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The genetic improvement of protein quality in common bean (*Phaseolus vulgaris* L.)

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in
Biology

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Abstract

The common bean (*Phaseolus vulgaris* L.) has a high seed protein content, between 20 and 30%. The protein quality in common is considered low because of the suboptimal levels of methionine and cysteine in the seed. Phaseolin, the main seed storage protein, accounts for 30-50% of the total seed protein content. Phaseolin only contains about 0.5 to 0.80% methionine. The suggested nutritional requirements for methionine-cysteine in the human diet are between 2.5 and 2.6 %. Previous studies on the germplasm SMARC1N-PN1 showed that deficiency in phaseolin and lectins leads to increased methionine-cysteine up to 2.6% in the bean seed. Dr. Hou, the bean breeder in Manitoba, made a cross between SMARC1N-PN1 and Morden-003. One Hundred and eighty-five recombinant inbred lines (RILs) F_{2:8} were obtained of this cross through eight generations of inbreeding. In this study I used SDS-PAGE to assess the protein profiles of the RILs according to the phenotypic expression for phaseolin and lectins. The RILs deficient in phaseolin and lectins increased their total methionine-cysteine seed content up to 3.4%. Field trials were conducted to assess the impact of the protein deficiency on the RILs' agronomic traits. The RILs deficient in phaseolin and lectins had a similar agronomic performance as Morden-003 thus can be considered Canadian elite germplasm to develop common bean cultivars with improved protein.

Key-words: *Phaseolus vulgaris*, SDS-PAGE, phaseolin, methionine, cysteine, recombinant inbreed lines

Dedication

To my beloved ones in my family for being the core of my life

To Moisés for bringing countless joy to my life

To René for being a special part in my life

To Luis Pérez Castro for his sincere and everlasting friendship

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List of Abbreviations

ANOVA	analysis of variance
°C	degrees Celsius
CBC	cystathione β -synthase
CaCl ₂	calcium chloride
CGL	cystathione γ -lyase
cm	centimeter
DTF	days to flowering
DTM	days to maturity
DTT	dithiothreitol
FDL	fluorescence Detector
g	grams
G×E	genotype by environment
GT	growth type
ha	hectare
HCl	hydrochloric acid
hr	hour
HPLC	high performance liquid chromatography
HSW	hundred seed weight
kDa	kilodaltons
KCl	potassium chloride
m	meter

m ²	square meter
MAT	methionine adenosyltransferase
MB	Manitoba
MES	2-(N-morpholino) ethanesulfonic acid
mg	milligram
min	minute
ml	milliliter
mM	millimolar
mm	millimeters
N	normal
mRNA	messenger ribonucleic acid
MS	methionine synthase
ON	Ontario
OPA	<i>o</i> -phthalaldehyde
PDCAAS	protein digestibility corrected amino acid score
PDH	pod height
pH	potential of hydrogen
PMSF	phenylmethylsulfonyl fluoride
PHT	plant height
PITC	phenylisothiocyanate
pmoles	picomoles
PTC	phenylthiocarbamyl
RIL	recombinant inbred line

SAM	<i>S</i> -adenosylmethionine
SAH	<i>S</i> -adenosylhomocysteine
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SSD	single seed descent
-SH	functional group composed of a sulphur and hydrogen atom
SPARC Biocentre	Sickkids Proteomics, Analytics, Robotics and Chemical Biology Centre
SPSS	Statistical Package for the Social Sciences
S-S	disulfide bridges
tRNA	transfer ribonucleic acid
μl	microliter
μm	micrometer
w/v	weight/volume
YLD	yield

Chapter 1: Introduction

1.1 Research question

The common bean seed has a high protein content, between 20 and 30% (Kelly and Bliss, 1975; Sathe, 2002). The seed protein quality is considered low because of the suboptimal levels of the protein sulphur amino acids. The common bean seed contains about 1.9% of total methionine-cysteine depending on the cultivar (Ma and Bliss, 1978; Sathe, 2002). Phaseolin is the main storage protein in common bean seed. Phaseolin represents up to 50% of the seed protein content (Chagas and Santoro, 1997); therefore phaseolin is the main source of methionine in the bean seed. The methionine content in phaseolin is between 0.5 and 0.80% depending on the commercial cultivar (George et al., 1993; Sathe, 2002; Montoya et al., 2010), which is considered low. The nutritional requirements for methionine-cysteine in the human diet are between 2.6 and 2.5%, equivalent to between 26 and 25 mg methionine-cysteine g^{-1} protein (Young and Pellett, 1991; Montoya et al., 2010).

Since phaseolin is the main storage protein in common bean seed, former common bean programs to improve the seed protein quality were designed to increase the phaseolin content. Previously, increased phaseolin contents were achieved through crosses between two common bean cultivars with high seed protein content (Gepts and Bliss, 1984). Through this strategy Gepts and Bliss (1984) were able to improve the methionine content in phaseolin up to 0.9% (9 mg g^{-1} phaseolin protein) in some common bean commercial cultivars. However, this increase is still below the nutritional requirements. In this thesis research, an alternative approach on improving the seed protein quality in common bean is evaluated based on the work of Taylor et al. (2008). The research of Taylor et al. (2008) revealed that the total methionine and cysteine content was raised from 1.8% (18.9 mg g^{-1} protein) in the bean germplasm SARC1 to 2.6% (26.8 mg g^{-1} protein) in the bean germplasm SMARC1N-PN1. The methionine and cysteine content in SMARC1N-PN1 is within the requirements for human nutrition. SARC1 has all three seed storage proteins: phaseolin, lectins and

arcelin, whereas SMARC1N-PN1 is deficient in all three seed storage proteins (Hartweck and Osborn, 1997). In the present work, the protein quality of Recombinant Inbred Lines (offspring of two genetically distant parents obtained through eight generations of inbreeding) of common bean, deficient in phaseolin and lectins, are evaluated for their protein quality. The Recombinant Inbred Lines (RILs) with increased methione and cysteine will constitute an elite germplasm for developing commercial common bean cultivars with improved protein quality.

1.2 Background

The common bean, *Phaseolus vulgaris* L., is widely cultivated for its edible seeds (Bliss, 1980). *P. vulgaris* is a diploid annual species ($2n = 2x = 22$), and is predominantly self-pollinating (De Ron et al., 2015). Wild forms of *P. vulgaris* occur from northern Mexico to northwestern Argentina. There are three distinguishable gene pools: from Mesoamerica, the Andean region and the northern Peru-Ecuador (Debouck et al., 1993). The Mesoamerican and the Andean are the main gene pools (Gepts, 1998; Bellucci et al., 2014). *Phaseolus* beans are the most important grain legume for direct human consumption (Gepts et al., 2008). Common bean is grown in diverse regions such as Latin America, Africa, the Middle East, China, Europe, the United States and Canada (FAOSTAT, 2014).

Latin America is the largest producer of common bean, with about 5.5 million metric tons per year accounting for nearly 45% of the total world production (CGIAR, 2012; FAOSTAT, 2014). The estimate of mean per capita consumption in Latin America is between 10 and 13.3 kg/year (Leterme and Munoz, 2002; Nedumaran et al., 2015). Africa is the second most important region, producing about 2.5 million metric tons yearly. The mean per capita consumption of dry bean in this area is 31.4 kg (CGIAR, 2012; FAOSTAT, 2014). Because of its high content of protein, the common bean is a major source of dietary protein in developing countries where animal proteins are scarce (Broughton et al., 2003; Salcedo, 2008). In Brazil the common bean consumption supplies 28% of the daily protein intake (Silva et al.,

2010), whereas in Central Africa it represents 50% of the dietary protein (Namugwanya et al., 2014). The common bean complements carbohydrate-rich sources such as rice, maize and cassava and is also a rich source of micronutrients such as iron, zinc, thiamin and folic acid (Beebe et al., 2000; Gepts et al., 2008). Dry beans on average contain, about 8 to 10% moisture, 50 to 60% total carbohydrates, 3 to 5% fiber, 3 to 4% ash (Deshpande and Damodaran, 1990) and 15 to 30% protein, depending on the cultivar (Kelly and Bliss, 1975; Deshpande and Damodaran, 1990; Messina, 1999; Sathe, 2002). Despite the high content of protein in common bean seed, its quality is considered poor because of its low levels of the sulphur amino acids methionine and cysteine, about 1%, depending on the cultivar (Evans and Bandemer, 1967; Johnson et al., 1982).

1.3 Seed storage proteins of common bean

Many different names and criteria of classification have been given to the seed storage proteins of *Phaseolus* bean (Danielsson, 1949; McLeester et al., 1973). This has led to a confusing nomenclature of the common bean seed storage proteins. Some of this confusion was partially due to comparison of phaseolin, the major bean globulin protein, to globulins commonly named as vicilins of other species of legumes like *Vicia faba*, and *Pisum sativum* (Jackson et al., 1969; McLeester et al., 1973) and of species of gramineae like: *Hordeum vulgare*, and *Secale cereale* (Danielsson, 1949). In the present thesis, the composition of the common bean seed storage proteins follows the nomenclature of the research work of Romero et al. (1975) and Sun and Hall (1975) who do not use the name of the phaseolin as analogous to vicilin.

1.3.1 The common bean seed storage protein composition

The common bean seed storage protein fractions are: G-1 globulin, G-2 globulin, the alkali-soluble fraction, containing glutelin and albumin, and the prolamin fraction (Romero et al., 1975; Ma and Bliss, 1978). The storage protein resolution corresponds to the cotyledon protein isolation of twenty-seven common

bean lines. Isolated cotyledons were dried and ground. Because globulins of *P. vulgaris* are soluble at acid pH values, Ma and Bliss (1978) made the extractions with L-ascorbic acid and NaCl following McLeester's (1973) procedure. After centrifugation of the extract, they obtained two phases: a pellet and a clear supernatant. The clear supernatant contained the albumin, globulin and free amino acids. Prechilled distilled water was added to the supernatant. The white, cloudy precipitate which formed was separated by means of centrifugation and was referred to as G-1 globulin protein. The clear supernatant containing the albumins, G-2 and the free amino acids was dialyzed in distilled water and later centrifuged. The pellet and the supernatant of the dialysate were referred to as G-2 and albumins fractions, respectively. The pellet phase from the ascorbate-NaCl extraction was separated using ethanol and NaOH solution and the separated fractions were referred to as prolamin and glutelin fractions, respectively. The major protein fractions are the globulins and the albumins. They account for up to 50 and 30% of the total seed storage protein, respectively (Montoya et al., 2010; Oliveira et al., 2017), thus providing the major contribution to the nutritional quality of the seed (Vitale and Bollini, 1995; Oliveira et al., 2017).

1.3.1.1 The G-2 globulin fraction

The G-2 globulin fraction is composed of lectins (Romero et al., 1975); they are also referred to as phytohemagglutinins for their ability to agglutinate cells (Bliss and Brown, 1983; Osborn et al., 1983; Martínez-Aragón et al., 1995; Vitale and Bollini, 1995; Sathe, 2002). Among the different legumes, *P. vulgaris* seed contains the highest amount of lectin. Lectins represent 10% of the total seed protein, (Pusztai et al., 1979; Vitale and Bollini, 1995; Nasi et al., 2009) and have less than 0.5% of protein sulphur-amino acids (Pusztai et al., 1979; Osborn and Brown, 1988; Vitale and Bollini, 1995). Their coefficient of sedimentation is 6.6 S (Itoh et al., 1980). Lectins are a family of highly homologous proteins. They are composed of polypeptides with a molecular weight in a range of 27 to 37 kDa (Bliss and Brown, 1983; Sathe, 2002). Lectins can be divided into single-chain lectins and two-chain

lectins. The single-chains are tetramers of identical or nearly identical monomers. *P. vulgaris* lectins are an example of this class (Bollini and Chrispeels, 1978). The α -amylase inhibitor lectin is processed post-translationally into α and β -chains (Moreno and Chrispeels, 1989). Lectins are glycosylated proteins. The sugars involved in the glycosylation of this protein are: glucosamine, xylose, and mannose (Ericson and Chrispeels, 1973). Lectin proteins of raw beans are toxic to mammals (Pusztai et al., 1975; Pusztai and Palmer, 1977; Grant et al., 1985), and birds (Jayne-Williams and Burgess, 1974). In addition of their toxicity, bean flour contains antinutritional factors such as trypsin and chymotrypsin inhibitors, and has poor digestibility and low nitrogen retention (Pusztai et al., 1979). Several biological functions such as host specificity in *Rhizobium* nodulation (Diaz et al., 1989) and in defense against seed-eating insects have been attributed to lectins. It appears that resistance to proteolytic degradation by the insect digestive enzymes and binding to insect gut structures are the requisites for lectins to exert their deleterious effects on insects (Murdock et al., 1990; Vasconcelos and Oliveira, 2004). Arcelin is an example of an insecticidal lectin. Arcelin has been named after Arcelia, a town in the state of Guerrero, Mexico, where one of the accessions was collected. Arcelin is mostly found in seed proteins of wild bean accessions (Osborn et al., 1986). Marsolais et al. (2010) identified six arcelin isoforms in the seed of the *P. vulgaris* germplasm SARC1. Lectin is produced during seed maturation, starting about 14 days after flowering (Sun et al., 1978).

1.3.1.2 The prolamin fraction

The prolamin has little impact on the total seed storage protein composition. It constitutes a minor fraction, between 2 to 4% of the total seed storage protein (Bliss and Brown, 1983; Oliveira et al., 2017).

1.3.1.3 The albumin fraction

The albumin fraction accounts for 12 to 30% of the total seed storage protein. It comprises protein subunits in the range of 25 to 35 kDa. The albumin fraction is rich in the sulphur amino acids methionine and cysteine. The proteomic analysis of

Phaseolus seeds conducted by Natarajan et al. (2013) reports 8.4% of cysteine and 2.4 % of methionine in the albumin fraction. Ma and Bliss (1978) found an average of 2% of methionine in the albumin fraction of ten F₅ lines of *Phaseolus vulgaris*. Chagas and Santoro (1997) quantified an average of 2% of cysteine and between 1.3 to 2% of methionine in the albumin of three Brazilian cultivars of *Phaseolus*. Bhatti (1982) found 1.2% of methionine in the albumin fraction of the *P. vulgaris* cultivar Saxa.

1.3.1.4 The G-1 globulin fraction

The G-1 globulin fraction comprises the phaseolin and the legumin. The legumin is a hexameric unglycosylated globulin with a sedimentation coefficient of 11S (Vitale and Bollini, 1995). Legumin is formed from six pairs of polypeptides with a molecular weight of about 60 kDa each. The components of each of the pairs are an acidic α subunit with a molecular weight of 40 kDa and a β subunit with 20 kDa (Vitale and Bollini, 1995; Mühling et al., 1997; Sathe, 2002; Yin et al., 2011). Legumin represents 10% of the total seed storage proteins and has a low concentration of the sulphur amino acids methionine and cysteine, 0.6% and 0.8%, respectively (Derbyshire et al., 1976).

Phaseolin is the major globulin storage protein. It constitutes 40 to 60% of the total seed storage proteins (Bliss and Brown, 1983; Chagas and Santoro, 1997). Phaseolin's nutritional value is limited by its low content of the sulphur amino acid methionine, less than 1% and no cysteine at all (Chagas and Santoro, 1997; Natarajan et al., 2013). Phaseolin is a glycoprotein with glucosamine, xylose and mannose forming part of its structure (Ericson and Chrispeels, 1973; Bliss and Brown, 1983; Sturm et al., 1987; Osborn and Brown, 1988). Phaseolin's coefficient of sedimentation is 7S. Phaseolin consists of 2 to 6 polypeptides with molecular weights between 43 and 54 kDa (Osborn and Brown, 1988). The differences in molecular weight and isoelectric point among the polypeptides reflect differences in DNA sequences, coding for two different polypeptide sub-families, α -phaseolin

polypeptides with 435 to 444 amino acid residues, and β -phaseolin polypeptides with 421 amino acid residues (Slightom et al., 1983). Early studies conducted by Brown et al. (1981) using SDS-polyacrylamide gel electrophoresis identified three different profiles for phaseolin. The profiles were named after the cultivars Tendergreen (T), Sanilac (S), and Contender (C). Montoya et al. (2010) on their bean domestication research found that for all the cultivars studied nearly 90% have the Tendergreen and the Sanilac phaseolin type. De La Fuente et al. (2012) reported more than forty different phaseolin profiles in wild and cultivated beans according to the polypeptide composition. The Mesoamerican bean accessions exhibit the “S” (Sanilac), “M” (Middle America), or “B” (Boyaca) types, while the Andean beans have the “T” (Tendergreen), “C” (Contender), “H” (Huevo de Huanchaco), “A” (Ayacucho), “J” (Jujuy) or “I” (Inca) phaseolin types. The “S” phaseolin type is mainly present in the cultivars of Central America, from Mexico to the North of Colombia. The “T” phaseolin type is mainly present in cultivars of the Andes, including the south of Peru, Bolivia, Argentina and Chile (Beebe et al., 2001). Acquaah et al. (1994) studied 650 common bean accessions from Malawi in Africa for their phaseolin profile and found that 49.2% had the “T” type, 24.8% the “C” type, 23.5% the “S” type and 2.5% the “H” type.

The characterization of the Tendergreen, Sanilac and Contender phaseolin protein bands is given by Bliss and Brown (1983). The “T” type has three polypeptide subunits with different molecular weights (α 51, β 48, and γ 45.5), and a minor subunit (γ 46). The “S” type consists of five major polypeptide subunits (α 49, α 48.5, β 48, β 47, and γ 45), and one minor subunit (γ 46). The “C” type has common subunits to both “T” and “S” type. It consists of six major subunits (α 51, α 49, β 48, β 47, γ 45.5, and γ 45) and one minor subunit (γ 46). The phaseolin subunits variation can be explained in terms of mRNAs giving rise to polypeptides of different molecular weights (Hall et al., 1978) and due to differential glycosylation of the polypeptides (Hall et al., 1980). Two asparagine residues Asn²⁵² and Asn³⁴¹ are present as the N-glycosylation sites. Phaseolin polypeptides with two glycans have

Man-7 (mannose) attached to Asn²⁵² and Man-9 attached to Asn³⁴¹, whereas polypeptides with only one glycan have a complex oligosaccharide Xylose-Man-3 at Asn²⁵² (Emani and Hall, 2008).

Phaseolin is encoded by a gene family formed by 6 to 10 co-dominant interrupted genes (five introns and six exons) per haploid genome, organized in a single close cluster on chromosome 7 (Talbot et al., 1984). Thus the genes controlling the polypeptides of each of the phaseolin types are tightly linked, inherited in a block like a single Mendelian gene (De La Fuente et al., 2012). Slightom et al. (1985) reported that the family gene for phaseolin is divided into two sub-families: the α and β family genes that encode the α and β phaseolin type, respectively. In the Tendergreen variety these two gene families α and β differ in their coding regions by the presence or absence, respectively, of 27 bp and 15 bp direct repeats (Slightom et al., 1985; Anthony et al., 1990). The α genes encode fourteen additional amino acids. Aside from these differences the α and β phaseolin genes show around 98% identity (Slightom et al., 1985; Anthony et al., 1990), which supports that most of the heterogeneity in the phaseolin polypeptides are due to post-translational processing, as mentioned above. Also the polypeptide units α , β and γ of the Tendergreen cultivar have similar amino acid composition, suggesting that they are encoded by a relatively small multigene family (Slightom et al., 1985). Thirteen cDNA clones were grouped according to the type of phaseolin polypeptide they encode, either α or β type; within each group the phaseolin cDNA clones have the same pattern of restriction sites, indicating that they are internally homologous (Slightom et al., 1985). Slightom et al. (1983) reported three methionine and two cysteine residues for phaseolin out of a total of 409 amino acid residues, including the signal peptide. The cysteines are removed after proteolytic processing by removal of the signal peptide; (Cramer et al., 1985; Cramer et al., 1987). Anthony et al. (1990) reported four methionine residues for the Sanilac variety. The production of phaseolin in the seed was detectable 14 days after flowering, with the major accumulation continuing for 12 to 14 days (Sun et al., 1978). The research of Hall et al. (1980) supports the findings that the

production of phaseolin in the seed of *P. vulgaris* is detectable fourteen days after flowering. They extracted proteins from the whole seed and from the cotyledon alone as well from seeds that started to form between 10 to 31 days after flowering. The electrophoretic separation showed phaseolin protein bands in seeds and cotyledons formed fourteen days after flowering, with continuing increase in this protein for seventeen days. The exponential accumulation of the phaseolin in the maturing seeds corresponds to the time of rapid proliferation of the rough endoplasmic reticulum in the bean cotyledon cells (Bollini and Chrispeels, 1979).

1.4 The importance of sulphur in the human body

Sulphur is the seventh most abundant mineral in the tissues of higher vertebrates (Ingenbleek, 2006; Emsley, 2011). The skin, muscles, connective tissues and bones are rich in sulphur (Soetan et al., 2010). The hair and nails that are made of the protein keratin contain a large share of sulphur (Rogers et al., 2004). The element sulphur plays a critical role in detoxification and is a built in molecule of glutathione, one of the most important antioxidants that the body produces (Anderson, 1998; Wu et al., 2004). It is well documented that sulphur compounds play a protective role during immune response (Grimble, 2006). Sulphur is a non-metal element. It is the second component in group VI of the periodic table of elements, situated just below oxygen (Palego et al., 2015). It has 8 valences and oxidation states ranging from +6 (sulphate, SO_4^{2-}) to -2 (sulphide, S^{2-}). The most common oxidized form is sulphate (SO_4^{2-}) (Ingenbleek, 2006). Plants, microorganisms and fungi, unlike animals, are able to reduce sulphate to sulphide (Bick and Leustek, 1998; Davidian and Kopriva, 2010; Takahashi, 2010) and to incorporate sulphur into organic metabolites, producing a large variety of organic compounds (Parcell, 2002; Palego et al., 2015). Among the organic compounds are the sulphur-containing amino acids methionine and cysteine (Wirtz and Droux, 2005; Ingenbleek, 2006; García et al., 2015). They constitute almost an exclusive dietary source of sulphur to the body (Parcell, 2002; Ingenbleek, 2006). Plants are the most important source of methionine and cysteine for humans and animals (Wirtz and Droux, 2005).

1.5 Methionine and cysteine in proteins

Methionine and cysteine are encoded by the standard genetic code. They are called proteinogenic amino acids because they are incorporated into proteins (Brosnan and Brosnan, 2006), thus they are taken into the human body as dietary protein constituents. Methionine is an essential amino acid that cannot be synthesized *de novo* in the human body and thus must be supplied in the diet (Wu, 2009; Palego et al., 2015). Cysteine enters into the human body in two ways: through cysteine-containing foods or through a metabolic pathway that converts methionine to cysteine. In the human body, the methionine metabolism begins with its activation to *S*-adenosylmethionine (SAM) by methionine adenosyltransferase (MAT), (Figure 1.5). In mammals, SAM is the main methyl donor substrate (Griffith, 1987; Stipanuk et al., 2006; Palego et al., 2015). SAM can donate its methyl group to a wide variety of acceptors, including amino acid residues in proteins, DNA, RNA and small molecules (Chiang et al., 1996; Laxman et al., 2013). SAM transfers the methyl group to an acceptor and gets converted to *S*-adenosylhomocysteine (SAH). SAH is hydrolyzed to adenosine and homocysteine. Homocysteine can be remethylated to methionine by methionine synthase (MS); in this manner, methionine can be regenerated for reuse. The methionine cycle occurs in all body cells (Brosnan and Brosnan, 2006). Homocysteine can also condense with serine to form cysteine by the combined action of cystathione β -synthase (CBS) and cystathione γ -lyase (CGL). The formation of cysteine from homocysteine is restricted to the liver, kidney, intestine and pancreas (Brosnan and Brosnan, 2006). The conversion of methionine to cysteine is an irreversible process. For this reason, methionine is a dietary essential amino acid, regardless of cysteine availability. Methionine is vital in making proteins in the body. Methionine is the initiation of mRNA translation by formyl-methionyl-tRNA. Methionine is a constituent of nearly all polypeptidic molecules with greater abundance in extracellular than in intracellular proteins (Ingenbleek, 2006). Cysteine is unique among the twenty standard amino acids in that it contains a thiol group or sulfhydryl group, that is to say, a functional group composed of a sulphur atom and a

hydrogen atom (-SH). The sulfhydryl group is able to form disulphide bridges (S-S). Disulfide bridge interactions are important in determining protein tertiary structure (Wirtz and Droux, 2005; Wu, 2009; Palego et al., 2015). The biological function of a protein as well its native state is dependent on the tertiary structure. If the native state of a protein is disrupted its biological function is lost. Extracellularly, disulfide bridges between cysteines increase the molecular stability of a protein. Disulphide bridges are present in proteins linked to defense against bacteria and viruses like immunoglobulins and T-cell receptors or structural proteins like keratin (Palego et al., 2015).

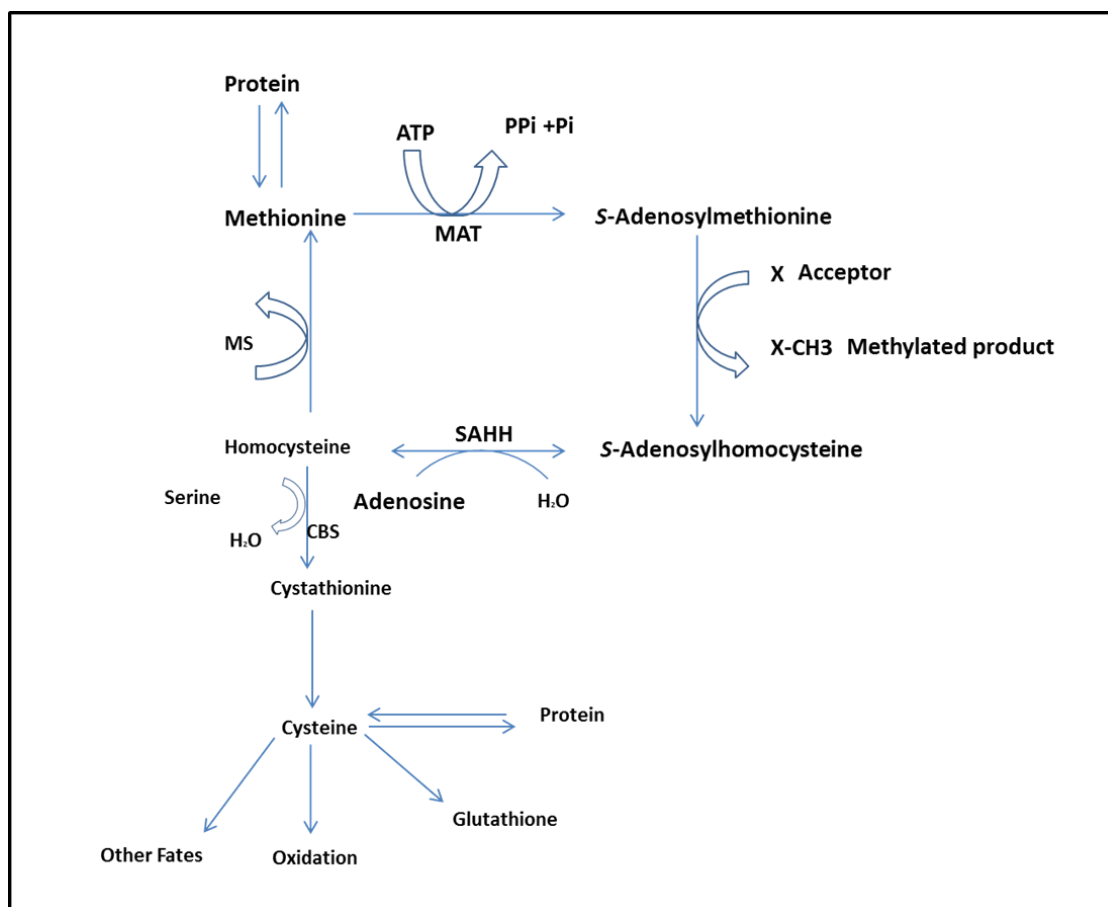


Figure 1.5 Major pathways of methionine and cysteine metabolism in the human body. ATP = adenosine triphosphate, PPi = inorganic pyrophosphate, Pi = inorganic orthophosphate, MAT = methionine adenosyltransferase, SAHH = *S*-adenosylhomocysteine hydrolase, MS = methionine synthase, CBS = cystathionine β -synthase, X = acceptor molecule

1.6 Quantitative variation of phaseolin for genetic improvement of seed protein quality in common bean

Depending on the cultivar, the total protein in common bean seed comprises 20 to 30% of total seed content (Delaney and Bliss, 1991; Sathe, 2002). Of the total seed protein content, 40 to 50% corresponds to the globulin protein phaseolin (Ma and Bliss, 1978; Bliss and Brown, 1983; Chagas and Santoro, 1997). A large

variation in seed protein content with minimum values of 19% and maxima of 32% was found within a collection of seventy-three common bean genotypes from the bean bank germplasm at the National Research Centre of Rice and Beans in southern Brazil (Pereira et al., 2009). Mutschler and Bliss (1981) categorized the bean seed protein content as: low when the seed protein content was around 19% or less, moderate content was referred to as 25% seed protein, and high seed protein content was considered above 28%. Most *P. vulgaris* lines with high seed protein content are snap bean cultivars, grown usually for their edible pods, or tropical dry bean accessions (Bliss and Brown, 1983). Kelly (1971) first reported the snap bean ‘Bush Blue Lakes 240 (‘BBL240’), to be high in total protein content. Other snap bean cultivars with high protein content are ‘Endogava Z.N.’ and PI229815 with around 27.5% (Mutschler and Bliss, 1981). Some tropical common bean cultivars with high seed protein content include ‘Bonita’ with 24% (Mutschler and Bliss, 1981), 15R-148 and ‘Porrillo 70’ with around 29% (Gepts and Bliss, 1984). Barampama and Simard (1993) on a research study of nutrient composition and protein quality of thirteen tropical common bean cultivars found an average of 22% of seed protein content with one of the cultivars with as high as 27.5%. More recent studies on seed protein content of common bean support the previous findings that tropical varieties have high protein content. Silva et al. (2012) in a study of seed protein quantification of one hundred common bean Brazilian cultivars found an average protein content of 25%, ranging from 19.6 to 30.4%. The studies on nutritional and mineral variability in 52 accessions of common bean cultivars from Madeira Island revealed an average seed protein content of 23% with variations between 18.5 to 29.6% (Gouveia et al., 2014).

Since phaseolin constitutes the main storage protein fraction in the common bean seed, studies on the quantification of phaseolin content have also been conducted. A large variation in this globulin protein was found among different common bean cultivars studied. Mutschler and Bliss (1981) quantified the total seed protein and the globulin G-1 protein content of six cultivars: Swedish Brown, WI74-

2047, Oregon 58, Bonita, PI 229815, and Endogava Z.N. The percentage of total seed protein ranged from 17.4 for Swedish Brown to 27.8% for Endogava Z.N. Variation in the content of G-1 protein was also found within these cultivars, ranging from 32 to 50.8%. No correlation between total seed protein and G-1 protein content was found among these cultivars. Oregon 58 and Bonita had a similar percentage of seed protein 23.6 and 24%, respectively. However, Oregon 58 had a significantly higher G-1 protein content (50.8%) than Bonita (45.1%). A similar situation was found for PI229815 with 27.5% of total seed protein and Endogava Z.N. with 27.8%. The latter has lower G-1 protein (47%) than PI229815 (49.6%). WI74-2047 has higher total seed protein (18.5%) than Swedish Brown (17.4%) but lower G-1 protein, 32.9 and 43.3%, respectively.

A basic function of protein in nutrition is to supply adequate amounts of needed amino acids. Despite phaseolin being the main storage protein in the common bean seed, its quality is limited by the low content of the sulphur amino acid methionine. Depending on the cultivars, the methionine content in the phaseolin fraction is less than 1%, with ranges between 0.50 to 0.90% (Gepts and Bliss, 1984; Montoya et al., 2010). The protein quality of food is assessed based on the amount of essential amino acids relative to nutritional requirements and on the digestibility of the proteins (Friedman, 1996; Vaz Patto et al., 2015). The Protein Digestibility Corrected Amino Acid Score (PDCAAS) has been used for the past 20 years to evaluate the protein quality based on the amino acid requirements of humans and their ability to digest it (Boye and Ma, 2012). PDCAAS value ranges from 0 to 100%. For common beans the PDCAAS values are lower, between 53 and 67%, depending on the cultivar (Vaz Patto et al., 2015). Sgabrieri et al. (1979) found a digestibility percentage of 60.5 to 65.5 in cooked beans of four Brazilian cultivars of dry beans. The low digestibility of common bean proteins is attributed to trypsin enzyme inhibitors, which contain a large number of disulfide bonds that are heat stable (Sathe, 2002).

Another measurement for determining protein quality is the biological value of the protein. The biological value measures the percentage of the digested protein that can be used in protein synthesis in the cells of the body. Biological values can range from 0 to 100%. These values are related to the percentage of essential amino acids required by the body. When one or more essential amino acids are missing or present in low quantities, the protein has a low biological value. The common bean has an average biological value of 58%, depending on the cultivar (Vaz Patto et al., 2015). Sgabrieri et al. (1979) found biological values between 38.3 to 58.9% in four Brazilian dry bean cultivars. Methionine and cysteine requirements (both together considered) for an adult are between 25 to 26 mg g⁻¹ of total protein (2.5 to 2.6%) (Young and Pellett, 1991; Montoya et al., 2010). Total methionine is present in common bean seed in an average of 12 mg g⁻¹ (1.2%) of total seed protein (Montoya et al., 2010). The average methionine content in the phaseolin fraction is about 9 mg g⁻¹ protein (0.9%) (Montoya et al., 2010), which is way far below the recommended dietary intake.

The high variation in seed protein content between different cultivars suggested a considerable opportunity for protein quality improvement through traditional breeding (Bliss and Brown, 1983). Since phaseolin is the main seed protein fraction, it constitutes the major contributor to available methionine in all common bean cultivars developed hitherto. The strategy of previous conventional common bean breeding programs on improving the sulphur amino acid content was to increase the percentage of phaseolin. Gepts and Bliss (1984) studied the relationship between available methionine concentration and the levels of phaseolin using three groups of genetic material: a) F₂ progeny of interspecific crosses between twelve *P. vulgaris* cultivars and a *P. coccineus* cultivar ('Mexican Red Runner') which lacks phaseolin; b) F₂ progeny of the cross between five *P. vulgaris* cultivars and a wild Mexican accession ('PI325690-3'), which has a dominant gene encoding for low phaseolin; c) F₂ progeny of the cross between the *P. vulgaris* cultivars: 'Sanilac' and 'Bush Blue Lake 240', the later is a high methionine line; and between

‘Sanilac’ and ‘15R-148’, which is a high non-phaseolin protein line. For each cross, the F₂ progenies were analyzed for total seed protein content, phaseolin content (the presence or absence of phaseolin was detected by rocket immunoelectrophoresis), and available methionine concentration, as percent of seed flour. A paired *t*-test was conducted (Gepts and Bliss, 1984). For the intergeneric cross with *P. coccineus* only three F₁ lines had viable seeds that produced F₂ progeny. The F₂ seeds without phaseolin showed a small but significant reduction in total protein concentration ranging from 26.1 to 27.7% compared to the 26.8 to 31.6% for those containing phaseolin: Similarly, seeds containing phaseolin (35 to 45.7%) had significantly higher available methionine (0.68 to 0.82%) compared to seeds without phaseolin (0.59 to 0.69%). In the F₂ progeny of the second cross, no significant differences in protein concentration were found between the seeds with high phaseolin (50.8 to 61.6%) and low phaseolin (25.2 to 33.6%). The ranges of protein concentrations for seeds with high phaseolin were between 26 to 29.7%. The seeds with low phaseolin had a percentage of protein content between 23 and 30. As for the methionine, the seeds with higher phaseolin levels had higher available methionine in the range of 0.74 to 0.90%. The available methionine for the seeds with lower phaseolin content was between 0.57 to 0.60%. For the F₂ progenies of the third cross between ‘Sanilac’ and ‘Bush Blue Lake 240’, and between ‘Sanilac’ and ‘15R-148’, Gepts and Bliss (1984) provide the coefficient of correlation for the results of the protein, phaseolin and methionine quantification. The F₂ progenies from the cross between ‘Sanilac’ and ‘Bush Blue Lake 240’ show a high coefficient of correlation (0.78) between available methionine and protein content. There is also a high coefficient of correlation (0.74) between available methionine and phaseolin content. The F₂ progenies of the cross between ‘Sanilac’ and ‘15R-148’ show a low correlation (0.45) between available methionine and protein content. No correlation was found between available methionine and non-phaseolin protein concentration. This suggests that the reduction in available methionine in the non-phaseolin seed was due primarily to the absence of phaseolin. Gept and Bliss (1984) concluded that available methionine was positively correlated with phaseolin content.

The more recent findings of Taylor et al. (2008) that increased methionine and cysteine in some common bean lines was due to a deficiency of phaseolin and lectins opened a new possibility to increase the protein quality in common bean. Thus, the bean breeding program in Canada took the strategy of decreasing the percentage of phaseolin for improving the sulphur amino acid content in the bean seed. Taylor et al. (2008) evaluated the protein profile, the protein content, the total protein amino acids, and free amino acids in mature seeds of the germplasm lines: SARC1, SMARC1-PN1, and SMARC1N-PN1. The three common bean lines are genetically related; Sanilac is the parental background. They share approximately 85% of the parental genetic background. The three lines integrate a progressive deficiency in major seed storage proteins: phaseolin, phytohemagglutinin and arcelin. SARC1 contains the “S” type phaseolin derived from Sanilac (Taylor et al., 2008) and phytohemagglutinin and arcelin derived from the wild parent due to tight linkage between phytohemagglutinin and arcelin genes (Osborn et al., 1986; Kami et al., 2006). Recessive null alleles conferring phaseolin or phytohemagglutinin deficiency from *P. coccineus* and “Great Northern 1140”, respectively, were introgressed in the SARC1 genetic background. The introgression of the arcelin-1 allele can lower the phaseolin content from about 50 to 14% of total protein (Romero et al., 1986). SAMARC1-PN1 is deficient in phaseolin; SMARC1N-PN1 is deficient in all three major seed storage proteins (Hartweck and Osborn, 1997). The analysis of the protein profile by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) revealed the presence of two major bands of 49 and 46 kDa in SARC1, corresponding to γ and α - β glycopolypeptides of “S” type phaseolin, respectively. However, the amount of phaseolin in SARC1 was much less than in the parental line Sanilac. These two bands were absent in the phaseolin deficient lines SMARC1-PN1 and SMARC1N-PN1. Two bands of 35 kDa and 32 kDa corresponding to arcelin (Hartweck et al., 1991) and phytohemagglutinin (Vitale and Bollini, 1995), respectively, were found in SARC1 and SMARC1-PN1 but were absent in SMARC1N-PN1. The total seed protein content for the three lines was between 14.8 to 17.8%, not significantly different. The percentage of soluble seed protein was significantly different among

the three lines. SMARC1N-PN1 showed a significantly higher content (7.40%) compared to SMARC1-PN1 (5.82%) and SARC1 (4.64%). The higher soluble protein content in SMARC1N-PN1 was associated with phaseolin, phytohemagglutinin, and arcelin deficiency. The total free amino acid content increased by 52.94% and 104.4% in SMARC1-PN1 and SMARC1N-PN1, respectively, as compared with SARC1. The individual free amino acid quantification showed only a slight increase in free methionine in SMARC1-PN1, and SMARC1N-PN1 as compared with SARC1. Free *S*-methylcysteine decreased by 2% and 25% in SMARC1-PN1 and SMARC1N-PN1, respectively, as compared with SARC1. Free γ -Glu-*S*-methylcysteine notably decreased by 45.9% in SMARC1N-PN1. SMARC1-PN1 showed a reduction by 18.9% of free γ -Glu-*S*-methylcysteine. The total methionine-cysteine content increased from 1.8% (18.9 mg g⁻¹ protein) in SARC1 to 2.6% (26.8 mg g⁻¹ protein) in SMARC1N-PN1. The total methionine-cysteine content in SMARC1N-PN1 is slightly above the 2.5% (25 mg g⁻¹ protein) of the nutritional requirements in the human diet (Young and Pellett, 1991). SMARC1-PN1 and SMARC1N-PN1 showed decreased total *S*-methylcysteine, 0.9% and 0.6%, respectively, as compared with SARC1 (1.1%).

The most important finding of Taylor et al. (2008) was the increase in the protein sulphur amino acids, as a response to seed storage protein deficiency. The sulphur from *S*-methylcysteine and γ -Glu-*S*-methylcysteine was shifted preferentially to the protein cysteine pool in the storage protein deficient lines. To date, this is the first report of a significant improvement in sulphur amino acid content in seeds of common bean based on natural genetic variation in seed storage protein composition. This opens the possibility to develop commercial common bean cultivars with improved protein quality. Based on these findings, the bean breeder in Manitoba (at the Morden Research and Developing Centre), Dr. Anfu Hou, made a cross between Morden-003 (a commercial dry bean cultivar, well adapted to Manitoba and with good agronomic performance) and the germplasm line SMARC1N-PN1. This cross gave rise to 185 (F_{2:8}) recombinant inbred lines (RILs) (offspring of two genetically

distant parents that were inbred through eight generations). Through genetic recombination, some RILs will be deficient in the main storage proteins and therefore with increased sulphur amino acid content and will also have the desirable agronomic traits of Morden-003. The RILs with increased sulphur amino acid content also have to meet the standards of desirable agronomic traits for dry beans. This is a relevant pre-requisite in order to consider the RILs as elite lines for developing new cultivars from them with increased protein quality.

1.7 Agronomic characteristics of the navy bean cultivar Morden-003

Morden-003 is a navy bean (*Phaseolus vulgaris* L.) cultivar developed at the Lethbridge Research and Development Centre, in Alberta (Mündel et al., 2004). The final selection and testing was performed at the Morden Research and Development Centre, in Manitoba. Morden-003 was issued the registration number 5603 by the Variety Registration Office, Plant Production Division, Plant Products Directorate, Canadian Food Inspection Agency, Nepean, Ontario, Canada. It was released as a cultivar in 2003, after two years of narrow-row field trials testing (Mündel et al., 2004). Morden-003 has desirable agronomic traits (Mündel et al., 2004). It is well adapted to Manitoba for narrow-rows (23 cm spacing between rows) production. It is an early maturity cultivar with an average of 101 days. The average number of days to flower is 48. Morden-003 is resistant to the fungal disease anthracnose caused by *Colletotrichum lindemuthianum*. Morden-003 is a high yielding cultivar with an average production of 2.9 tn ha⁻¹. The hundred seed weight average of Morden-003 is 18.5 g. Acceptable weight of hundred seeds for commercial navy bean cultivars ranges from 17.5 to 20.5 g (Adams et al., 1986; Uebersax and Siddiq, 2012). Morden-003 has a determinate bush growth Type I with strong and erect stem and branches (Mündel et al., 2004). The cultivated *Phaseolus* species have been classified into four growth habits: Type I bush determinate with erect stem, Type II bush indeterminate with erect stem, Type III prostrate indeterminate, and Type IV climbing indeterminate (Singh, 1982). Morden-003 has a lodging score of 1.3 (scored on a 1 to

5 scale, where 1 is upright and 5 prostrate). Lodging is defined as the tendency of a plant to bend (Shah et al., 2016). Plants that are decumbent and with indeterminate growth habit, tend to lodge during pod filling, resulting in pods very close to the ground making them difficult to harvest (Eckert et al., 2011), and more exposed to soilborne diseases (Botelho et al., 2013) like the white mold (*Sclerotinia sclerotiorum*). Plants with a growth Type I and II strongly favor the efficiency of harvesting (Eckert et al., 2011).

1.8 Agronomic characteristics of the bean germplasm

SMARC1N-PN1

The bean germplasm SMARC1N-PN1 was developed and released by the Wisconsin Agricultural Experiment Station and the University of Wisconsin (Osborn et al., 2003). It has the registration number GS-21. SMARC1N-PN1 has an average of 20.7 g 100 seeds⁻¹, which is in the range of the market class of dry beans. It has a Type II growth habit, indeterminate bush with erect stem and branches. It is resistant to anthracnose. SMARC1N-PN1 is a late maturing cultivar with an average of 115 days. It has low yield with an average of 1.5 tn ha⁻¹ (Osborn et al., 2003).

1.9 Hypothesis

By modifying seed protein composition using selective breeding, protein quality in the common bean seed can be improved.

1.10 Research objectives

1 To determine the protein profiles of a total of 177 RILs grown in the field, by SDS-PAGE;

2 To quantify methionine, cysteine, and S-methylcysteine of a total of 185 RILs grown in the greenhouse, by HPLC (High Performance Liquid Chromatography);

3 To assess the effect associated with seed storage protein deficiency on the agronomic trait performance of the RILs;

4 To evaluate the genotype by environment interaction (G×E) on the RILs' agronomic traits in two different locations: Morden, MB and London, ON.

Chapter 2: Materials and Methods

2.1 Plant material

Germplasm line SMARC1N-PN1 was crossed with the navy bean cultivar Morden-003 by Dr. Anfu Hou at the Morden Research and Development Centre in Morden, MB. Initially there were 3 F₁ plants of this cross, which were self-fertilized giving F₂ plants. Each F₂ plant gave rise to a “population”. Through eight generations of inbreeding in the greenhouse, via Single Seed Descent (SSD), Dr. Hou obtained a total of 185 RILs for the 3 populations (RILs are F_{2:8}). The Population 1 has fifty-five RILs, the Population 2 sixty-seven RILs, and the Population 3 sixty-three RILs. Dr. Hou planted in the field the 185 RILs he obtained in the greenhouse. The planting took place in May of 2013 at the Morden Research and Development Centre. From the 185 RILs that initially were planted, the seeds of 177 RILs were harvested in late September (Some RILs were lost in the field, the seeds did not germinate). In January of 2014 the seeds of the 177 RILs were sent to Dr. Marsolais’ laboratory at the London Research and Development Centre in London, ON to determine their protein profile for phaseolin and lectins.

2.2 Determination of phaseolin and lectin protein profiles of the 177 field RILs

2.2.1 Soluble protein extractions

Seed samples (1.2 g) of each of the 177 RILs were ground with a Kleco ball mill. Soluble proteins were extracted from 50 mg ground tissue of each of the RILs using 1 ml of cold extraction buffer (50 mM Tris-HCl pH 8.0, 50 mM KCl, 1mM CaCl₂, 10% glycerol) containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM dithiothreitol (DTT). Extracts were centrifuged twice at 25,000 × g at 4°C for 20 min. Supernatants were placed in a 1.5 ml tube on ice (Vanden Bosch et al., 1989) to later run the SDS-PAGE gels.

2.2.2 Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis of the 177 field RILs

Five μl of $4 \times$ SDS protein sample buffer was added to 1.5 μl of the protein extract in a 1.5 ml tube. Samples were vortexed, centrifuged for 2 min at room temperature and heated at 99°C for 5 min. Five μl of these protein samples were loaded on a pre-cast NuPage Novex 4-12% gradient Bis-Tris gel with $\times 1$ MES running buffer (*life technologies*) and visualized by Coomassie staining (Taylor et al., 2008).

2.2.3 Determination of the phaseolin and lectin protein profiles of the 185 RILs grown in the greenhouse in Morden

The 185 RILs grown in the greenhouse in Morden were sent in 2011 to the London Research and Development Centre to determine their protein profile for phaseolin and lectins. The assessment of the protein profiles was performed by the laboratory technician Agnieszka Pajak following the same protocol described above for the assessment of the protein profiles of the 177 field RILs.

2.3 Methionine, cysteine, and S-methylcysteine quantification of the 185 RILs grown in the greenhouse at the Morden Research and Development Centre

Seeds of the RILs grown in Morden in the greenhouse were sent to the London and Research Development Centre for the quantification of methionine, cysteine and S-methylcysteine. Of the 185 RILs, twenty-seven were sent in 2012 to the SPARC BioCentre in Toronto for the same purpose of quantifying methionine, cysteine, and S-methylcysteine. The samples sent to the SPARC BioCentre were chosen based on their protein profile determined in 2011 by SDS-PAGE gels.

2.3.1 Hydrolysis phase

Hydrolysis needs to be performed in order to break the peptide bonds in the proteins for amino acid quantification. Ten mg of ground seeds of each RIL (three biological replications per sample; seeds were collected from three different plants of the same genotype) were hydrolyzed in shell vials (8×40 mm) in the presence of 450 μ l of 6 N HCl and 1% phenol (w/v) and 50 μ l of 12 mM norvaline (it was used as an internal standard). The shell glass vials were placed inside the reaction vials. Using an Eldex Workstation the reaction vial was placed under vacuum for 1-2 seconds. Thereafter the reaction vial was flushed with pre-purified nitrogen for 5 seconds. The above steps were repeated twice. After the third flushing, the reaction vial was sealed under vacuum and nitrogen flow. The reaction vial was placed in an oven (Johns Scientific) for 24 hr at 110 °C. After 24 hr the samples were taken out of the oven and transferred to 1.5 Eppendorf tubes. The samples in the Eppendorf tubes were centrifuged for 15 min at $20,000 \times g$. The supernatants were transferred to new 1.5 ml Eppendorf tubes. A 100 μ l aliquot of each sample was placed in shell glass vials in the speedvacuum evaporator (LABCONCO) for 1 hr. When the samples were dry, 500 μ l of HPLC water was added to each sample and left to stand for 10 min. Thereafter the samples were transferred to 0.2 μ m microfilter tubes (Life Sciences), and centrifuged for 2 min at $14,000 \times g$; the filters were removed (Fountoulakis and Lahm, 1998; Jafari et al., 2016).

2.3.2 Oxidation phase

Oxidation was performed only for cysteine, prior to the hydrolysis phase. Oxidation is necessary because decomposition of cysteine residues occurs during protein hydrolysis (Manneberg et al., 1995). Therefore, cysteine was determined as cysteic acid (Hirs, 1967). Performic acid (oxidation solution) was prepared by adding 100 μ l of 30% hydrogen peroxide to 900 μ l of 88% formic acid in a screw-capped 1.5 ml centrifuge tube. The oxidation solution was allowed to stand at room temperature for 60 min. Ten mg of ground seeds of each RIL were oxidized in shell glass vials

and heated at 50 °C for 5 min. One hundred µl of the prepared oxidation solution was added to each shell vial and heated for 30 min (Hirs, 1967). Thereafter, each vial was placed in the speedvacuum evaporator for 90 min to dry the samples. When the samples were dry they underwent the hydrolysis phase following the same protocol as detailed above.

2.3.3 Separation and quantification of the amino acids by HPLC (High Performance Liquid Chromatography)

The amino acids were analyzed according to Jafari et al. (2016). The filtered samples were diluted 1:10, and 1 µl was loaded in the HPLC. The amino acids underwent a pre-column derivatization with *o*-phthalaldehyde (OPA). They were separated on a Zorbax column of a chromatography Agilent 1290 Infinity HPLC system (Agilent Technologies, Mississauga, ON). The fluorescence detector 1260 FLD detected the OPA derivatized amino acids. A calibration file consisting of the response factor of each amino acid was used to calculate the concentration of amino acids present in the sample. The calculation of the concentration detected by the HPLC involves dividing the peak area corresponding to a given amino acid, (methionine, cysteine and *S*-methylcysteine) by its response factor to give a value in pmol/µl (Rutherford and Gilani, 2009). The amino acids in the SPARC BioCentre were analyzed according to Taylor et al. (2008). The amino acids underwent a pre-column derivatization using phenylisothiocyanate (PITC) to produce phenylthiocarbamyl (PTC) amino acids. The amino acids were quantified as phenylisothiocyanate derivatives (PITC)

2.4 Assessment of the agronomic traits of the parent lines Morden-003 and SMARC1N-PN1 and of the RILs of Population 1, Population 2, and Population 3

2.4.1 Seed material and planting methodology

The seeds of the parent lines Morden-003 and SMARC1N-PN1 and of the 185 RILs were sent to London from the Morden Research and Development Centre, in early May of 2014 and 2015. The planting for the field trials of 2014 and 2015 took place in two different locations: in Morden, MB (49.1923° N, 98.0977° W) at the Morden Research and Development Centre, in late May, and in London, ON (42.9849° N, 81.2453° W) at the London Research and Development Centre, the first week of June. The seeds were planted using a three funnel planter. In each location, the seeds of each RIL were planted along with their parent lines, according to their respective population. The experimental design within each population was a randomized complete block design with three replications. Each plot size was 3.5 m² (5 m length by 0.70 m width, with 0.70 m spacing between plots). One hundred twenty seeds of each genotype were planted in each plot. The planting methodology was followed for the two year field trials in the two locations. The traits tested for each genotype in the two locations and for the two year trials were: days to flowering (DTF), plant height (PHT), lodging (the tendency of a plant to bend), pod height (PDH), growth type (GT), days to maturity (DTM), yield (YLD), and hundred seed weight (HSW). Days to flowering was designated as the number of days after planting when 50% of the plants in the plot had at least one open flower. The plant height data correspond to the average height (cm) of three plants randomly chosen in the plot. The plant height was measured at flowering stage. Days to maturity were determined as the number of days after planting when 90% of the plants in the plot were ready to harvest. Lodging was determined at the time of maturity on a 1 to 5 scale; 1 = upright plants, 5 = plants lie flat on the ground. Growth type was measured on a scale of 1= determinate bush with strong and erect stem and branches, 2 =

indeterminate bush, with erect stem and branches, 3 = indeterminate bush with weak and prostrate stem and branches, 4 = indeterminate climbing habit with weak, long and twisted stem and branches. Pod height, was scored as the percentage of pods in the plant above 5 cm from the ground. Pod height was measured when the leaves of the plants were fallen. Following harvest, yield and hundred seed weight (g 100 seeds⁻¹) were measured. The yield was measured on a plot basis (g plot⁻¹) and converted to tn ha⁻¹ for the statistical analyses.

2.5 Data analyses

2.5.1 Determination of the phaseolin and lectin protein profiles of the 177 field RILs

The SDS-PAGE gels allowed me to visualize the phaseolin and lectin profiles of each of the RILs. On the gels I included protein samples of the parent lines Morden-003 and SMARC1N-PN1. I classified the RILs into four groups according to the phenotypic expression for phaseolin and lectins when compared to the parental lines protein profile. The four groups were classified as: MM, MS, SM, and SS. M and S stand for the protein phenotypic expression of the parent lines Morden-003 and SMARC1N-PN1, respectively.

2.5.2 Methionine, cysteine, and S-methylcysteine quantification

I quantified the concentration of the amino acids in nmoles/mg. The mean values of the sulphur amino acid concentrations for each of the RILs were compared descriptively against the sulphur amino acids concentrations of the parental lines Morden-003 and SMARC1N-PN1, used as control lines in my study.

2.5.3 Data analyses on the assessment of the agronomic traits

I conducted three-way ANOVAs to test for differences in agricultural traits based on genotype (RILs and parental lines), year (2014 and 2015), location (Morden and London), and all possible interaction terms. Differences were significant at the probability level less than 0.05. The pattern analysis was performed using SPSS 11.0 on the basis of observed means. In the present study, the independent variable of primary interest is the genotypes, therefore they were considered as the moderators. If significant effects of genotype were identified, I used Dunnett's *post-hoc* test to compare individual RILs and the parent line SMARC1N-PN1 to the parent line Morden-003. The growth type and lodging were analyzed as descriptive categories.

Chapter 3 Results

3.1 Phaseolin and lectin protein profiles of the 185 RILs grown in the greenhouse in Morden

The protein profiles of the parental lines Morden-003 and SMARC1N-PN1 and of the 185 RILs were analyzed by SDS-PAGE of soluble protein extract from mature seeds. Figure 3.1 shows the different electrophoresis bands for the proteins: phaseolin and lectins. Phaseolin consists of 2 to 6 polypeptides of molecular weights between 43 and 54 kDa (Osborn and Brown, 1988). The parental Morden-003 showed two major bands in the range of 43 to 55 kDa that correspond to the globulin protein phaseolin. Lectin polypeptides have a molecular weight in the range of 27 to 37 kDa (Bliss and Brown, 1983; Sathe, 2002). Morden-003 showed two bands in a range of 27 and 34 kDa; the latter appears as a prominent band. The two bands correspond to lectins. The parental SMARC1N-PN1 is deficient in phaseolin and lectins (Hartweck and Osborn, 1997) consequently the bands for phaseolin and lectins on the SDS-PAGE were absent in this germplasm line. The protein profile for phaseolin and lectins classified the RILs grown in the greenhouse as: 61 with the parent line Morden-003 profile both for phaseolin and lectins (MM), 39 with the parent line SMARC1N-PN1 profile for phaseolin and with the parent line Morden-003 profile for lectins (SM), 58 with the parent line Morden-003 profile for phaseolin and with the parent line SMARC1N-PN1 profile for lectin (MS), and 25 with the parent line SMARC1N-PN1 profile both for phaseolin and lectins (SS). An additional three lines had the MM profile with abundant lectin (mM) or a subset of the MM grouping.

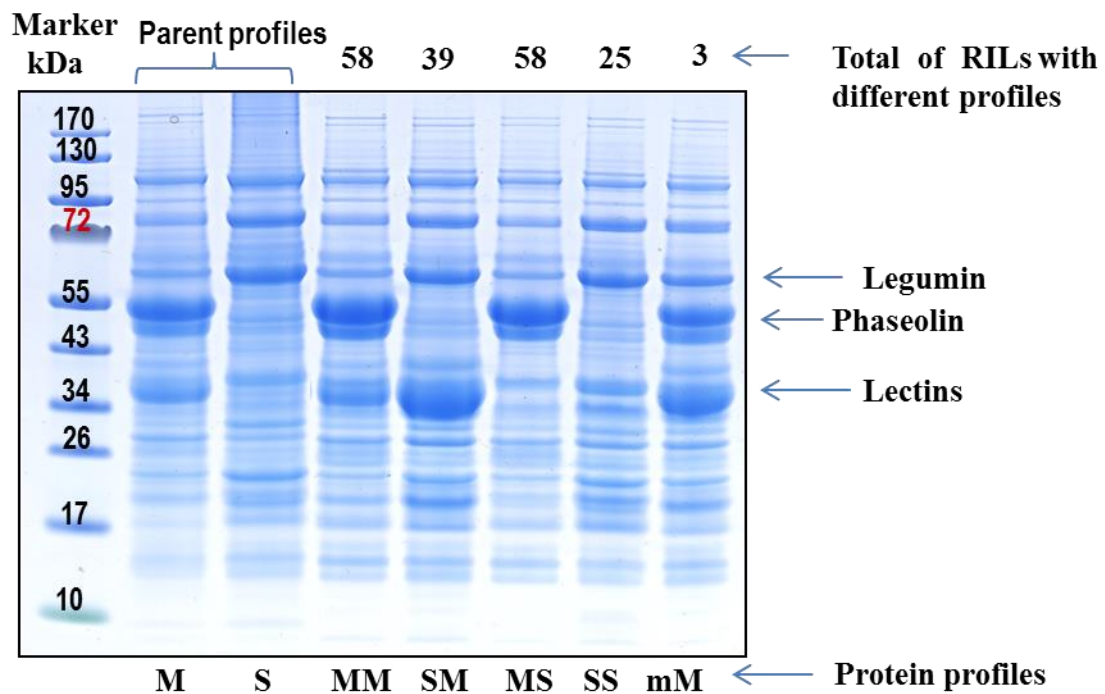


Figure 3.1 SDS-PAGE of phaseolin and lectin profiles for the parent lines Morden-003 and SMARC1N-PN1 and the RILs grown in the greenhouse in Morden. The gel percentage used was 4-12 acrylamide and stained with Coomassie Brilliant Blue. The RILs' were classified according to the phenotypic expression for phaseolin and lectins when compared to the protein profiles of the parental lines. M and S stand for the protein phenotypic expression of the parent lines Morden-003 and SMARC1N-PN1, respectively. MM = Morden-003 protein profile both for phaseolin and lectins, SS = SMARC1N-PN1 protein profile both for phaseolin and lectins, SM = SMARC1N-PN1 protein profile for phaseolin and Morden-003 protein profile for lectins, MS = Morden-003 protein profile for phaseolin and SMARC1N-PN1 protein profile for lectins. mM = RILs with the MM protein profile, but with abundant lectins.

3.1.1 Phaseolin and lectin protein profiles of the 177 RILs grown in the field in Morden

Seeds of the 185 RILs were planted in the field in Morden, Manitoba (49.1023° N, 98.0977° W) in the cropping season of 2013 for field trials. Seeds of a total of 177 RILs were harvested. The SDS-PAGE of soluble protein extracts from mature seeds classified the RILs as: 94 with the parent line Morden-003 protein profile both for phaseolin and lectins (MM), 7 with the parent line SMARC1N-PN1 both for phaseolin and lectins (SS), 24 with the parent line SMARC1N-PN1 protein profile for phaseolin and with the parent line Morden-003 protein profile for lectins (SM), and 52 with the parent line Morden-003 protein profile for phaseolin and with the parent line SMARC1N-PN1 protein profile for lectins (MS) (Figure 3.1.2).

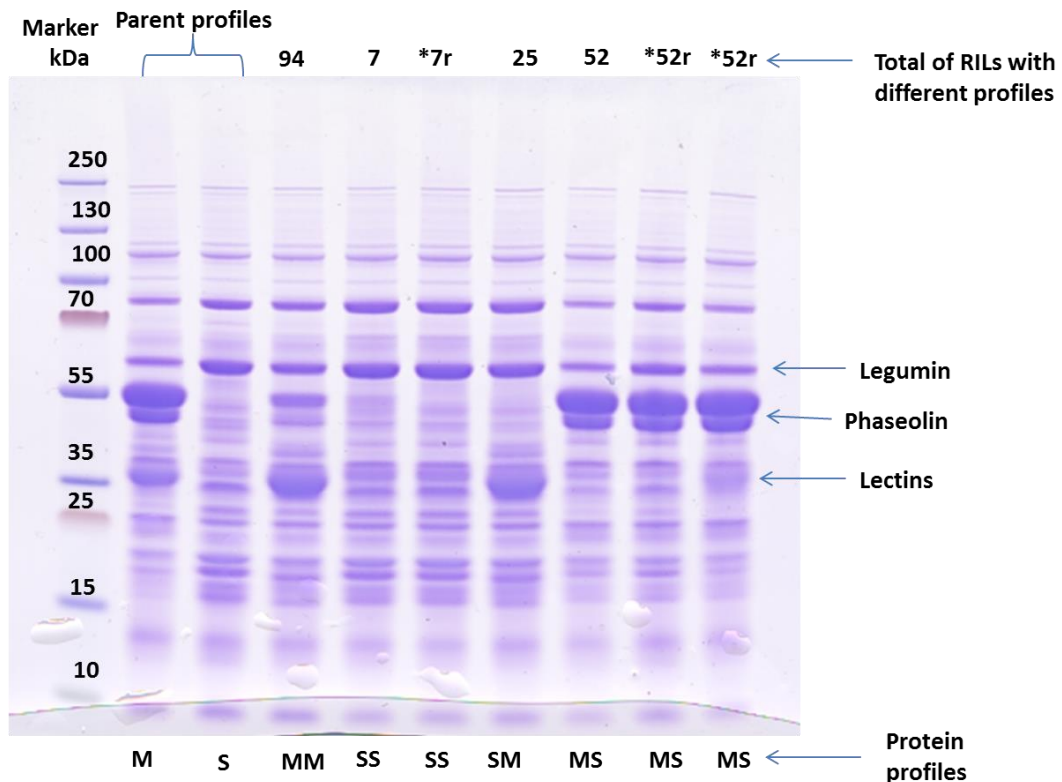


Figure 3.1.1 SDS-PAGE of phaseolin and lectin profiles for the parent lines Morden-003 and SMARC1N-PN1 and the RILs grown in the field in Morden in 2013 (*7r and *52r are repetitions of the neighbour profile). The gel percentage used was 4-12 acrylamide and stained with Coomassie Brilliant Blue. The RILs' were classified according to the phenotypic expression for phaseolin and lectins when compared to the protein profiles of the parental lines. M and S stand for the protein phenotypic expression of the parent lines Morden-003 and SMARC1N-PN1, respectively. MM = Morden-003 protein profile both for phaseolin and lectins, SS = SMARC1N-PN1 protein profile both for phaseolin and lectins, SM = SMARC1N-PN1 protein profile for phaseolin and Morden-003 protein profile for lectins, MS = Morden-003 protein profile for phaseolin and SMARC1N-PN1 protein profile for lectins.

3.2 Quantification of methionine and cysteine and *S*-methylcysteine of the parent lines Morden-003 and SMARC1N-PN1 and the RILs grown in the greenhouse at the Morden Research and Development Centre

Methionine, cysteine and *S*-methylcysteine concentrations were quantified in the mature seeds of a total of 185 RILs with different protein profiles, grown in the greenhouse, at the Morden Research and Development Centre in Morden, MB. The sulphur amino acid concentration of the two parental lines: Morden-003 and SMARC1N-PN1 were used as controls for quantifying variations in the sulphur amino acid concentration of the RILs. The previous results on the protein profiles by SDS-PAGE revealed the presence of phaseolin and lectins in the cultivar Morden-003. Hartweck and Osborn (1997) have previously established the absence of phaseolin, phytohemagglutinin, and arcelin in SMARC1N-PN1. My study confirmed the absence of the storage proteins in SMARC1N-PN1 (Figure 3.1). Taylor et al. (2008) found that the deficiency in all three seed storage proteins in the germplasm SMACR1N-PN1 was related to increased methionine and cysteine and a decrease in *S*-methylcysteine. Increased methionine and cysteine occurs due to a redirection of the sulphur from *S*-methylcysteine, mostly to the cysteine protein pool and to a lesser extent to the methionine protein pool. The results on the sulphur amino acid extractions and quantifications, performed by myself and the extractions performed at the SPARC BioCentre in Toronto, are presented separately. The concentration of methionine and cysteine is presented as a summation of both amino acids. The dietary requirements consider both sulphur amino acids together (Young and Pellett, 1991; Montoya et al., 2010).

3.2.1 Quantification of total methionine and cysteine and, total *S*-methylcysteine performed at the London Research and Development Centre

Total methionine-cysteine, and total *S*-methylcysteine concentrations in mature seeds of the parental lines Morden-003 and SMARC1N-PN1 and the RILs with different protein profiles are shown in Figures 3.2.1-a, and 3.2.1-b, respectively. They correspond to mean values of three biological repetitions as explained in the Materials and Methods. The total methionine-cysteine concentration of the parent line Morden-003 was 42.4 nmoles mg⁻¹ (22.4 mg g⁻¹ protein). The parental SMARC1N-PN1 had a concentration of total methionine-cysteine of 51.6 nmoles mg⁻¹ (27.3 mg g⁻¹ protein). SMARC1N-PN1 had a lower content of total *S*-methylcysteine, 9.6 nmoles mg⁻¹ (5.1 mg g⁻¹ protein), in response to a redirection of the sulphur to the methionine and cysteine protein pool. Morden-003 had a higher concentration of total *S*-methylcysteine, 16.8 nmoles mg⁻¹ (9.1 mg g⁻¹ protein). All sixteen RILs with the SMARC1N-PN1 protein profile (SS) both for phaseolin and lectins showed increased methionine-cysteine concentrations as compared to the parental Morden-003. The RILs with the SS protein profile showed increased methionine-cysteine concentrations between 48.8 and 64.9 nmoles mg⁻¹ (25.8 to 34.4 mg g⁻¹ protein) as compared with Morden-003. Despite the increase in total methionine-cysteine in all sixteen RILs with the SS protein profile, only ten showed decreased total *S*-methylcysteine, between 9.1 and 16.3 nmoles mg⁻¹ (4.9 to 8.8 mg g⁻¹ protein), as compared with the parental Morden-003. Among the RILs with the SM protein profile, seven out of thirty showed increased methionine-cysteine content, between 43.7 and 51.6 nmoles mg⁻¹ (23.1 to 27.1 mg g⁻¹ protein), as compared with the parental Morden-003. Among the seven RILs with increased total methionine-cysteine only two showed a decrease in total *S*-methylcysteine 11.1 and 13.3 nmoles mg⁻¹ (5.9 and 1.7 mg g⁻¹ protein), respectively, as compared with Morden-003. As for the RILs with the MS protein profile, one among fifty-two showed increased total methionine-cysteine 45.7 nmoles mg⁻¹ (24.1 mg g⁻¹) with respect to Morden-003.

Despite the increase in total methionine-cysteine in this RIL, there was no decrease in total *S*-methylcysteine as compared with the parental Morden-003. The RILs with the Morden-003 protein profile both for phaseolin and lectins (MM) showed no increased total methionine-cysteine, as compared with the parental Morden-003. The mean values quantified for methionine-cysteine in the RILs with the MM protein profile ranged from 23.5 to 41.0 nmoles mg⁻¹ (12 to 21.4 mg g⁻¹ protein). The lower sulphur amino acid content in the RILs with the MM protein profile was associated with the presence of phaseolin and lectins in the seed. Some of the RILs with the MM protein profile showed decreased total *S*-methylcysteine, between 6.8 and 16.7 nmoles mg⁻¹ (3.6 to 9 mg g⁻¹ protein) as compared with Morden-003.

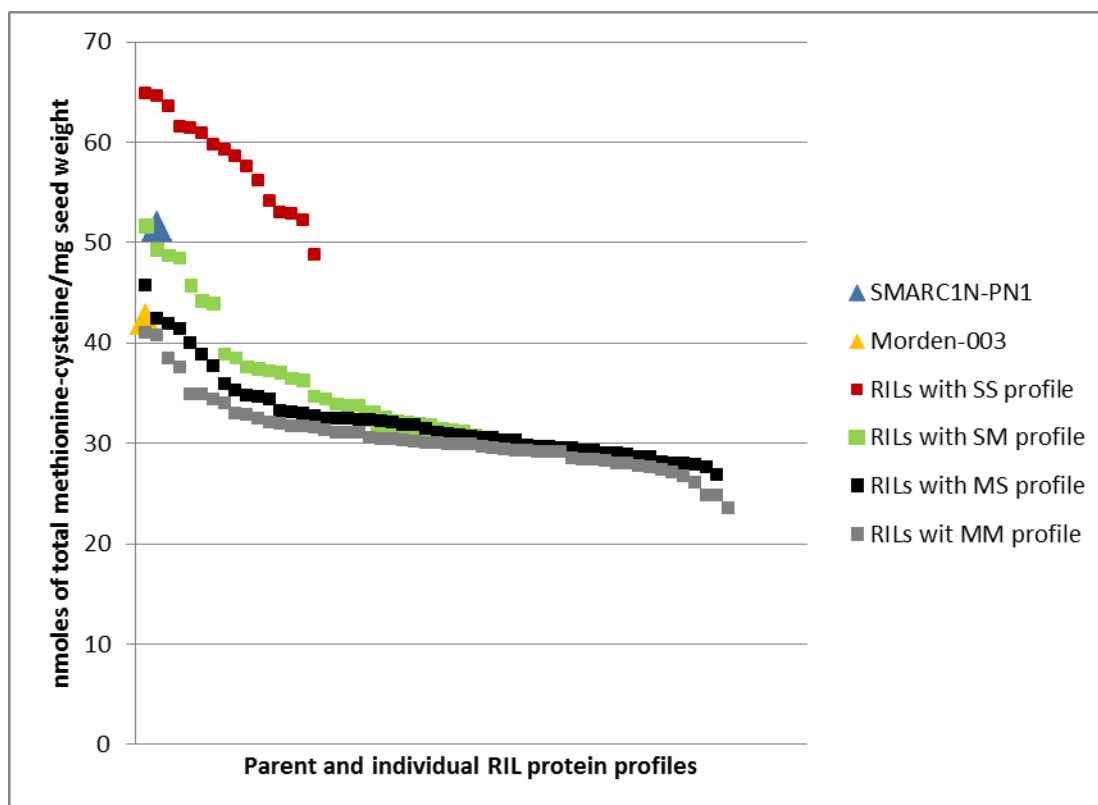


Figure 3.2.1-a Total methionine-cysteine concentration in mature seeds of the parent lines Morden-003 and SMARC1N-PN1, and the RILs with different protein profiles grown in the greenhouse in Morden. Values are expressed in nmoles mg^{-1} of seed weight; $n = 3$. MM = Morden-003 protein profile both for phaseolin and lectins, SS = SMARC1N-PN1 protein profile both for phaseolin and lectins, SM = SMARC1N-PN1 protein profile for phaseolin and Morden-003 protein profile for lectins, MS = Morden-003 protein profile for phaseolin and SMARC1N-PN1 protein profile for lectins.

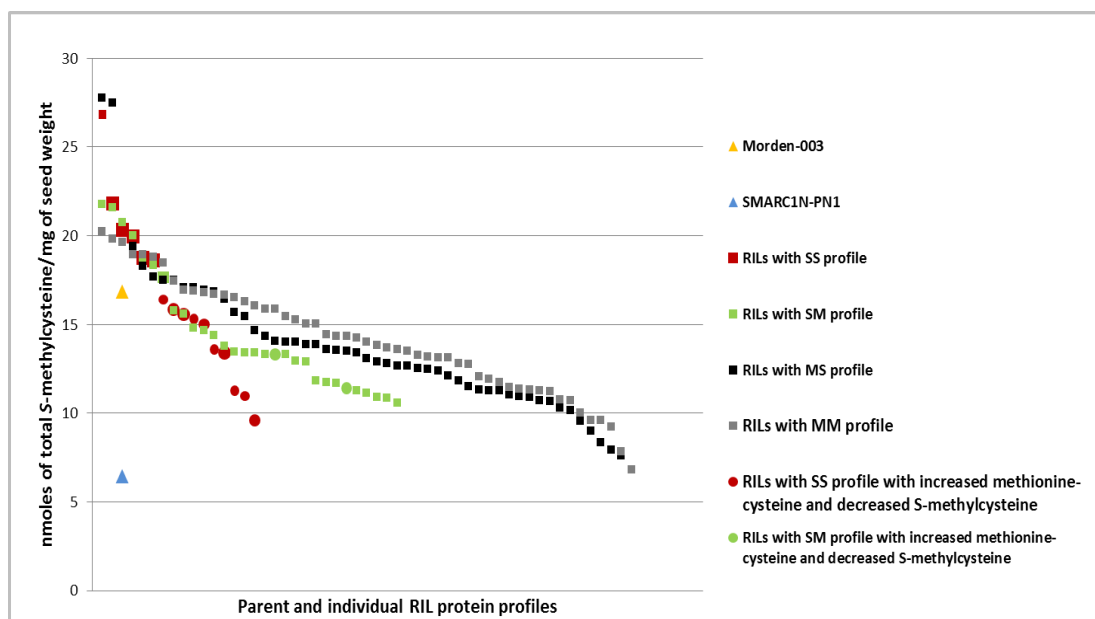


Figure 3.2.1-b Total *S*-methylcysteine concentration in mature seeds of the parent lines Morden-003 and SMARC1N-PN1, and the RILs with different protein profiles grown in the greenhouse in Morden. Values are expressed in nmoles mg⁻¹ of seed weight; n=3. The circles for the RILs with SS and SM protein profiles (red and green colour, respectively) represent the RILs that have both increased methionine-cysteine and decreased *S*-methylcysteine. MM = Morden-003 protein profile both for phaseolin and lectins, SS = SMARC1N-PN1 protein profile both for phaseolin and lectins, SM = SMARC1N-PN1 protein profile for phaseolin and Morden-003 protein profile for lectins, MS = Morden-003 protein profile for phaseolin and SMARC1N-PN1 protein profile for lectins.

3.2.2 Quantification of total methionine, cysteine, and total *S*-methylcysteine performed at the SPARC Biocentre

Based on the SDS-PAGE results of the protein profiles of the 185 RILs, seeds of twenty-seven RILs with different protein profiles together with seeds of the parent lines Morden-003 and SMARC1N-PN1 were sent to the SPARC Biocentre in

Toronto for quantification of methionine, cysteine and *S*-methylcysteine. Total methionine-cysteine, and total *S*-methylcysteine concentrations in mature seeds of the parent lines and the RILs are shown in Figure 3.2.2-a, and 3.2.2-b, respectively. Total methionine-cysteine concentration of the parent line Morden-003 was 50.8 nmoles mg^{-1} (27.5 mg g^{-1}). The parental SMARC1N-PN1 had 45.26 nmoles mg^{-1} (13.7 mg g^{-1} protein). SMARC1N-PN1 had a lower content of total *S*-methylcysteine, 19.8 nmoles mg^{-1} (10.6 mg g^{-1} protein), than Morden-003, 34.4 nmoles mg^{-1} (18.5 mg g^{-1} protein). Eight out of ten RILs with SS protein profile had increased total methionine-cysteine concentration between 52 and 59.8 nmoles mg^{-1} (27.3 and 31.2 mg g^{-1} protein) as compared with Morden-003. Among the eight RILs with increased total methionine-cysteine, seven out of eight RILs showed decreased total *S*-methylcysteine, in the range of 23.4 to 25.0 nmoles mg^{-1} (12.6 to 13.5 mg g^{-1} protein). Among the five RILs with the SM profile, three had increased total methionine-cysteine, between 52.6 and 54.5 nmoles mg^{-1} (27.7 and 28.7 mg g^{-1} protein) as compared with Morden-003. Two of the three RILs had decreased total *S*-methylcysteine, 31.4 nmoles mg^{-1} and 33.48 nmoles mg^{-1} (16.5 and 18 mg g^{-1} protein), respectively. Three out of six RILs with the MS protein profile increased the total methionine-cysteine content between 50.9 and 54.4 nmoles mg^{-1} (27.32 and 29.12 mg g^{-1} protein). Only one of the three RILs had decreased total *S*-methylcysteine, 30.5 nmoles mg^{-1} (16.4 mg g^{-1} protein), as compared with Morden-003. Two of the six RILs with the MM protein profile showed increased total methionine-cysteine concentration of 51.6 and 54.5 nmoles mg^{-1} (27.39 and 29.32 mg g^{-1} protein), respectively. None of the two RILs decreased the total *S*-methylcysteine concentration with respect to Morden-003.

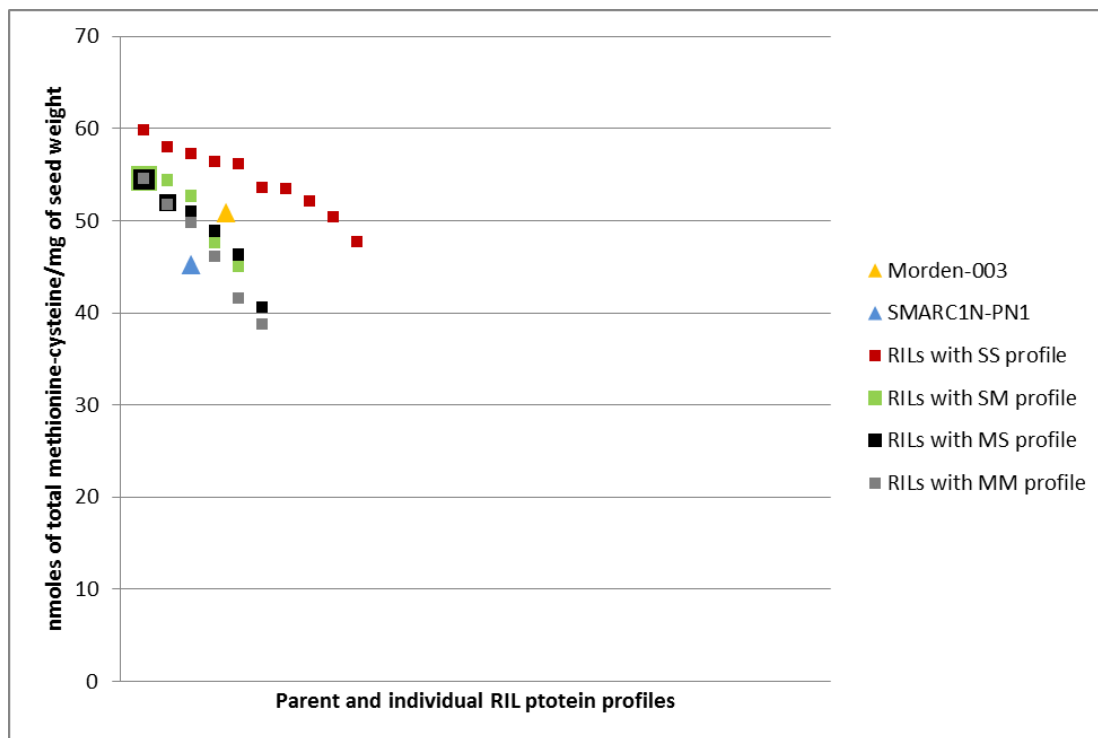


Figure 3.2.2-a Total methionine-cysteine concentration in mature seeds of the parent lines Morden-003 and SMARC1N-PN1, and the twenty-seven RILs with different protein profiles grown in the greenhouse in Morden. Values are expressed in nmoles mg^{-1} of seed weight; $n = 3$. MM = Morden-003 protein profile both for phaseolin and lectins, SS = SMARC1N-PN1 protein profile both for phaseolin and lectins, SM = SMARC1N-PN1 protein profile for phaseolin and Morden-003 protein profile for lectins, MS = Morden-003 protein profile for phaseolin and SMARC1N-PN1 protein profile for lectins.

3.2.3 Quantification of total methionine, cysteine, and total *S*-methylcysteine of the RILs with SS protein profile grown in the field in London and Morden in the cropping season of 2014

Five out of the seven field RILs previously identified as having the SS protein profile by SDS-PAGE were randomly chosen for sulphur amino acid quantification. The seeds of the 2014 field trials conducted in London and Manitoba were used for the amino acid analysis in Dr. Marsolais' laboratory. The total methionine-cysteine, and total *S*-methylcysteine concentration in mature seeds of the parent lines Morden-003 and SMARC1N-PN1 and the field RILs with SS protein profile are shown in Figures 3.2.3-a, and 3.2.3-b, respectively. They correspond to mean values of three technical replicates of seeds of the same experimental plot. The parent Morden-003 had 30.7 nmoles mg⁻¹ (16 mg g⁻¹ protein) of methionine-cysteine and 17.5 nmoles mg⁻¹ of *S*-methylcysteine (9.4 mg g⁻¹ protein). As expected, SMARC1N-PN1 had a higher content of total methionine-cysteine, 41.4 nmoles mg⁻¹ (21.7 mg g⁻¹ protein) and decreased total *S*-methylcysteine, 11.1 nmoles mg⁻¹ (5.9 mg g⁻¹ protein). All five RILs with SS profile had increased total methionine-cysteine, between 33.0 and 41.6 nmoles mg⁻¹ (17.1 and 21.8 mg g⁻¹ protein) as compared with the parent Morden-003. All five RILs with SS protein profile showed decreased total *S*-methylcysteine content in the range of 9.0 to 12.6 nmoles mg⁻¹ (4.8 to 6.8 mg g⁻¹ protein); as compared with Morden-003.

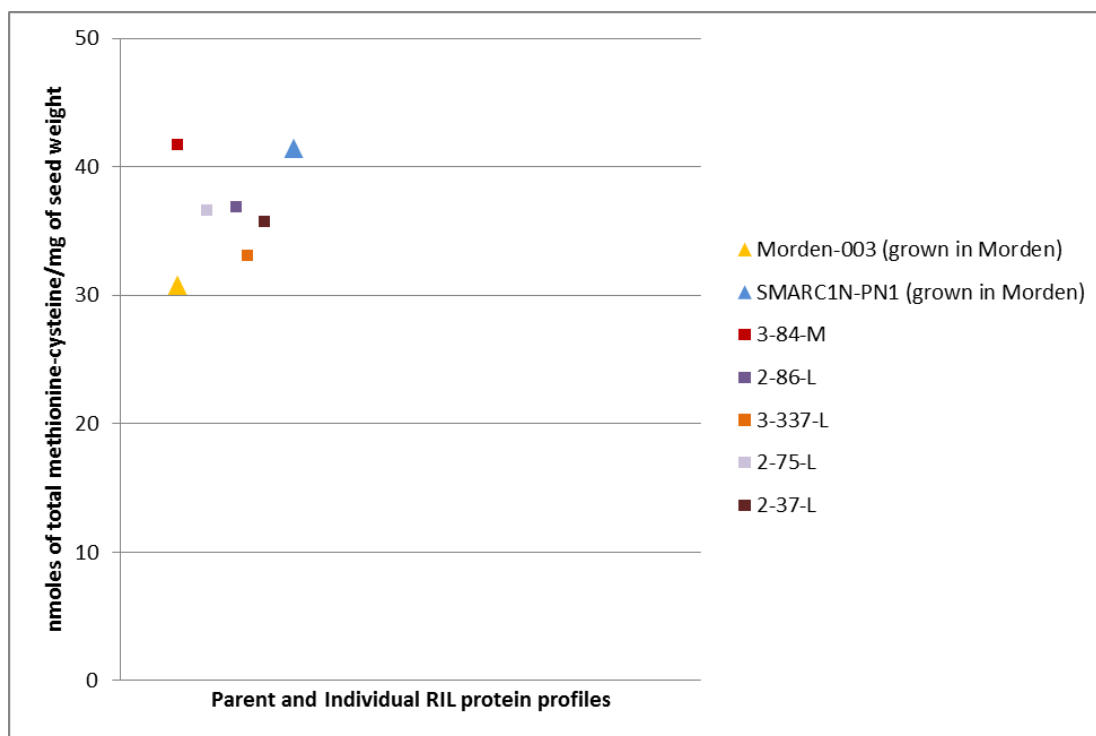


Figure 3.2.3-a Total methionine-cysteine concentration in mature seeds of the parent lines Morden-003 and SMARC1N-PN1, and the RILs with SS protein profile grown in the cropping season of 2014 in Morden and London. The numbers 2 and 3 that appear before the number of the RIL identify the population they correspond to. M = Morden, L = London. Values are expressed in nmoles mg^{-1} of seed weight; $n = 3$. MM = Morden-003 protein profile both for phaseolin and lectins, SS = SMARC1N-PN1 protein profile both for phaseolin and lectins, SM = SMARC1N-PN1 protein profile for phaseolin and Morden-003 protein profile for lectins, MS = Morden-003 protein profile for phaseolin and SMARC1N-PN1 protein profile for lectins.

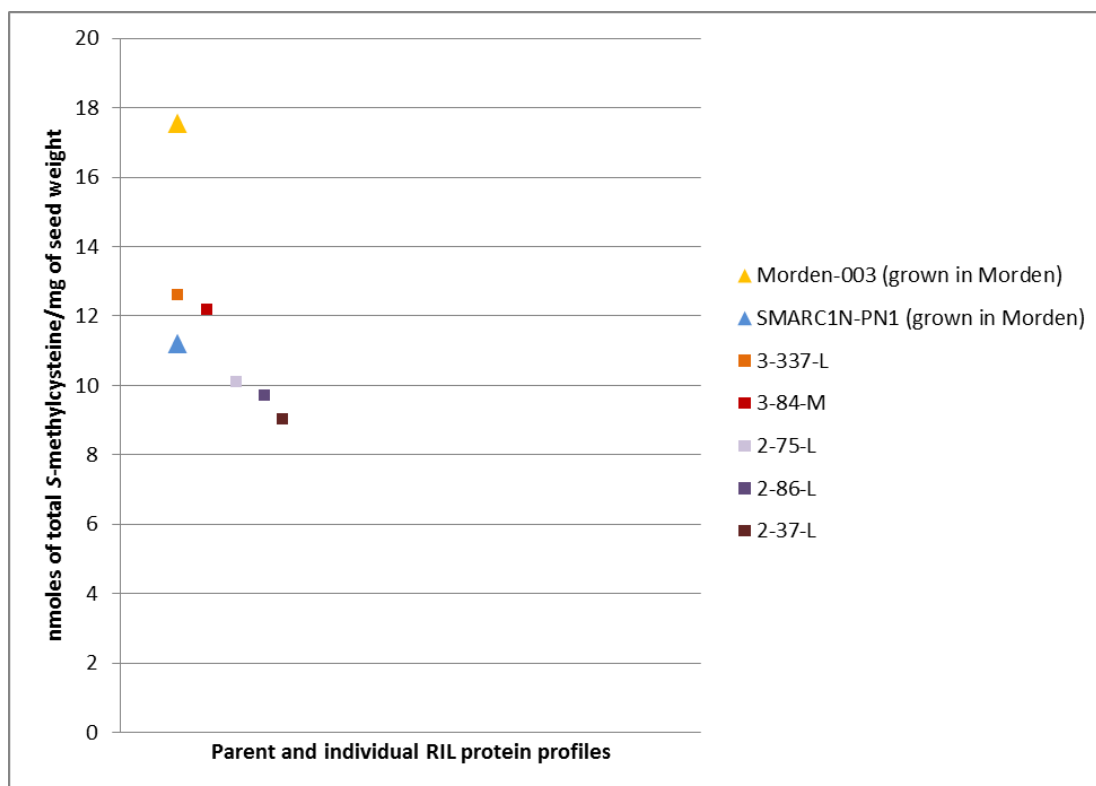


Figure 3.2.3-b Total S-methylcysteine concentration in mature seeds of the parent lines Morden-003 and SMARC1N-PN1, and the RILs with SS protein profile grown in cropping season of 2014 in Morden and London. The numbers 2 and 3 that appear before the number of the RIL identify the population they correspond to. M=Morden, L = London. Values are expressed in nmoles mg⁻¹ of seed weight; n =3. MM = Morden-003 protein profile both for phaseolin and lectins, SS = SMARC1N-PN1 protein profile both for phaseolin and lectins, SM = SMARC1N-PN1 protein profile for phaseolin and Morden-003 protein profile for lectins, MS = Morden-003 protein profile for phaseolin and SMARC1N-PN1 protein profile for lectins.

3.3 Assessment of the impact of major protein deficiency on the agronomic trait performance of the RILs with SS protein profile in two different locations

The SDS-PAGE for the field RILs showed that a total of seven RILs were deficient in phaseolin and lectins (RILs with SS protein profile). The previous results revealed that the RILs deficient in phaseolin and lectins have increased total methionine-cysteine. The agronomic traits are affected by the growing environment as well as by the genotype (Mohammadi et al., 2011). Therefore the objective of the field trials was to assess the agronomic performance of the genotypes deficient in phaseolin and lectins, in two different environments. Eight agronomic traits were measured. The three-way ANOVA of differences in agricultural traits based on genotype and environment and all possible interactions is presented in Tables 3.3.1-a, and 3.3.1-b, for Population 2 and 3, respectively. A descriptive analysis was performed for growth type and lodging for the genotypes in both populations. The results are presented in Tables 3.3.1.8-a, 3.3.1.8-b, and 3.3.1.9-a, and 3.3.1.9-b, respectively. As mentioned in the background section the RILs with increased sulphur amino acid content have to meet the standards of desirable agronomic traits for dry beans. That is to say, in the present study, the agronomic performance of the RILs has to be similar to the parental Morden-003 which is a cultivar well-adapted to Manitoba and with good agronomic performance. I conducted a Dunnett's *post-hoc* test to compare the mean agronomic trait values of the RILs and the parental SMARC1N-PN1 that were lower than Morden-003 (control line) to see if these differences might indicate a lower agronomic performance. Significant variations for each agronomic trait were detected at the 0.05 level of probability. The mean values for each agronomic trait of the genotypes of Population 2 and 3 are shown in the Tables 3.3.1.1-a, and 3.3.1.1-b, respectively. The mean agronomic performance of the parental Morden-003 tested in the present study is compared descriptively with the previous study by Mündel et al. (2004) on the same genotype (Table 3.3.1.1-c).

3.3.1 Genotype by environment interaction

For the analyses of genotype by environment interaction the results of the agronomic traits of both years and both locations were analyzed together. An interaction exists when the effect of one independent variable on a dependent variable depends on another independent variable. In Population 2 and 3 there was no significant interaction between genotype, year and location ($P > 0.05$) except for days to flowering, for both populations ($P < 0.05$), and pod height for Population 3 ($P < 0.05$). In Population 2 and 3, the main effects of genotype, location, and trial year were the most important source of the significant variation ($P < 0.05$) in the agronomic traits.

Table 3.3.1-a. A three-way ANOVA of agronomic traits between genotypes, location, year and their interactions for the RILs of Population 2 across the two year trials (2014-2015) and the two different locations (London and Morden).

Effects	Agronomic traits					
	DTF (days)	DTM (days)	PHT (cm)	YLD (tn ha ⁻¹)	PDH (%)	HSW (g 100 seeds ⁻¹)
Genotype (G)	.001*	.001*	.021*	.001*	.066	.270
Year (Yr)	.002*	.001*	.001*	.001*	.207	.688
Location (Loc)	.001*	.001*	.334	.001*	.001*	.001*
G × Yr	.001*	.001*	.920	.016*	.362	.957
G × Loc	.001*	.334	.143	.382	.279	.421
Yr × Loc	.001*	.061	.023*	.618	.001*	
G × Yr × Loc	.001*	.280	.342	.215	.526	

*Effects are significant at 0.05 level of probability; DTF=days to flowering, DTM = days to maturity, PHT = plant height, YLD = yield, PDH = pod height, HSW = hundred seed weight (blank cells = data not recorded).

Table 3.3.1-b. A three-way ANOVA of agronomic traits between genotypes, location, year and their interactions for the RILs of Population 3 across the two year trials (2014-2015) and the two different locations (London and Morden).

Effects	Agronomic Traits					
	DTF (days)	DTM (days)	PHT (cm)	YLD (tn ha ⁻¹)	PDH (%)	HSW (g 100 seeds ⁻¹)
Genotype (G)	.001*	.001*	.001*	.014*	.001*	.001*
Year (Yr)	.001*	.001*	.001*	.001*	.115	.002*
Location (Loc)	.001*	.061	.594	.001*	.001*	.001*
G × Yr	.001*	.002*	.966	.461	.252	.015*
G × Loc	.001*	.929	.013*	.635	.009*	.060
Yr × Loc	.001*	.349	.001*	.931	.001*	
G × Yr × Loc	.001*	.504	.288	.087	.008*	

*Effects are significant at 0.05 level of probability; DTF = days to flowering, DTM = days to maturity, PHT = plant height, YLD = yield, PDH = pod height, HSW = hundred seed weight (blank cells = data not recorded).

3.3.1.2 Days to flowering

Days to flowering is considered an important trait of bean cultivars because it is positively correlated to days to maturity (González et al., 2016). The seed of *P. vulgaris* start to form between 10 to 31 days after flowering (Hall et al., 1980). This means, the earlier a crop flowers, the earlier it is going to reach the maturity stage and be ready for harvesting. Therefore, days to flowering can be used as an indirect marker for maturity (González et al., 2016). A short-cycle bean genotype has the advantage of reducing disease occurrence, lowering soil “use” time and thus facilitating crop rotation (Zilio et al., 2013). In Population 2 and 3 of the present research, Morden-003 had an average number of days to flowering of 46. This mean value is close to the 48 days of flowering reported by Mündel et al. (2004) for Morden-003. The RILs of both populations had a mean value of days to flowering in the range of 44 to 47. Only the RIL 3-59 had significantly fewer days to flowering ($P < 0.05$). The parental SMARC1N-PN1 also had a mean value similar to Morden-003.

Table 3.3.1.1-a Two-year (2014-2015) average of days to flower, days to maturity, plant height, yield, pod height, and hundred seed weight, for the genotypes of Population 2 over two different trial sites: London, ON, and Morden, MB.

Genotypes	Agronomic traits					
	DTF (days)	DTM (days)	PHT (cm)	YLD (tn ha ⁻¹)	PDH (%)	HSW (g 100 seeds ⁻¹)
Morden-003 (control line)	46 ± 2.5	95 ± 5.5	43 ± 5.2	1.9 ± 0.6	73 ± 16.1	17.2 ± 1.5
SMARC1N-PN1	48 ± 2.2	109 ± 9.3	50 ± 10.2	1.9 ± 0.8	69 ± 14.7	17.2 ± 1.1
2-37	46 ± 3.1	101 ± 7.3	48 ± 7.5	1.9 ± 0.6	70 ± 9.5	18.3 ± 1.0
2-75	46 ± 2.5	98 ± 5.7	45 ± 5.9	1.5 ± 1	69 ± 18.8	18.1 ± 1.9
2-86	45 ± 1.0	99 ± 6.1	41 ± 11.1	1.1* ± 0.2	62* ± 18.6	17.6 ± 1.5

t-Dunnett (< control); * Effects are significant at 0.05 level of probability; n = 3; DTF = days to flowering, DTM = days to maturity, PHT = plant height, YLD = yield, PDH = pod height, (percentage of pods above 5 cm from the ground), HSW = hundred seed weight

Table 3.3.1.1-b Two-year (2014-2015) average of days to flower, days to maturity, plant height, yield, pod height, hundred seed weight, for the genotypes of Population 3 over two different trial sites: London, ON, and Morden, MB.

Genotypes	Agronomic traits					
	DTF (days)	DTM (days)	PHT (cm)	YLD (tn ha ⁻¹)	PDH (%)	HSW (g 100 seeds ⁻¹)
Morden-003 (control line)	46 ± 3	99 ± 5.9	41 ± 5.9	2.25 ± 0.9	76 ± 13.0	18.7 ± 1.2
SMARC1N-PN1	47 ± 2	109 ± 9	50 ± 8.6	2.15 ± 0.9	70* ± 14.2	17.9 ± 1.2
3-59	44* ± 0.7	100 ± 3.3	44 ± 7.9	1.91 ± 0.9	68* ± 13.5	17* ± 0.9
3-84	46 ± 3.1	97 ± 3.9	41 ± 6.0	1.63* ± 0.7	67* ± 13.8	15.5* ± 0.7
3-301	47 ± 2.3	100 ± 4.5	47 ± 8.3	2.16 ± 0.8	73 ± 8.3	17.1* ± 0.7
3-337	46 ± 2.7	101 ± 4.6	45 ± 8.5	1.93 ± 1.0	70* ± 9.6	18 ± 1.7

t-Dunnett (< control); * Effects are significant at 0.05 level of probability; n = 3; DTF = days to flowering, DTM = days to maturity, PHT = plant height, YLD = yield, PDH = pod height (percentage of pods above 5 cm from the ground), HSW = hundred seed weight

Table 3.3.1.1-c Two-year (2001-2002) average of days to flower, days to maturity, yield, hundred seed weight, growth type, and lodging for Morden-003 navy bean cultivar over seven trial sites in the Eastern Prairie.

Genotype	DTF (days)	DTM (days)	YLD (tn ha ⁻¹)	HSW (g 100 seeds ⁻¹)	Growth type (1-4)	Lodging (1-5)
Morden-003	48	100	2.95	18.2	1	1.3

Lodging was measured on a scale of 1 = upright to 5 = prostrate; Growth type was measured on a scale of 1 = determinate bush to 4 = indeterminate climbing habit with weak, long and twisted stem and branches. DTF = days to flowering, DTM = days to maturity, YLD = yield, HSW = hundred seed weight.

3.3.1.3 Days to maturity

Early maturity is related to yield. It has been reported that a reduction of one day of the cycle caused a 33 kg ha⁻¹ increase in yield (Zilio et al., 2013). Also, late-maturing lines can be adversely affected by frost before harvest. Common bean is susceptible to frost. The days to maturity for Morden-003 were 95 and 99 for Population 2 and 3, respectively. Mündel et al. (2004) reported an average of 100 days to maturity for Morden-003. The days to maturity for the RILs ranged from 97 to 101. The RIL 3-84 had less number of days to maturity (97) than Morden-003 but no significant differences were detected for this variation ($P > 0.05$). SMARC1N-PN1 had a slightly higher number of days to maturity (109) than Morden-003.

3.3.1.4 Plant height

Plant height is a major agronomic characteristic because of its association with lodging (Zilio et al., 2013). Taller plants are more susceptible to lodging. The plant height for Morden-003 was 43 and 41 cm for Population 2 and 3, respectively. The RILs plant height was between 41 and 48. The mean plant height of the RIL 2-86 in Population 2 was lower than Morden-003 but no significant differences ($P > 0.05$) were detected. SMARC1N-PN1 plant height was 50 cm in both populations.

3.3.1.5 Yield

Crop yield is one of the most important agronomic traits since it is related to cost effectiveness and food security (Gelin et al., 2004). Mündel et al. (2004) reported a mean yield of 2.95 tn ha⁻¹ for Morden-003. The Morden-003 genotype tested in the present research had a lower performance, 1.92 and 2.25 tn ha⁻¹ for Population 2 and 3, respectively. The RILs, in both populations, had a similar lower performance as the parental Morden-003. The yield of the RILs ranged from 1.63 to 2.16 tn ha⁻¹. The RILs 2-86 and 3-84 had a significantly lower yield ($P < 0.05$) than the control line Morden-003. SMARC1N-PN1 yields in both populations were similar to Morden-003.

3.3.1.6 Pod height

Pod height is important as a means of avoiding soilborne diseases. Crops are susceptible to losses due to soilborne pathogens because of the close association of the pods with the soil (Botelho et al., 2013). For Morden-003, 73 and 76% of the pods in Population 2 and 3, respectively, were above 5 cm from the ground. Sixty-two to 73% of the pods of the RILs in both populations were 5 cm above the ground. Significant differences were found for the RILs 2-86, 3-59, 3-84, and 3-337 when compared to the parent line Morden-003 ($P < 0.05$). Sixty-nine and 70% of the pods of SMARC1N-PN1 in Population 2 and 3, respectively, were 5 cm above the ground. Significant differences were detected ($P < 0.05$) for SMARC1N-PN1 when compared to Morden-003 in Population 3.

3.3.1.7 Hundred seed weight

Seed weight is correlated to yield (Monpara and Gaikwad, 2014). Mündel et al. (2004) reported 18.2 g for hundred seed weight for Morden-003. The mean weight for one hundred seeds for Morden-003 was 17.2 and 18.7 g for Population 2 and 3, respectively. All RILs of Population 2 performed similar to the parental Morden-003. In Population 3, the RILs 3-59, 3-84, and 3-301 had a significantly lower seed weight, when compared to the parental Morden-003 in the same population ($P < 0.05$). The hundred seed weight for SMARC1N-PN1 was similar to Morden-003 in both populations.

3.3.1.8 Growth type

Erect plant types allow mechanized harvest and prevent pods from coming in contact with the ground thus reducing the incidence of soilborne diseases (Silva et al., 2013). The mean values for growth type for the genotypes of Population 2 and 3 are presented in Tables 3.3.1.8-a, and 3.3.1.8-b, respectively, for each location and trial year. The parental line Morden-003 in Population 2 and 3 had the expected growth Type I for both trial years in both experimental sites as described by Mündel et al. (2004). SMARC1N-PN1 had the growth Type II in Population 2 and 3 in the London

site and was consistent for both trial years. In the Morden site SMARC1N-PN1 has the growth Type II in both populations for the 2014 trial year, but was not consistent in the 2015 trial year. In the 2015 trial year SMARC1N-PN1 showed the indeterminate growth Type IV in both populations. The RILs of Population 2 and 3 in the London site had the growth Type I or II and were consistent between the two trial years. The RILs 2-75 and 3-84 had the growth Type I similar to Morden-003. The RILs 2-37, 2-86, 3-301, and 3-337 had the growth Type II similar to SMARC1N-PN1. The RILs of Population 2 and 3 in the Morden site had a growth type in the range of I to III and were not consistent between both trial years.

Table 3.3.1.8-a Growth type for the parent lines and the field RILs with SS protein profile of Population 2

	London					Morden				
	Parent lines		RILs			Parent lines		RILs		
	Morden-003	SMARC1N-PN1	2-37	2-75	2-86	Morden-003	SMARC1N-PN1	2-37	2-75	2-86
Trial year 2014	1	2	2	1	2	1	2	2	1	1
Trial year 2015	1	2	2	1	2	1.6	4.6	2	2.6	2

Mean values; n = 3; Growth type was measured on a scale of 1 = determinate bush to 4 = indeterminate climbing habit with weak, long and twisted stem and branches

Table 3.3.1.8-b Growth type for the parent lines and the field RILs with SS protein profile of Population 3

	London						Morden					
	Parent lines		RILs				Parent lines		RILs			
	Morden -003	SMARC1N- PN1	3-59	3-84	3-301	3-337	Morden -003	SMARC1N- PN1	3-59	3-84	3-301	3-337
Trial year 2014	1	2	2	1	2	2	1	2	2	1	2	2
Trial year 2015	1	2	2	1	2	2	1	4	3	2.6	2.6	3

Mean values; n = 3; Growth type was measured on a scale of 1 = determinate bush to 4 = indeterminate climbing habit with weak, long and twisted stem and branches

3.3.1.9 Lodging

As mentioned in the materials and methods, lodging is the tendency of a plant to bend. Lodging is the most important trait to avoid the fungal disease white mold (*Sclerotinia sclerotiorum*) which is a devastating disease of common bean (Miklas et al., 2013). Genotypes with a low lodging can avoid white mold because the pods are not in contact with the ground. The mean values for lodging for the genotypes of Population 2 and 3 are presented in Tables 3.3.1.9-a, and 3.3.1.9-b, respectively for each location and trial year. In the London site, in Population 2 and 3, the parental Morden-003 had a mean value for lodging in the range of 1 to 1.5 for both trial years. The mean values were similar to the mean value of 1.3 reported by Mündel et al. (2004) for Morden-003. In the Morden site, in Population 2, Morden-003 showed a slight tendency to bend with a mean value of 1.6 and 2.3, for 2014 and 2015, respectively. In Population 3, Morden-003 had a mean value of 2 for both trial years. In the London site, in both populations, SMARC1N-PN1 had a consistent mean value of 2 for both trial years. In the Morden site SMARC1N-PN1 showed a strong tendency to bend with mean values between 3 and 4 in both populations for both trial years. The RILs in the London site in both populations were consistent in their

performance between the two trial years. The RILs 2-75 and 3-59 had a lodging of about 1.5 similar to the parental Morden-003. The RILs 2-37, 2-86, 3-84, 3-301, and 3-337 had a performance similar to SMARC-1N-PN1. In the Morden site, the RILs of the two populations had a similar performance as SMARC1N-PN1 with more tendency to bend.

Table 3.3.1.9-a Lodging for the parent lines and the field RILs with SS protein profile of Population 2

	London					Morden				
	Parent lines		RILs			Parent lines		RILs		
	Morden-003	SMARC1N-PN1	2-37	2-75	2-86	Morden-003	SMARC1N-PN1	2-37	2-75	2-86
Trial year 2014	1.5	2	2	1.5	2	1.6	4	3	2	2.6
Trial year 2015	1	1.5	2.3	1.7	1.8	2.3	3	3	2.6	3

Mean values; n = 3; Lodging was measured on a scale of 1 = upright to 5 = prostrate.

Table 3.3.1.9-b Lodging for the parent lines and the field RILs with SS protein profile of Population 3

	London						Morden					
	Parent lines		RILs				Parent lines		RILs			
	Morden-003	SMARC1N-PN1	3-59	3-84	3-301	3-337	Morden-003	SMARC1N-PN1	3-59	3-84	3-301	3-337
Trial year 2014	1.5	2	1.5	2	2	2	2	3.7	2.3	2	1.7	2.3
Trial year 2015	1.2	1.7	1.8	2	1.3	1	2	3.7	3.7	3.3	3.7	3.3

Mean values; n = 3; Lodging was measured on a scale of 1 = upright to 5 = prostrate.

Chapter 4: Discussion

4.1 Protein quality of common bean

Common bean (*Phaseolus vulgaris* L.) has high seed protein content (Kelly and Bliss, 1975), but its nutritional quality is limited due its low content of the protein sulphur amino acids methionine and cysteine (Montoya et al., 2010). The research work of Taylor et al. (2008) revealed that a deficiency in phaseolin and phytohemagglutinin is associated with a nearly two-fold increase in methionine and cysteine seed content in genetically related lines of common bean (*Phaseolus vulgaris* L.). A redirection of the sulphur of *S*-methylcysteine, a non-protein amino acid, to the methionine and cysteine seed protein pool takes place leading to increased protein sulphur amino acids (Taylor et al., 2008). In the present research, the Recombinant Inbred Lines (RILs) deficient in phaseolin and lectins showed increased methionine-cysteine up to 3.4% in the common bean seed which is consistent with the report of Taylor et al. (2008). The agronomic performance of the RILs deficient in phaseolin and lectins was similar to the parental Morden-003. This means that the deficiency in major seed storage proteins in the RILs did not have a negative impact on the agronomic performance. This study evaluated, for the first time, a different approach to improve the protein quality in common bean. Previous efforts of common bean breeding programs on improving the seed protein quality were through increasing the content of phaseolin, the main seed storage protein in common bean. The RILs deficient in seed storage proteins with increased protein sulphur amino acid content, and with good agronomic performance will constitute Canadian unique elite germplasm to develop common bean cultivars with increased protein quality.

4.2 Protein profiles of the common bean genotypes

SDS-PAGE is a useful proteomic tool to separate proteins based on their molecular weight. In my study SDS-PAGE of soluble protein extract of mature seeds permitted separation and identification of the proteins of the navy bean Morden-003 and the germplasm SMARC1N-PN1, and of the 185 RILs obtained from the cross

between the two parental lines. As expected, the parental Morden-003 showed the phaseolin and lectin bands whereas these two protein bands were absent in the germplasm SMARC1N-PN1 (Figure 3.1 and 3.1.1). The later was consistent with the previous report by Hartweck and Osborn (1997) that SMARC1N-PN1 is deficient in both storage proteins. As a result of genetic recombination of the cross between the two parental lines, the RILs showed the protein profile for phaseolin and lectins of either parent (Figure 3.1). The decreased number of RILs with SS protein profile (Figure 3.1.1) that were grown in the field as compared to the same RILs grown in the greenhouse (Figure 3.1) may be attributed to cross-pollination in the field, although common bean cultivars are predominantly self-pollinating (De Ron et al., 2015). Cross-pollination is more common in wild types (Muñoz et al., 2017). The decreased number of SS field RILs could also be due to mishandling of the individual seed packages during planting or harvesting in the field. Another reason might be that the odds of getting RILs with SS protein profile for the small sample size of the RILs relative to all the recombination that can occur < 200 RILs, is a small number and 25 greenhouse RILs with SS protein profile compared to 7 field RILs with SS protein profile might not be significant.

4.3 Phaseolin as a biochemical and genetic marker

Former studies using SDS-PAGE for different phaseolin types provided evidence that the Mesamerican bean accessions have the “S”, “B” or “M” phaseolin type (Beebe et al., 2001). I compared the phaseolin banding pattern of Morden-003 (Figures 3.1 and 3.1.2) with the protein bands of the Sanilac, Tendergreen and Contender cultivars showed in Gepts et al. (1986) work. The protein banding comparison, classified Morden-003 as “S” type. Morden-003 is derived from Mesoamerican bean accessions. The result is supported by Beebe et al (2001) studies that the “S” type phaseolin is found within Mesoamerican bean lines and in accordance with about 90% of the common bean cultivars have the “S” type phaseolin (Montoya et al., 2010). Phaseolin has also been used as a genetic marker in the selection of bean germplasm to increase protein sulphur amino acids (Kelly and

Miklas, 1999). In former common bean breeding programs, crosses were made between genotypes identified as having high phaseolin content, to increase the protein sulphur amino acid in common bean seeds (Gepts and Bliss, 1984). By contrast, in the work of Taylor et al. (2008) phaseolin deficiency in genetically related common bean lines was associated with increased methionine and cysteine. In my research, I identified by SDS-PAGE, common bean genotypes deficient in phaseolin and lectins. As presented in the results section, the RILs identified as deficient in phaseolin and lectins showed increased methionine-cysteine. This confirms the findings of Taylor et al. (2008). Thus, any common bean line deficient in phaseolin and lectins might be considered to have increased protein sulphur amino acids.

4.4 Sulphur amino acid content in the parent lines Morden-003 and SMARC1N-PN1, and the RILs

Taylor et al. (2008) analyzed SARC1, SMARC1-PN1 and SMARC1N-PN1, three genetically related lines, integrating a progressive deficiency in phaseolin, phytohemagglutinin and arcelin. The study revealed that seed storage protein deficiency is associated with increased methionine and cysteine content. The previous SDS-PAGE results (Figure 3.1) on the genotypes grown in the greenhouse showed that SMARC1N-PN1 and twenty-five RILs were deficient in phaseolin and lectins. Based on the SDS-PAGE results and in agreement with Taylor et al. (2008) findings, I would have expected SMARC1N-PN1 with higher content of total methionine-cysteine than Morden-003. This was not the case in all seed samples analyzed of the parental SMARC1N-PN1. The seeds of the parental SMARC1N-PN1 that were sent to the SPARC BioCentre in Toronto had less total methionine-cysteine content than the parental Morden-003. According to Taylor et al. (2008) increased methionine and cysteine is due to a redirection of the sulphur from *S*-methylcysteine to the methionine and cysteine protein pool. The lower content of total methionine-cysteine in the seeds of the parental SMARC1N-PN1 sent to the SPARC BioCentre would indicate that no redirection of the sulphur from *S*-methylcysteine had taken place. Nevertheless, SMARC1N-PN1 showed decreased total *S*-methylcysteine as compared

with Morden-003 (Figure 3.2.2-b). The quantification performed by myself on the sulphur amino acid content in the seeds of the parental line SMARC1N-PN1 is consistent with Taylor et al. (2008). The seeds of SMARC1N-PN1 had increased total methionine-cysteine as compared with Morden-003 (Figure 3.2.1-a). As presented in the results section SMARC1N-PN1 had similar sulphur amino acid content as reported by Taylor et al. (2008) for the same genotype. My results showed that the total methionine-cysteine content in SMARC1N-PN1 is slightly above the 25 to 26 mg g⁻¹ protein suggested for human nutrition (Young and Pellett, 1991; Montoya et al., 2010). The redirection of the sulphur from *S*-methylcysteine was evident in the seeds of SMARC1N-PN1. The total *S*-methylcysteine content decreased in SMARC1N-PN1 as compared with Morden-003 (Figure 3.2.1-b). The parental SMARC1N-PN1 grown in the field also showed increased total methionine-cysteine content as compared with Morden-003 (Figure 3.2.3-a). Total *S*-methylcysteine decreased in SMARC1N-PN1 as a redirection of the sulphur to the methionine and cysteine protein pool (Figure 3.2.3-b). The content of total methionine-cysteine in the seeds of SMARC1N-PN1 grown in the field is lower than the content of the seeds grown in the greenhouse, 41.4 and 51.6 nmoles mg⁻¹ (27.3 and 21.7 mg g⁻¹ protein), respectively. This decrease might be because of suboptimal levels of sulphur in the soil. Sulphate is absorbed from the soil by the root system of the plant and reduced to sulphide to incorporate sulphur into organic metabolites (García et al., 2015). Experiments of potato plants grown in soil pots differing in sulphate concentrations showed that contents in all essential amino acids, were reduced in soil pots deficient in sulphur (Eppendorfer and Eggum, 1994). Thus a soil sulphate deficiency might be a possible reason for decreased total sulphur amino acid content in seeds of SMARC1N-PN1 grown in the field as compared with the seeds grown in the greenhouse. Pandurangan et al. (2015) evaluated the total sulphur amino acid contents in the seeds of SMARC1N-PN1 plants grown in soil pots with low and high sulphate content, respectively. The results showed that there was an increase by 13% of total methionine-cysteine content in the seeds of plants grown in high sulphate soil as compared with the seeds of plants grown in low sulphate soil pots.

As for the SS RILs grown in the greenhouse that were deficient in phaseolin and lectins, all genotypes were consistent with Taylor et al. (2008). The group of SS genotypes quantified by myself showed increased methionine-cysteine as compared with the parental Morden-003 (Figure 3.2.1-a). However, increased total methionine-cysteine in this group of genotypes was not correlated with decreased total *S*-methylcysteine content in all the genotypes. The group of SS genotypes sent to the SPARC Biocentre also showed increased total methionine-cysteine as compared with the parental Morden-003 (Figure 3.2.2-a). Total *S*-methylcysteine decreased in these RILs with the exception of one RIL (Figure 3.2.2-b). One RIL showed increased total *S*-methylcysteine as compared with Morden-003. The RILs with SS protein profile showed a total methionine-cysteine content above the requirements in the human diet.

Increased total methionine-cysteine was also evident in the SS RILs grown in the field (Figure 3.2.3-a). As expected, all SS field RILs showed decreased total *S*-methylcysteine as compared with Morden-003 (Figure 3.2.3-b). The seeds of the RILs with SS protein profile grown in the field had less total methionine-cysteine content as compared with seeds of the same RILs grown in the greenhouse (Figures 3.2.1-a, 3.2.2-a, and 3.3.2-a). As discussed above a possible deficiency of sulphate in the soil might be the reason for lower levels of sulphur amino acid content in the field seeds as compared with the greenhouse seeds.

My results also confirmed that deficiency in either phaseolin or lectins led to increased total methionine-cysteine. Seven RILs deficient in phaseolin (SM) showed increased methionine-cysteine as compared to Morden-003 (Figure 3.2.1-a). One RIL deficient in lectins (MS) increased the total methionine-cysteine content (Figure 3.2.1-a). However, increased total methionine-cysteine in the RILs with SM and MS profile was lower as compared with the increase in the RILs with the SS protein profile (deficient both for phaseolin and lectins).

4.5 Agronomic trait performance of the RILs deficient in phaseolin and lectins

The goal of the Canadian common bean breeding program is to improve the protein quality of the navy bean cultivars. However, improving the protein quality should be without compromising the desirable agronomic characteristics that the navy bean cultivar Morden-003 possesses. The results on the eight agronomic traits assessed are discussed regarding to their correlation to each other. The randomized block design used in the present study assured to avoid bias in the assessment of the agronomic traits.

4.5.1 Days to flowering

In Populations 2 and 3, the number of days to flowering for Morden-003 was two days earlier (Table 3.3.1.1-a, and 3.3.1.1-b) as compared with the report of Mündel et al. (2004) for the same genotype (Tables 3.3.1.1-c). This difference may seem small. Nevertheless, a reduction of eight days in flowering corresponds to a reduction of approximately 10% of the cycle (Mendes et al., 2008). The number of days to flowering is positively correlated to days to maturity (González et al., 2016). In my study, the positive correlation between days to flowering and days to maturity was evident for Morden-003 tested in Population 2 (Table 3.3.1.1-a). Morden-003 reached the maturity stage five days earlier as compared with the report of Mündel et al. (2004) (Table 3.3.1.1-c). In Population 3 the fewer number of days to flowering for Morden-003 had less impact on the number of days to maturity as it reduced the cycle by only one day (Table 3.3.1.1-a) as compared with the report of Mündel et al. (2004) (Table 3.3.1.1-c). There is no evidence for a positive correlation between days to flowering and days to maturity in SMARC1N-PN1. The numbers of days to flowering in SMARC1N-PN1 was similar to Morden-003 but the number of days to maturity was between 10 to 15 days later (Tables 3.3.1.1-a, and 3.3.1.1-b).

A high heritability has been reported for days to flowering in common bean (Arriél et al., 1990; Scully et al., 1991; Mendes et al., 2008). This was evident in my

study since the RILs had an average number of days to flowering similar to the parental line Morden-003 tested in each population (Tables 3.3.1.1-a, and 3.3.1.1-b). Also, the number of days to flowering of the RILs was below the 48 days to flowering for Morden-003 reported by Mündel et al. (2004). Despite the fewer number of days to flowering in all the RILs, only the RILs 2-75, 2-86, and 3-84, reduced the number of days to maturity as compared with Morden-003 in the work of Mündel et al. (2004) (Table 3.3.1.1-c). Notable is the RIL 3-59, which had the shortest number of days to flowering (44) among the RILs, but no reduction in the number of days to maturity (Table 3.3.1.1-b).

González et al. (2016) stated that the number of days to flowering can be used as an indirect phenotypic marker for days to maturity. Vaid et al. (1985) reported a positive coefficient of correlation of 0.83 between days to flowering and days to maturity in common bean. The previous results are supported by the work of Tar'an et al. (2002) who found a significant positive correlation between days to flowering and days to maturity in a population of 142 common bean lines studied. In my research study the RILs and the parental Morden-003 had an average number of days to flowering between 44 and 47 and an average number of days to maturity in the range of 95 and 101. Based on the high coefficient of correlation between these two traits reported in the previous studies mentioned above, I might expect that any common bean line that has an average number of 45 ± 3 days to flowering might be a short-cycle line, with between 85-100 days to maturity (Kelly and Cichy, 2012). However this positive correlation is not evident for SMARC1N-PN1. Conversely a negative correlation might be suggested for this genotype. SMARC1N-PN1 showed similar numbers of days to flowering as Morden-003 and the RILs but reached the maturity stage between 12 to 14 days later. The phenotypic expression of days to flowering varies widely between locations and sowing dates (Mendes et al., 2008). This is highly evident in the present research. There was a significant interaction ($P < 0.05$) between genotype, year and location for this trait (Tables 3.3.1-a, and 3.3.1-b).

4.5.2 Days to maturity

A common bean cultivar is considered an early maturity cultivar when the number of days to maturity is between 85 to 100 (Kelly and Cichy, 2012). Morden-003 is within this category of early cultivars with an average of 100 days to maturity (Mündel et al., 2004). Morden-003 tested in my research (Tables 3.3.1.1-a, and 3.3.1.1-b) was consistent with Mündel et al. (2004) results. The number of days to maturity of the RILs, in both populations, was similar to the parental line Morden-003. The RILs' number of days to maturity was also close to the report of Mündel et al. (2004). Thus I might consider the RILs as early maturing lines. The heritability for days to maturity is reported to be high (White and Singh, 1991). This might support my results for the similar number of days to maturity of the RILs to the parental line Morden-003. SMARC1N-PN1 is considered a late maturing cultivar (Osborn et al., 2003) and this is evident in my results. SMARC1N-PN1 matured between 10 to 14 days later than Morden-003.

There is controversy whether a positive or negative correlation exist between days to maturity and yield. Zilio et al. (2013) in the study on twenty-six common bean lines found that short-cycle genotypes produced increased yields. By contrast, Tar'an et al. (2002) found a significant positive correlation between days to maturity and yield in the study on 142 common bean lines and the two parental lines. The parental line with longer days to maturity showed higher yields. Scully and Wallace (1990) evaluated eight agronomic traits on 112 common bean (*Phaseolus vulgaris* L.) cultivars, and found that the lines with an average of 112 days to maturity showed the highest yield. Nevertheless, some exceptions did exist in the study. Four lines with shorter days to maturity, between 104 and 100, showed similar yields as the lines with longer days to maturity (Scully and Wallace, 1990). In my study, Morden-003 in both populations showed a lower yield than the expected yield of 2.9 tn ha⁻¹ (Mündel et al., 2004). Therefore, for my study I cannot discuss whether a positive or negative correlation exists between days to maturity and yield. SMARC1N-PN1 is a late maturing cultivar (Osborn et al., 2003). In the present work the parental

SMARC1N-PN1, in both populations, matured nine days later (Tables 3.3.1.1-a, and 3.3.1.1-b) than Morden-003 and the RILs. It needs to be considered that common bean is regarded as susceptible to frost (Holubowicz and Dickson, 1989), thus a late harvest needs to be avoided. Early maturing common bean cultivars also have an economic value for farmers because of a rapid marketable product (White and Singh, 1991).

4.5.3 Plant height

Quantitative estimates for an optimum plant height in common bean are lacking (White and Izquierdo, 1991). White and Izquierdo (1991) concluded that “for a given set of growth conditions there is an optimal bean plant height”. Mündel et al. (2004) in his work on characterizing the navy bean cultivar Morden-003 did not present results on plant height. In my research, Morden-003 had an average height around 40 cm in both populations. The mean plant height of the RILs was similar to Morden-003. The average plant height of the parent line SMARC1N-PN1 was higher than Morden-003 (Tables 3.3.1.1-a, and 3.3.1.1-b). This is in accordance with indeterminate growth type cultivars being taller than determinate growth type cultivars (Nleya et al., 1999). Zilio et al. (2013) found genotype by environment interactions for plant height in twenty-six common bean lines assessed for this trait. However, in my research the three-way ANOVA did not detect any genotype by location by year interactions ($P > 0.05$) in any of the two populations, for this trait (Tables 3.3.1-a, and 3.3.1-b).

Tar'an et al. (2002) found a significant positive correlation between lodging and plant height in 142 common bean lines assessed. Zilio et al. (2013) state that there is a greater tendency to bend in plants with higher plant height. Morden-003 showed a shorter height than the RILs in both populations (Tables 3.3.1.1-a, and 3.3.1.1-b). In the London site, Morden-003 showed a lodging of 1, that is to say no tendency to bend. Nevertheless, this positive correlation between plant height and

lodging was not evident in the Morden site. In the Morden site, Morden-003 showed a slight tendency to bend in both populations and in both trial years.

A positive correlation between plant height and lodging was evident for the RILs and the parental line SMARC1N-PN1. SMARC1N-PN1 was 5 cm higher than the average height of Morden-003 and the RILs, in both populations. This might explain the greater tendency to bend in both populations for both trial years (Tables 3.3.1.9-a, and Table 3.3.1.9-b). The RILs had a slight higher height than the parental Morden-003 (Tables 3.3.1-a, and 3.3.1-b). This might explain the slight tendency to bend in both populations and in both trial years for the London and Morden site (Tables 3.3.1.9-a, and Table 3.3.1.9-b).

There is controversy in the findings for the association between plant height and yield. Tar'an et al. (2002) and Zilio et al. (2013) found a positive correlation between these two traits. Silva Cabral et al. (2011) found that the plant height did not present a correlation with the yield. In my research it is difficult to say whether a correlation exists between plant height and yield. Morden-003, a high yielding cultivar (Mündel et al., 2004), had lower yields in both populations. The RILs in both populations also had a similar lower yield than Morden-003. Since the yield performance of Morden-003 was not consistent with the previous report, it is hard to establish whether a correlation exists between the RILs' height and their yield performance.

4.5.4 Lodging

Lodging was lower for Morden-003 than for SMARC1N-PN1 and the RILs. For the same reasons discussed in the previous subheading, it is difficult to establish a correlation between lodging and yield for my results. White and Izquierdo (1991) reported that a negative correlation exists between lodging and yield. They tested eight common bean cultivars grown with and without trellises. The use of trellises helped to increase the yield by 5 to 509 kg ha⁻¹ in five out of the eight cultivars tested.

Tar'an et al. (2002) found no correlation between lodging and yield in 142 common bean lines evaluated for fourteen different agronomic traits.

A main concern of common lines with high lodging is that they are prone to fungal diseases because of the proximity to the ground (Miklas et al., 2013). White and Izquierdo (1991) also state mechanical damage due to high lodging and subsequently disease infection. The lodging of the genotypes I tested showed a lower lodging in both trial years and in both locations, except for SMARC1N-PN1 in the Morden site. Low lodging in the parental lines and the RILs might have been one factor contributing to no disease occurrence during my field trials.

4.5.5 Pod height

One main concern is the soilborne pathogens that can diminish the pod quality and thus affect yield (Botelho et al., 2013). Therefore, pod height is a very important agronomic trait. In the bean crop, good agronomic performance for pod height is considered when the pods are 5 cm above the ground level. In my research, 60 to 73% of the pods for all the genotypes that I tested were above 5 cm from the ground (Figure 3.3.1.1-a, and 3.3.1.1-b). These results mean that the genotypes had a good agronomic performance for this trait. Therefore, the significant differences detected in some of the RILs should not be considered as a lower performance. Zilio et al. (2013) found a positive correlation between percentage of pods above the ground level and yield, whereas Tar'an et al. (2002) did not find any correlation between these two traits.

4.5.6 Growth type

Desirable growth types are I and II since they ease the mechanical harvest for the farmers. The growth Type I that the plants of Morden-003 showed in both trial years and sites (Tables 3.3.1.8-a, and 3.3.1.8-b) was consistent with the report of Mündel et al. (2004) for the same genotype. The growth Types I and II that was assessed among the RILs in both populations showed that the RILs had a good agronomic performance for this trait and that they accomplished with the

requirements of the farmers. The growth type of the RILs and parental lines was consistent in both locations for the trial year of 2014 (Tables 3.3.1.8-a, and 3.3.1.8-b). These consistent results, that I found, are supported by the work of Zilio et al. (2013). Zilio et al. (2013) tested twenty-six common bean lines for growth type in two different locations across two trial years. Twenty-three out of twenty-six genotypes were consistent with their growth type between the two locations. In my research, the growth type of the genotypes was also consistent between the two different trial years in the London site. In the Morden site there was a variation in the growth type for the same genotype between the two trial years (Table 3.3.1.8-a, and 3.3.1.8-b). Zilio et al. (2013) stated that growth type is highly influenced by the environment. Although this is not evident in the London site since the growth type of the lines was consistent between the two trial years, there might have occurred a genotype by environment interaction in the Morden site for the growth type variation between the two trial years. In the Morden site, the parental SMARC1N-PN1 showed a growth Type IV in both populations for the trial year 2014. It should be noted that the growth Type IV is mostly found in the wild relatives of common beans (Checa et al., 2006).

There is a controversy whether erect stem cultivars (growth Type I and II) or prostrate cultivars (growth Type III) have higher yields. Silva et al. (2010) evaluated fifteen common bean lines and found that the lines with prostrate stem had higher yields than the lines with erect stem. Zilio et al. (2013) found that among twenty-six common bean lines, differing in growth Type I, II, III and IV, the lines with growth Type II, that is to say with erect stems, had the highest yield. In my research, the parental lines and the RILs that I tested, showed the growth Type I or II, that is to say had erect stems, except for the RILs 3-59 and 3-337 in the Morden site for the trial year of 2015. The RILs had a similar yield to the parental Morden-003 suggesting a positive correlation between growth type and yield. More important than trying to establish a correlation between growth type and yield, is to consider the growth type that fulfills the farmer's requirements. Farmers demand high yielding cultivars with growth types that provide easy mechanical harvest, like Type I and II (Silva et al.,

2010). Therefore, even that prostrate cultivars might have higher yields, they do not accomplish the farmer's needs.

4.5.7 Hundred seed weight

The average of 18 g 100 seeds⁻¹ of the cultivar Morden-003 I tested in both populations (Tables 3.3.1.1-a, and 3.3.1.1-b) was consistent with Mündel et al. (2004) for the same genotype. The average of hundred seed weight for SMARC1N-PN1 is similar to Morden-003 in both populations. The RILs that I tested had an average hundred seed weight similar to Morden-003. The average hundred seed weight of the RILs also fell within the range of 17 to 20.5 g 100 seeds⁻¹ proposed by Adams et al. (1986) and Uebersax and Siddiq (2012) for navy bean cultivars. The only exception was the RIL 3-301 that showed a significant lower performance for this trait as compared with the parental Morden-003. The data for hundred seed weight of the Morden location for the 2015 trial year was not available. Therefore, it was not possible to analyze the interaction for genotype by year by location, for the two populations. The interaction of genotype by location was not significant in the two populations ($P > 0.05$) (Tables 3.3.1-a, and 3.3.1-b) suggesting that the genotypes do not perform differentially to location influences. There was no main effect of genotype in Population 2 ($P > 0.05$) (Table 3.3.1-a); all five genotypes of Population 2 had a similar performance for hundred seed weight (Table 3.3.1.1-a). In contrast, in Population 3 there was a main effect of genotype ($P < 0.05$) for hundred seed weight (Table 3.3.1-b). As discussed above, the RIL 3-84 had a significantly lower performance as compared to Morden-003 and also did not fall within the hundred seed weight range established for navy bean cultivars. Regardless of the significant difference found when comparing the RILs 3-59, and 3-301, (Table 3.3.1.1-a) with the parent line Morden-003, those RILs still had the hundred seed weight of 17 g which is within the range for the small seeded navy bean cultivars.

The trait hundred seed weight is reported to have high heritability (White et al., 1994). This explains that all RILs, except 3-84 had a very similar performance

compared to Morden-003. Hundred seed weight is one of the main components of yield (White and Izquierdo, 1991). This means that a correlation between these two traits exists (Nienhuis and Singh, 1988; White and Izquierdo, 1991). Nevertheless, Tar'an et al. (2002) did not find a significant correlation between these two traits in the study on 142 common bean lines. In my research work, the similar hundred seed weight of the RILs as compared to the parent line Morden-003, might have contributed to the RILs having achieved a similar yield as the parental Morden-003. By contrast, the RIL 3-84 with a lower performance for hundred seed weight also had the lowest yield performance among all the RILs. These results suggest a positive correlation between these two traits.

4.5.8 Yield

Yield is the most important trait in common bean and in all crops because of its relatedness with profits for the crop producers. High yielding cultivars are of great economic value (Kelly and Cichy, 2012). Many efforts have been made in common bean programs to increase yield in this crop (Kelly and Cichy, 2012). The small seed dry beans have an expected potential yield of between 3 and 2.5 tn ha⁻¹ (Nienhuis and Singh, 1988; White and Izquierdo, 1991). Mündel et al. (2004) reported a yield of 2.9 tn ha⁻¹ for Morden-003 which is in accordance with potential yields for small seed dry beans. In my research, Morden-003 in both populations produced between 1 and 0.65 tn ha⁻¹ less (Tables 3.3.1.1-a, and 3.3.1.1-b). There is no significant interaction between genotype by year by location in any of the two populations that might explain this lower performance. Hundred seed weight, which is one of the major components of yield that I assessed, was in accordance with the report of Mündel et al. (2004). The probable reason for the lower yield is that no testing was performed for adequate levels of nutrients in the soil of the planting plots. Optimum field bean production requires high fertility soil with 30% of Nitrogen, 24% phosphate, 17% potassium, 22% calcium, 6% magnesium (Fageria, 2002). Mündel et al. (2004) do not give details about the fertilization practices performed before the trials they conducted, but since Morden-003 produced the expected yields, it might be

most likely that they used soil fertilizers before planting. The yield for SMARC1N-PN1 in both populations was below the 2.9 tn ha⁻¹. This is in accordance with SMARC1N-PN1 as a low yielding bean line (Osborn et al., 2003).

The lower yield of the RILs might be because of suboptimal levels of nutrient in the soil in the field plots as explained above. Lower yields of the RILs might be also due high inbreeding in the RILs. It is well documented that inbreeding lowers the yield potential (Souza Jr and Fernandes, 1997). The RILs underwent eight generations of inbreeding. This means that the RILs are about 99% homozygous at each locus. Since SMARC1N-PN1 is a low yielding bean line. The alleles of SMARC1N-PN1 for low yield might have been fixed in these advanced inbreed lines. Also the main effects of year and location on yield are significant ($P < 0.05$) in both populations (Table 3.3.1-a, and 3.3.1-b). Thus, differences in yield among the RILs might also be due to environmental factors. One of these environmental factors might be suboptimal levels of nutrients in the field plots. Low yields in the RILs might be also due a low general combining ability. Combining ability is the capacity of an individual to transmit superior performance to its offsprings. Nienhuis and Singh (1988) reported that small seed cultivars, like Morden-003, have a low general combining ability. Generally in plant breeding programs the best performing lines for a specific trait are used as parental lines (Fasahat et al., 2016). Normally, when the breeding program aims to increase the yield, crosses between two high yielding parents are made. In my study, Morden-003 is a high yielding cultivar whereas SMARC1N-PN1 is a low yielding bean germplasm. In all the other agronomic traits, the RILs in both populations showed a good agronomic performance. In breeding programs any RIL with good agronomic performance is considered an elite germplasm. This means that the elite germplasm is used to make crosses between another commercial cultivar in order to improve a specific agronomic trait. In my study, the RILs are going to be crossed with a high yielding commercial cultivar, for example Morden-003, in order to improve the yield.

Chapter 5 Conclusions

5.1 Concluding remarks

Common bean seed constitutes an important source of protein in the human diet. However its quality is limited by the low content of the protein sulphur amino acids methionine and cysteine in the bean seeds. All research studies showed that phaseolin is the main seed storage protein in common bean, thus constituting the main source of methionine in the seed. To date, none of the breeding efforts on increasing the phaseolin content in the bean seed, led to improve the seed protein quality in commercial bean cultivars, according to levels required in the human diet.

Previous reports on increased methionine-cysteine in the germplasm SMARC1N-PN1 to slightly above the requirements in the human diet opened the possibility to a new approach to improve the protein quality in common bean seeds of commercial common bean cultivars. Increased methionine-cysteine was due to a response to a deficiency in phaseolin, phytohemagglutinins, and arcelin and a redirection of the sulphur from *S*-methylcysteine to the methionine and cysteine protein pool (Taylor et al., 2008). Thus, the bean breeder in Manitoba, Dr. Anfu Hou, made a cross between the germplasm line SMARC1N-PN1 and the parental line Morden-003, a commercial cultivar well-adapted to Manitoba and with good agronomic trait performance. A hundred and eighty five RILs with different protein profiles were obtained from this cross through eight generations of inbreeding.

The research reported in this thesis has investigated the impact of seed protein deficiency on sulphur amino acid composition and on agronomic traits of the RILs. To my knowledge this is the first study to assess the possibility of using bean germplasm deficient in phaseolin and lectins to release new commercial bean cultivars with improved seed protein quality. The SDS-PAGE used in my thesis was a useful proteomic tool to identify the RILs with different protein profiles. The quantification of the sulphur amino acids in the seeds of the RILs showed that the RILs deficient in phaseolin and lectins had increased the methionine-cysteine content

in levels above the requirements for human nutrition. The difference in the content of sulphur amino acids between the seeds grown in the greenhouse and in the field might be due to a sulphate deficiency in the soil of the field plots.

One question mark in my research was what kind of impact the protein deficiency would have on the agronomic trait performance of the RILs. As discussed in the discussion chapter, the improvement of a specific characteristic should not alter other desirable characteristics that a cultivar possesses. The results for the eight agronomic traits assessed showed that the RILs deficient in phaseolin and lectins had a good agronomic performance, similar to Morden-003. Suboptimal levels of nutrients in the planting plots limited the expected yield for the parental Morden-003 and the RILs.

5.2 Future prospects

In my study, all the RILs deficient in phaseolin and lectins showed increased methionine-cysteine and had a similar agronomic performance as the parent line Morden-003. Thus, the RILs can be considered a Canadian elite germplasm to develop commercial bean cultivars with improved protein quality. Assuming that the required soil nutrient contents are accomplished in future field trials the challenging part for the bean breeding program will be to obtain a high yielding commercial bean cultivar with improved protein quality.

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