GENOTYPE BY ENVIRONMENT ANALYSIS OF THE PERFORMANCE OF TWO LOW PHYTATE PEA LINES

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ABSTRACT

Reducing phytate concentration in crops may result in an increase in the bioavailability of phosphorus and micronutrients and create more environmentally friendly production. Two low-phytate pea lines (1-2347-144 and 1-150-81) were recently developed at the University of Saskatchewan. These lines were grown in field trials at three diverse locations in Saskatchewan in 2009 and 2010 in comparison to CDC Bronco, the parent variety, and two other widely grown varieties (CDC Golden and Cutlass). The low-phytate lines had similar seedling emergence counts, plant height, mycosphaerella blight score and lodging score when compared with CDC Bronco. The low-phytate lines had somewhat later days to flowering and days to maturity, and somewhat lower grain yield and seed weight than CDC Bronco. Harvested seeds of the low-phytate lines had substantially higher inorganic phosphorus (1.21-1.28 mg/g) concentration than CDC Bronco (0.24-0.25 mg/g) and the other normal-phytate varieties. The concentration of phytate phosphorus was reduced in low-phytate lines by about 60% of CDC Bronco. The total phosphorus concentration was similar in all lines and ranged from 3.50-3.80 mg/g. The low-phytate lines had similar Se concentration, but slightly higher Zn and Fe concentration than CDC Bronco. Crude protein concentration was significantly higher than CDC Bronco, while ether extract, acid detergent fibre, neutral detergent fibre and starch concentrations did not differ significantly between the low-phytate lines and CDC Bronco. The low-phytate lines had germination rates similar to CDC Bronco under normal conditions; however, their germination rate was reduced after the seeds had been stressed by accelerated aging or cold treatment.

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DEDICATION

This thesis is dedicated to my dear father, Delgerjav Manlai, who passed away on March 24, 2012. My father encouraged and supported me to continue my education, and I only wish that he had lived long enough to see me complete my Masters degree.

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1. INTRODUCTION

Approximately 60-80% of total phosphorus (P) is stored in crop seeds as phytate (myo-inositol 1,2,3,4,5,6-hexa*kis*phosphate, InsP6, syn. phytic acid), also known as phytic acid, an organic compound. The high concentration of phytate in crops leads to several problems. Phytate is not readily available to humans and non-ruminant livestock because of their lack of phytase enzyme (Erdman, 1979). Surface water pollution may occur as the result of excretion of the non-digested phytate P (Parry, 1998). In addition, some essential micronutrients are not useable for humans and non-ruminant animals as they bound to phytate (Fernandez, et al., 1997).

Reducing phytate concentration in crops should result in an increase in the bioavailability of P and micronutrients (Wilcox, et al., 2000) and create environmentally friendly production (Bruno, et al., 2009). Thus, development of low-phytate varieties is desirable. Over the last several years, mutants with reduced concentrations of phytic acid have been described in a number of crop species, including maize (*Zea mays* L.) (Raboy, et al., 2000), rice (*Oryza sativa* L.) (Larson, et al., 2000), soybean (*Glycine max* L. Merr.) (Wilcox, et al., 2000), wheat (*Triticum aestivum* L.) (Guttreri, et al., 2004), and barley (*Hordeum vulgare* L.) (Larson, et al., 1998; Raboy, et al., 2001; Rasmussen and Hatzack, 1998; Rossnagel et al., 2008). In these lines, reduction in phytate concentration is offset by increased inorganic P concentration without changing the amount of seed total P (David, et al. 2007). The phytate reduction may be brought about by the lack of synthesis of myo-inositol-3-phosphate. The ABC transporter gene in low-phytate maize *lpa1* (Shi, et al., 2007) discontinues the phytic acid development by causing the blockage of the phytic acid pathway.

Two low-phytate pea lines (1-2347-144 and 1-150-81) were recently developed using chemical mutagenesis of the variety CDC Bronco by the Crop Development Centre (CDC), University of Saskatchewan (Warkentin, et al., 2012). The purpose of this study was to study the agronomic attributes of three normal-phytate varieties and two low-phytate lines grown over two years at three locations for a total of six environments in Saskatchewan. This work was conducted in collaboration with Dr. Victor Raboy (U. S. Department of Agriculture, Agricultural Research Service, National Small Grains Germplasm Research Facility, Aberdeen, Idaho).

The objectives of this study were 1) to determine the concentration of phytate P and inorganic P in two low-phytate lines compared to CDC Bronco and two other widely grown

varieties, 2) to determine whether the low-phytate trait in pea is associated with any other pleiotropic effects on performance, and 3) to determine seed germination rate of two low-phytate lines compared to CDC Bronco and two widely grown varieties under normal and stressed conditions.

The first two of these objectives were addressed using multi-location, multi-year field trails in Saskatchewan, so that the effects of variety, environment and their interaction could be assessed. The third objective was assessed under controlled environment conditions.

The hypotheses tested were 1) the two low-phytate pea lines had substantially reduced phytate P concentrations compared to their parent and two other widely grown varieties, 2) the agronomic performance of two low-phytate pea lines was not substantially different from that of their parent and two widely grown varieties, and 3) the seedling germination rate of two low-phytate lines was not substantially different from that of their parent and two widely grown varieties.

2. LITERATURE REVIEW

2.1 Field pea production and utilization

Field pea (*Pisum sativum* L.) is a pulse crop with 7 pairs of chromosomes. Pea was one of the earliest cultivated plants and was domesticated before 6000 BC in near Eastern and Greek Neolithic societies (Zohary and Hopf, 1973). Pea is a cool season crop and is widely grown in the cooler temperate zones of the world. The total world pea production is currently estimated to be 12 million tonnes per year on eight million hectares (Fig. 2.1). The most important pea producing countries are Canada, France China, Russia, India and Ukraine.

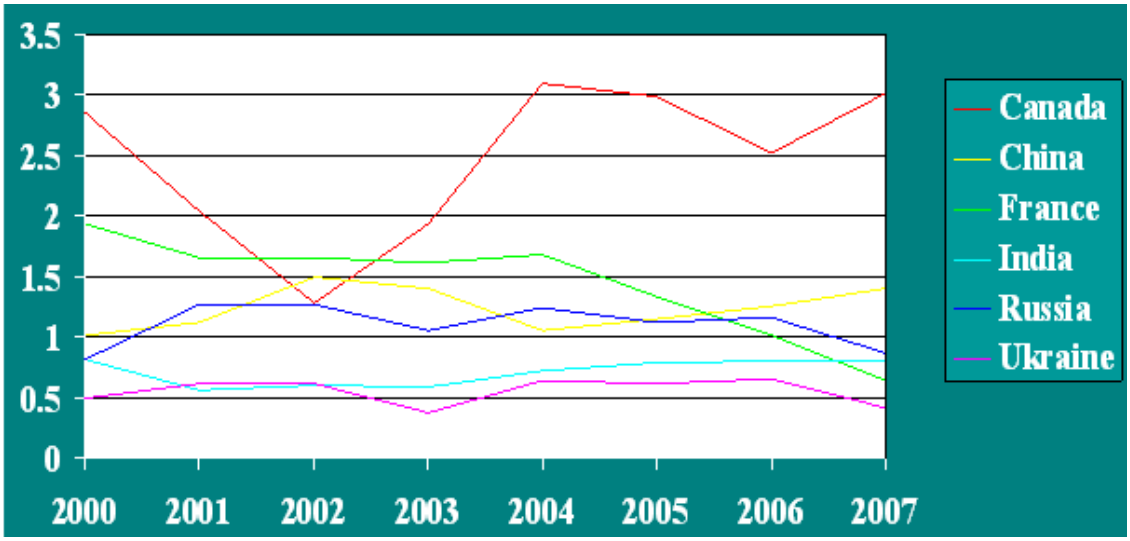

Figure 2.1 World dry pea production (million tonnes). Source: FAOSTAT, May 2009

Field pea has been a successful pulse crop since 1985 in Canada. Canadian producers grew peas on 1.0 to 1.6 million ha with mean annual production of 3.1 million tonnes from 2005 to 2009 (Saskatchewan Ministry of Agriculture, 2011). Canada is the top exporter of pea in the world with annual exports ranging from 1.8 to 2.8 million tonnes between 2006 and 2010 (Agriculture and Agri-Food Canada, 2011). India, China, and Bangladesh are the largest importers of Canadian peas.

2.1.1 Agronomic value of field peas

Field pea is an important pulse crop in many countries including Canada. Several agronomic benefits are known in using crop rotations that include field pea or other pulse crops. Pulse crop provide a rotational break from cereal diseases provide a good opportunity for management of grassy weeds, and improve soil tilth and fertility through symbiotic nitrogen fixation (McPhee, 2007). Pea is not a host crop for wheat midge or wheat stem

sawfly and the crop environment is not suitable for grasshopper growth (McVicar et al., 2006). Pulses require less nitrogen fertilizer because they can convert atmospheric nitrogen to forms that can be used by the plant through a symbiotic relationship with a group of bacteria known as rhizobia. Using pulses in a rotation contributes to increases in soil nitrogen which to some extent will be available to subsequent crops, reducing the need to use nitrogen fertilizer (van Kessel and Hartley, 2000).

2.1.2 Human and animal consumption

Field pea contains a substantial concentration of protein, complex carbohydrates, vitamins and minerals (Wang and Daun, 2004). Pulses are valuable sources of dietary protein for both humans and animals. Several potential health benefits are associated with consuming pulses including reduced risk of diabetes due to their low glycemic index (Viswanathan, et al., 1989), reduced risk of colon cancer (Sharma and Kawatra, 1995), protection against cardiovascular diseases (Lee, et al., 1992; Sharma and Kawatra, 1995) and decreased serum cholesterol concentration.

Field pea seeds are harvested as dry mature seed while garden peas are harvested as immature seeds. Several types of markets are known for each of these two products. The dry mature seeds are widely used in as whole seeds in various soups and stews, dhal (dehulled and split seeds), flour, snack foods and sprouted. Residual supplies are used in livestock feed. Garden peas are consumed as a fresh vegetable, canned, dehydrated or frozen (Davies, 1993). In this market seed color, texture, sugar and starch contents and seed size vary among cultivars in response to demands.

Pea seeds contain high quality protein, a high level of starch, fibre, B vitamins and minerals (K, Mg, Ca and Fe) and thus a good source of energy and protein (Wang and Duan, 2004). The crude protein concentration of field pea is typically 22-26% over 70% of which is soluble, and starch concentration is typically 40-50% (Christensen and Mustafa, 2000).

2.2 Chemical structure of phytate

Phytate was discovered by Hartig in 1855-1856 in the form of small, non-starch grains from seeds of various plants, which were considered to be important substances for the germination of seeds and the growth of plants (Rose, 1912). These particles were categorized into three groups, i.e., 1) crystals of calcium oxalate, 2) a protein substance, and 3) a compound, which gave no reaction for protein, fat, or inorganic salts (Pfeffer, 1872). The

third group of particles named by Pfeffer as "globoids" was identified as a combination of phosphate and carbohydrate. The globoid substances were very similar to the compound that was later studied by Schulze and Winterstein (1896), and the name was changed to "inosite-phosphoric acid" because it yields inosite and phosphoric acid on hydrolysis.

The chemical structure of this compound was characterized by several researchers (Neuberg, 1908, Levene, 1909, Starkestein 1911, and Anderson 1920) over 20 years of studies. Nevertheless, for more than the following 50 years the exact chemical structure of the compound was not definitely established (Shridhar and Reddy, 2002). *Myo*-inositol 1,2,3,4,5,6 hexa*kis*phosphate is currently the accepted name of phytic acid and is generally called "phytate(s)" (Reddy, et al., 1982).

2.3 Biosynthesis and dephosphorylation of phytate

Three possible explanations of biosynthesis of phytate pathway are suggested (Cosgrove, 1966). These include 1) phosphorylation of phosphoinositide intermediates and subsequent hydrolysis to generate corresponding inositol phosphates, 2) successive phosphorylation in the absence of free intermediates at each step, and 3) direct stepwise phosphorylation of free myo-inositol and/ or myo-inositol monophosphate by kinase type of reaction (Reddy, et. al., 1989). The first two mechanisms of generating phytate are generally not favoured in the literatures (Loewus and Loewus, 1980). The third mechanism, the direct stepwise phosphorylation pathway from myo-inositol to the myo-inositol hexa*kis*phosphate, is presently the accepted form of phytate biosynthesis (Asada, et. al., 1968). Biosynthesis of phytate has been studied in different parts of organisms including whole plant, organ, and cell culture by many researchers (Scott and Loewus, 1986). The injection of labelled myo-inositol or labelled inorganic phosphorus was used to analyse phytate biosynthesis in immature pea pods (Ahuja, 1962), ripening rice (Scott and Loewus, 1986), media containing cultured rice cells (Igaue, et al., 1980), various duck weeds (Robert and Loewus, 1968) and mung bean seeds (Majumder and Biswas, 1972). Biosynthesis of phytate involves addition of phosphate to the myo-inositol molecule through a series of steps (Fig. 2.2) (Reddy, et. al., 1989). During seed development phytate biosynthesis starts with glucose-6-phosphate through the enzyme, 1L-myoinositol-1-phosphate synthase (Loewus, 1983) and myoinositol-2-phosphate (Igaue, et. al., 1980.; Igaue, et. al., 1982. and Tanaka, et. al., 1971). Stepwise phosphorylation begins with myoinositol-1-phosphate and the end product is myo-inositol pentaphosphate with the use of the phosphoinositol kinase enzymes, and when stepwise

phosphorylation begins with myoinositol-2-phosphate the end product is myo-inositol hexa*kis*phosphate (Chakrabarti and Biswas, 1981). If myoinositol-1-phosphate is used as the substrate during phosphorylation the required enzyme is phytate-ADP-phosphotransferase, and this enzyme is required for myo-inositol pentaphosphate to myo-inositol hexakisphosphate synthesis from myo-inositol pentaphosphate (De and Biswas, 1979). In the phosphorylation initiated with myoinositol-1-phosphate the intermediate forms were identified as myoinositol-1,3-diphosphate, myoinositol-1,3,5-triphosphate, myoinositol-1,2,3,4,5-tetraphosphate and myoinositol-1,3,4,5,6-pentaphosphate, whereas the phosphorylation initiates with myoinostil-2-phosphate the intermediate forms were identified as myoinostil-2,4-diphophate, myoinositol-2,4,5-triphosphate, myoinositol-1,2,4,5-tetraphosphate and myoinositol-1,2,4,5,6-pentaphosphate (I) and myoinositol-1,2,3,4,5-pentaphosphate (Cosgrove, 1980).

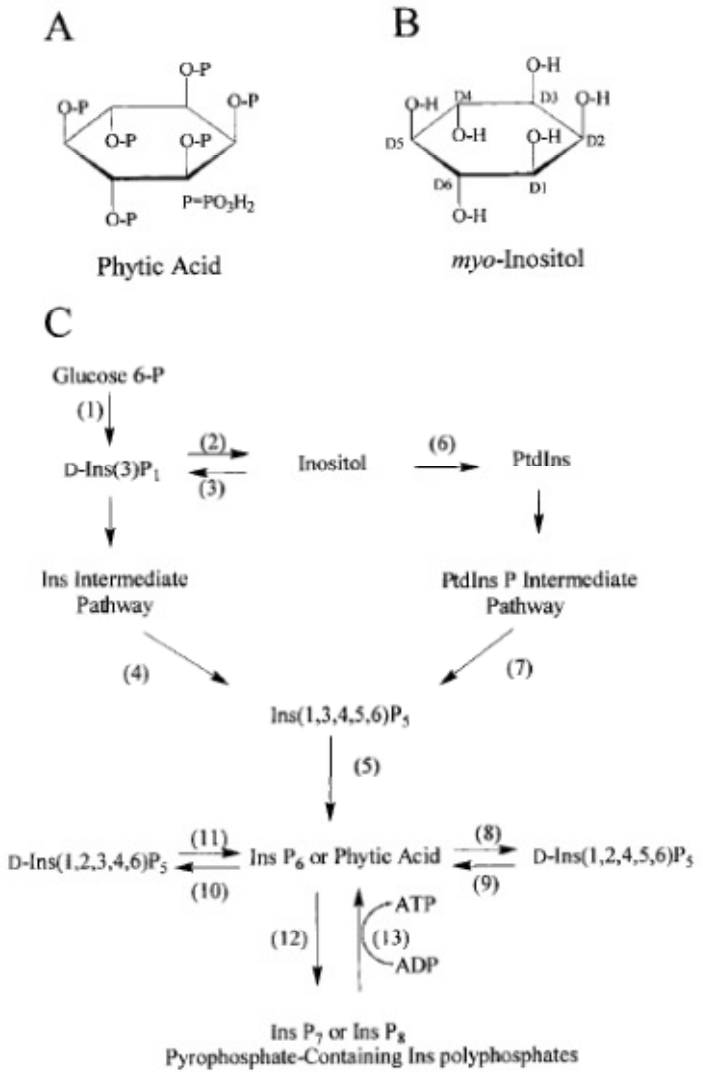

Figure 2.2 Biosynthetic pathways to phytic acid (myo-inositol-1,2,3,4,5,6-hexakisphosphate or Ins P6) in the eukaryotic cell. A, Structure of phytic acid. B, Structure of Ins. The numbering of the carbon atoms follows the "D-Convention" (Loewus and Murthy, 2000). C, Biochemical pathways:(1), D-Ins(3)-P1 (or L-Ins[1]-P1) synthase; (2), D-Ins 3-phosphatase (or L-Ins 1-phosphatase); (3), D-Ins 3-kinase (or L-Ins 1-kinase); (4), Ins P- or polyP kinases; (5), Ins (1,3,4,5,6) P5 2-kinase or phytic acid-ADP phosphotransferase; (6), PtdIns synthase; (7), PtdIns and PtdIns P kinases, followed by PtdIns P-specific phospholipase C, and Ins P kinases; (8), D-Ins(1,2,3,4,5,6) P6 3-phosphatase; (9) D-Ins(1,2,4,5,6) P5 3-kinase; (10), D-Ins(1,2,3,4,5,6) P6 5-phosphatase; (11), D-Ins(1,2,3,4,6) P5 5-kinase; (12), pyrophosphate forming Ins P6 kinases; (13), pyrophosphate-containing Ins PolyP-ADP phosphotransferases

Dephosphorylation of phytates occurs during seed germinations. In order to supply the growing tissues dephosphorylation of phytates releases inorganic phosphate and free myo-inositol by one or more phytase enzymes (Scott and Loewus, 1986). Phytase dephosphorylation activities are slightly different in plants, fungi, and bacteria (Cosgrove,

1980). In plants the dephosphorylation of phytate starts with phytase acting on L-myoinositol-1,2,3,4,5-pentaphosphate. However, the initial product of dephosphorylation of phytate in micro-organism is D-myoinositol-1,2,4,5,6 (Tomlinson and Ballou, 1962.; Maiti and Biswas, 1979). Two phytases, F1 (6-phytase) and F2 (2-phytase) detected in wheat bran (Tomlinson and Ballou, 1962), facilitate F1 phytase dephosphorylation of phytate to produce the first product 1L-myoinositol-1,2,3,4,5 pentaphosphate. It then removes phosphate from carbons as 5,4,3 or 5,4,1 to end up with the ultimate product myoinositol-2-phosphate in wheat bran, whereas the myoinositol-1-phosphate is the end product if F2 phytase is the enzyme used for dephosphorylation of phytate (Lim and Tate, 1971). In mung bean studies phytase dephosphorylates phytate to produce the first product myo-inositol 1,2,3,4,5-pentaphosphate by removing phosphate from carbon 6 and continually removes phosphates from carbon 5,4,1, and 3 to produce the end product myoinositol-2-phosphate (Maiti, 1979). The end product of dephosphorylation of phytate could be changed to glucose-6-phosphate and used for the PO4 is available in the form of ADP to provide energy for germination (Biswas, et al., 1978).

2.4 Physiological function of phytate

Phytate plays an important role in during seed germination. Phytate provides the reserve phosphate, inositol and micronutrients to seeds which supports seed growth and supplies the biosynthetic needs in the growing tissues (Gosgrove, 1980). Several physiological activities have been suggested for phytate in seeds. Phytate is a store of phosphorus (Hall and Hodges, 1966), energy (Biswas, et al., 1978) and involved in the breaking of dormancy (Williams, 1970).

2.5 Occurrence, distribution, content and dietary intake of phytate

2.5.1 Occurrence of phytate

Phytate exists in seeds and a variety of other places in plants such as roots and pollen in some species (Reddy, 2002). It occurs in the form of mono- and divalent cations in many plant seeds (Lott and Ockenden, 1986). Phytate is found in subcellular proteins and cotyledons of dicotyledonous seeds. During seed maturation phytate concentration increases and occurs with starch, protein and lipids (Lott and Buttrose, 1978). Phytate accumulates in the subcellular single membrane called the aleurone layer in cereal seeds (Tanaka and Kasai, 1981). In legumes phytate accumulates in the single-membrane storage organelles referred to

as globoids. The globoids are present in the proteinaceous matrix of protein bodies (Reddy, et al 1989). Lott et al. (1984) indicated that these globoid crystals exist in the cotyledons of legumes not in the seed coats. In the protein bodies, globoid crystals are structurally and chemically different. Depending on the species these substances vary in size and number. The size of isolated soy globoids varied from 0.1 to 1.0 µm, which were smaller than protein bodies (2 to 20µm) (Prattley and Stanley, 1982). Some dicotyledonous species including pea contain phytate although they lack globoids within the protein bodies (Lott, et al., 1984). The development and biogenesis of globoids could be controlled by some minerals, including calcium, magnesium and potassium concentration (Lott and Ockenden, 1986). In many seeds including rice (Tanaka and Kasai, 1981), wheat (Tanaka, et al., 1974), broad beans (Lott and Buttrose, 1978) and sesame (O'Dell and deBoland, 1976), soybeans (Lott and Buttrose, 1978) and Great Northern beans (Reddy and Pierson, 1987) phytate accumulates in the form of a potassium-magnesium salt. In soybean, Prattley and Stanley (1982) found that some phytate forms were water soluble and some are water insoluble. Reddy and Pierson (1987) indicated that phytate salts of Great Northern beans with a molecular weight less than 1000 daltons were water soluble, but those with molecular weight greater than 1000 daltons were not water soluble. The phytate form in pea is a potassium salt, and it is water soluble (Lott, et al., 1984).

2.5.2 Phytate distribution and concentration

Phytate forms during seed development in most plant seeds and grains, and its concentration increases until seed maturity (Tanaka and Kasai, 1981). Welch et al. (1974) indicated the phytate concentration increased from 0.16-1.23% during pea seed development. Phytates are distributed differently in monocotyledon (cereals) compared to dicotyledonous (legumes) crops. In cereal seeds such as wheat and rice phytates are present in the germ, aleurone layers and endosperm (O'Dell, et al., 1972). On the other hand, phytates are present in the cotyledons of legumes (Lott and Ockenden, 1986). Ferguson and Bollard (1976) reported that in dry peas 99% of the total phytate is in the cotyledon and 1% is in the embryo axis. Phytate P represents about 65% of the total P in pea cotyledons and 20% of the total P in the pea embryo axis. The remaining is present as inorganic phosphate in seed. There is almost no phytate in the seed coat fractions.

Phytate concentration ranged from 0.06 to 2.22% (Table 2.1) in dry cereal seeds (Wise, 1983). In dry legume seed phytate concentration ranged from 0.22-2.90% (Table 2.2).

The highest phytate containing seeds were kidney beans and broad beans with 0.61-2.38% (Harland and Oberleas, 1987) and 0.37-2.90% (Harland, 2001), respectively. In pea the average phytate concentration ranged 0.22-1.22% (Harland, 2001). These wide ranges in phytate concentration in all crops were attributed to the effects of differing cultivars, environmental conditions, and stages of seed maturation (Schlemmer, et al., 2009).

Table 2.1 Phytate concentration in cereals (from Schlemmer, et al., 2009)

Names	Taxonomic names	Phytate %	References
Corn	Zea mays	0.72-2.22	(Harland & Oberleas, 1987)
Corn germ		6.39	(Reddy et al., 2002)
Wheat	Triticum spp. (about 25 species)	0.39-1.35	(Harland & Spiller, 2001)
Wheat bran		2.1-7.3	(Reddy et al., 2002)
Wheat germ		1.14-3.91	(Reddy et al., 2002)
Rice	Oryza glaberrima/sativa	0.06-1.08	(Harland & Oberleas, 1987)
Rice bran		2.56-8.7	(Wise, 1983)
Barley	Hordeum vulgare	0.38-1.16	(Kasim & Edwards, 1998)
Sorghum	Sorghum spp. (about 30 species)	0.57-3.35	(Ravindran et al., 1994)
Oat	Avena sativa	0.42-1.16	(Kasim & Edwards, 1998)
Rye	Secale cereale	0.54-1.46	(Harland & Oberleas, 1987)
Millet	Pennisetum sp.	0.18-1.67	(Ravindran et al., 1994)
Triticale	Triticale secale	0.50-1.89	(Simwemba et al., 1984)
Wild rice	Zizania sp.	2.2	(Harland et al., 1979)

Table 2.2 Phytate concentration in legumes (from Schlemmer, et al., 2009)

Names	Taxonomic names	Phytate %	References
Kidney beans	Phaseolus vulgaris	0.61-2.38	(Harland & Oberleas, 1987)
Haricot beans			
Pinto beans			
Navy beans			
Black-eyed beans			
Broad beans	Vicia faba	0.51-1.77	(Kon & Sanshuck, 1981)
Peas	Pisum sativum	0.22-1.22	(Harland, 2001)
Dry cowpeas	Vigna unguiculata	0.37-2.90	(Harland, 2001)
Black-eyed peas			
Chickpeas	Cicer arietinum	0.28-1.60	(Harland, 2001)
Lentils	Lens culinaris	0.27-1.51	(Ravindran et al., 1994)

2.5.3 Dietary intake of phytate

The main sources of dietary phytate for humans are cereals, legumes, oilseeds and nuts. Estimation of human daily intake of phytate is presented in Table 2.3 from several countries. Daily phytate intake for average Americans with a weight of 75 kg was estimated to be about 750 mg (Harland and Peterson, 1978). Vegetarian adults consume a higher mean daily phytate intake of 1250 and 1550 (Ellis, et al., 1987).

Table 2.3 Daily intake of phytate in various countries (from Schlemmer, et al., 2009)

Country	Groups	Mean of phytate (mg/day), mean ±SD or range	References
Unites States	Children (1-5 years)	390-501	(Arsenault & Brown, 2003)
	Men & women	538-1293	(Held et al., 1988)
	Women (18-24 years)	395±334	(Ellis et al., 1987)
	Vegetarian men	~1550±550	(Ellis et al., 1987)
	Vegetarian women	~1250 ±450	(Ellis et al., 1987)
	Average American (75 kg)	750	(Harland & Peterson, 1978)
Canada	Boys (4-5)	320 (203-463)	(Gibson et al., 1991)
	Girls (4-5)	250 (132-318)	(Gibson et al., 1991)
Mexico	Infants (18-30m)	1666±650	(Murphy et al., 1992)
	Children (7-9 years)	3380±1070	(Murphy et al., 1995)
Guatemala	Women (15-37)	2254	(Fitgerald et al., 1993)
India	Men & women	670	(Grewal & Hira, 1995)
	Children (4-9 years)	720-1160	(Khokhar et al., 1994)
	Adolescents (10-19 years)	1380-1780	(Khokhar et al., 1994)
	Men & women	1290-2500	(Khokhar et al., 1994)
China	Men	690±330	(Wang et al., 1992)
	Women	915±189	(Wang et al., 1992)
Thailand	Men	1104-1304	(Nititham et al., 1999)
	Women	997-1139	(Nititham et al., 1999)
South Korea	Men	839±400	(Joung et al., 2004)
	Women	725±407	(Joung et al., 2004)
Egypt	Children (7-9 years)	1270±280	(Murphy et al., 1995)
Kenya	Children (7-9 years)	2390±480	(Murphy et al., 1995)
Nigeria	Men & women	2200	(Harland et al., 1988)

The countries that are presented in Table 2.3 suggest that a mean daily phytate intake varies with sex and age and diet (vegetarian or not).

2.6 Phytate digestion and bioavailability

Phosphorus in phytate is not readily available for humans and non-ruminant livestock because of their lack of the phytase enzyme (Erdman, 1979). Absorption of phytate by rats using C^{14} labelled phytate showed the distribution of radioactivity in blood, organs, bones, urine and expired CO_2 (Nahapetian and Young, 1980). Sakamoto et al. (1993) also used labelled phytate in rat phytate absorption studies and reported similar results as the radioactivity was widely distributed in the organs (liver, kidney and gut), with only traces in the blood and urine. The detected radioactivity in chromatographic studies showed most of the inositol phosphates in the gastric epithelial cells were Ins P3, while InsP5 and InsP6 were absent. When sodium phytate was added to the diet, plasma concentration of phytate was higher than that of a phytate free diet (Grases et al 2001). Urine concentration also increased indicating the body has the capacity of absorption limited amount of phytate but much is excreted in the urine. The phosphorus is not readily available as the phytate is not metabolized. Veum et al. (2002) studied bioavailibity of normal-phytate barley and low-phytate barley using in vitro procedure designed to mimic the digestive system of the pig. The pigs fed by low-phytate barley had higher ($p<0.05$) bone ash weight, bone breaking strength, P absorption and retention, and Ca absorption and retention compared to pigs fed normal-phytate barley. P availibility was 52% for low-phytate barley and 32% for normal-phytate barley for pigs.

2.7 Potential benefits of low-phytate crops for food and feed

Several potential benefits are associated with low-phytate crops. Low-phytate crops may increase the bioavailability of phosphorus and several important nutrients such as iron (Raboy, et al., 2000). Zn absorption was studied in children in Guatemala, where the zinc supply was reported to be low and about 50% of the caloric intake comes from maize (Mazariegos, et al., 2006). A low-phytate maize *lpa1-1* and normal phytate local landrace maize were given to school children for ten days. The absorption of zinc fraction was 14% for children that consumed the low-phytate maize, whereas the absorption of the zinc fraction was 10% for children that consumed the local maize line. Hambidge et al., (2005) compared calcium absorption of *lpa1-1* and normal-phytate maize in humans. Although total calcium concentration was equal for the tortillas made from *lpa1-1* and normal-phytate maize, the absorption of calcium was 0.50% for *lpa1-1* and 0.35% for normal-phytate maize consumed by the five adult females.

Low-phytate crops will reduce the phosphorus surface water pollution created by the excretion of non-digested phytate phosphorus. Veum et al. (2002) found that use of low-phytate barley in pig diets reduced P excretion in swine waste by 55% and 16% in their semi-purified and practical diets, respectively, compared with normal-phytate barley. The low-phytate barley and normal-phytate barley were equal in nutritional value after supplementation of normal-phytate barley with inorganic P to equal the estimated available P in the low-phytate barley diet. The differences in pig growth were not significant for variables such as fresh bone weight, fat-free dry bone weight, bone ash, bone breaking strength, or N utilization (Veum et al. 2002).

A study carried out at the University of Saskatchewan examined the effects of phytase supplementation to barley based swine diets (Thacker et al. 2006). Diets containing two new low-phytate lines of barley were compared with diets based on Harrington barley with and without the addition of dicalcium phosphate. Calcium and phosphorus digestibility were both higher in diets containing supplemental phytase at 1000 phytase units per gram FTU kg^{-1}. The addition of phytase tended to improve weight gain and significantly improved feed conversion. Two feeding trials were carried out at University of Alberta (Htoo et al, 2007) to study the effects of low-phytate barley or phytase supplementation on barley-soybean meal swine diets. The digestibility of phosphorus was higher and the phosphorus excretion was lower for the diets containing the low-phytate lines of barley. In the second trial, the addition of inorganic phosphorus did not increase the digestibility of phosphorus, but did increase the amount retained and excreted. Addition of phytase to diets based on normal phytate barley or diets based on low-phytate barley resulted in decreased levels of phosphorus excretion. Therefore, use of low-phytate barley was as effective as the addition of supplemental inorganic phosphorus or phytase.

2.8 Effects of phytic-acid on cancer and other diseases

Shamsuddin (2002) summarized reports of a number of *in vitro* and *in vivo* studies that indicated that myo-inositol or inositol in combination with phytic acid has properties that prevent both the formation and incidence of various types of cancer cells. One of the mechanisms that have been proposed is the anti-angiogenic activity of phytic acid. Angiogenesis is the process of blood vessel formation that occurs under certain physiological and pathological conditions and is associated with the growth and metastasis of solid tumours. Phytic acid inhibited the proliferation of tumour cells *in vivo* by anti-angiogenic activity (Vucenik et al., 2004). Fox and Eberl (2002) reported that phytic acid had anti-

neoplastic properties in heart, colon, liver and skin. Idiopathic hypercalciuria is associated with a high frequency of renal stones. A diet containing high levels of phytic acid has been used to treat hypercalciuria and binding stones (Ohkawa et al., 1984). High levels of phytic acid in the diet were associated with the prevention of hypercholesterolemia and heart disease (Reddy, 2002).

2.9 Breeding low-phytate crops

Plant breeding is an effective method of reducing phytate concentration in crop seeds, enhancing available phosphorus and other nutrients in food and feed. Phytate reduced lines have been developed for a number of cereal and legume crops. Phytate reduced maize was identified by Raboy, et al., 2000. Other examples of low-phytate lines have been reported in barley (Larson, et al., 1998, Rossnagel et al., 2008), rice (Larson et al., 1998), soybean (Wilcox et al., 2000), wheat (Guttieri et al., 2004) and common bean (Campion et al., 2009). The breeding method used in each case was mutagenesis followed by screening for the low phytate phenotype. A colorimetric assay for phytic acid concentration in single seeds that is rapid and accurate was developed by Gao et al. (2007) which makes screening much easier.

A number of low-phytate mutants (*lpa*) have been reported for each of the species of cereals and legumes mentioned above. Cichy and Raboy (2009) reported that 24 *lpa* mutants representing different alleles at a minimum of six *lpa* loci were identified in barley. Mutants having similar effects have been found in many other crops. For example the *lpa-2* mutant in barley resulted in increased concentration of Ins phosphates such as Ins P_4 and Ins P_5. These are the same as the changes caused by the *lpa-2* mutant found in maize.

Most variables such as seed yield, time to maturity, disease resistance are not affected in the low-phytate lines. However, some reports state that field emergence and seedling vigour are reduced in low-phytate lines. Meis et al, (2003) found the low-phytate soybean produced in a tropical environment had field emergence rate (8%), as compared with the field emergence rate of seeds of the same genotype produced in a temperate region (63%), or normal-phytate seeds produced in a tropical environment (77%), and a temperate environment (83%). In low-phytate soybean with *pha1* and *pha2* alleles, the emergence rate was reduced about 20% (Oltmans et al., 2005). Spear and Fehr (2007) studied low-phytate soybean lines selected from a population developed by additional backcrosses to normal-phytate soybean B019 beyond that of the population studied by Hulke at al. (2004). They compared 36 low-phytate BC_3F_3-derived lines with low saturated fat to the low-phytate parent, CX1834, the normal-phytate recurrent parent B019, and a normal-phytate line

IA3023. They found 15 of the low-phytate lines were not significantly different for field emergence than B019. Based on this result they suggested that breeding of low-phytate soybean cultivars with acceptable field emergence should be possible.

Low-phytate barley CDC Lophy-1 was developed at the Crop Development Centre, University of Saskatchewan (Rossnagel et al., 2008). CDC Lophy-1 originated from the initial cross M2-635/CDC Freedom. M2-635 originated from a low-phytate M2 selection developed at the USDA-ARS (Aberdeen, ID) derived from chemically mutagenized (sodium azide) Harrington barley, which was subsequently crossed once to non-mutagenized Harrington (Dorsch et al., 2003). CDC Lophy-1 was intended for production in western Canada and targeted as a feed for the hog industry. This low-phytate barley showed acceptable agronomic performance for yield, threshability, maturity, plant height, straw strength, test weight, kernel weight and grain plumpness. Also, it showed resistance to loose smut and moderate resistance to Fusarium head blight. Phytate reduction was 60-65% in CDC Lophy-1 seed (Rossnagel et al., 2008), which resulted in greater phosphorus availability for monogastric animals and improved mineral uptake and growth performance (Veum et al., 2002). In swine feeding trials CDC-Lophy1 had higher digestibility of phosphorus than normal phytate barley. It also showed a higher level of digestibility and higher standardized ileal digestibility of crude protein and amino-acids than normal phytate barley. The results suggested that using low-phytate barley in swine diets may have economical and environmental benefits (Ige et al., 2010).

3. MATERIALS AND METHODS

3.1 Development of low-phytate field pea lines

Two low-phytate field pea lines were developed using chemical mutagenesis of variety CDC Bronco (Warkentin et al., 2012). Briefly, CDC Bronco (Warkentin et al., 2005) was selected from a set of field pea varieties as the target for chemical mutagenesis based on its desirable agronomic and seed quality traits in Western Canadian trials. Two kg of seed (approximately 9000 seeds) of CDC Bronco was divided into two lots with one lot exposed to sodium azide (1 mM) for 4 hours, and the second exposed to 1.5% ethyl methane sulfonate (EMS) for 4 hours. This work was conducted in collaboration with Dr. Victor Raboy (USDA, Aberdeen, Idaho) who previously developed low-phytate varieties in maize, soybean and barley.

Treated seed (M0) was planted in a contra-season nursery near Yuma, Arizona in November 2003 and approximately 500,000 M1 seed were bulk harvested in April 2004. Approximately 7000 M1 seed were sown in a space-planted field nursery at Saskatoon, SK in summer 2004, with remaining seed held in reserve. Approximately 2500 single plants were harvested in September 2004 from each of the sodium azide and EMS treatments. Plants were threshed individually with each plant yielding approximately 25-100 M2 seed. In summer 2005, three M2 seeds per plant, derived from the 5000 harvested plants were tested for the low-phytate trait using Chen's reagent in a colorimetric assay (Raboy, 1997). Putative low-phytate mutants were re-tested using the same assay in autumn 2005, and also by HPLC using the method of Newkirk and Classen (1998).

In 2006, M2 seeds of 20 putative low-phytate lines, 12 putative normal-phytate lines, CDC Bronco and several commonly grown pea varieties were grown in the greenhouse for seed increase. Approximately 75 M3 seeds of each of these lines were planted in 1 m^2 microplots in the field at Saskatoon. Five individual plants were harvested from each line, with the remainder of the plot harvested in bulk. Twelve M4 seeds per line, from the bulk harvest, were tested by Chen's reagent. Two lines were identified with the highest inorganic P concentration, i.e., lines 1-2347 and 1-150 (note that 1- indicates they were derived from the sodium azide mutagenesis treatment).

In 2007, M4 seeds of lines 1-2347 (750 seeds) and 1-150 (500 seeds) and CDC Bronco (750 seeds) were planted in the field at Saskatoon for seed multiplication. Approximately 200 single plants were harvested from each line, with the rest of the plot harvested in bulk. In 2008, six M5 seeds of each plant individually harvested in 2007 were tested by Chen's reagent. Seeds from plants 1-2347-144 and 1-150-81 uniformly displayed

the low-phytate phenotype and were used in all future research. M6 and M7 generations of 1-2347-144 and 1-150-81 as well as CDC Bronco were multiplied in the greenhouse, followed by field multiplication of the M8 generation in 2009.

3.2 Field experimental methods

A randomized complete block experiment (POYT-PHY) was designed with three replications to evaluate the two low-phytate pea lines (1-2347-144, and 1-150-81), in comparison to their progenitor CDC Bronco, and two other check varieties which are widely grown in Saskatchewan, i.e., CDC Golden (Warkentin et al., 2004) and Cutlass (Blade et al., 2004). The seed of the low-phytate lines and CDC Bronco used in the 2009 trials was produced in the University of Saskatchewan Agriculture Greenhouse in the winter of 2008-2009, while the seed of Cutlass and CDC Golden was from field seed produced in 2008. All seed used in 2010 was derived from the 2009 field trial at Rosthern. The experiment was conducted at three locations in Saskatchewan: Outlook (Dark Brown soil zone, irrigated), Rosthern (Black soil zone) and Saskatoon (Saskatchewan Pulse Growers land near Saskatoon, Dark Brown soil zone) in 2009 and 2010. The previous crops planted at the sites were: wheat for Outlook, wheat for Rosthern and fallow (wheat was planted before fallow) for Saskatoon. Soil sampling and soil probes inserting were done at each location 2-3 weeks after planting. Soil probes were at the field for 3 weeks. Plots consisted of 4 rows with 30 cm row spacing and 4 m row length. Seeding rate was 75 seeds/m^2. Seeding in both years was conducted between May 11 and May 17, harvesting was conducted between August 27 and September 17. Small plot research drills and combines were used for planting and harvesting of each experiment.

During the experimental season several agronomic characteristics were evaluated including: emergence counts, plant height (vine length), days to flowering, mycosphaerella blight score (1-9), lodging score (1-9), days to maturity, grain yield and 1000 seed weight. The first emergence count (%) was collected from 1 m^2 in each plot three weeks after planting, and the second emergence count was collected two weeks after the first count. Plant height was measured when the plants in the plot had completed pod set. Days to flowering was recorded as the number of days from planting until at least 10% of the plants in a plot had at least one open flower. Mycosphaerella blight caused by the fungus *Mycosphaerella pinodes* was assessed approximately 10 days after the start of flowering and the second scoring was recorded two weeks later according to Xue, et al., (1996). Lodging was assessed at physiological maturity using a 1-9 scale where 1 indicated completely erect

plants, while 9 indicated completely lodged plants. Days to maturity was assessed as the number of days from planting until 80% of the pods turned to a tan color. After harvest, grain yield per plot was converted to tonnes/ha. Thousand seed weight was determined using a representative seed sample which had been cleaned of straw and broken seeds.

3.3 Laboratory experimental methods

3.3.1 Wet ashing method

The wet ashing method was employed to determine the total phosphorus concentration in each line. A total of 50 mg of 0.5 mm ground pea seeds were placed in 10 mL digestion tubes and 1 mL of concentrated H_2SO_4 was added. The samples were incubated overnight in a fume hood then 200 µl of 30% H_2O_2 was added, and the tubes were placed on a heating block in a fume hood. When the block reached a temperature of 220°C the samples were removed from the heating block and allowed to cool for 15 minutes. Another 200 µl of 30% H_2O_2 was added and the samples were placed on the heating block (between 220°C and 250°C) for 30 minutes. Then they were removed from the heating block and allowed to cool for 15 minutes. The cycle was repeated several times until the sample became clear. In the final step of wet ashing 200 µl of 30% H_2O_2 were added and the samples were placed on the heating block for an hour. If the digestion remained clear after 30 to 45 minutes of heating the samples were then removed from the heating block and allowed to cool. The volume in the digestion tubes was brought up to 6.25 mL with double distilled water to allow for a proper range of colorimetric readings. Chen's reagent method was used to analyse total phosphorus after digestion (Raboy, V., personal communication, 2011).

3.3.2 Modified colorimetric (Wade's reagent) method

Phytate phosphorus determinations were conducted using the Wade's reagent method (Gao, et al., 2007). The chemicals used were: 1) 0.8N HCl:10% NA_2SO_4, 2) 10% NaCl and 3) Wade's reagent (0.03% $FeCl_3$ $6H_2O$:0.3% sulfosalicylic acid). A total of 50 mg of ground (Retsch Model ZM200, Newtown, PA, USA 0.5 mm particle size 1,800 grm) sample from each line was placed in a tube and 1 mL of 0.8N HCl:10% Na_2SO_4 was added. The aliquots were put on a shaker for 16 hours, then centrifuged at 3000 g for 20 minutes. Thirty µl of extract was placed in a new tube and 720 µl of double distilled water and 250 µl of Wade's Reagent was added and the tube was vortexed for 10 seconds. A 200 µl aliquot was placed in microtitre plate wells, and the absorbance values were read at 540 nm using a microplate reader (Bio-Rad Benchmark, Hercules, CA, U.S.A.).

A stock solution containing 1 mg phytic acid phosphorus per mL was prepared by dissolving 549.9 mg phytic acid dodecasodium salt hydrate (Sigma-Aldrich Co., St. Louis, MO, U.S.A.) in 100 mL water. This stock solution was used to prepare standard solutions with 25, 50, 100, 200, 300, 400, 500 and 600 uL phytic acid phosphorus per mL. The standard curve was used to obtain the value for phytic acid phosphorus per 30 µl of the 1 mL total extract. This value was converted to mg of phytate phosphorus (PAP) g^{-1} as follows:

mg PAP g^{-1} =(µg PAP in assay) x (1mLs extract/0.03mLs assayed colorimetrically) x (1/50mg tissue) x (1/1000)

3.3.3 Chen's reagent method

Chen's reagent method was employed to determine the concentration of inorganic phosphorus for each sample (Chen, et al., 1956). Chen's reagent consists of 1 volume of 6N H_2SO_4, 1 volume of 2.5% ammonium molybdate, 1 volume of 10% ascorbic acid and 2 volumes of double distilled water. Fifty mg of 0.5 mm ground sample was placed in a tube for each sample and 1 ml of 0.4M HCl was added. The aliquots were incubated overnight at 4°C then vortexed for 10 seconds. After further 30 minutes incubation, 10 µl extracts were placed in microtitre plate wells with 90 µl of double distilled H_2O and 100 µl of Chen's reagent. The mixtures were incubated for two hours before reading the absorbance values on a microplate reader at 655 nm. A standard curve was calculated based on the concentration of standards and their absorbance values. The absorbance value for each well was converted to µL of 1 mM K_2HPO_4. Seeds with normal-phytate concentration produced colourless solutions, while low-phytate seeds produced blue solutions depending on the amount of inorganic phosphorus.

3.3.4 Atomic absorption spectrophotometry method

Micronutrients (Zn, Fe and Se) were measured by atomic absorption spectrophotometry (AAS) using the method of Gawalko et al. (1997). One gram of 0.5 mm ground pea seeds was used for all samples. Each sample was placed in a digestion tube with 3 mL of concentrated HNO_3. The samples were digested at 80°C, and then 0.5 mL of 30% H_2O_2 was added. The total iron and zinc was determined directly using a NovAA330 spectrophotometer (Analytik-Jena AG, Jena, Thuringia, Germany) using air-acetylene flame after digestion. In the case of selenium analysis, after digestion, 3 ml of the solution was taken from all samples and 9 mL 0.72 M HCl was added. Then the samples were placed in a water bath at 78°C for 45 minutes. The total selenium concentration was determined by

atomic absorption on a NovAA330 spectrophotometer using the hydride system (model HS60, Kundendienst, Germany). The iron, zinc and selenium concentrations were calculated by comparing with standard solutions.

3.3.5. Near infrared spectroscopy method

The spectra of whole field pea seeds were determined using the Foss NIRSystems 6500 Near Infrared Spectrophotometer (FOSS NIRSystems, Inc., Laurel, MD, USA), using a 60 gram sample. The spectra of the samples were obtained from 400 to 2498 nm at 2 nm intervals. Twenty-five scans were determined and averaged for each measurement. Crude protein, ash, ether extract (EE), acid detergent fibre (ADF), neutral detergent fibre (NDF), and starch were predicted using equations developed at the Crop Development Centre (Arganosa et al. 2006).

3.4 Germination test procedure

All pea varieties were evaluated for germination rate under control conditions, accelerated aging and cold temperature conditions. Each test, replicated two times, was performed on samples of 50 seeds per line.

Under control conditions, seeds were placed in 15 cm diameter Petri dishes lined with filter paper with 20 ml of distilled water to complete germination at 20°C in the dark in a 70% humidity chamber at the Crop Sciences Field Laboratory

The accelerated aging (AA) test was also conducted according to Association of Official Seed Analyst (AOSA), (2009) to determine if seeds maintain their vigour after aging. AA was achieved by incubating seeds in a thermostatic chamber with 100% relative humidity at 41°C for 72 hours. The inner aging chamber was a plastic box (11.0 x 11.0 x 3.5 cm) with a lid, into which was placed a plastic try with a 10.0 x 10.0 x 0.3 cm wire mesh screen (mesh 14 x 18 micron) (AOSA, 2009). After incubation the germination test was conducted under control conditions as described above.

Exposing seeds to cold conditions is another approach to test seed vigour according AOSA, 2009. In the cold germination test, seeds were placed in 15 cm diameter Petri dishes lined with filter paper and with 20 ml of distilled water at 5°C for 7 days and then moved to 20°C for 5-7 days using the modified test for cold wet temperate region (AOSA, 2009).

3.5 Statistical analysis

Five varieties were studied using a randomized complete block design with three replications carried out at three locations and two years to give six environments. Varieties and environments were considered to be fixed effects and replications were considered a random effect. Mixed model of analysis of variance (ANOVA) was carried out using R statistical packages (Michael, 2007). Fisher's LSD (Least Significant Difference) was employed to compare treatment group means. Traits which displayed a significant genotype X environment interaction were described in figures in the Results section of this thesis. Phenotypic correlations among traits with significant P values were also estimated using the raw data. Days to maturity data at Outlook were not recorded in 2009 as maturity developed faster than expected, leaving only five environments for this trait. Growing season precipitation was much greater than average in 2010 causing high coefficient of variation (CV) for yield and thousand seed weight at Outlook and Saskatoon, and thus data for these two traits were omitted from the analysis for these two environments.

4. RESULTS

4.1 Field experimental results

Table 4.1 shows the soil nutrient status at the Outlook, Rosthern and Saskatoon, SK trial sites in 2009 and 2010. Samples were taken 2-3 weeks after planting. Soil P concentration was moderate at all sites except Saskatoon 2009 where it was low. Available soil P was moderate at all sites except Rosthern 2009 where it was low (Table 4.1).

Table 4.1 Soil nutrient status at Outlook, Rosthern and Saskatoon, SK trial sites in 2009 and 2010

Environment	NO^3-N*	P*	K*	SO^4-S*	Available P** (n)
	kg/ha				µg/10cm2
Outlook 2009	89	60	627	74	21 (4)
Rosthern 2009	15	46	629	19	6 (4)
Saskatoon 2009	14	11	332	29	12 (4)
Outlook 2010	8	63	394	10	12 (9)
Rosthern 2010	9	46	520	5	34 (9)
Saskatoon 2010	11	48	672	13	11 (9)

*ALS Laboratory Group Agricultural Services
**Western Ag Canada; n=number of replication

Table 4.2 shows monthly mean temperature and monthly total precipitation for May, June, July and August in 2009 and 2010 for Outlook, Rosthern and Saskatoon in Saskatchewan.

Table 4.2 Mean temperature and total precipitation of growing season (May–August) at Outlook, Rosthern and Saskatoon, SK in 2009 and 2010

Environment	Soil zone	Mean Temperature (°C)	Total Precipitation (mm)
Outlook 2009*	Dark Brown (irrigated)	15	144
Rosthern 2009**	Black	14	247
Saskatoon 2009*	Dark Brown	14	215
Outlook 2010*	Dark Brown (irrigated)	15	322
Rosthern 2010**	Black	15	NA
Saskatoon 2010*	Dark Brown	15	387

* based on data from Environment Canada, ** based on data from the Weather Network

The objectives of the field study were to determine the concentration of phytate P and inorganic P in two low-phytate lines compared to CDC Bronco and two other widely grown

varieties, and to determine whether the low-phytate trait in pea is associated with any other pleiotropic effects on performance. These two objectives were addressed by evaluating the five varieties using a randomized complete block design with three replications carried out at three locations over two years for a total of six environments.

Table 4.3 summarizes the mean squares of the combined ANOVA for emergence counts at two stages, plant height, days to flowering, mycosphaerella blight assessed at two stages, and lodging score for the five field pea varieties grown in six environments in Saskatchewan. Table 4.4 summarizes the mean squares of the combined ANOVA for days to maturity for the five field pea varieties with useful data from five environments in Saskatchewan. Table 4.5 summarizes the mean squares of the combined ANOVA for grain yield and thousand seed weight for the five field pea varieties with useful data from four environments in Saskatchewan.

Table 4.3 Mean squares of combined ANOVA for EC1, EC2, plant height, days to flowering, MP1, MP2 and lodging of field pea varieties grown in six environments in Saskatchewan

Source	Mean squares				
	Environment (E)	Replication in Environment	Variety (G)	G x E	Residuals
Df	5	12	4	20	48
Emergence count 1	565**	146.11	45.7	87	80.9
Emergence count 2	598**	108.64	67.7	121.4	82.4
Plant height	1165**	24.91	69.7**	47.7**	16.7
Days to flowering	171**	0.76	51.5**	3.54**	1.59
Mycosphaerella blight 1	2.9**	0.14	0.29**	0.29**	0.05
Mycosphaerella blight 2	6.5**	0.38	3.12**	0.56*	0.31
Lodging	50.2**	1.76	1.47*	26.2**	0.55

Note: *P<0.01; **P<0.05

Table 4.4 Mean squares of combined ANOVA for days to maturity of field pea varieties grown in five environments in Saskatchewan

Source	Mean squares				
	Environment (E)	Replication in Environment	Variety (G)	G x E	Residuals
Df	4	10.00	4	16	40.00
Days to maturity	3876**	0.85	34.1**	4.9**	1.52

Note: *P<0.01; **P<0.05

Table 4.5 Mean squares of combined ANOVA for grain yield and thousand seed weight of field pea varieties grown in four environments in Saskatchewan

Source	Mean squares				
	Environment (E)	Replication in Environment	Variety (G)	G x E	Residuals
Df	3	8	4	12	32
Grain yield	7.25**	0.43	2.35**	0.14	0.22
Thousand seed weight	2598**	120	1460**	47.2	49.2

Note: *P<0.01; **P<0.05

Table 4.6 Mean of EC1, EC2, PH, DTF, MP1, MP2, and lodging of field pea varieties grown in six environments, mean of DTM of field pea varieties grown five environments, and mean grain yield and thousand seed weight of field pea varieties grown in four environments in Saskatchewan

Variety	EC1	EC2	PH (cm)	DTF (days)	MP1 (1-9 score)	MP2 (1-9 score)	Lodging (1-9 score)	DTM (days)	Grain Yield (t/ha)	1000 Seed Weight
1-150-81	61	66	68	57	3.4	6.0	3.9	97	5.15	203
1-2347-144	58	63	67	57	3.3	6.1	3.8	96	5.53	204
CDC-Bronco	59	63	69	54	3.3	6.3	4.6	95	6.02	217
CDC-Golden	62	68	72	54	3.4	6.9	3.9	93	4.86	216
Cutlass	60	64	68	53	3.6	6.7	4.0	94	5.22	230
Mean	60	65	69	55	3.4	6.4	4.1	95	5.36	214
CV (%)	14.9	14	6	2	6.6	3.7	18.3	1	8.77	3
LSD=0.05	6	6	3	1	0.1	0.4	0.5	1	0.39	6
Outlook 2009	64	67	72	54	3.0	5.8	4.0	NA	5.94	195
Outlook 2010	51	54	63	51	3.6	6.9	2.3	73	NA	NA
Rosthern 2009	58	62	63	58	3.1	6.1	5.2	112	5.43	222
Rosthern 2010	63	67	82	55	3.8	6.2	3.4	101	4.36	222
Saskatoon 2009	59	65	58	60	3.0	5.9	2.4	112	5.70	217
Saskatoon 2010	68	73	75	52	4.0	7.5	7.1	97	NA	NA
Mean	61	65	69	55	3.4	6.4	4.1	95	5.16	220
LSD=0.05	10	8	4	1	0.3	0.5	1.1	1	0.552	4.9

Note: NA-not applicable, CV-coefficient of variation, LSD-least significant difference

Varieties did not differ in emergence count 1 (EC1) taken 3 weeks after planting, environments differed, and the variety X environment interaction was not significant (Table 4.3). Mean EC1 was 60% (Table 4.6). The Outlook 2010 environment had the lowest EC1 (51%), significantly lower than EC1 at Outlook 2009, Rosthern 2010, and Saskatoon 2010. Similarly, when emergence was assessed 5 weeks after planting (EC2), varieties did not differ, environments differed, and the variety X environment interaction was not significant (Table 4.3). Mean EC2 was 65% (Table 4.6). Similar to EC1, Outlook 2010 had the lowest EC2 (54%), significantly lower than EC2 at all other environments.

Varieties and environments differed significantly for plant height, and the variety X environment interaction was also significant (Table 4.3). CDC Golden was slightly taller than all other varieties. The two low-phytate lines did not differ in height from CDC Bronco (Table 4.6). Mean plant height was greatest at Rosthern 2010 and shortest at Saskatoon 2009. Variety differences in plant height were quite small at Outlook 2009, Rosthern 2010 and Saskatoon 2009, but greater at the other environments (Fig 4.1).

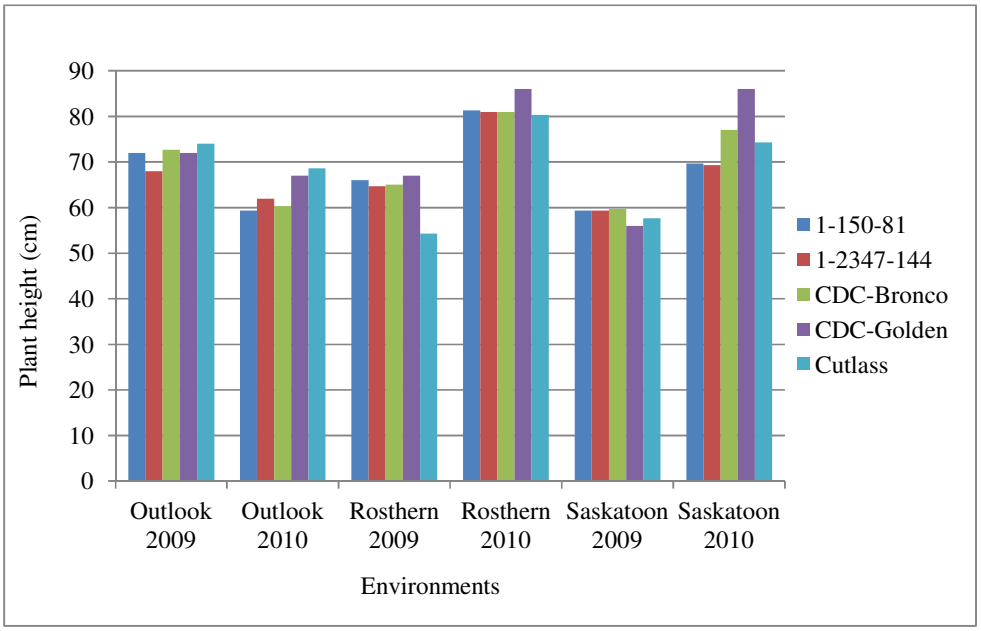

Figure 4.1 Plant height of five field pea varieties grown in six environments in Saskatchewan (Grand mean=69 cm; SE of interaction means=5).

Varieties and environments differed significantly for days to flowering, and the variety X environment interaction was also significant (Table 4.3). The two low-phytate lines were 3 days later to flower than CDC Bronco and CDC Golden and 4 days later than Cutlass (Table 4.6). Days to flowering was shortest (51 days) at Outlook 2010, and longest

(60 days) at Saskatoon 2009. Variety differences in days to flowering were quite small at Saskatoon 2010, but greater at the other environments (Fig 4.2).

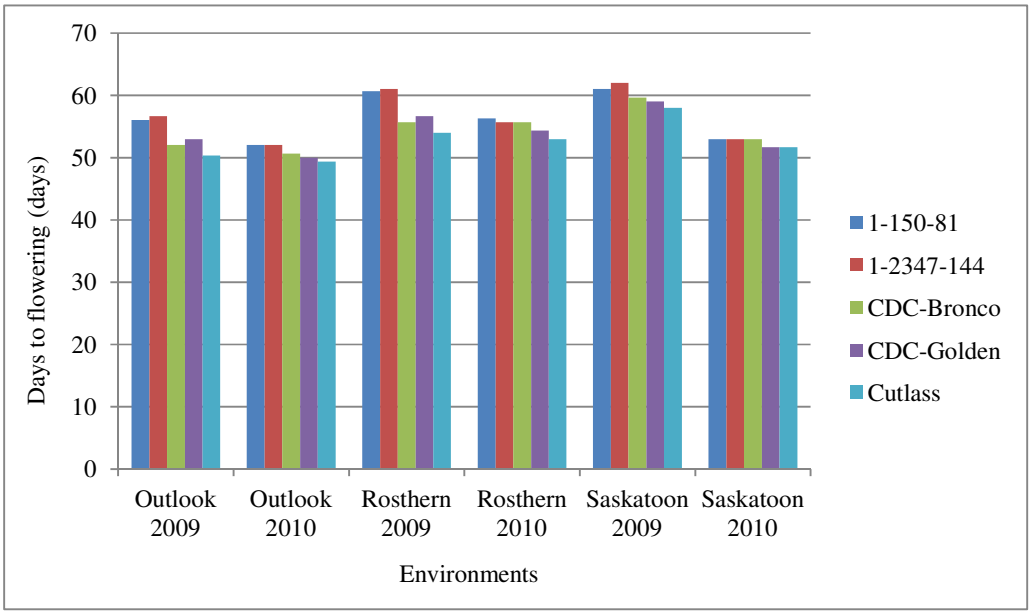

Figure 4.2 Days to flowering of five field pea varieties grown in six environments in Saskatchewan (Grand mean=55 days; SE of interaction means=3).

Varieties and environments differed significantly for the first mycosphaerella blight score (MP1) taken approximately 10 days after the start of flowering, and the variety X environment interaction was also significant (Table 4.3). Cutlass had a slightly greater score (3.6) than all other varieties. The two low-phytate lines did not differ in MP1, and did not differ from CDC Bronco (Table 4.6). MP1 was greater at all three environments in 2010 compared to 2009. Variety differences in MP1 were zero or small at all three environments in 2009 and at Saskatoon 2010, but were greater at Outlook 2010 and Rosthern 2010 (Fig 4.3). Most plots had a score of 3 (Fig 4.3) in MP1 indicating that the disease was not present in the upper portion of the canopy, was light (1-20%) in the middle portion, and the lower part of the canopy had moderate (21-50%) disease severity.

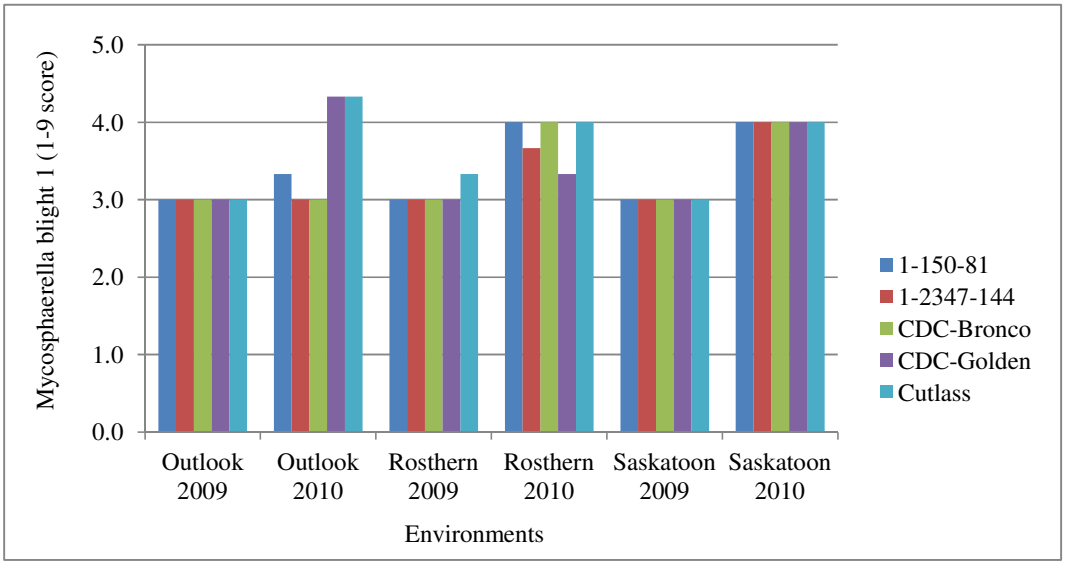

Figure 4.3 Mycosphaerella blight 1 (MP1) of five field pea varieties grown in six environments in Saskatchewan (Grand mean=3.4; SE of interaction means=0.2).

Varieties and environments differed significantly for the second mycosphaerella blight score (MP2) taken approximately 24 days after the start of flowering, and the variety X environment interaction was also significant (Table 4.3). CDC Golden and Cutlass had greater scores (6.9 and 6.7 respectively) than the other varieties. The two low-phytate lines did not differ in MP2, and did not differ from CDC Bronco (Table 4.6). MP2 was greater at Outlook 2010 than Outlook 2009 and greater at Saskatoon 2010 than Saskatoon 2009, while Rosthern 2009 and Rosthern 2010 did not differ. Variety differences in MP2 were small at all three environments in 2009, but were greater at all three environments in 2010 (Fig 4.4). Most plots had a score of 6 (Fig 4.4) in MP2 indicating that the disease was present in the upper portion of the canopy, was moderate (21-50%) in the middle portion, and the lower part of the canopy had high (51-100%) disease severity.

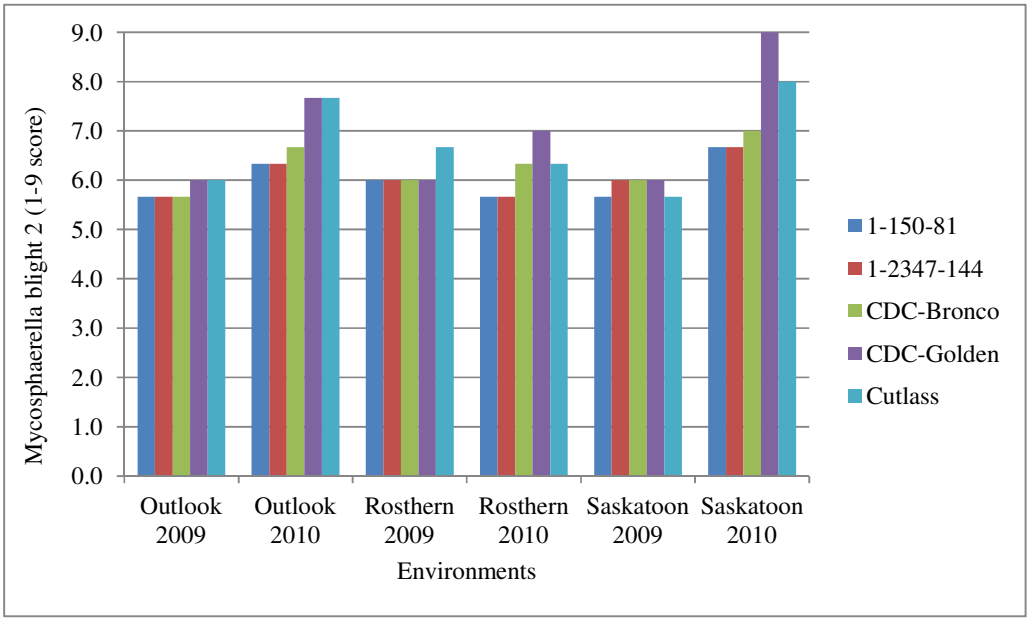

Figure 4.4 Mycosphaerella blight 2 (MP2) of five field pea varieties grown in six environments in Saskatchewan (Grand mean=6.4; SE of interaction means=0.6).

Varieties and environments differed significantly for lodging score, and the variety X environment interaction was also significant (Table 4.3). CDC Bronco had greater mean lodging score (4.6) than the other varieties (Table 4.6). The two low-phytate lines did not differ from each other in lodging score. Lodging was least severe at Outlook 2010 (2.3) and Saskatoon 2009 (2.4) and was most severe at Saskatoon 2010 (7.1) followed by Rosthern 2009 (5.2). Variety differences in lodging score were small at Saskatoon 2010 where all varieties had scores near 7, but were greater at all other environments (Fig 4.5).

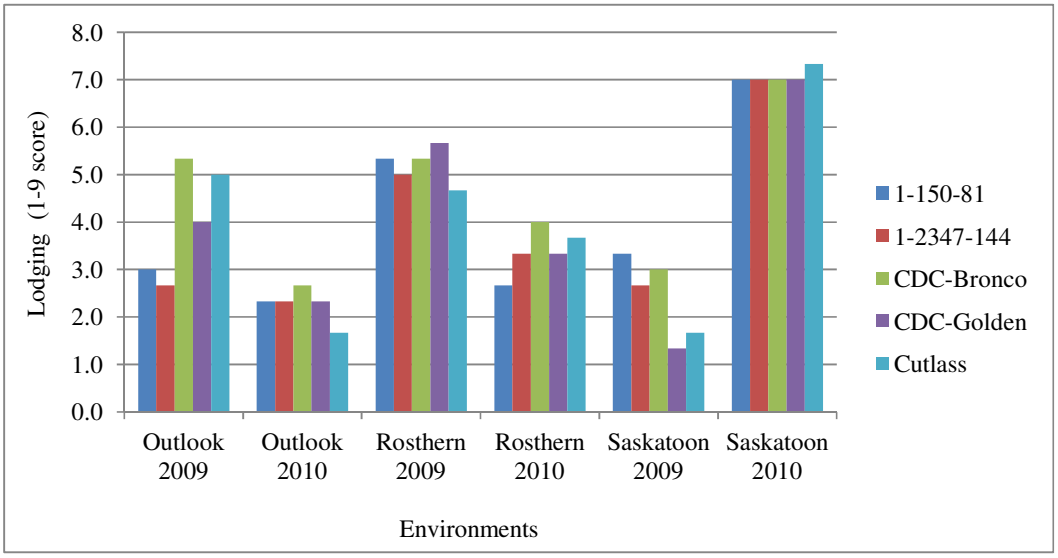

Figure 4.5 Lodging score of five field pea varieties grown in six environments in Saskatchewan (Grand mean=4.1; SE of interaction means=0.8).

Varieties and environments differed significantly for days to maturity, and the variety X environment interaction was also significant (Table 4.4). Line 1-150-81 (97 days) was one day later maturing than line 1-2347-144 which was one day later than CDC Bronco, which was one day later than Cutlass which was one day later than CDC Golden (Table 4.6). Mean days to maturity was shortest in Outlook 2010 (73) followed by Saskatoon 2010 (97), Rosthern 2010 (101), then Rosthern 2009 and Saskatoon 2009 (both 112 days). Days to maturity was not assessed at Outlook 2009. Variety differences in days to maturity were small within each environment, so although the variety X environment interaction was significant, it nearly trivial in effect (Fig 4.6).

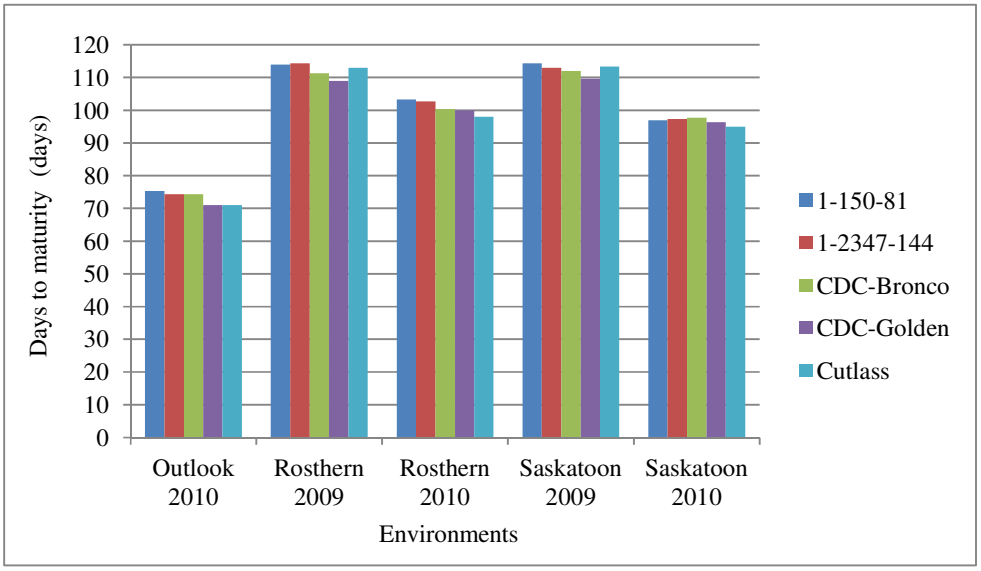

Figure 4.6 Days to maturity of five field pea varieties evaluated in five environments in Saskatchewan (Grand mean=95; SE of interaction means=1).

Varieties and environments differed significantly for grain yield; the variety X environment interaction was not significant (Table 4.5). CDC Bronco had the highest mean yield (6.02 t/ha) significantly greater than all other varieties (Table 4.6). Lines 1-2347-144 (5.53 t/ha), 1-150-81 (5.15 t/ha) and Cutlass (5.22 t/ha) did not differ significantly in grain yield, while CDC Golden had the lowest mean yield (4.86 t/ha). The three 2009 environments did not differ in mean yield, but they were greater than Rosthern 2010. Due to the excessively wet conditions in 2010, the coefficient for variation for grain yield was unacceptable at Outlook 2010 and Saskatoon 2010, so these environments were excluded from the combined ANOVA.

Varieties and environments differed significantly for thousand seed weight; the variety X environment interaction was not significant (Table 4.5). Cutlass had the greatest mean thousand seed weight (230g), significantly greater than CDC Bronco (217 g) and CDC Golden (216 g), which were significantly greater than lines 1-2347-144 (204 g) and 1-150-81 (203 g) (Table 4.6). The Rosthern 2009 and Rosthern 2010 environments had the greatest mean seed weight followed by Saskatoon 2009, then Outlook 2009. Thousand seed weight was not assessed for the Outlook 2010 and Saskatoon 2010 environments.

4.2 Laboratory experiment results

Varieties and environments differed significantly for total P concentration of harvested seeds, and the variety X environment interaction was also significant (Table 4.7). Line 1-150-81 (3.80 mg/g) had greater mean total P concentration than the other four varieties whose mean total P concentration ranged from 3.50-3.60 mg/g (Table 4.8). Environments differed substantially in mean total P concentration of harvested seeds. Saskatoon 2010 (4.69 mg/g) had the greatest total P concentration, followed by Outlook 2010 and Outlook 2009, then by Rosthern 2010

Table 4.7 Mean squares of combined ANOVA for total P, phytate P, inorganic P, Zn, Fe, Se, crude protein, ash, ether extract, ADF, NDF, starch of five field pea varieties grown in six environments in Saskatchewan

Source	Environment (E)	Replication in Environment	Variety (G)	G x E	Residuals
Df	5	12	4	20	48
Total P	5.87**	0.11	0.24**	0.12*	0.06
Phytate P	3.97**	0.07	17.4**	0.41**	0.06
Inorganic P	1.04**	0.01	5.39**	0.35**	0.01
Zn	514**	20.8	36.59**	10.6*	5.17
Fe	1003**	10.6	25.57*	24.7**	6.89
Se	13.09**	0.23	0.11	0.11	0.14
Crude protein	135.2**	0.60	14.14**	1.86	0.39
Ash	1.23**	0.02	0.04	0.02	0.01
Ether extract	0.23**	0.02	0.14**	0.06	0.03
Acid detergent fibre	6.3**	0.03	0.16	0.02	0.04
Neutral detergent fibre	8.31**	0.17	0.98**	0.20**	0.08
Starch	6.58**	0.70	0.83	1.28	0.54

Note: *P<0.01; **P<0.05

Table 4.8 Means of total P, phytate P, inorganic P, Zn, Fe, Se, CP, ash, EE, ADF, NDF and starch concentration of five field pea varieties evaluated in six environments in Saskatchewan

Variety	Total P (mg/g)	Phytate P (mg/g)	Inorganic P (mg/g)	Zn (ppm)	Fe (ppm)	Se (ppm)	CP (%)	Ash (%)	EE (%)	ADF (%)	NDF (%)	Starch (%)
1-150-81	3.80	1.20	1.28	28.9	42.1	0.78	24.4	1.49	0.96	6.7	17.1	43.4
1-2347-144	3.56	1.13	1.22	28.0	40.8	0.89	24.6	1.48	0.99	6.7	17.1	43.7
CDC-Bronco	3.57	2.99	0.24	25.2	39.4	0.67	23.4	1.54	0.92	6.5	16.8	43.7
CDC-Golden	3.60	2.94	0.26	28.1	40.1	0.83	22.9	1.57	1.09	6.5	16.8	44.0
Cutlass	3.50	2.95	0.26	27.1	42.1	0.80	22.6	1.59	1.13	6.6	16.6	43.8
Mean	3.61	2.24	0.65	27.5	40.9	0.79	23.6	1.5	1.0	6.6	16.9	43.7
CV (%)	6.8	11	13	8.2	6.4	60.9	2.6	6.7	17.3	3.0	1.7	1.7
LSD=0.05	0.16	0.16	0.06	1.52	1.75	0.32	0.5	0.07	0.12	0.14	0.19	0.7
Outlook 2009	3.70	2.22	0.68	24.9	35.7	0.18	22.5	1.59	0.86	7.0	17.0	43.4
Outlook 2010	3.82	2.78	0.46	16.6	35.1	0.38	23.8	1.79	0.98	5.8	16.3	43.6
Rosthern 2009	2.94	1.70	0.50	29.6	37.4	0.16	22.3	1.53	1.01	6.8	16.2	44.0
Rosthern 2010	3.28	1.92	0.52	31.4	40.0	0.26	24.1	1.87	1.06	5.7	16.3	44.5
Saskatoon 2009	3.19	1.82	0.58	30.0	40.3	1.28	19.7	1.36	0.97	7.1	17.3	44.2
Saskatoon 2010	4.69	2.95	1.16	32.1	57.0	2.49	28.8	1.09	1.23	7.1	18.1	42.6
Mean	3.60	2.23	0.65	27.4	40.9	0.79	23.6	1.54	1.02	6.6	16.9	43.7
LSD=0.05	0.26	0.21	0.07	3.6	2.59	0.38	0.60	0.11	0.11	0.14	0.33	0.67

Rosthern 2009 and Saskatoon 2009 had the lowest mean total P concentration. Line 1-150-81 had the greatest mean total P concentration at all environments except Rosthern 2009 and Saskatoon 2009 (Fig 4.7).

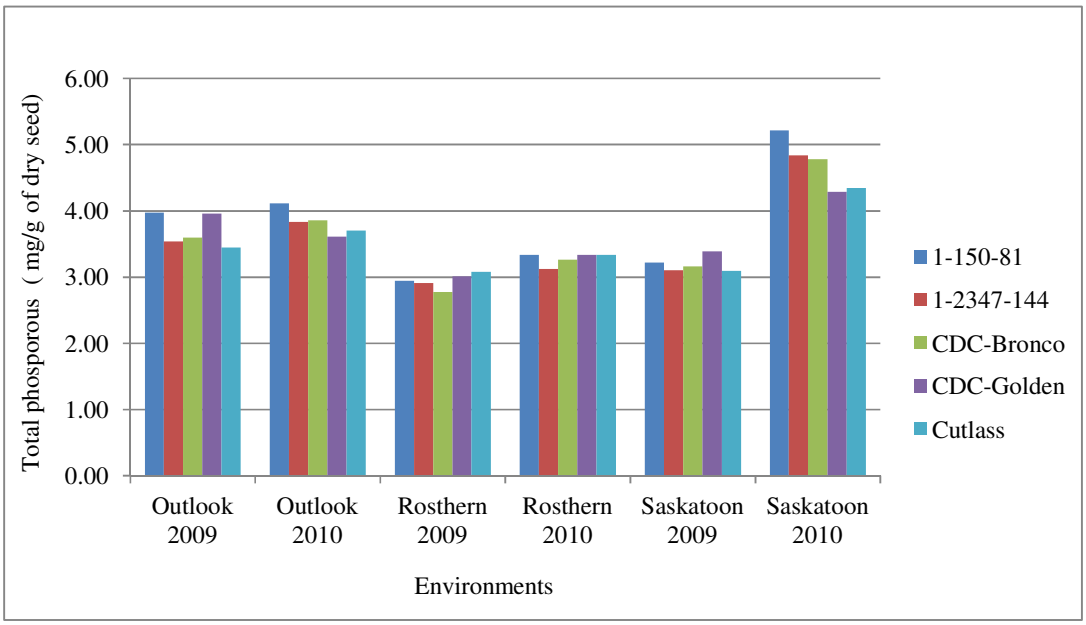

Figure 4.7 Total phosphorus concentration of five field pea varieties grown in six environments in Saskatchewan (Grand mean=3.61 mg/g; SE of interaction means=0.28).

Varieties and environments differed significantly for phytate P concentration of harvested seeds, and the variety X environment interaction was also significant (Table 4.7). Lines 1-150-81 (1.20 mg/g) and 1-2347-144 (1.13 mg/g) had substantially lower concentration of phytate P than the three other varieties which ranged from 2.94-2.99 mg/g (Table 4.8). Saskatoon 2010 and Outlook 2010 had the greatest mean phytate P concentration, followed by Outlook 2009. The variety X environment interaction was significant but small and related to changes in rank among the low-phytate P lines at the six environments, and similarly changes in rank among the normal-phytate P lines at the six environments (Fig 4.8).

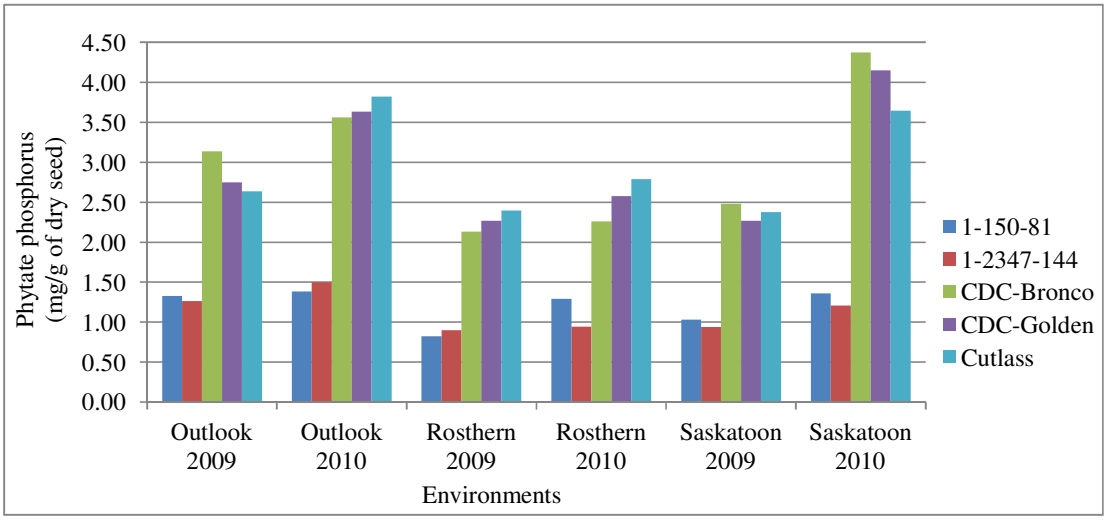

Figure 4.8 Phytate phosphorus concentration of five field pea varieties grown in six environments in Saskatchewan (Grand mean=2.24 mg/g; SE of interaction means=0.28)

Mirroring the results for phytate P, varieties and environments differed significantly for inorganic P concentration of harvested seeds, and the variety X environment interaction was also significant (Table 4.7). Lines 1-150-81 (1.28 mg/g) and 1-2347-144 (1.22 mg/g) had substantially greater concentration of inorganic P than the three other varieties which ranged from 0.24-0.26 mg/g (Table 4.8). Saskatoon 2010 had substantially greater mean inorganic P concentration than the other environments. The variety X environment interaction was significant but small and related to changes in rank among the normal phytate P lines at the six environments (Fig 4.9).

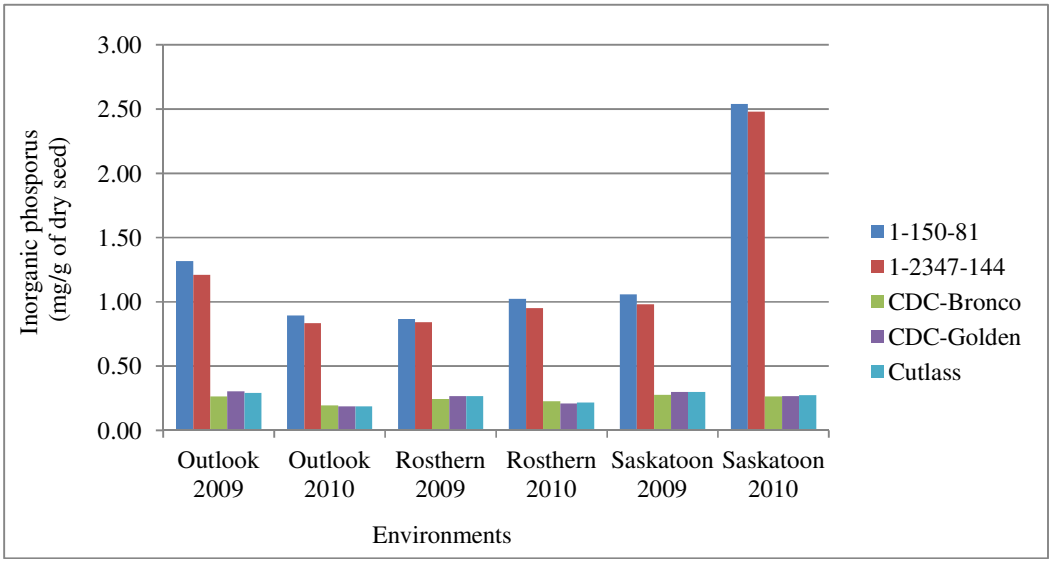

Figure 4.9 Inorganic phosphorus concentration of five field pea varieties grown in six environments in Saskatchewan (Grand mean=0.65 mg/g; SE of interaction means=0.12)

Varieties and environments differed significantly for Zn concentration of harvested seeds, and the variety X environment interaction was also significant (Table 4.7). CDC Bronco (25.2 ppm) had lower mean Zn concentration than the other four varieties whose mean total Zn concentration ranged from 27.1-28.9 ppm (Table 4.8). Environments differed substantially in mean total Zn concentration of harvested seeds. The two Saskatoon and two Rosthern environments had greater Zn concentration than Outlook 2009, which was greater than Outlook 2010. CDC Bronco had the lowest mean Zn concentration at all environments except Saskatoon 2009 (Fig 4.10).

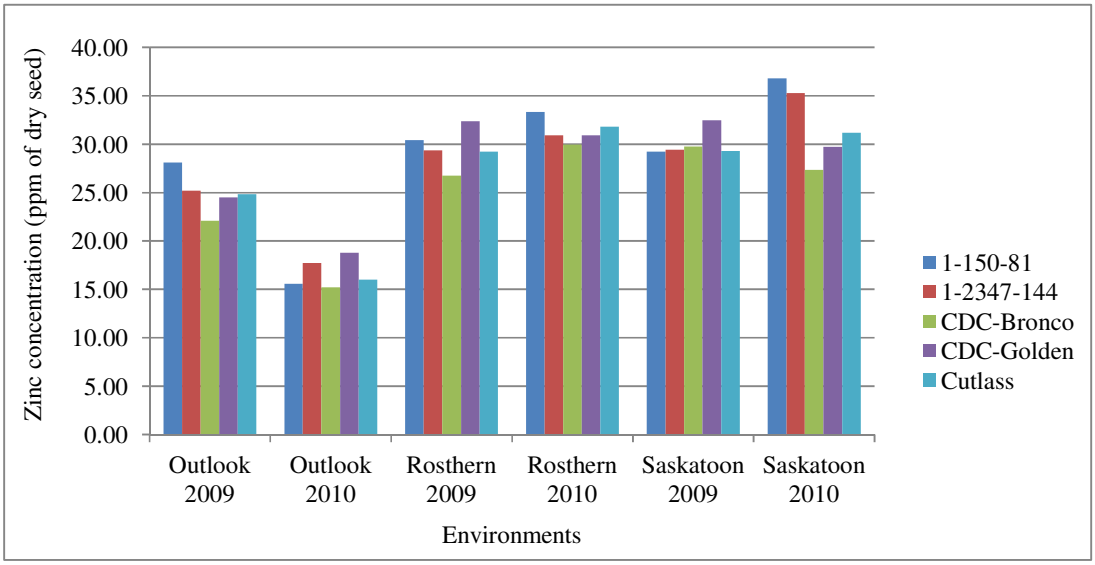

Figure 4.10 Zinc concentration of five field pea varieties grown in six environments in Saskatchewan (Grand mean=27.4 ppm; SE of interaction means=2.6)

Varieties and environments differed significantly for Fe concentration of harvested seeds, and the variety X environment interaction was also significant (Table 4.7). Variety means for mean Fe concentration fell into a relatively narrow band, with line 1-150-81 and Cutlass (both 42.1 ppm) having greater concentration than CDC Bronco (39.4 ppm), with the other two varieties intermediate (Table 4.8). Environments varied more widely with mean Fe concentration ranging from 35.1 ppm (Outlook 2010) to 57.0 ppm (Saskatoon 2010). Variety differences in Fe concentration were small at all environments except Saskatoon 2010 (Fig 4.11).

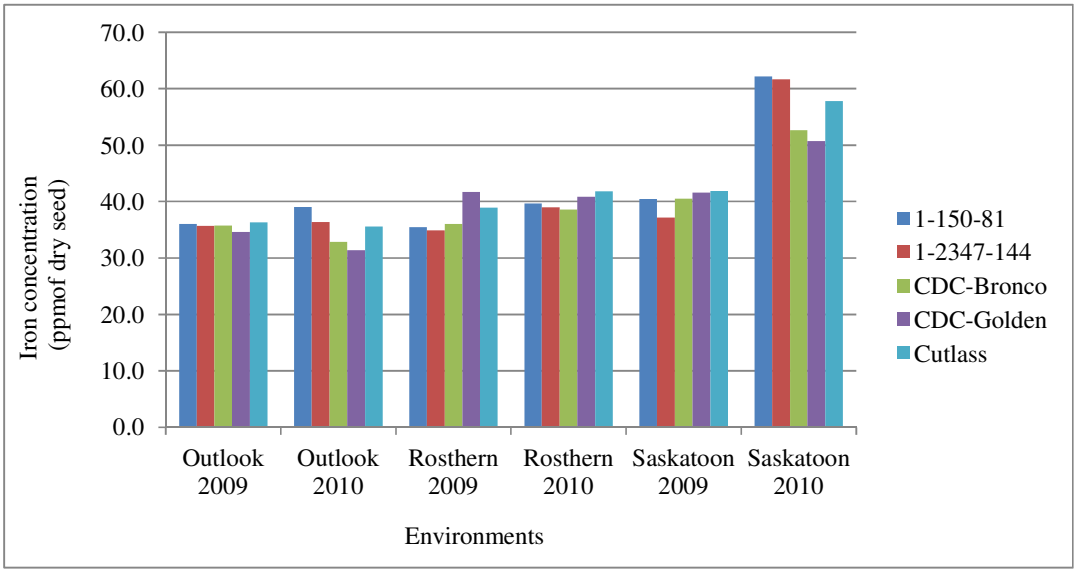

Figure 4.11 Iron concentration of five field pea varieties grown in six environments in Saskatchewan (Grand mean=40.9 ppm; SE of interaction means=3.0)

Environments differed significantly for Se concentration of harvested seeds, while varieties did not differ and the variety X environment interaction was not significant (Table 4.7). Se concentration was greatest at Saskatoon 2010 (2.49 ppm) followed by Saskatoon 2009 (1.28 ppm) (Table 4.8). The Rosthern and Outlook environments produced substantially lower Se concentration.

Varieties and environments differed significantly for crude protein concentration of harvested seeds, while the variety X environment interaction was not significant (Table 4.7). Lines 1-150-81 (24.4%) and 1-2347-144 (24.6%) had greater crude protein concentration CDC Bronco (23.4%) which was greater than the other two varieties (Table 4.8). Saskatoon 2010 (28.8%) had the greatest mean crude protein concentration, while Saskatoon 2009 had the lowest (19.7%) with the other environments being intermediate.

Environments differed significantly for ash concentration of harvested seeds, while varieties did not differ and the variety X environment interaction was not significant (Table 4.7). Ash concentration was greatest at Rosthern 2010 (1.87%) and Outlook 2010 (1.79%) and lowest at Saskatoon 2010 (1.09%) (Table 4.8).

Varieties and environments differed significantly for ether extract concentration of harvested seeds, while the variety X environment interaction was not significant (Table 4.7). Cutlass (1.13%) and CDC Golden (1.09%) had greater ether extract concentration compared to the other varieties which ranged in concentration from 0.92-0.99% (Table 4.8). Saskatoon

2010 (1.23%) had the greatest ether extract concentration, while Outlook 2009 had the lowest (0.86%) with the other environments being intermediate.

Environments differed significantly for ADF concentration of harvested seeds, while varieties did not differ and the variety X environment interaction was not significant (Table 4.7). ADF concentration was greatest at Saskatoon 2009 and 2010 (7.1% for both) and Outlook 2009 (7.0%) and lowest at Outlook 2010 (5.8%) (Table 4.8).

Varieties and environments differed significantly for NDF concentration of harvested seeds, and the variety X environment interaction was also significant (Table 4.7). Lines 1-150-81 and 1-2347-144 (17.1% each) had greater NDF concentration than the other three varieties which ranged in concentration from 16.6-16.8% (Table 4.8). Saskatoon 2010 (18.1) had greater NDF concentration than the other environments which ranged in concentration from 16.2-17.3%. Lines 1-150-81 and 1-2347-144 had greater NDF concentration than all the other varieties in all environments except Rosthern 2009 and Saskatoon 2009 (Fig 4.12).

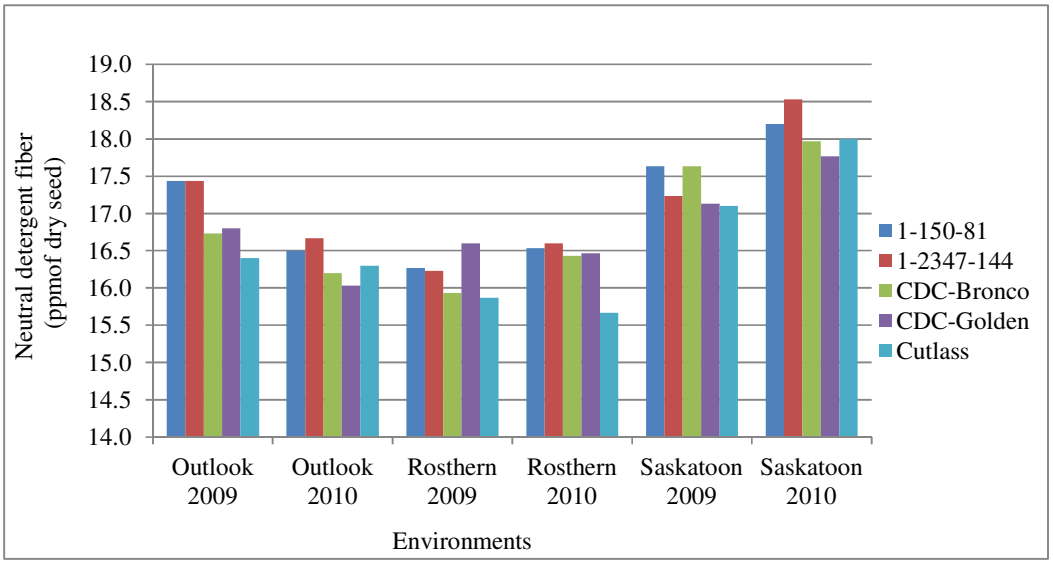

Figure 4.12 Neutral detergent fibre concentration of five field pea varieties grown in six environments in Saskatchewan (Grand mean=16.9 ppm; SE of interaction means=0.3).

Environments differed significantly for starch concentration of harvested seeds, while varieties did not differ and the variety X environment interaction was not significant (Table 4.7). Starch concentration was greatest at Rosthern 2010 (44.5%), Saskatoon 2009 (44.2%) and Rosthern 2009 (44.0%) and lowest at Saskatoon 2010 (42.6%) (Table 4.8).

Varieties and environments differed significantly for germination rate under control conditions, and the variety X environment interaction was also significant (Table 4.9)

Table 4.9 Mean squares of combined ANOVA for control, accelerated aging, and cold stress germination of five field pea varieties grown in six environments in Saskatchewan

Source	Environment (E)	Replication in Environment	Variety (G)	G x E	Residuals
Df	5	12	4	20	48
Control germination	399**	59.39	238.9**	108.8*	57
Accelerated aging germination	1005**	533.6	960.9**	200.7	146.6
Cold stress germination	1558**	111.2	907.6**	88.6	75.9

Note: *P<0.01; **P<0.05

Table 4.10 Means of control, accelerated aging and cold stress germination rate of five field pea varieties evaluated in six environments in Saskatchewan.

Variety	Control Germination (%)	Accelerated Aging Germination (%)	Cold stress Germination (%)
1-150-81	80	61	55
1-2347-144	80	56	53
CDC-Bronco	83	72	67
CDC-Golden	82	69	48
Cutlass	74	72	58
Mean	79	66	56
CV (%)	9.5	18	16
LSD=0.05	5	8	6
Outlook 2009	82	65	52
Outlook 2010	77	51	44
Rosthern 2009	85	71	62
Rosthern 2010	73	65	73
Saskatoon 2009	75	73	54
Saskatoon 2010	85	71	52
Mean	79	66	56
LSD=0.05	6	18	8

Cutlass (74%) had lower mean germination rate under control conditions than the other four varieties, whose mean germination rate ranged from 80-83% (Table 4.10). Outlook 2009 and Saskatoon 2010 (85% each) had greater mean germination rate under control conditions than Outlook 2010, Rosthern 2010 and Saskatoon 2009, while Outlook 2009 was intermediate. Cutlass had the lowest germination rate under control conditions at all environments except Outlook 2010 and Rosthern 2010 (Fig 4.13).

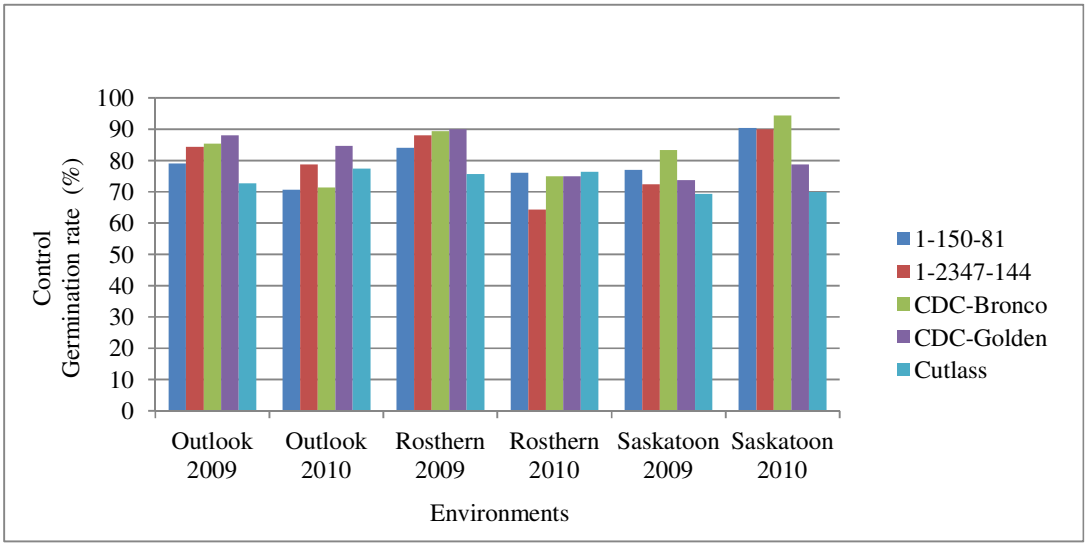

Figure 4.13 Germination rate under control conditions for five field pea varieties evaluated in six environments in Saskatchewan (Grand mean=79%; SE of interaction means=9).

Varieties and environments differed significantly for germination rate under accelerated aging conditions; the variety X environment interaction was not significant (Table 4.9). Lines 1-150-81 (61%) and 1-2347-144 (56%) had lower mean germination rate under accelerated aging than CDC Bronco (72%), CDC Golden (69%), and Cutlass (72%) (Table 4.10). Saskatoon 2009 (73%), Saskatoon 2010 (71%), and Rosthern 2009 (71%) had greater mean germination rate under accelerated aging conditions than Outlook 2010, while Outlook 2009 and Rosthern 2010 were intermediate

Varieties and environments differed significantly for germination rate under cold conditions; the variety X environment interaction was not significant (Table 4.9). CDC Bronco (67%) had the highest mean germination rate under cold conditions, followed by Cutlass (58%); CDC Golden (48%) had the lowest rate, while lines 1-150-81 (55%) and 1-2347-144 (53%) were intermediate (Table 4.10). Rosthern 2010 (73%) had the greatest mean germination rate under cold conditions, followed by Rosthern 2009 (62%); Outlook 2010 had the lowest mean germination rate, while the other three environments had intermediate mean germination rates.

The relationship between phytate phosphorus and several traits was studied (Table 4.11). DTF and DTM were negatively correlated with phytate phosphorus concentration. MP1 and MP2 were positively correlated with phytate phosphorus. Total phosphorus concentration and ether extract were positively correlated with phytate phosphorus.

Inorganic phosphorus and zinc concentration were negatively correlated with phytate phosphorus.

Table 4.11 Correlation coefficients among phytate P concentration and several other traits of five field pea varieties grown in six environments in Saskatchewan

Field traits		Chemical traits	
Variables	Phytate P	Variables	Phytate P
Emergence count 1	ns	Total phosphorus	0.275*
Emergence count 2	ns	Inorganic phosphorus	-0.639**
Days to flowering	-0.602**	Zn	-0.281*
Height	ns	Fe	ns
Mycosphaerella blight 1	0.366**	Se	ns
Mycosphaerella blight 2	0.560**	Crude protein	ns
Lodging	ns	Ash	ns
Days to maturity	-0.416**	Ether extract	0.254*
Yield	ns	Acid detergent fibre	ns
1000 seed weight	ns	Neutral detergent fibre	ns
Control germination	ns	Starch	ns
Accelerated aging	ns		
Cold germination	ns		

Significance: ** $p<0.01$; *$p<0.05$.

Blank (ns) indicates no significance.

5. DISCUSSION

The total P concentration in the harvested seeds of the low-phytate lines and check varieties did not differ substantially, with line 1-150-81 having a slightly greater concentration than the other varieties. The environment had a much greater effect on total P concentration than did variety. In contrast, differences in phytate P and inorganic P concentrations were substantial among varieties, much greater than the effect of environment.

Mean concentration over six environments in Saskatchewan for phytate P was 1.13 mg/g in line 1-2347-144, 1.20 mg/g in line 1-150-81 and 2.99 mg/g in CDC Bronco. Thus, phytate P concentration was reduced by greater than 50% in the two low-phytate lines in comparison to their progenitor variety. In contrast the mean concentration over six environments in Saskatchewan for inorganic P was 1.22 mg/g in line 1-2347-144, 1.28 mg/g in line 1-150-81 and 0.24 mg/g in CDC Bronco. Thus, inorganic P concentration was increased 5-fold in the two low-phytate lines in comparison to their progenitor variety. The increased inorganic P concentration in seeds has been reported to be equal to the increase in phytate P in low phytate crops (Cichy and Raboy, 2009).

Low-phytate varieties are currently available for wheat, rice, maize, soybean and barley (Lott et al., 2002). This study shows that low-phytate lines of pea have been developed that also have suitable agronomic and quality characteristics. The development of low-phytate lines of crops such as pea provides a particularly interesting opportunity as there are problems associated with the availability of phosphorus to humans and other non-ruminant animals when the phosphorus is in the form of phytate. However, recognizing the required human dietary intake of nutrients is very important. For example, the phosphorus requirement for infants is 300-500 mg per day and for children and adults between 800-1200 mg per day (Reddy, 2002). Recommended average dietary intake of P is 1000 mg per person per day, which equals 365 g of P per person per year. Lott, et al. (2002) estimated that the annual global total uncorrected P yield from all crop seeds and fruits was over 12 million tonnes. If we assume all the P found in crop seeds is bioavailable for humans, then each of the 6 billion humans on earth may obtain about 1399 grams of P per year. Unfortunately, not all of the phosphorus from crop seeds and fruits is available to humans because 60-80% of the total P is in the form of phytate. If more of the phosphorus in seeds or grains were available to non-ruminants, the environmental problems associated with P pollution would be reduced. If 25% of all the world cereal and legume crops were replaced by low-phytate varieties having a 66% reduction in phytate P, the world total for phytate P in seeds and fruits

would be reduced by about 3.4 million tonnes or about 15% of the total (Lott, et al., 2002). This would reduce the amount of excess P that enters the water supply.

Deficiencies of iron and zinc affect over half of the world's population. Reducing phytate concentration of crop seeds will contribute to the biofortification of crops for human and animal diets (Nesel et al., 2006). Iron bioavailability of the two low-phytate pea lines and CDC Bronco were recently tested using the Caco-2 mammalian cell bioassay, with preliminary results indicating greater bioavailability of iron in the two low-phytate lines than CDC Bronco (unpublished data, X. Liu and T. Warkentin).

The rate of plant development as measured by days to flowering and days to maturity differed among the varieties used in this study. The low-phytate lines were slightly later in both mean days to flower and mean days to maturity than CDC Bronco, CDC Golden and Cutlass. From a production point of view in Saskatchewan a one or two day delay in field pea maturity is not substantial. Similar to days to flower and days to maturity, the variety differences in plant height, MP1, MP2, and lodging score were relatively small in this study, smaller than the effect of environment. The low-phytate lines did not display phenotypes for these traits that would be considered detrimental to their performance as varieties. The mean grain yield of line 1-150-81 was 86% of that of CDC Bronco, the progenitor variety, while the yield of line 1-2347-144 was 92% of CDC Bronco. However, the yield of CDC Bronco also exceeded the yield of the widely grown check cultivars CDC Golden (81% of CDC Bronco) and Cutlass (87% of CDC Bronco) over the two years of this research. However, it should be noted that long-term mean yields of CDC Bronco, CDC Golden, and Cutlass in Saskatchewan are similar based on testing at many diverse locations in the province (Saskatchewan Ministry of Agriculture, 2012). Several low-phytate crops had lower grain yield compared to their progenitor (Cichy and Raboy, 2009). Lines 1-150-81 and 1-2347-144 likely carry many additional mutations which could have negative effects on grain yield. Seed weight was lower for the low-phytate lines than for CDC Bronco. Since seed weight is an important component of grain yield, this may explain a portion of the reduced grain yield.

Recurrent selection for yield can be used to select low-phytate lines with an increased yield and earlier maturity. This method can reduce drag from other unwanted mutations caused by chemical mutagenesis. Evaluation of the inheritance of the low-phytate trait in field pea is currently in progress. Lines 1-150-81 and 1-2347-144 were crossed with normal-phytate lines and with each other to determine the number of genes involved and whether they are allelic. The deposition of phosphorus compounds in low-phytate and normal-phytate pea lines during seed development is under evaluation. Mapping of the low-phytate trait in

the pea genome and evaluation of the performance of low-phytate pea in mammalian nutrition is also in progress (Warkentin, et al., 2012).

Variety differences in macronutrient (crude protein, ash, ether extract, ADF, NDF and starch) and micronutrient (Zn, Fe, and Se) concentrations were relatively small to absent in this study. In contrast, environment differences for these traits were often substantial, likely reflecting local weather and soil conditions. Thus, the low phytate pea lines were not deleteriously affected for nutrient concentration.

The negative nutritional effects of phytate in crop seeds are of interest worldwide (Lott, et al., 2002). The ability of phytate bind with trace minerals (Ca, Fe, Zn etc.) can be a major factor in limiting mineral bioavailability, resulting in trace mineral deficiencies (Schlemmer, et al., 2009). Over three billion humans have micronutrient deficiencies, mainly in developing countries that lead to increased health issues such as learning disabilities among children, increased morbidity and mortality rates, lower worker productivity and higher health care costs. The concentration of minerals in field pea seeds measured in this study (Zn, Fe and Se) are important, however, the proportion of these minerals that are absorbed in the intestine and utilized by humans is of greater importance. The low phytate pea lines are expected to allow for increased bioavailability of zinc and iron, in addition to phosphorus, when fed to humans or monogastric animals. The Indian subcontinent is a major export market for Canadian peas. The phosphorus and micronutrient status of Indian consumers could be improved by consumption of low phytate pea and other grains.

Crude protein and NDF concentration in the low-phytate lines was higher than for CDC Bronco and the other check varieties. However, Wang and Daun, (2004) reported that phytate P concentration was not correlated with crude protein and NDF in field pea.

Approximately 1.9 million tonnes of feed are required to produce 5.1 million hogs per year in western Canada. Since only approximately 30% of the phosphorus in the feed is digestible, 1.5% dicalcium phosphorus must be added to the feed to meet the dietary requirements. At $600 per tonne, the cost of added dicalcium phosphorus would be $17 million per year. If low-phytate barley CDC Lophy-1, developed at the University of Saskatchewan (70% of ration), and low-phytate pea (30% of ration) were used in hog rations in western Canada, phosphorus excretion into the environment could be reduced by approximately 3000 tonnes compared to rations with normal-phytate barley and normal-phytate pea because of improved digestibility of phosphorus. Another cost saving could occur by eliminating the need to add phytase enzyme to hog rations. Thacker et al, (2006) used 0.02 % phytase in hog diets (200 g per tonne of feed) at a cost of $17.60/kg. Thus, the

cost of phytase enzyme would be approximately $6.7 million per year to feed 5.1 million hogs. Reducing phosphorus pollution by 3000 tonnes per year would be a great benefit to the people of western Canada. Greg McCullough (University of Manitoba) reported that Lake Winnipeg, one of the largest fresh water lakes in the world, is compromised due to phosphorus pollution (Manitoba Agriculture news releases, 2011). Phosphorus levels in this lake are three times higher than that of Lake Erie when it was described as dead. One of the sources of phosphorus pollution in Lake Winnipeg could be manure from livestock production in the region, and legislation has been developed to limit further hog industry expansion.

The seedling vigour of low-phytate soybean and corn is sometimes less than that of control varieties (Meis et al., 2003). Emergence counts, seedling vigour and germination rate are related, but these variables do not measure exactly the same characteristics. In the present study, the low-phytate lines had a similar rate of field emergence as the normal-phytate lines. Estimates of emergence performance were unbiased because uniform seed sources were used in each season. However, variety differences were observed for germination rates among the five varieties considered. The ranking of varieties for germination rate depended on the method used to measure germination. It was stated earlier that phytate is believed to play a role in breaking of dormancy and in germination. When the accelerated aging method was used, the low-phytate lines had a lower germination rate than the normal-phytate varieties. Similarly, the low-phytate lines had a lower germination rate than CDC Bronco under cold temperature conditions. Germination and field emergence were reduced in many but not all low-phytate lines of soybean (Cichy and Raboy, 2009). The amount of reduction in emergence or germination rate therefore appears to be dependent on the specific mutation(s) that gave rise to the low-phytate phenotype and the type of stress encountered by the seed.

6. SUMMARY AND CONCLUSION

In general, lines 1-150-81 and 1-2347-144 performed successfully when compared to the normal-phytate varieties over six environments in Saskatchewan. Agronomic performance of the two low-phytate pea lines and normal-phytate lines was similar with a few differences. The two low-phytate lines did not differ from the normal-phytate lines in field emergence counts, plant height, mycosphaerella blight score, and lodging score. Days to flowering and days to maturity were slightly slower for the low-phytate pea lines and yield (7-15%) and seed weight (6%) were reduced compared to CDC Bronco.

The phytate P concentration in lines 1-150-81 and 1-2347-144 was approximately 60% lower than for the progenitor line CDC Bronco. This reduction corresponded to a proportional increase in inorganic P, with similar total P concentrations for both low-phytate and normal-phytate lines. For most traits measured, the effect of environment was greater than the effect of variety, except for phytate P and inorganic P concentration where the effect of variety was greater.

Micronutrient (iron, zinc and selenium) concentrations and macronutrient (crude protein, ash, ether extract, acid detergent fibre, neutral detergent fibre and starch) of the low-phytate lines were similar to CDC Bronco, Cutlass and CDC Golden.

The low-phytate lines had similar germination rate to the checks under control conditions, but somewhat reduced rates under cold and accelerated aging conditions.

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