.74	18.62	3.96	<0.0001***
Block	4	57.89	14.47	3.08	0.0197*
PM‡					
Genotype	255	9839.71	38.59	4.12	<0.0001***
Block	4	62.87	15.72	1.68	0.1610NS
Vine length					
Genotype	255	81114.01	318.09	7.99	<0.0001***
Block	4	613.71	153.43	3.86	0.00059***
FlwrNd§					
Genotype	255	1576.17	6.18	2.84	<0.0001***
Block	4	23.75	5.94	2.73	0.0336*
RNd¶					
Genotype	255	445.93	1.75	1.05	0.4012NS
Block	4	9.90	2.47	1.48	0.2135NS
PodsPd††					
Genotype	255	39.07	0.15	1.38	0.0328*
Block	4	1.88	0.47	4.24	0.0033**
Stand count					
Genotype	255	3676.46	14.42	1.49	0.0116*
Block	4	137.19	34.30	3.54	0.0096**
Seed yield					
Genotype	255	141702559.0	555696.3	2.15	<0.0001***
Block	4	5810836.9	1452709.5	5.62	0.0004***
TSW‡‡					
Genotype	255	98994.23	388.21	3.70	<0.0001***
Block	4	2639.84	659.96	6.30	0.0001***

NS, not significant; *, *P<0.05*; **, *P<0.01*; ***, *P<0.001*
† first flower date
‡ physical maturity date
§ number of nodes to first flower
¶ number of reproductive nodes
†† number of pods per peduncle
‡‡ thousand seed weight

Table 13. Combined statistical data from GLM analysis for agronomic characteristics in 2017 field season.

Variable	R-square	CV	Root MSE	Mean
FlwrDt†	0.90	2.47	1.30	52.76
Bloom period	0.91	12.90	2.17	16.81
PM‡	0.92	3.47	3.06	88.13
Vine length	0.96	8.81	6.31	71.57
FlwrNd§	0.90	8.55	1.48	17.27
RNd¶	0.73	20.02	1.29	6.46
PodsPd††	0.80	18.32	0.33	1.82
Stand count	0.81	14.50	3.11	21.45
Seed yield	0.86	20.74	508.56	2451.89
TSW‡‡	0.93	4.61	10.24	221.97

† first flower date
‡ physical maturity date
§ number of nodes to first flower
¶ number of reproductive nodes
†† number of pods per peduncle
‡‡ thousand seed weight

The number of days from planting to first flower ranged from 47 to 59 days with an average of 53 days (Table 14). The average bloom period was 17 days and ranged from 10 to 34 days (Table 14). The least days to physiological maturity was 78 days and the majority (53%) reached maturity over a 6 day period from 84 to 90 days (Figure A5).

Individual plots reached heights of 46 cm to 137 cm and averaged 72 cm. The majority (36%) reached heights from 64 to 73 cm (Figure A6). The vine lengths of Agassiz and CDC Striker at Minot, Carrington, and Dickinson REC during the 2017 field season are comparable to those recorded in the current trial. The average vine length for Agassiz was 62, 68, 82, and 41 cm for Prosper, Minot, Carrington, and Dickinson, North Dakota, respectively (NDSU-VTR, 2017). CDC Striker has a short vine length in general and was 52, 61, 62, and 36 cm long at Prosper, Minot, Carrington, and Dickinson, North Dakota, respectively. Lifter has a long average vine length of 74 cm compared to all susceptible parents except for PS07ND0190 (73.8 cm). The

average vine length in the current experiment was 72 cm, which is comparable to the average length of 73 and 67 cm for Carrington and Minot, respectively.

The number of nodes to first flower averaged 17 nodes ranging from 13 to 23 nodes (Table 14). The number of reproductive nodes ranged from 4 to 10 nodes with an average of 6 reproductive nodes per plant (Table 14). The parents averaged 1.92 pods per peduncle, and the overall population had an average of 1.82 (Table 14). The average yield was 2452 kg ha^{-1} consisting of a wide range of 4357 kg ha^{-1} from a low of 33 kg ha^{-1} to the highest yielding line with 4390 kg ha^{-1} (Table 14). Most lines (28%) had yields between 2212 to 2648 kg ha^{-1} represented in Figure A7. Lifter and Agassiz yielded 3062 and 2680 kg ha^{-1}, respectively, out preforming all of the recurrent parents. CDC Striker yielded 2431 kg ha^{-1} and was only surpassed by NDP121361, which yielded 2609 kg ha^{-1} (Figure 6). The seed yield of 44 test entries exceeded that of Lifter. Some lines had a better germination and field survival rate than others, which may have influenced the yields for some of the plots. The average stand count was 21 of 25 total seeds planted per plot. At the end of the season the lowest line had 3 plants to the most having 26 plants. The lowest 1000 seed weight was 167 grams to the highest being 280 grams with a difference of 113 (Table 14). Nearly 25% of the lines fell within 218 to 228 grams with a mean of 222 grams (Figure A8).

Table 14. Combined LS means data for agronomic characteristics in 2017 field season.

Trait	Average	Minimum value	Maximum value	Range
FlwrDt†	52.76	47.12	58.63	11.51
Bloom period	16.81	9.98	33.98	24.00
PM‡	88.13	78.00	107.59	29.59
Vine length (cm)	71.57	46.16	136.84	90.68
FlwrNd§	17.27	12.69	22.53	9.84
RNd¶	6.46	3.59	10.42	6.83
PodsPd††	1.82	0.88	2.19	1.31
Stand count	21.45	2.68	26.45	23.77
Seed yield (kg ha^{-1})	2451.89	33.31	4390.35	4357.04
TSW‡‡ (g)	221.97	166.52	279.77	113.25

† first flower date
‡ physical maturity date
§ number of nodes to first flower
¶ number of reproductive nodes
†† number of pods per peduncle
‡‡ thousand seed weight

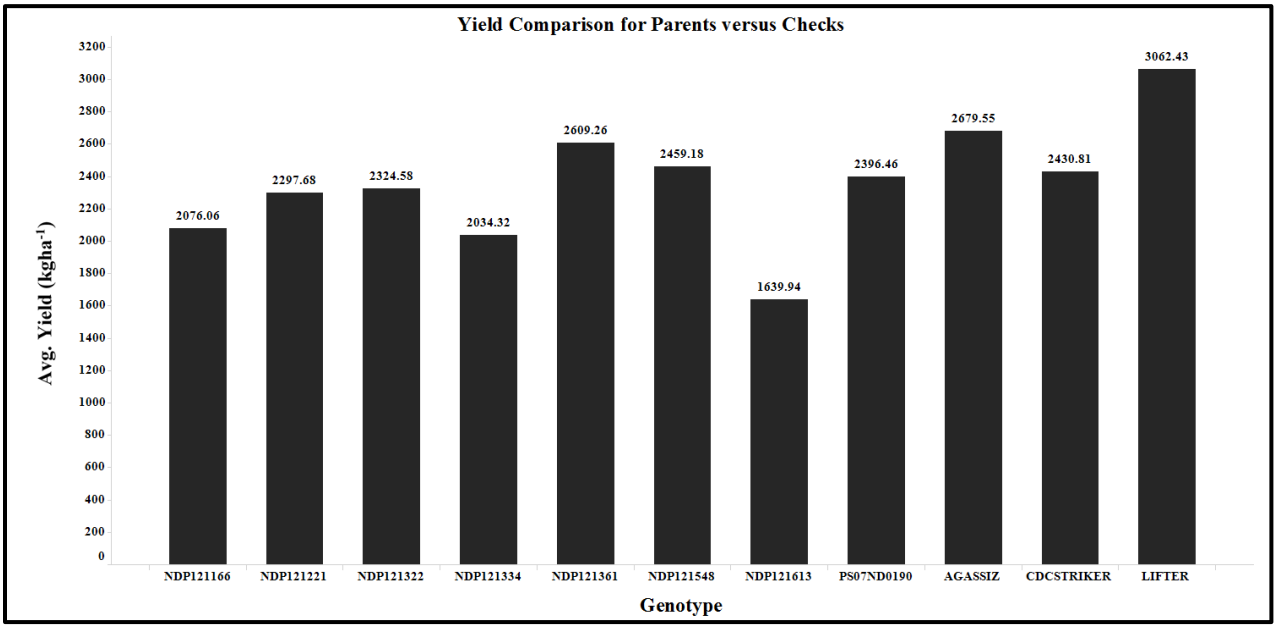

Figure 6. Yield comparison of parental lines and check cultivars from 2017 field season.

To expand on the analysis conducted for each genotype tested, a SAS PROC GLM procedure including the probability of difference option was conducted. The probability of difference was used to calculate a t-test between the recurrent parents and the populations/families derived from each recurrent parent. The three traits analyzed were vine length, seed yield, and thousand seed weight. Significant t-tests at $P<0.05$ were detected for vine

length for NDP121361, NDP121548, and NDP121613; for seed yield for NDP121334 and NDP121613; and for thousand seed weight for NDP121322 (Table 15).

Table 15. T-test conducted between the populations/families derived from a recurrent parent compared to their respective recurrent parent from 2017 field season.

Recurrent parent	Vine length	Seed yield	TSW†
NDP121166	0.3725	0.8674	0.4879
NDP121221	0.0789	0.5698	0.2266
NDP121322	0.5705	0.0759	0.0049*
NDP121334	0.2031	0.0227*	0.6686
NDP121361	0.0330*	0.7149	0.6406
NDP121548	0.0156*	0.7608	0.8286
NDP121613	0.0376*	0.0076*	0.2230
PS07ND0190	0.9049	0.9645	0.9592

*, *P<0.05*

† thousand seed weight

The 2017 field experiment was designed as a partially replicated incomplete block with diagonal checks to conduct a spatial analysis allowing for row and column effects. The reported results are based on an incomplete block analysis, but row and column were omitted. Spatial analysis was also conducted using PROC GLM in SAS version 9.4 (SAS Institute, Cary, NC) considering row and column effects, where experiment and genotype were considered fixed effects (Figure A2). The LS means for genotype estimated in the non-spatial analysis were compared to those of the spatial analysis. The correlation values for each trait indicates a strong relationship between the two analyses (Table A3). The simplest model that best explained the results was used, which was the non-spatial incomplete block analysis. The results from the spatial analysis including row and column did not justify the use of that type of analysis to report the results. As this experiment was small in size covering approximately 0.093 ha and only conducted at one location in one year. A spatial analysis may be more appropriate where more locations, years, and larger experimental designs were conducted to adjust the genotypic means accordingly to the checks and surrounding neighbors. In addition, PROC MIXED was run in

SAS version 9.4 (SAS Institute, Cary, NC) for yield, where block was fixed and genotype was random (Figure A2). It too was compared to both the spatial and non-spatial LS means, and resulted in high correlation value for both not justifying the use of the analysis.

Discussion

This study demonstrates the introgression of a *Pea Seed-borne Mosaic Virus* resistant gene (*sbm-1*) from cv. Lifter into PSbMV susceptible lines adapted to the Northern Great Plains region through backcross breeding. Lifter was selected as the donor parent for multiple disease resistance and its high yield performance (McPhee and Muehlbauer, 2002). The recessive allele (*sbm-1*) conferring resistance to PSbMV was successfully introduced using marker assisted backcross selection minimizing time, cost, and other resources. However, Lifter has the dominant allele (*Af*) for a normal leaf type morphology and has a delayed flowering period that matures later than cultivars grown in the NGP region. The susceptible parents have the homozygous dominant form of the allele (*Sbm-1*), but have the recessive allele (*af*) for semi-leafless morphology and were selected from advanced breeding lines developed in the NDSU Pulse Crops Breeding Program.

Marker assisted backcross selection is beneficial over traditional backcrossing since it hastens the backcrossing process. The recurrent parent is continuously backcrossing until a desired number of backcrosses is achieved compared to having a progeny test after each backcross in the traditional backcross breeding for a recessive gene conferring resistance. Various markers have been shown to be closely linked to the gene of interest, but Gao et al. (2004a) developed a 'perfect' PCR marker located within the gene which was used in this study. Smýkal et al. (2010) expanded the work of Gao et al. (2004a) and determined the complete sequence of *eIF4E* gene. The results from Gao et al. (2004a) and Smýkal et al. (2010) showed a

50 bp difference between the susceptible and resistant allele. What is consistent in the work of Smýkal et al. (2010) and Gao et al. (2004a) is the intron size of the *sbm-1* at 1151 bp, and Smýkal et al. (2010) was able to detect the *sbm-1* resistant allele in Lifter. Smýkal et al. (2010) used the complete *eIF4E* gene sequence to identify a missing intron and exon which resulted in an intron length of 1201 bp in susceptible accessions versus a 1257 bp length found by Gao et al. (2004a). Despite the difference, the marker accurately identified the polymorphism in this study.

Several studies have shown the effectiveness of using marker assisted backcross selection methods as a means to optimize breeding efforts which include introgression of both dominant and recessive genes (Neeraja et al., 2007; Yi et al., 2009; Chu et al., 2011). Neeraja et al. (2007) utilized marker assisted selection for a major QTL (*Sub 1*) to develop submergence tolerant rice cultivars using markers tightly linked and flanked the *Sub 1* gene. In peanut, a dominant gene for nematode resistance (*Rma*) was introduced simultaneously with a recessive trait for high oleic:linoleic acid ratio (*olol*) (Chu et al., 2011). Yi et al. (2009) introgressed a recessive gene (*badh2*) via marker assisted backcross selection for rice fragrance, and simultaneously introduced a dominant gene, *Wx*, controlling amylose content. Irrespective of the mode of inheritance for the gene of interest, i.e. recessive or dominant, the number of backcrosses required is particular to the study depending on the divergent level between the two parents. Yi et al. (2009) conducted four backcrosses due to the differences among the donor and recurrent parent. The recurrent parent is adapted, superior in yield, grain quality, and milling characteristics. Despite the gene of interest from the donor, they were more interested in recovering the recurrent parent to a homozygosity level of 96.9 %, and eliminating negative characteristics by breaking linked genes that are associated with the donor parent. However, the parents used in this study mainly differ in agronomic adaptation, leaf type and other disease

resistant genes from Lifter, which is why only two backcrosses were performed. Progeny were only selected for PSbMV resistance, but was important to retain additional resistance genes from Lifter since the progeny have the potential to segregate for race 1 of Fusarium wilt, pea enation mosaic virus, and powdery mildew (McPhee and Muehlbauer, 2002). Lifter as a relatively tall and high yielding parent is desirable, and it was not necessary to preform additional backcrosses compared to that of Yi et al. (2009).

After each generation of DNA testing, a chi-square analysis was preformed to identify if the developed populations segregate for the *sbm-1* resistance gene as expected. In a BC_1F_1 population, it was expected that a single gene segregates in a 1:1 ratio of homozygous dominant (*Sbm-1Sbm-1*) to heterozygous (*Sbm-1sbm-1*). In this generation of testing, there were 73 plants tested consisting of 25 homozygous dominant and 48 heterozygous plants. From the chi-square analysis, the observed values do not fit the expected 1:1 ratio for *sbm-1* gene. The skewed data observed in this study was in favor of heterozygous individuals, which were used in development of the second backcross populations. The small sample size may explain the observed segregation pattern. This was the only generation that did not segregate as expected.

The second X^2 test conducted was on the BC_2F_1 populations which also have a 1:1 ratio consisting of 377 individuals tested containing 190 homozygous dominant to 187 heterozygotes. There were more BC_2F_1 individuals tested, increasing the sample size to properly show the segregation frequency. The final BC_2F_2 populations tested represent all three genotypic classes since the BC_2F_1 populations were allowed to self-pollinate. This study focused on one pair of segregating alleles and when self-pollinated the segregation ratio should fit the expected genotypic ratio of 1:2:1. There were 2080 self-pollinated individuals tested resulting in 569 homozygous dominant, 1031 heterozygotes, and 480 homozygous recessive individuals. Using

the chi-square analysis, the observed values do fit the expected segregating frequency of 1:2:1 with a calculated Chi-square value of 7.772, which is lower than the critical value of 9.210.

The resistant BC_2F_2 individuals were inoculated with PSbMV according to the procedure used by (Pasche, personal communication, 2017) to verify the reaction of resistant versus susceptible individuals. Smýkal et al. (2010) used mechanical inoculation with the PSbMV-P1 isolate and confirmed the reaction by DAS-ELISA similar to the protocol used to inoculate the BC_2F_2 individuals. Unfortunately, their ELISA results did not identify the same susceptible individuals as the PCR results did, they found some of the susceptible individuals escaped infection. Their results are comparable to those in this study where there was not a 100% success rate. Among 469 test entries inoculated, 35 susceptible checks and 10 susceptible parents were also tested, and found three that tested negative for infection despite being inoculated twice resulting in a 93.5% infection rate. The phenotypic characteristics of virus infectivity are not a reliable measure when assessing resistance because visual symptoms are not always expressed (Hagedorn and Gritton, 1973; Smýkal et al., 2010). Symptom expression can be dependent on age and titer of inoculum, the age of the plants being inoculated, and the amount of time after inoculation before tissue is collected for ELISA (Hagedorn and Gritton, 1973). However, 100% success in inoculation is possible as Provvidenti and Alconero (1988b) show by having all susceptible *Sbm-4* individuals test positive for virus infectivity when inoculated with PSbMV-P4. They maintained PSbMV-P4 inoculum that was later rubbed on the first two fully expanded leaves, and then a week later a second inoculation was performed. They observed systemic infection which was confirmed with ELISA.

The three studies by Hagedorn and Gritton (1973), Provvidenti and Alconero (1988b), and Smýkal et al. (2010) conducted experiments where a single resistant gene conferred

resistance to a single pathotype, similar to this study. A 'perfect' marker for *sbm-1* identified homozygous recessive individuals and were then inoculated with a PSbMV-P4 isolate (Pasche, personal communication, 2017), which has been shown to have resistance by Gao et al. (2004b). This study indicated the effectiveness of the marker developed by Gao et al. (2004b) to identify individuals that confer resistance to the PSbMV-P4 isolate. The isolate from Pasche (personal communication, 2017) was collected from field pea grown in North Dakota in 2014, and had the highest sequence homology (99%) to PSbMV-P4. The symptoms observed in the NGP region tend to resemble those of the isolate used for inoculation.

The design chosen for the field study allows for spatial analysis which improves the precision of estimating line effects (Burgueño et al., 2000). A custom PROC GLM SAS version 9.4 program was used allowing spatial analysis to account for association of neighboring plots within the row and column (Gleeson and Cullis, 1987). Many studies support spatial analysis using a mixed linear approach to increase precision of variety estimates using software packages such as ASReml (Gleeson and Cullis, 1987; Cullis and Gleeson, 1991; Gilmour et al., 1997; Gilmour et al., 2015). It was confirmed that they provide a more precise estimate than incomplete block analyses based on studies by Brownie et al. (1993); Stroup et al. (1994); and Grondona et al. (1996). Spatial analysis for yield in this study did not increase the level of precision. The relatively small number of individuals tested and the test site spanning a small area with minimal environmental variation contributed to the reduced impact of the spatial analysis. Increasing the level of complexity by adding additional parameters such as row and column effects, would increase precision as indicated by Gilmour et al. (2015). Gilmour et al. (2015) tested an unreplicated early generation variety trial in wheat, and observed differences in model analyses. However, there were more lines tested over a larger space, which could have

had more variation due to environmental conditions. Increasing the number of parameters could explain more variation resulting in a truer estimate of the lines being tested within the experiment.

The LS means for each agronomic trait analyzed was comparable to those tested at research trials at Research Extension Centers (REC) located across North Dakota. The average yield observed in this early generation trial was 2452 kg ha^{-1}, which was lower than the average yield (2628 kg ha^{-1}) for all variety trials around North Dakota for a two year yield average in 2016 and 2017 (NDSU-VTR, 2017). Yield estimates for Agassiz and CDC Striker during the 2017 field season at three RECs are comparable to the early generation results at Prosper, ND. Agassiz yielded 2680 kg ha^{-1} for Prosper, ND, during the 2017 field study compared to 3719, 3816, and 1582 kg ha^{-1} for Minot, Carrington, and Dickinson, respectively. A yield trial was not assessed in Prosper, ND during the 2017 field season, but the average yield of Agassiz in 2014, 2015, and 2016 was 3541 kg ha^{-1}. CDC Striker had similar results, the 2017 field study in Prosper, ND yielded 2431 kg ha^{-1}, which was lower than the three year average of 2014, 2015, and 2016 at 2891 kg ha^{-1}. This difference could be attributed to the small plot size with a limited number of seeds per plot. The 2017 growing season was also drier than the 2014, 2015, and 2016 growing seasons at Prosper, ND. Across the state in Minot, Carrington, and Dickinson during the 2017 field season, CDC Striker yielded 2879, 3400, and 1772 kg ha^{-1}, respectively. The overall yield ranges from 33 kg ha^{-1} to 4390 kg ha^{-1} at Prosper, ND for the 2017 field study. The trial averages in 2017 for Carrington, Minot, and Dickinson were 3319, 3715, and 1647 kg ha^{-1}, respectively, and trial averages in Prosper, ND, for 2014, 2015, and 2016 were 2895, 3142, and 3192 kg ha^{-1}, respectively. Thus indicating that the lines tested to have PSbMV resistance are

comparable to yields observed statewide in 2017 and three advanced yield trial years in Prosper, ND.

There was significant variation among genotypes represented in the early generation trial as indicated before with a range of test entries yielding from 33 kg ha^{-1} to 4390 kg ha^{-1}. Forty-four test entries produced yields greater than Lifter, which had a yield of 3062 kg ha^{-1}. Agronomic performance of the material in this study is comparable to results from the statewide variety trials, but further testing of these lines in larger plots will provide better estimates of their relative performance and value in the breeding program or as new varieties.

Thirty test entries across seven of the eight families are considered superior to the checks and parents based on yield, vine length, 1000 seed weight, days to first flower, and days to physiological maturity (Table A4). Both green and yellow cotyledon types were represented in this group, as well as most are semi-leafless with a few that are still segregating for semi-leafless and normal leaf type. No lines from family NDP121548 were selected due to low yield, and the average yield for test entries of the NDP121548 family was 2300 kg ha^{-1}. The NDP121166 family had a lower yield average of 2244 kg ha^{-1}. However, test entries N16P291-35-0 and N16P284-14-0 are progeny of NDP121166 and were selected for their superior yield, early flowering, long bloom period, semi-leafless leaf morphology, high number of reproductive nodes, and vine lengths comparable to cultivars tested in NDSU variety trials. The highest yielding family was NDP121322 with 3436 kg ha^{-1}, however, this is based on only 3 lines, of which only line N16P357-6-0 was selected for its high yield, early flowering, long bloom period, semi-leafless leaf morphology, high number of reproductive nodes, and comparable vine length. Although it is noted that all test entries from NDP121322 in the field of 2017 were susceptible to powdery mildew (PM). The selected lines along with additional years of data will identify

excellent sources for PSbMV resistant material adapted to the NGP region to be used as a foundation for the NDSU Pulse Crops breeding program and other scientific communities. The developed adapted lines can be used for pyramiding additional disease resistance genes, improve yield, and lodging resistance for cultivar enhancement in the Northern Great Plains region.

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CHAPTER IV. CONCLUSION

Overall, there is still research and testing needed to fully understand the performance of the resistant material developed. The inclusion of a PSbMV resistant cultivar would be beneficial in a cropping rotation to provide a source of nitrogen, reduce inoculum buildup of small grains, and disrupt weed and pest pressures. There has been an increase in pea production, but yield and quality of the seed is limited due to PSbMV (Coutts, 2016).

The first year of the early generation trial has provided an initial understanding of how the progeny perform against the parents and checks in a single location. An additional year(s) of agronomic data to continue evaluation are necessary to consider the best lines for advancement. More importantly, superior lines must be identified to be considered for germplasm release and the potential for cultivar release. To make accurate selections of the best lines, a breeder must evaluate the resistant material in multiple environments and years. Enough seed was generated during the summer of 2017 for generation advancement and collection of additional agronomic data to continue evaluation in multiple environments for most of the plots.

The use of a molecular marker was demonstrated by successfully introducing the resistant *sbm-1* gene through marker assisted backcross breeding procedure into susceptible NDSU Pulse Crops breeding lines and retained to achieve homozygous recessive PSbMV resistant individuals. This procedure reduced time, cost, and resources compared to traditional breeding methods to introduce a desirable trait. The developed lines also stood up to virus inoculations to valid resistance in the AES greenhouse, minus three individuals, and under field conditions. Further studies are needed to confirm and understand why three individuals from ELISA testing in the AES greenhouse were positive. The gene introduced from Lifter was *sbm-1* (McPhee and Muehlbauer, 2002; Smýkal et al., 2010), and the BC_2F_2 individuals were inoculated with

PSbMV-P4 since that is the known isolate in North Dakota (Pasche, personal communication, 2017).

From a breeding stand point, the analysis from the first year of early generation testing identified breeding lines that have the potential to have a higher seed yield than the parents and the checks. Although, many of the lines with high seed yield have the normal leaf type or are still segregating, a number of them were semi-leafless and had yields better than their respective recurrent parent (Table A4). It is important to growers in North Dakota to have semi-leafless cultivars for easier harvestability, and less disease with more air flow and light penetration into the canopy (McPhee, 2003; Kielpinski and Blixt, 1982). Those selected for germplasm release to the scientific community will aid in providing a PSbMV resistant line that is adapted to NGP region. The germplasm can be utilized to pyramid additional disease resistant genes or improve yield by crossing to elite lines within a breeding program that lack PSbMV resistance. The material developed has not been evaluated for additional disease resistance genes, and may be considered when crossing to additional lines. Perfect markers are available allowing breeders to efficiently transfer the recessive allele into elite lines (Gao et al., 2004; Smýkal et al., 2010), thus minimizing the threat of PSbMV to grower in important pea producing states in the Northern Great Plains region.

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APPENDIX

Table A1. BC_1F_1 individual plants labeled within each plot possessing the homozygous recessive allele used to develop BC_2F_1 populations.

BC_1F_1 Line	HO† dominant plant ID	Heterozygous plant ID	Total plants tested
N16P095	2,4	1,3	4
N16P097	0	1-4	4
N16P098	0	1-3	3
N16P099	0	1	1
N16P105	1	2,3	3
N16P106	4	1-3	4
N16P107	0	1	1
N16P108	0	1-3	3
N16P115	1,2	0	2
N16P116	1	2	2
N16P117	1	0	1
N16P118	1	2	2
N16P132	1,3	2	3
N16P133	2,5	1,3,4,6,7	7
N16P134	0	1-3	3
N16P135	1	2	2
N16P136	3	1,2,4	4
N16P137	1	0	1
N16P138	2,3	1	3
N16P140	0	1-6	6
N16P141	1	2,3	3
N16P142	1-3	0	3
N16P143	0	1	1
N16P153	2	1	2
N16P154	1,5	2,3,4	5
Total	25	48	73

† homozygous

Table A2. LS means for each genotype in the 2017 early generation yield trial conducted using non-spatial analysis.

Name	FlwDt†	Bloom Period	PM‡	Vine Length	FlwNd§	RNd¶	PodsPd††	Stand Count	Seed Yield	TSW‡‡
AGASSIZ	51.70	17.19	84.72	61.54	16.33	6.09	1.92	23.57	2679.55	232.54
ARAGORN	52.12	17.01	85.82	61.05	16.69	6.42	1.86	7.78	640.92	193.00
CDCSTRIKER	50.27	15.23	82.34	52.08	13.87	6.36	1.57	22.45	2430.81	197.13
LIFTER	52.34	24.33	97.47	74.29	14.32	8.16	1.85	19.47	3062.43	226.83
N16P212-12-0	53.26	13.98	86.00	67.16	13.85	7.13	1.19	25.32	3205.60	233.52
N16P212-5-0	50.26	14.98	83.00	65.16	16.85	5.13	2.19	23.32	1782.20	218.02
N16P213-2-0	53.26	14.98	91.00	65.16	15.85	6.13	1.19	12.32	1740.90	244.52
N16P213-4-0	49.26	18.98	79.00	76.16	14.85	8.13	2.19	21.32	2868.20	206.02
N16P213-9-0	52.26	12.98	106.00	55.16	15.85	5.13	2.19	21.32	2688.10	235.52
N16P214-1-0	52.26	12.98	85.00	61.16	15.85	4.13	2.19	21.32	3452.50	242.52
N16P214-20-0	51.26	11.98	84.00	54.16	17.85	5.13	1.19	19.32	1925.40	242.02
N16P214-6-0	51.26	14.98	87.00	73.16	17.85	7.13	2.19	21.32	3879.50	237.52
N16P215-3-0	53.26	16.98	88.00	80.16	19.85	6.13	2.19	23.32	3460.40	206.52
N16P215-4-0	52.26	16.98	86.00	110.16	17.85	7.13	2.19	16.32	1088.90	198.02
N16P217-10-0	52.26	11.98	85.00	51.16	16.85	4.13	2.19	18.32	1747.00	202.52
N16P217-12-0	53.26	15.98	93.00	72.16	16.85	7.13	1.19	17.32	1646.00	222.52
N16P217-13-0	54.26	15.98	88.00	70.16	18.85	7.13	2.19	22.32	3776.70	216.52
N16P217-5-0	52.26	21.98	88.00	89.16	16.85	8.13	2.19	22.32	2961.40	212.02
N16P219-16-0	54.26	13.98	92.00	82.16	19.85	7.13	1.19	25.32	1906.10	211.02
N16P219-17-0	55.26	12.98	87.00	69.16	17.85	6.13	2.19	25.32	3088.80	206.52
N16P219-21-0	52.26	13.98	81.00	70.16	19.85	5.13	2.19	21.32	2203.10	206.02
N16P219-31-0	53.26	20.98	98.00	85.16	16.85	7.13	1.19	22.32	2037.00	214.02
N16P219-35-0	52.26	12.98	81.00	72.16	17.85	5.13	2.19	25.32	3223.20	209.02
N16P219-36-0	53.26	17.98	98.00	70.16	17.85	6.13	2.19	19.32	2530.80	200.52
N16P219-37-0	52.26	12.98	85.00	85.16	19.85	6.13	2.19	22.32	1816.50	202.02
N16P228-15-0	52.26	18.98	88.00	61.16	15.85	6.13	2.19	14.32	1450.90	166.52
N16P228-18-0	51.26	14.98	78.00	59.16	16.85	4.13	2.19	23.32	2387.60	202.02
N16P282-4-0	53.26	10.98	88.00	57.16	16.85	7.13	2.19	21.32	1870.90	225.52
N16P282-6-0	52.26	12.98	86.00	63.16	14.85	6.13	1.19	24.32	2718.00	251.02
N16P283-18-0	55.26	10.98	91.00	69.16	19.85	7.13	2.19	21.32	3151.10	230.52
N16P284-14-0	52.26	18.98	86.00	67.16	16.85	7.13	2.19	22.32	3076.50	215.52
N16P284-19-0	50.26	13.98	84.00	55.16	17.85	6.13	2.19	23.32	2927.10	223.52
N16P284-21-0	52.26	13.98	92.00	56.16	15.85	8.13	2.19	23.32	2616.90	232.52
N16P284-35-0	53.26	17.98	87.00	46.16	14.85	5.13	2.19	20.32	2442.90	223.52
N16P284-37-0	52.26	11.98	83.00	65.16	14.85	7.13	2.19	22.32	2169.70	226.02
N16P285-1-0	52.26	13.98	87.00	84.16	19.85	6.13	2.19	23.32	2710.90	229.52
N16P285-19-0	53.26	15.98	85.00	72.16	16.85	8.13	2.19	21.32	2443.80	236.52
N16P285-30-0	52.26	12.98	83.00	61.16	14.85	6.13	2.19	22.32	2119.60	227.52
N16P286-5-0	52.26	14.98	98.00	60.16	14.85	8.13	2.19	19.32	1268.20	205.02
N16P291-14-0	52.26	13.98	82.00	66.16	17.85	5.13	2.19	25.32	2484.20	208.52
N16P291-3-0	52.26	15.98	83.00	74.16	19.85	7.13	2.19	18.32	2057.20	216.02
N16P291-34-0	53.26	12.98	86.00	53.16	18.85	6.13	2.19	19.32	1801.50	221.52
N16P291-35-0	52.26	17.98	90.00	74.16	20.85	8.13	2.19	25.32	3462.20	224.02
N16P291-4-0	53.26	22.98	98.00	64.16	17.85	7.13	2.19	19.32	1843.70	188.02
N16P291-41-0	53.26	10.98	91.00	53.16	17.85	4.13	2.19	20.32	1543.20	234.52
N16P291-5-0	52.26	33.98	92.00	69.16	17.85	8.13	2.19	20.32	2325.20	201.52
N16P293-11-0	55.26	9.98	98.00	94.16	17.85	7.13	1.19	12.32	770.90	202.02
N16P293-17-0	54.26	21.98	95.00	78.16	18.85	9.13	2.19	21.32	2413.10	215.52
N16P293-18-0	52.26	14.98	86.00	62.16	19.85	5.13	2.19	23.32	2007.10	220.02
N16P293-5-0	52.26	16.98	86.00	80.16	12.85	8.13	2.19	20.32	2598.50	234.02
N16P293-9-0	54.26	14.98	91.00	58.16	18.85	7.13	1.19	18.32	1976.40	239.02

Table A2. LS means for each genotype in the 2017 early generation yield trial conducted using non-spatial analysis (continued).

Name	FlwDt†	Bloom Period	PM‡	Vine Length	FlwNd§	RNd¶	PodsPd††	Stand Count	Seed Yield	TSW‡‡
N16P294-11-0	52.99	16.20	88.59	69.61	16.85	6.77	2.10	19.45	2452.05	223.77
N16P294-18-0	51.99	13.20	82.59	56.61	17.85	4.77	2.10	26.45	2474.05	228.77
N16P294-29-0	51.99	13.20	83.59	69.61	19.85	6.77	2.10	22.45	1929.25	231.27
N16P294-35-0	52.99	24.20	104.59	113.61	17.85	9.77	2.10	9.45	670.15	214.77
N16P294-8-0	52.26	15.98	83.00	60.16	15.85	6.13	2.19	25.32	2759.30	230.02
N16P300-10-0	50.99	21.20	83.59	109.61	17.85	6.77	1.10	18.45	1453.85	226.77
N16P300-14-0	51.99	14.20	93.59	80.61	15.85	4.77	2.10	23.45	2168.25	275.27
N16P300-2-0	52.99	15.20	107.59	55.61	15.85	3.77	2.10	18.45	1618.15	261.77
N16P300-23-0	49.99	14.20	78.59	58.61	17.85	3.77	2.10	23.45	1864.25	231.77
N16P300-27-0	50.99	14.20	83.59	69.61	19.85	4.77	2.10	26.45	2488.95	257.27
N16P300-4-0	51.99	14.20	86.59	71.61	16.85	5.77	2.10	24.45	2977.45	248.27
N16P301-13-0	52.99	19.20	89.59	71.61	18.85	4.77	1.10	24.45	2471.35	237.77
N16P301-15-0	51.99	18.20	86.59	123.61	19.85	8.77	1.10	13.45	1396.75	221.77
N16P301-2-0	51.99	15.20	104.59	74.61	15.85	5.77	2.10	23.45	3154.95	246.77
N16P301-30-0	51.99	17.20	88.59	92.61	16.85	6.77	2.10	21.45	2198.95	232.77
N16P301-33-0	50.99	14.20	91.59	67.61	18.85	4.77	2.10	22.45	2036.45	244.77
N16P301-34-0	50.99	15.20	88.59	74.61	14.85	5.77	2.10	22.45	3122.45	233.77
N16P301-36-0	50.99	16.20	88.59	83.61	19.85	6.77	2.10	21.45	2513.55	234.27
N16P301-41-0	51.99	13.20	98.59	75.61	18.85	4.77	2.10	25.45	1699.05	242.77
N16P301-44-0	52.99	14.20	92.59	72.61	14.85	6.77	2.10	24.45	2778.05	241.77
N16P301-45-0	50.99	14.20	87.59	67.61	18.85	4.77	2.10	20.45	2160.35	231.77
N16P301-5-0	51.99	19.20	107.59	124.61	17.85	4.77	2.10	14.45	1156.05	242.27
N16P301-8-0	52.99	16.20	88.59	80.61	18.85	6.77	2.10	23.45	3348.25	243.77
N16P302-13-0	52.99	16.20	91.59	83.61	19.85	4.77	2.10	23.45	3343.85	274.27
N16P302-14-0	52.99	14.20	91.59	78.61	17.85	7.77	1.10	18.45	2051.35	265.77
N16P302-18-0	50.99	14.20	91.59	85.61	18.85	5.77	2.10	13.45	1536.45	261.77
N16P302-4-0	51.99	13.20	91.59	58.61	16.85	4.77	2.10	19.45	2064.55	279.77
N16P302-8-0	50.99	15.20	92.59	74.61	18.85	5.77	2.10	24.45	3064.45	271.27
N16P303-14-0	52.99	16.20	91.59	67.61	13.85	6.77	2.10	24.45	2621.65	246.27
N16P306-11-0	51.99	15.20	91.59	78.61	18.85	5.77	2.10	25.45	3104.05	278.27
N16P306-8-0	52.99	13.20	98.59	70.61	18.85	4.77	2.10	25.45	3188.35	246.27
N16P306-9-0	52.99	18.20	92.59	77.61	16.85	5.77	2.10	15.45	2190.25	258.77
N16P356-10-0	48.99	27.20	93.59	49.61	12.85	5.77	2.10	17.45	2924.75	231.27
N16P356-6-0	47.99	21.20	83.59	62.61	15.85	5.77	2.10	24.45	4144.35	256.27
N16P357-6-0	47.99	18.20	84.59	71.61	18.85	8.77	2.10	24.45	3237.55	237.77
N16P366-1-0	55.99	18.20	91.59	81.61	18.85	5.77	2.10	24.45	3453.75	216.77
N16P367-11-0	52.99	21.20	91.59	102.61	19.85	6.77	2.10	21.45	3751.55	218.77
N16P367-19-0	54.99	15.20	88.59	61.61	14.85	5.77	1.10	25.45	3603.05	234.27
N16P367-24-0	54.99	16.20	86.59	69.61	17.85	6.77	2.10	25.45	3306.95	218.27
N16P367-28-0	54.99	15.20	88.59	78.61	18.85	4.77	2.10	21.45	2948.45	201.77
N16P367-3-0	55.99	14.20	86.59	70.61	16.85	5.77	1.10	25.45	3369.35	227.77
N16P367-38-0	55.99	14.20	84.59	71.61	17.85	6.77	1.10	25.45	2648.85	210.77
N16P367-53-0	55.99	14.20	86.59	81.61	20.85	4.77	2.10	24.45	2872.05	207.27
N16P367-7-0	51.99	16.20	83.59	79.61	21.85	5.77	1.10	22.45	3415.95	227.27
N16P367-9-0	55.99	16.20	89.59	69.61	21.85	4.77	1.10	24.45	4354.35	236.27
N16P368-24-0	55.99	18.20	86.59	79.61	20.85	5.77	1.10	24.45	4390.35	221.27
N16P368-29-0	52.99	19.20	87.59	128.61	21.85	6.77	1.10	7.45	497.05	210.27
N16P368-3-0	51.99	14.20	82.59	65.61	17.85	4.77	2.10	26.45	3456.35	232.77
N16P368-53-0	52.99	17.20	84.59	85.61	19.85	5.77	2.10	22.45	2905.45	221.77
N16P368-54-0	54.99	12.20	86.59	63.61	15.85	4.77	1.10	26.45	2580.35	207.77
N16P370-2-0	56.99	15.20	90.59	70.61	15.85	5.77	2.10	22.45	3980.05	197.77

Table A2. LS means for each genotype in the 2017 early generation yield trial conducted using non-spatial analysis (continued).

Name	FlwDt†	Bloom Period	PM‡	Vine Length	FlwNd§	RNd¶	PodsPd††	Stand Count	Seed Yield	TSW‡‡
N16P370-5-0	55.63	15.48	85.68	81.84	16.08	6.59	0.97	22.77	2820.23	205.27
N16P370-6-0	53.63	13.48	86.68	68.84	17.08	4.59	0.97	21.77	2429.23	226.27
N16P371-5-0	58.63	16.48	87.68	65.84	20.08	4.59	0.97	20.77	2535.53	202.27
N16P374-1-0	52.63	16.48	84.68	76.84	19.08	6.59	1.97	21.77	1666.53	215.27
N16P375-14-0	54.63	10.48	83.68	70.84	20.08	6.59	0.97	24.77	2626.03	233.77
N16P375-25-0	54.63	17.48	83.68	87.84	20.08	6.59	1.97	25.77	3451.13	207.77
N16P375-27-0	54.63	17.48	87.68	77.84	18.08	5.59	1.97	25.77	2328.23	204.77
N16P375-30-0	55.63	13.48	88.68	73.84	18.08	6.59	1.97	23.77	2786.83	231.27
N16P375-37-0	56.63	11.48	93.68	69.84	20.08	7.59	0.97	22.77	2835.13	219.77
N16P375-39-0	56.63	12.48	89.68	61.84	21.08	5.59	0.97	23.77	2559.23	224.27
N16P375-42-0	55.63	13.48	83.68	67.84	20.08	5.59	1.97	25.77	2681.43	246.77
N16P375-46-0	53.63	12.48	83.68	67.84	18.08	6.59	1.97	25.77	1880.03	205.27
N16P375-52-0	52.63	15.48	86.68	72.84	19.08	7.59	1.97	20.77	2568.93	222.77
N16P376-13-0	54.63	13.48	85.68	61.84	14.08	6.59	1.97	24.77	2639.23	196.27
N16P376-16-0	54.63	17.48	86.68	66.84	18.08	5.59	0.97	24.77	3248.13	221.27
N16P376-17-0	56.63	14.48	92.68	98.84	16.08	6.59	1.97	18.77	1961.83	189.27
N16P376-20-0	55.63	14.48	83.68	79.84	18.08	5.59	1.97	22.77	2826.43	215.77
N16P376-21-0	55.63	14.48	87.68	62.84	17.08	5.59	0.97	21.77	3336.93	199.27
N16P376-22-0	54.63	15.48	88.68	69.84	18.08	5.59	1.97	21.77	2027.73	209.27
N16P376-3-0	52.63	18.48	83.68	73.84	17.08	4.59	1.97	25.77	2753.43	186.27
N16P376-38-0	55.63	15.48	91.68	72.84	17.08	5.59	1.97	18.77	1366.03	207.27
N16P376-4-0	58.63	16.48	98.68	91.84	21.08	3.59	1.97	17.77	2391.43	221.77
N16P376-41-0	56.63	18.48	86.68	79.84	21.08	4.59	1.97	22.77	1779.03	201.27
N16P376-5-0	54.63	12.48	89.68	75.84	18.08	6.59	1.97	18.77	1710.53	213.77
N16P378-23-0	52.63	14.48	83.68	61.84	15.08	4.59	1.97	21.77	2069.83	225.27
N16P378-39-0	54.63	12.48	83.68	76.84	22.08	6.59	0.97	20.77	1834.43	239.27
N16P378-4-0	54.63	10.48	82.68	66.84	20.08	3.59	1.97	25.77	2559.23	232.27
N16P378-41-0	51.63	27.48	94.68	130.84	21.08	3.59	0.97	12.77	1024.23	263.27
N16P379-10-0	55.63	19.48	98.68	136.84	22.08	5.59	0.97	16.77	891.63	203.27
N16P379-13-0	55.63	14.48	87.68	75.84	18.08	3.59	1.97	19.77	2139.23	223.27
N16P379-17-0	55.63	15.48	88.68	66.84	21.08	4.59	1.97	22.77	2850.13	195.77
N16P379-18-0	55.63	11.48	86.68	74.84	17.08	4.59	1.97	21.77	2911.63	204.27
N16P379-20-0	54.63	16.48	83.68	86.84	20.08	3.59	1.97	18.77	1391.53	191.27
N16P379-35-0	55.63	16.48	89.68	85.84	21.08	6.59	1.97	21.77	1731.53	222.27
N16P379-39-0	55.63	12.48	87.68	82.84	22.08	4.59	1.97	19.77	1623.43	218.27
N16P379-6-0	55.63	17.48	88.68	128.84	22.08	6.59	1.97	13.77	1312.43	202.27
N16P380-7-0	55.63	10.48	86.68	71.84	20.08	5.59	1.97	22.77	2548.73	229.77
N16P381-19-0	55.63	16.48	86.68	65.84	17.08	5.59	1.97	23.77	2494.23	213.77
N16P381-4-0	56.63	14.48	85.68	79.84	21.08	5.59	1.97	24.77	2811.43	228.27
N16P381-6-0	55.63	11.48	87.68	69.84	21.08	4.59	0.97	24.77	3003.83	243.27
N16P383-12-0	52.63	21.48	95.68	119.84	16.08	7.59	0.97	10.77	412.73	222.77
N16P383-13-0	52.63	15.48	88.68	75.84	19.08	5.59	1.97	24.77	2830.73	232.77
N16P383-16-0	53.63	11.48	85.68	70.84	21.08	5.59	1.97	22.77	2554.03	218.77
N16P383-20-0	50.63	26.48	96.68	116.84	15.08	4.59	0.97	14.77	515.53	224.27
N16P383-23-0	50.63	14.48	85.68	83.84	19.08	6.59	0.97	23.77	2892.33	220.27
N16P383-29-0	52.63	12.48	86.68	69.84	20.08	5.59	1.97	21.77	2364.23	224.77
N16P383-30-0	53.63	10.48	83.68	77.84	21.08	5.59	0.97	24.77	2170.93	194.77
N16P383-31-0	54.63	13.48	86.68	61.84	18.08	5.59	1.97	24.77	2521.53	208.27
N16P383-37-0	54.63	15.48	85.68	65.84	19.08	6.59	1.97	23.77	2613.73	220.77
N16P383-42-0	51.99	14.34	80.91	62.34	18.53	6.09	1.88	23.68	2392.51	212.43
N16P383-5-0	55.63	17.48	91.68	76.84	20.08	7.59	1.97	18.77	2242.03	221.27

Table A2. LS means for each genotype in the 2017 early generation yield trial conducted using non-spatial analysis (continued).

Name	FlwDt†	Bloom Period	PM‡	Vine Length	FlwNd§	RNd¶	PodsPd††	Stand Count	Seed Yield	TSW‡‡
N16P383-50-0	54.99	13.34	85.91	75.34	19.53	7.09	1.88	23.68	2112.21	204.93
N16P383-53-0	54.99	13.34	89.91	75.34	19.53	6.09	1.88	23.68	2990.81	218.93
N16P383-54-0	52.99	14.34	84.91	69.34	19.53	7.09	1.88	23.68	2556.81	217.43
N16P383-59-0	54.99	14.34	84.91	72.34	19.53	5.09	1.88	22.68	2370.51	209.43
N16P383-60-0	51.99	14.34	79.91	65.34	19.53	6.09	1.88	23.68	2139.41	205.93
N16P383-61-0	52.99	16.34	87.91	61.34	18.53	5.09	0.88	22.68	1776.51	223.43
N16P383-62-0	53.99	13.34	87.91	65.34	20.53	6.09	1.88	23.68	2042.81	208.43
N16P383-64-0	53.99	14.34	85.91	58.34	18.53	4.09	1.88	22.68	1823.11	224.43
N16P386-102-0	54.99	16.34	87.91	74.34	19.53	7.09	1.88	22.68	2278.31	191.93
N16P386-11-0	54.99	22.34	87.91	126.34	18.53	10.09	0.88	10.68	665.01	195.43
N16P386-14-0	53.99	13.34	87.91	83.34	18.53	7.09	1.88	22.68	2432.91	220.43
N16P386-19-0	52.99	15.34	84.91	116.34	20.53	7.09	0.88	13.68	578.11	225.43
N16P386-20-0	55.99	14.34	88.91	74.34	15.53	6.09	1.88	19.68	2586.71	211.43
N16P386-24-0	52.99	18.34	86.91	68.34	20.53	6.09	1.88	23.68	2166.71	200.43
N16P386-3-0	53.99	13.34	80.91	60.34	19.53	4.09	1.88	23.68	2000.61	200.43
N16P386-37-0	53.99	15.34	87.91	73.34	17.53	6.09	1.88	23.68	3170.11	218.93
N16P386-38-0	52.99	20.34	91.91	65.34	18.53	8.09	1.88	23.68	2416.61	221.43
N16P386-47-0	54.99	14.34	89.91	72.34	21.53	6.09	1.88	23.68	2663.11	207.93
N16P386-55-0	54.99	20.34	90.91	65.34	16.53	7.09	1.88	23.68	3142.01	214.43
N16P386-56-0	52.99	17.34	89.91	72.34	18.53	8.09	1.88	22.68	2609.51	198.93
N16P386-57-0	52.99	14.34	86.91	71.34	19.53	6.09	1.88	23.68	2390.71	214.43
N16P386-62-0	52.99	16.34	79.91	68.34	19.53	7.09	1.88	22.68	2200.11	219.93
N16P386-67-0	53.99	13.34	86.91	72.34	21.53	5.09	1.88	23.68	2368.81	206.93
N16P386-69-0	53.99	15.34	84.91	69.34	16.53	7.09	0.88	23.68	2379.31	198.43
N16P386-7-0	54.99	19.34	89.91	71.34	17.53	6.09	1.88	19.68	2157.01	217.93
N16P386-71-0	52.99	14.34	79.91	68.34	18.53	6.09	1.88	23.68	2558.61	209.93
N16P386-72-0	54.99	17.34	91.91	78.34	20.53	7.09	1.88	23.68	2470.71	187.43
N16P386-77-0	54.99	15.34	85.91	71.34	17.53	7.09	1.88	20.68	2741.31	225.93
N16P386-79-0	53.99	15.34	86.91	73.34	18.53	8.09	1.88	22.68	2615.71	209.43
N16P386-84-0	55.99	15.34	88.91	75.34	22.53	6.09	1.88	23.68	2566.51	204.93
N16P386-94-0	52.99	17.34	87.91	72.34	16.53	8.09	1.88	23.68	2545.41	206.43
N16P386-99-0	53.99	15.34	81.91	63.34	18.53	5.09	0.88	23.68	1694.01	204.43
N16P387-13-0	55.99	21.34	98.91	135.34	22.53	8.09	0.88	2.68	33.31	175.43
N16P387-14-0	54.99	12.34	86.91	73.34	20.53	6.09	1.88	22.68	2743.11	218.93
N16P387-17-0	53.99	15.34	80.91	71.34	19.53	6.09	1.88	23.68	2439.11	216.43
N16P387-28-0	54.99	18.34	88.91	75.34	20.53	7.09	1.88	22.68	2475.11	195.43
N16P387-3-0	55.99	26.34	105.91	128.34	18.53	8.09	0.88	16.68	1612.21	212.93
N16P387-39-0	54.99	15.34	87.91	83.34	18.53	7.09	1.88	17.68	2116.61	216.93
N16P387-41-0	55.99	15.34	88.91	73.34	18.53	7.09	1.88	19.68	2830.11	204.93
N16P387-5-0	54.99	21.34	94.91	85.34	19.53	8.09	1.88	19.68	2258.91	216.93
N16P387-8-0	51.99	20.34	84.91	118.34	18.53	5.09	1.88	16.68	948.81	232.43
N16P393-14-0	54.99	21.34	97.91	114.34	18.53	6.09	0.88	17.68	877.71	213.93
N16P393-20-0	54.99	14.34	87.91	65.34	17.53	7.09	1.88	20.68	1573.61	228.43
N16P400-10-0	51.99	17.34	91.91	80.34	14.53	6.09	1.88	22.68	2386.31	223.43
N16P400-11-0	52.99	22.34	89.91	100.34	17.53	6.09	1.88	11.68	2215.01	199.93
N16P400-14-0	53.99	17.34	87.91	86.34	17.53	7.09	1.88	22.68	2343.31	226.93
N16P400-3-0	52.99	20.34	94.91	88.34	18.53	8.09	0.88	21.68	2783.51	215.43
N16P400-31-0	52.99	16.34	87.91	101.34	17.53	7.09	1.88	23.68	2073.51	202.93
N16P400-37-0	52.99	21.34	88.91	92.34	16.53	9.09	1.88	20.68	3039.21	200.43
N16P400-38-0	52.12	15.01	87.82	74.05	13.69	6.42	1.86	22.78	2187.32	207.50
N16P400-44-0	56.12	25.01	100.82	94.05	15.69	7.42	1.86	16.78	2608.22	201.00

Table A2. LS means for each genotype in the 2017 early generation yield trial conducted using non-spatial analysis (continued).

Name	FlwDt†	Bloom Period	PM‡	Vine Length	FlwNd§	RNd¶	PodsPd††	Stand Count	Seed Yield	TSW‡‡
N16P400-49-0	53.12	27.01	94.82	79.05	16.69	7.42	1.86	19.78	2074.92	215.50
N16P400-55-0	53.12	20.01	89.82	83.05	15.69	7.42	1.86	18.78	2496.62	227.50
N16P400-57-0	55.12	22.01	90.82	69.05	16.69	6.42	1.86	23.78	3512.32	225.00
N16P400-67-0	53.12	23.01	94.82	64.05	16.69	6.42	1.86	20.78	2965.82	233.00
N16P401-8-0	50.12	15.01	88.82	73.05	15.69	4.42	1.86	20.78	1939.52	263.00
N16P403-44-0	53.12	19.01	88.82	84.05	15.69	6.42	1.86	21.78	2356.92	228.50
N16P404-16-0	53.12	18.01	89.82	81.05	19.69	7.42	1.86	20.78	2455.32	250.00
N16P404-22-0	52.12	21.01	87.82	77.05	17.69	7.42	1.86	18.78	2074.92	232.50
N16P404-24-0	49.12	20.01	87.82	64.05	16.69	5.42	1.86	21.78	1989.62	228.50
N16P404-6-0	52.12	15.01	86.82	68.05	20.69	5.42	1.86	21.78	2011.62	261.00
N16P404-7-0	53.12	14.01	88.82	75.05	19.69	5.42	1.86	20.78	2059.92	257.00
N16P405-10-0	52.12	14.01	88.82	75.05	16.69	6.42	1.86	15.78	1667.32	241.50
N16P405-2-0	54.12	16.01	89.82	53.05	15.69	6.42	1.86	21.78	2931.52	239.50
N16P405-9-0	50.12	18.01	87.82	49.05	14.69	7.42	1.86	23.78	2563.42	212.50
N16P407-15-0	50.12	21.01	89.82	76.05	15.69	8.42	1.86	18.78	2848.92	237.50
N16P407-34-0	53.12	21.01	92.82	69.05	16.69	8.42	1.86	16.78	1379.02	223.00
N16P407-7-0	50.12	16.01	79.82	82.05	16.69	8.42	1.86	22.78	1988.72	216.00
N16P410-14-0	52.12	16.01	84.82	62.05	15.69	6.42	1.86	23.78	1995.82	222.00
N16P410-2-0	51.12	16.01	84.82	74.05	17.69	7.42	1.86	21.78	2680.22	211.00
N16P410-28-0	53.12	17.01	94.82	83.05	18.69	6.42	1.86	22.78	2364.82	252.50
N16P410-29-0	52.12	15.01	87.82	68.05	14.69	8.42	1.86	22.78	2398.22	254.00
N16P412-36-0	50.12	15.01	79.82	69.05	15.69	7.42	1.86	21.78	2975.52	222.50
N16P413-29-0	49.12	16.01	82.82	70.05	16.69	7.42	1.86	18.78	2287.52	212.00
N16P413-34-0	51.12	15.01	86.82	68.05	16.69	6.42	1.86	21.78	2094.22	206.50
N16P414-18-0	52.12	14.01	82.82	78.05	17.69	5.42	1.86	23.78	2690.82	225.00
N16P414-24-0	52.12	16.01	87.82	76.05	14.69	7.42	1.86	20.78	2037.12	212.00
N16P417-14-0	51.12	20.01	84.82	73.05	16.69	7.42	1.86	17.78	2102.12	204.00
N16P417-2-0	53.12	18.01	85.82	61.05	14.69	8.42	1.86	22.78	3112.52	217.00
N16P417-33-0	50.12	22.01	87.82	80.05	18.69	9.42	1.86	21.78	3546.62	218.00
N16P418-13-0	52.12	24.01	93.82	97.05	15.69	10.42	1.86	20.78	3580.02	225.50
N16P418-20-0	52.12	20.01	94.82	64.05	12.69	8.42	1.86	18.78	2841.92	222.00
N16P418-3-0	52.12	19.01	83.82	87.05	16.69	6.42	1.86	21.78	3341.02	232.00
N16P447-10-0	51.12	19.01	92.82	63.05	12.69	7.42	1.86	23.78	3769.82	244.00
N16P447-4-0	56.12	15.01	87.82	72.05	16.69	6.42	1.86	23.78	2831.42	247.00
N16P448-38-0	53.12	16.01	89.82	71.05	18.69	6.42	1.86	17.78	2347.22	235.50
N16P449-13-0	51.12	24.01	94.82	66.05	12.69	9.42	1.86	23.78	4126.52	240.00
N16P449-31-0	56.12	20.01	94.82	55.05	14.69	8.42	1.86	20.78	2561.62	247.50
N16P450-5-0	49.12	26.01	97.82	79.05	14.69	8.42	1.86	19.78	2456.22	247.50
N16P451-37-0	53.12	24.01	92.82	101.05	20.69	7.42	1.86	11.78	1471.22	224.50
N16P458-18-0	51.12	25.01	90.82	69.05	14.69	6.42	1.86	23.78	3106.42	243.00
N16P458-36-0	47.12	27.01	87.82	70.05	15.69	8.42	1.86	20.78	3081.82	237.50
N16P458-42-0	50.12	23.01	87.82	70.05	16.69	7.42	1.86	19.78	2828.72	247.00
N16P458-46-0	48.12	28.01	92.82	79.05	18.69	8.42	1.86	19.78	2713.62	233.50
N16P459-22-0	49.12	20.01	87.82	74.05	15.69	6.42	1.86	23.78	3094.12	230.50
NDP121166	52.60	14.60	86.00	62.20	18.80	6.80	2.00	19.60	2076.06	227.40
NDP121221	51.80	12.40	86.60	65.20	18.20	5.40	2.00	22.80	2297.68	256.60
NDP121322	48.40	18.80	82.40	54.00	15.80	6.40	2.00	22.40	2324.58	212.20
NDP121334	53.60	18.00	92.20	65.80	17.00	7.20	2.00	17.60	2034.32	234.00
NDP121361	55.20	12.20	85.00	63.40	18.40	5.60	1.60	23.40	2609.26	218.50
NDP121548	53.60	13.20	86.60	63.60	18.20	6.20	2.00	21.40	2459.18	227.70
NDP121613	50.60	15.40	82.80	62.20	17.20	6.40	2.00	21.00	1639.94	211.60

Table A2. LS means for each genotype in the 2017 early generation yield trial conducted using non-spatial analysis (continued).

Name	FlwDt†	Bloom Period	PM‡	Vine Length	FlwNd§	RNd¶	PodsPd††	Stand Count	Seed Yield	TSW‡‡
PS07ND0190	53.80	16.40	88.20	73.80	15.80	7.00	1.80	19.80	2396.46	214.30

† first flower date
‡ physical maturity date
§ number of nodes to first flower
¶ number of reproductive nodes
†† number of pods per peduncle
‡‡ thousand seed weight

Table A3. Non-spatial versus spatial analysis correlation for each trait.

Trait	Correlation
FlwrDt†	0.98
Bloom period	0.98
PM‡	0.96
Vine length	0.98
FlwrNd§	0.98
R nodes¶	0.92
PodsPd††	0.95
Stand count	0.95
Seed yield	0.97
TSW‡‡	0.97

† first flower date
‡ physical maturity date
§ number of nodes to first flower
¶ number of reproductive nodes
†† number of pods per peduncle
‡‡ thousand seed weight

Table A4. Top thirty performing BC_2F_3 experimental test entries during the 2017 field season.

Name	FlwDt†	Bloom Period	PM‡	Vine Length	FlwNd§	RNd¶	PPd††	Seed Yield	TSW‡‡	Lf§§	Sd¶¶
N16P214-6-0	51.3	15.0	87.0	73.2	17.9	7.1	2.2	3879.5	237.5	+/-	-
N16P215-3-0	53.3	17.0	88.0	80.2	19.9	6.1	2.2	3460.4	206.5	-	-
N16P217-13-0	54.3	16.0	88.0	70.2	18.9	7.1	2.2	3776.7	216.5	-	-
N16P217-5-0	52.3	22.0	88.0	89.2	16.9	8.1	2.2	2961.4	212.0	-	-
N16P284-14-0	52.3	19.0	86.0	67.2	16.9	7.1	2.2	3076.5	215.5	-	-
N16P291-35-0	52.3	18.0	90.0	74.2	20.9	8.1	2.2	3462.2	224.0	-	-
N16P300-4-0	52.0	14.2	86.6	71.6	16.9	5.8	2.1	2977.4	248.3	-	+
N16P301-34-0	51.0	15.2	88.6	74.6	14.9	5.8	2.1	3122.4	233.8	-	+
N16P301-36-0	51.0	16.2	88.6	83.6	19.9	6.8	2.1	2513.5	234.3	-	+
N16P301-8-0	53.0	16.2	88.6	80.6	18.9	6.8	2.1	3348.2	243.8	-	+
N16P302-8-0	51.0	15.2	92.6	74.6	18.9	5.8	2.1	3064.4	271.3	-	+
N16P306-11-0	52.0	15.2	91.6	78.6	18.9	5.8	2.1	3104.0	278.3	+/-	+
N16P357-6-0	48.0	18.2	84.6	71.6	18.9	8.8	2.1	3237.5	237.8	-	+
N16P367-24-0	55.0	16.2	86.6	69.6	17.9	6.8	2.1	3306.9	218.3	+/-	-
N16P368-24-0	56.0	18.2	86.6	79.6	20.9	5.8	1.1	4390.3	221.3	+/-	+/-
N16P368-53-0	53.0	17.2	84.6	85.6	19.9	5.8	2.1	2905.4	221.8	-	+
N16P376-20-0	55.6	14.5	83.7	79.8	18.1	5.6	2.0	2826.4	215.8	-	+
N16P383-13-0	52.6	15.5	88.7	75.8	19.1	5.6	2.0	2830.7	232.8	-	+
N16P383-23-0	50.6	14.5	85.7	83.8	19.1	6.6	1.0	2892.3	220.3	-	+
N16P386-37-0	54.0	15.3	87.9	73.3	17.5	6.1	1.9	3170.1	218.9	-	+
N16P386-55-0	55.0	20.3	90.9	65.3	16.5	7.1	1.9	3142.0	214.4	-	+
N16P407-15-0	50.1	21.0	89.8	76.1	15.7	8.4	1.9	2848.9	237.5	+/-	+/-
N16P410-2-0	51.1	16.0	84.8	74.1	17.7	7.4	1.9	2680.2	211.0	-	+
N16P414-18-0	52.1	14.0	82.8	78.1	17.7	5.4	1.9	2690.8	225.0	-	+
N16P417-33-0	50.1	22.0	87.8	80.1	18.7	9.4	1.9	3546.6	218.0	-	+
N16P417-33-0	50.1	22.0	87.8	80.1	18.7	9.4	1.9	3546.6	218.0	-	+
N16P418-3-0	52.1	19.0	83.8	87.1	16.7	6.4	1.9	3341.0	232.0	+/-	+/-
N16P447-10-0	51.1	19.0	92.8	63.1	12.7	7.4	1.9	3769.8	244.0	-	+
N16P449-13-0	51.1	24.0	94.8	66.1	12.7	9.4	1.9	4126.5	240.0	-	+
N16P458-36-0	47.1	27.0	87.8	70.1	15.7	8.4	1.9	3081.8	237.5	-	+

† first flower date
‡ physical maturity date
§ number of nodes to first flower
¶ number of reproductive nodes
†† number of pods per peduncle
‡‡ thousand seed weight
§§ leaf type, "-" semi-leafless, "+/-" segregating normal and semi-leafless
¶¶ seed color, "-" green, "+"yellow, "+/-" mixed, segregating

Proc GLM Non-Spatial

```
proc glm;
class name r_expt ;
model firstflower bloomperiod physmature vinelength flowernode
     reprnodes podspedun standcount seedyield tsw= r_expt
     name/ss3;
lsmeans name;
title 'Proc GLM Non-Spatial';
run;
ods rtf close;
```

Proc GLM Non-Spatial by Recurrent Parent

```
proc glm;
class reparent r_expt ;
model firstflower bloomperiod physmature vinelength flowernode
     reprnodes podspedun standcount seedyield tsw= reparent
     r_expt/ss3;
lsmeans reparent/pdiff;
title 'Proc GLM Non-Spatial by Recurrent Parent';
run;
ods rtf close;
```

Figure A1. Non-spatial analysis SAS PROC GLM code used for 2017 field study analysis.

Proc GLM Spatial-Analysis

```
proc glm;
class name r_expt ;
model firstflower bloomperiod physmature vinelength flowernode
     reprnodes podspedun standcount seedyield tsw= r_expt
     prow(r_expt) pcol(r_expt) name/ss3;
lsmeans name;
title 'Proc GLM Spatial-Analysis';
run;
ods rtf close;
```

Blups Proc Mixed Analysis

```
proc mixed;
class name r_expt;
model seedyield=r_expt;
random name/solution;
title 'Blups Proc Mixed Analysis'
run;
ods rtf close;
```

Figure A2. Spatial analysis SAS PROC GLM and PROC MIXED code used for 2017 field study analysis.

Plate 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	35	77	109	128	153	209	264	327	357	385	442	465
B	44	82	116	129	163	216	265	329	359	386	443	467
C	49	83	121	132	167	219	287	336	360	399	444	470
D	56	96	122	142	168	221	297	340	364	418	445	471
E	59	98	123	148	169	231	306	341	370	419	448	474
F	60	100	124	149	179	242	315	343	375	433	454	475
G	62	105	126	150	204	248	316	349	382	436	458	P
H	67	106	127	151	206	295	318	356	383	439	463	N

Plate 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	477	513	543	580	631	656	699	742	767	827	865	935
B	478	514	547	589	634	661	708	743	777	832	870	940
C	481	522	549	593	635	662	718	745	792	843	879	941
D	485	525	553	603	637	666	720	746	798	848	883	942
E	497	528	559	606	642	681	729	748	800	849	919	944
F	501	530	568	609	645	684	732	750	802	854	921	947
G	505	532	570	614	646	696	736	758	819	861	930	P
H	507	534	576	616	652	697	738	763	824	864	932	N

Plate 3

	1	2	3	4	5	6	7	8	9	10	11	12
A	950	973	998	1042	1081	1110	1164	1188	1209	1226		
B	951	976	1001	1043	1084	1119	1165	1189	1211	1244		
C	956	977	1012	1047	1088	1121	1168	1192	1212	1247		
D	957	980	1013	1049	1089	1122	1172	1194	1213	1248		
E	963	981	1016	1056	1091	1125	1175	1195	1214	1252		
F	964	982	1020	1063	1098	1127	1181	1201	1215	1265		
G	965	985	1026	1065	1104	1140	1182	1202	1216	1266		P
H	970	988	1028	1077	1106	1157	1183	1205	1221	1275		N

Plate 4

	1	2	3	4	5	6	7	8	9	10	11	12
A	1185	1242	1297	1327	1349	1384	1449	1475	1536	1588	1622	1729
B	1186	1283	1299	1330	1360	1386	1452	1477	1551	1589	1627	1739
C	N1361†	1284	1300	1335	1371	1393	1457	1481	1558	1592	1633	2246
D	1206	1285	1305	2365	1373	ND1348	1464	1489	1562	2351	1635	1740
E	1231	2389	1307	1337	1375	1400	1469	Blank	1563	1605	1645	1742
F	1235	N0173†	1312	1340	1377	1404	1470	1495	1568	1609	1678	1749
G	1237	1290	1314	1345	1379	1424	2234	1505	P0190†	1615	1698	P
H	1239	1295	1322	1346	2232	1425	1472	1515	1581	1616	1717	N

Figure A3. ELISA analysis for BC_2F_2 individuals from the AES greenhouse mechanical inoculation.

Plate 5

	1	2	3	4	5	6	7	8	9	10	11	12
A	1754	1779	1800	1831	2341	1920	1962	1996	2033	2075	2112	2147
B	1755	1782	1803	N1166†	1874	1924	2256	2002	2038	2081	2119	2292
C	1757	1784	1815	1840	1877	1929	1967	2006	2049	2082	2122	2157
D	1761	1785	1818	1854	1880	1933	1974	2008	2050	2085	2129	2161
E	1771	1787	1821	1855	1901	1938	1979	2012	2057	2087	2263	2167
F	1776	2249	1822	1856	1904	1942	N1221†	2262	2058	2091	2130	2176
G	ND1145	1795	1828	1858	1909	1945	1990	2014	ND1283	2092	2135	P
H	1778	1797	1830	1859	1911	1950	1995	2024	2069	2102	N1322†	N

Plate 6

	1	2	3	4	5	6	7	8	9	10	11	12
A	2181	2217	2280	N1443†	2399	2436	2459	2484	2511	N1548†	580	1065
B	2316	2221	2287	2336	2407	2437	2462	2488	2512	287	593	1172
C	2194	2321	2291	2339	2327	2438	2464	2498	2513	264	606	1202
D	2195	2223	2300	2342	2413	2440	2470	ND1450	2337	370	624	1205
E	2196	2236	2308	2344	2424	2441	2471	2500	393	418	697	1216
F	2206	N1334†	2311	2356	2426	2444	2478	2506	722	436	732	Lifter
G	2213	2241	2329	2374	2431	2445	2330	2509	59	N1613†	879	P
H	2215	2279	2334	2381	2435	2455	2483	2510	131	445	940	N

Discard Escape PCR Resistant, ELISA Positive ELISA Positive

Figure A3. ELISA analysis for BC_2F_2 individuals from the AES greenhouse mechanical inoculation (continued).

† name of parental lines shortened

Plate 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	101	111	119	127	135	143	151	159	167	203	211	219
B	104	112	120	128	136	144	152	160	168	204	212	220
C	105	113	121	129	137	145	153	161	169	205	213	221
D	106	114	122	130	138	146	154	162	170	206	214	222
E	107	115	123	131	139	147	155	163	171	207	215	223
F	108	116	124	132	140	148	156	164	172	208	216	224
G	109	117	125	133	141	149	157	165	201	209	217	P
H	110	118	126	134	142	150	158	166	202	210	218	N

Plate 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	225	233	241	249	257	265	301	309	317	325	333	341
B	226	234	242	250	258	266	302	310	318	326	334	342
C	227	235	243	251	259	267	303	311	319	327	335	343
D	228	236	244	252	260	268	304	312	320	328	336	344
E	229	237	245	253	261	269	305	313	321	329	337	345
F	230	238	246	254	262	270	306	314	322	330	338	346
G	231	239	247	255	263	271	307	315	323	331	339	P
H	232	240	248	256	264	272	308	316	324	332	340	N

Plate 3

	1	2	3	4	5	6	7	8	9	10	11	12
A	347	355	363	371	407	415	423	431	439	447	455	463
B	348	356	364	372	408	416	424	432	440	448	456	464
C	349	357	365	401	409	417	425	433	441	449	457	465
D	350	358	366	402	410	418	426	434	442	450	458	466
E	351	359	367	403	411	419	427	435	443	451	459	467
F	352	360	368	404	412	420	428	436	444	452	460	468
G	353	361	369	405	413	421	429	437	445	453	461	P
H	354	362	370	406	414	422	430	438	446	454	462	N

Plate 4

	1	2	3	4	5	6	7	8	9	10	11	12
A	469	505	513	521	529	537	545	553	561	569		
B	470	506	514	522	530	538	546	554	562	570		
C	471	507	515	523	531	539	547	555	563	571		
D	472	508	516	524	532	540	548	556	564	572		
E	501	509	517	525	533	541	549	557	565			
F	502	510	518	526	534	542	550	558	566			
G	503	511	519	527	535	543	551	559	567			P
H	504	512	520	528	536	544	552	560	568			N

ELISA Positive

Figure A4. ELISA analysis for BC_2F_3 plots from the 2017 field study.

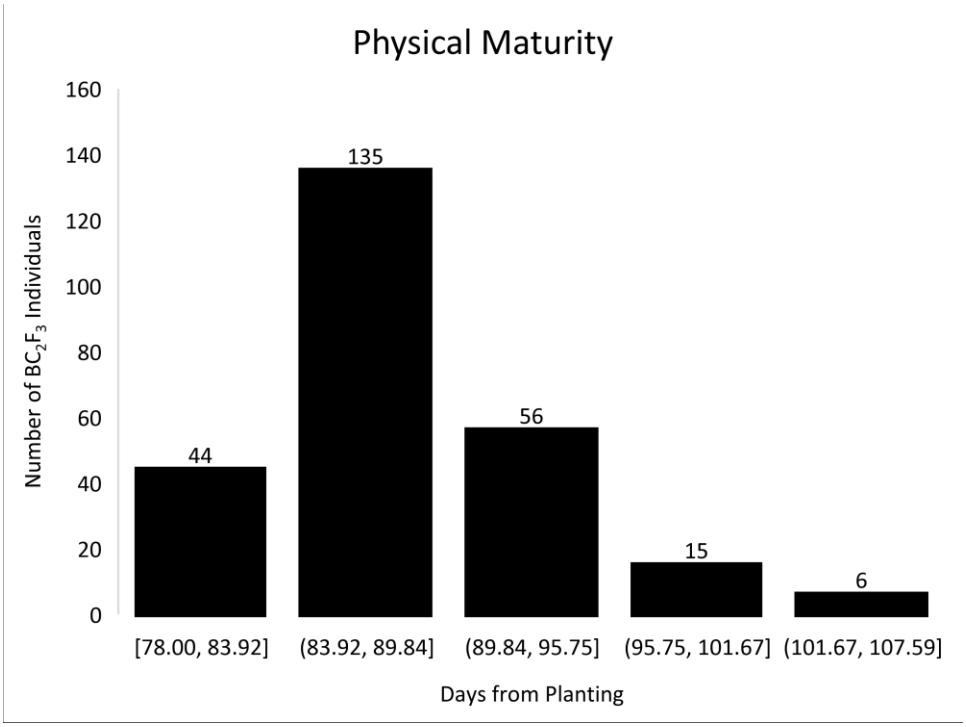

Figure A5. Histogram for days to physical maturity for BC_2F_3 individuals from the 2017 field trial.

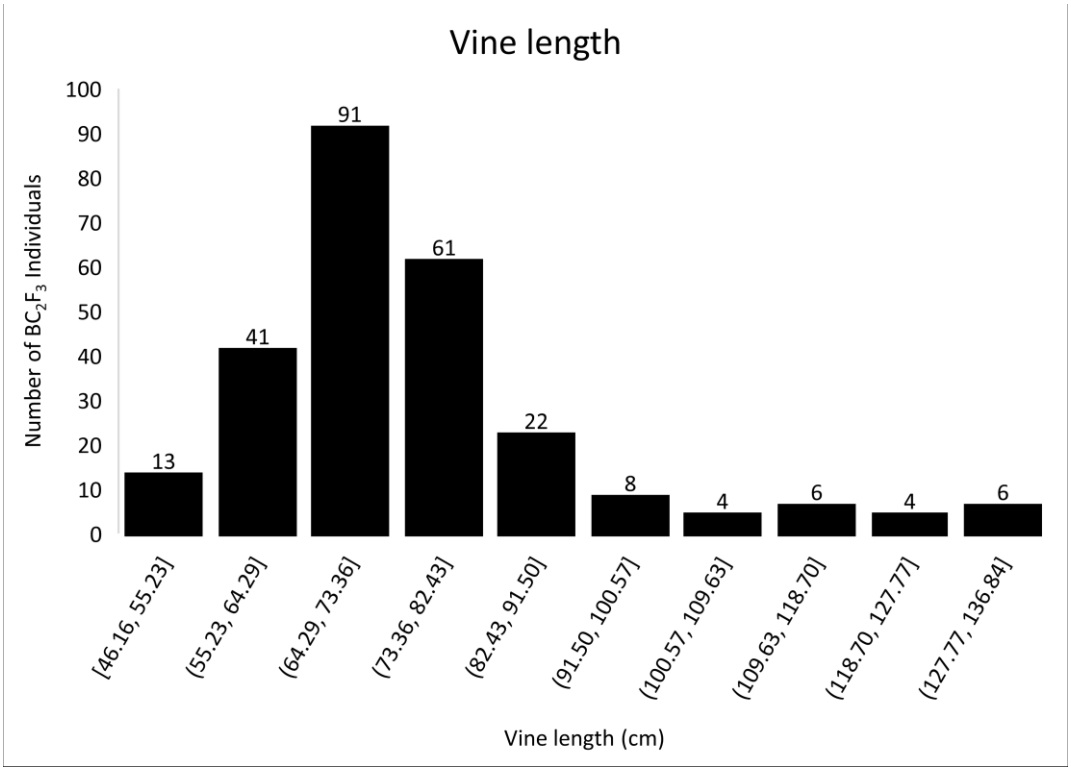

Figure A6. Histogram for vine length for BC_2F_3 individuals from the 2017 field trial.

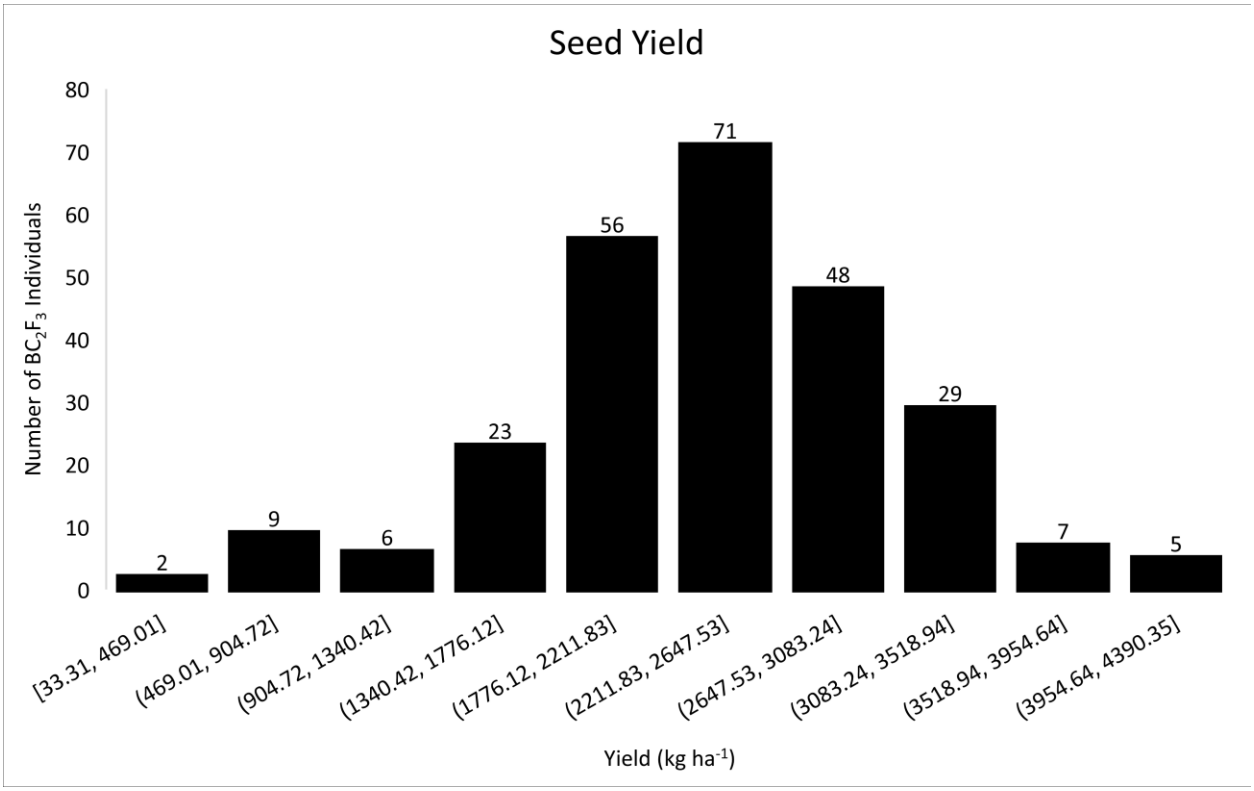

Figure A7. Histogram for seed yield for BC_2F_3 individuals from the 2017 field trial.

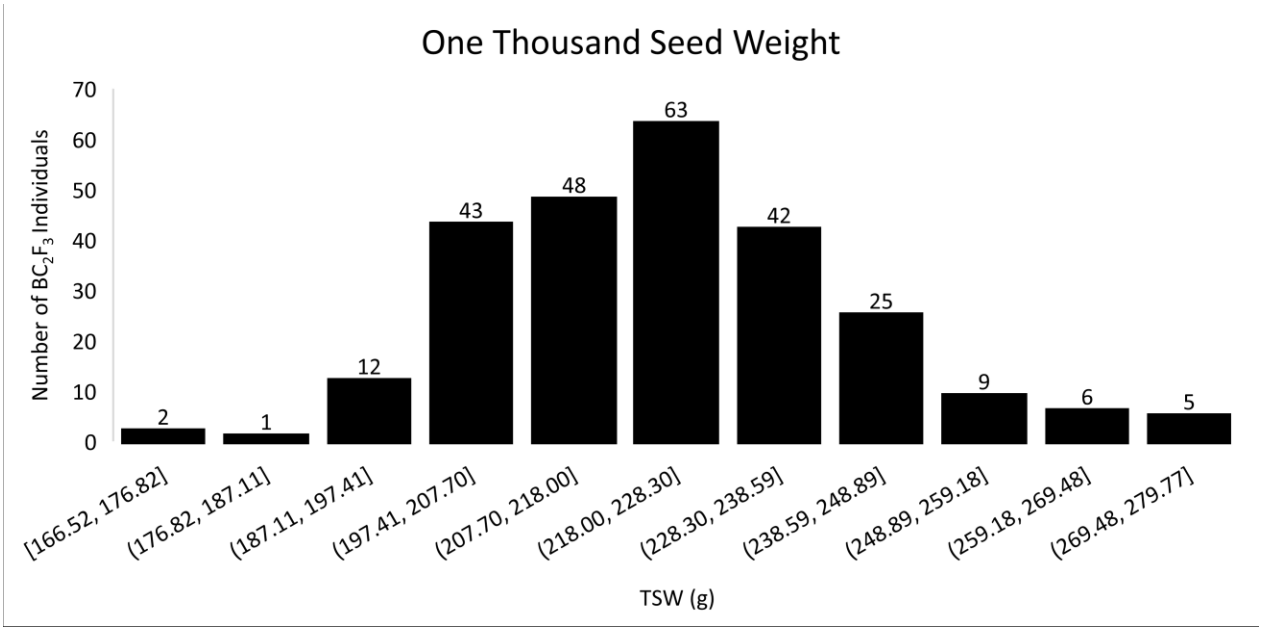

Figure A8. Histogram for one thousand seed weight for BC_2F_3 individuals from the 2017 field trial.