GREEN SEED COAT COLOUR RETENTION IN LENTIL (*Lens culinaris)*

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By
Blaine F. Davey

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ABSTRACT

Poor seed coat colour desirability in green lentil (*Lens culinaris*) costs lentil producers millions of dollars each year. The monetary value that Canadian lentil producers receive for their crop is based on the visual characteristics of the seed coat, mainly the colour. Higher value is given for samples described to have more desirable green seed coat colour. A breeding line, 1294M-23, has been noticed to consistently produce more desirable green lentil samples.

A cross was made between 1294M-23 and a less desirable breeding line 1048-8R with the goal of studying the heritability of green seed coat desirability measured by the Acurum® machine. The resulting progeny were taken to F_7 by single seed descent. In 2005 and 2006 the recombinant inbred lines (RILs) were seeded in a randomized complete block design at three sites in the current main lentil growing region of Saskatchewan. To measure the seed coat colour of the samples, the Acurum® machine, which is a colour analyzing machine developed for grain crops, was used to consistently compare the samples. The study illustrated that the trait has large environmental effects and is quantitative with a high broad sense heritability of 0.82, using this specific cross and environments. Transgressive segregation occurred for RILs that had more desirable green seed coats and lower index scores than the desirable parent, 1294M-23. A tester that included all registered green lentil cultivars set was grown with the RILs in all environments. The seed coat colour index scores of the tester set fit into a small section of the range of index scores. They all had relatively high mean index scores, meaning less

desirable, showing little genetic variation for the trait in current Canadian green lentil cultivars.

Chlorophyll was extracted from seed coats of some of the RILs. The amount of total chlorophyll, chlorophyll a, and chlorophyll b was compared to the Acurum® scores using regression analysis. The study found that there was significant relationship between chlorophyll a and b content and the index score, explaining 32 and 37 percent of the variation, respectively.

Another portion of the study was to determine if preharvest treatment of the green lentil crop has an effect on the green seed coat colour of the sample. A set of genotypes consisting of all registered green seed coat cultivars was grown at two locations in Saskatchewan in both 2005 and 2006. Prior to harvest a plot of each genotype was swathed, and a second plot was desiccated with diquat. After harvest the samples were analyzed for green seed coat colour using the Acurum® machine. In general, across most genotypes, sites, and years, swathing produced a significantly more desirable green lentil sample. The desirable green parent from the RILs, 1294M-23 produced the most desirable green lentil sample in this study. When the maturity rating was correlated to the Acurum® score a significant positive relationship was found in 2005 but not in 2006. This showed that lines with later maturity could be associated with more desirable green seed coat colour in some environments. Thus caution must be taken when selecting for more desirable phenotypes that genetic gains are being made rather than indirect selection for longer maturity.

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1.0 INTRODUCTION

Green lentil has become a major crop in Canada. The monetary value that a lentil grower receives is based on the desirability of the green seed coat colour of the sample. Currently all grading is done visually using human graders. Problems may arise when different graders assign different grade levels for the same sample within the same year. Another problem is that Canadian grade standards for the desirability of green seed coats differ from year to year (Pankewich, 2007). There is a need in Canada to have a more consistent method of classifying green lentil quality in the commercial sector. This same tool could then be used by Canadian lentil researchers to ensure that commercial qualities are being analyzed during research. DuPont™ has developed the Acurum® machine which is able to consistently and quickly classify green lentil into different quality categories.

As lentil in Canada has developed into a major crop, the breeding objectives have changed. Objectives like yield and disease resistance are still important but there has been a shift to breeding for improved seed quality. Dr. A. Vandenberg of the Crop Development Centre at the University of Saskatchewan found one

breeding line, 1294M-23, that consistently produced a more desirable green seed coat across many environments and weathering conditions. It is considered to have superior green seed coat colour meaning it appears to withstand weathering of the seed coat better than some lentil lines (Vandenberg, 2005). As with many traits, it was assumed to have some genetic control but the understanding of it was very poor. A cross between 1294M-23 and a line with a less desirable green seed coat was made to allow genetic analysis of the heritability of green seed coat colour differences in lentil. The results would then guide the breeding program on the most effective route to breeding more desirable green lentils. If green lentil could exhibit a more desirable green colour in the field it would allow more marketing flexibility for green lentil producers. It is not known if other traits are associated with differences in green seed coat colour, for example, chlorophyll content in the seed coat. Little research has been done on visual seed quality of green lentil and most seed coat quality traits are poorly understood. An understanding of the heritability would allow more efficient breeding for improved green seed coat colour desirability. This will allow quicker release of more desirable genotypes in Canada improving the probability of receiving top grade for individual producers.

Lentil producers in Saskatchewan have always attempted to produce the highest quality sample with the least amount of risk. Many producers have stopped desiccating their green lentil crops, a practice which was recommended by agronomists, and resorted to swathing the crop. The producers say that the swathing allows increased probability of producing higher quality green lentil samples. Swathing is a common practice in other crops in western Canada, for

example canola. In wheat it is used to shorten the harvest interval and allow even maturation of the crop for harvest. Producers need to know if desiccation or swathing produces a significantly more desirable sample consistently so that production risk can be reduced.

It was expected that with so little known about green lentil visual quality that many more questions will be raised than answers found from this studying the heritability and preharvest treatments. Even in areas of the world like Central Asia and the Middle East, where green lentil has been a staple of the diet and agriculture for thousands of years, little is known about green lentil visual seed coat quality because most of the research is related to increasing yield per unit area but not the quality of the sample harvested.

2.0 LITERATURE REVIEW

2.1 Introduction

2.1.1 Lentil (*Lens culinaris*)

Lentil (*Lens culinaris* Medik.) is a dicotyledonous legume plant. Lentil is the fifth largest pulse crop grown in the world (Hymowitz, 1990). It grows as an indeterminate annual in a semi-erect pattern. The leaves are compound and usually have tendrils at the leaf tips. Lentil is capable of obtaining nitrogen through a symbiotic relationship with nitrogen-fixing *Rhizobium* bacteria, thereby reducing requirements for added nitrogen. Depending upon the genotype and environment there may be one to four flowers per peduncle. The small blue and white flowers develop pods usually containing one or two lens-shaped seeds, but in some cases may have up to four.

Lentil is a diploid plant with a haploid chromosome number, n=7. It is native to the Fertile Crescent and has been an important food crop for over 8500 years (Oplinger et al., 1990). Hundreds of millions of people in the world have made lentil a staple in their diet and receive a portion of their daily protein, fiber,

carbohydrate, and micronutrient requirements from it. A balanced and nutritious diet can be formulated from cereals and lentil. Protein complementation occurs because the cereals are low in amino acids like lysine and leucine, while legumes like lentil have a relatively high content of these amino acids. Amino acids like methionine and cysteine that are low in lentil (Sell, 1993) are high in cereal making for balanced protein in the diet of monogastric mammals like humans and swine.

2.1.2 Canadian Lentil

Lentil is an important cash crop in Canada especially in Saskatchewan. Saskatchewan produces 99 percent of the Canadian lentil crop (SPG, 2007). In 2005, 2.16 million acres of lentil were planted in Saskatchewan which produced 1.26 million tonnes (SAFRR, 2007). Canada is the world's largest lentil exporter. In 2003 Canada accounted for 44 percent of total international lentil exports (AAFC, 2007).

There are two main types of lentil grown in Canada, red and green. They differ on the basis of seed coat colour and cotyledon colour. Green lentil has a light green seed coat with a yellow cotyledon while red lentil usually has a brown or grey seed coat with an orange to red cotyledon. Until 1997 green lentil was the prevalent type grown on over 95 percent of the seeded area. In 2006 Saskatchewan had more red lentil acres seeded than green lentil for the first time (Hursh, 2006). The value of the two market classes is somewhat independent as the markets and uses of the two market classes differ. Red lentil is primarily consumed after the seed coat is removed by abrasive dehulling while green lentil is usually eaten whole. Thus

green lentil value is based on the visual characteristics of the seed coat. Usually the more 'green' that a sample appears the higher the price it will command in the market place. The largest importers of the Canadian green lentil crop are South America, North Africa, and the Mediterranean regions of Europe (SPG, 2005).

2.2 Canadian lentil grading system

Canadian lentils are graded by standards set by the Canadian Grain Commission (CGC). The grading system for green lentils is based on the visual appearance of the seed (Table 2.1). Lentils that are described as having good natural colour exhibit little staining or seed coat discolouration and have undergone little field weathering or darkening from storage. Market classes are differentiated by seed diameter as well as by seed coat and cotyledon colour. The system is subjective and not always consistent. Each year the CGC sets grade standards depending upon the current Canadian crop. A sample designated a certain grade one year may fall into another grade category another year (Pankewich, 2007). Depending on environmental factors individual inspectors may grade the same samples differently (Michta, 2005). The price offered for green lentils decreases as the grade of the sample decreases. It is therefore important to accurately grade the sample for the individual lentil producer to have the best opportunity to receive the highest price. Up to 30 percent of Canadian lentil exports may have acceptance issues with foreign buyers (Vandenberg, 2005). It is important to have a more consistent grading system that accurately portrays the same sample year after year.

It would also be useful for lentil growers to have better varieties that withstand weathering in the field at their disposal.

Table 2.1 Colour descriptions of the four main green seed coat categories used for grading Canadian green lentil (Canadian Grain Commission, 2005).

Colour description used for grade determinants	Characteristics
Good natural colour	Lentils that are sound, well matured and have good natural colour.
Reasonably good natural colour	Lentils that are moderately immature, with light amounts of adhered soil or lightly discoloured from storage or other natural causes.
Fair colour	Lentils that are immature but not green, moderate amounts of adhered soil, or otherwise moderately discoloured from natural causes.
Poor colour	Lentils that do not meet the definition of fair colour, but are without severely adhered soil or are severely discoloured (dark brown).

2.3 How the human eye works and instrumental colour determination

The human eye is very a complex biological system for perception of images. It can only detect light in the wavelength range of 380-780 nm (Lew, 2001). Depending upon the person, the light perception range may be limited to 400-700 nm (Lew, 2001). The light passes through the cornea where it is deflected through the pupil. Depending upon the light intensity, the pupil can enlarge to allow more light to pass through it. Next the light passes through a lens that focuses the light on the retina (Steen-Hall Eye Institute, 2004). The photoreceptor cone and rod cells are in the retina. Rod cells allow sight in dark conditions and do not

perceive colour. Cone cells are used in brighter conditions and are responsible for perception of colour. People that are colour blind do not have fully functioning cone cells (Montgomery, 2007). The colour that is perceived by the cone cell of the eye is the wavelength that the object reflects (Lew, 2001).

Every person sees different colours somewhat differently. Across a population of people the range of colour perception is very large from complete colour blindness to an ability to perceive colours in the two extremes of the visible light range. This variability makes the use of machines intriguing. If machines are calibrated properly and have the ability to consistently classify colour, the opportunity exists to have reliable and repeatable measurement of colour across many different environments. With machines it is possible to have repeatable measurements across machines, which is not possible between humans.

Instruments can be used to accurately and consistently determine the colour of seed samples. The Acurum® machine (Figure 2.1) is a computerized colour analyzing machine developed by DuPont™. The computer used a Windows® based personal computer operating at 3 gigahertz. Before each sample was analyzed the machine calibrated itself using an internal colour card. This was important because the machine operated in a dusty environment that could affect analysis. The colour card had proprietary colour definitions, not from any international standards. The machine uses a camera to record digital images of one side of individual seeds as a sample passes by in a single layer on a conveyor belt. The camera captures digital images at a size of 768 × 1024 pixels as white LED strobe lights flash. The images are analyzed in a bitmap format with an eight bit colour display. Neural networks

process and categorize the data into predefined colour categories. As the machine analyzes each image it uses mathematical models to define each seed in the image. Each seed within the image is then subjected to colour analysis through the use of algorithms to classify each seed. The machine operates at a speed of 2.5 images per second. Approximately 30 lentil seeds are captured in each frame (Van Natto, 2007). The colour categories that were used in this research were defined by Larry Michta, a former Canadian Grain Commission grader (Table 2.2). The colour categories were designed to match commercial industry standards and were not all the same size in the 3-D colour spectrum. This was the basis of the colour categories used in the commercial interface for the machine. The desirability of the lentil seeds are measured numerically by the Acurum® machine. The categories are numbered one through 20 with the lowest numbers representing most desired colour. The higher numbered categories represent decreasing desirability and show definite yellow and brown colours.

Table 2.2 can be structured to show approximate grades that would be assigned by the CGC to the lentil sample. Scores 1 through 3 would be a Canada number 1. Scores 4 through 7 would be a Canada number 2. A Canada extra 3 grade would be 8 through 10. A Canada number 3 would be 11 through 13. Scores 14 through 20 would all be sample or feed grade lentils. Samples that are composed of many different lentils seeds from many scores are graded lower than the best individual seeds scores.

Figure 2.1 The Acurum® machine at the Pulse Field Lab.

Table 2.2 Descriptions of the colour categories used by the Acurum® machine. They were developed by Larry Michta, to classify green lentil from most desirable (1) to least desirable (20) seed coat colour.

Score	Definition
1	dark green bordering on immature
2	dark mature green (desirable)
3	medium green (desirable)
4	light green
5	light green showing signs of bleaching
6	very light green with bleaching present
7	light green in color containing bleaching and some oxidation
8	light green with oxidation
9	immature green with signs of oxidation
10	dark green with oxidation
11	salmon colored sun bleached
12	pale green bleached and oxidized
13	very pale green and bleached
14	uniform sample with light to moderate oxidation
15	moderate oxidation
16	moderate oxidation with tinge of green
17	moderate oxidation of bleached sample
18	moderate to severe oxidation
19	severely oxidized
20	oxidized non uniform sample

There has been no academic research conducted with the Acurum® machine in the past. There have been other efforts to develop machines that would classify green lentil by colour and variety. It is assumed that challenges identified in research with other machines would be similar to challenges encountered by the Acurum® machine. Shahin and Symons (2002) reported that a colour analyzing machine prototype was being field tested by the CGC. The prototype was manufactured by Hinz Automation (Saskatoon, Saskatchewan). This system uses a flatbed scanner and image analysis software which uses neural networks for data

analysis. The system assigns a grade for green lentil that is 95 percent consistent with the grade assigned by CGC inspectors. The system used a scanned sample image that was 512 pixels × 512 pixels with a resolution of 100 dots per inch (Shahin and Symons, 2001a). The system was also able to identify different varieties of lentil with reasonable accuracy (Table 2.3). The sizing ability of the image analysis software employed was within 0.2 mm of the actual seed size for lentils.

One drawback that was identified by Shahin and Symons (2001b) was that peeled or split lentils may skew the colour data that were analyzed by the system. These off colours affected the mean colour of the sample and could cause the system to misclassify the sample and place it into the wrong colour category. Shahin and Symons (2001b) used three colour categories to classify colour. One category received most of the incorrect classifications, due to the split and peeled lentils. It is expected that the larger the number of colour categories, the larger the error will be, unless the system is able to identify split or peeled lentils or if the operator was to manually remove these from the sample before analysis.

Table 2.3 Estimates for the accuracy of the Hinz Automation system at identifying different lentil market classes and varieties (Shahin and Symons, 2002).

Actual Variety	Percent Predicted Variety				
	Laird	Richlea	Eston	Crimson	CDC Redwing
Laird	98.5	1.5	0	0	0
Richlea	3.3	96.7	0	0	0
Eston	0	0	100	0	0
Crimson	0	0	0	100	0
CDC Redwing	0	0	0	0	100

2.4 Perceived seed colour in green lentil

There are two factors that determine the perceived colour of the seed: seed coat colour and cotyledon colour. The main cotyledon colours of lentil are orange, green, and yellow. Seed coat colours include black, brown, gray, light green, and tan (Vandenberg and Slinkard, 1990). It is also known that cotyledon colour may affect the seed coat colour in lentil (Emami and Sharma, 2000). The inner surface of the seed coat may be either brown or green depending on the colour of the cotyledons. This shows that the coloured pigment in the cotyledon can be absorbed by the seed coat tissue, thereby affecting its appearance. If the seed coat tissue does not develop a colour separately from the cotyledon, the cotyledon colour may stain the inside of the seed coat. The tan seed coat may have a green hue due to the leaching of the colour out of the cotyledons (Emami and Sharma, 2000). This has a smaller effect on perceived colour than the colour produced genetically. When green cotyledon is combined with green seed coat colour the resulting seed may appear to be a darker 'green' than if the cotyledon was yellow in colour.

The colour perceived in green lentil is due to the colour of the seed coat and to some extent the cotyledon colour. The seed coat is green while the cotyledons are yellow in colour. As seed colour becomes less desirable in green lentil it goes from green to yellow in colour. The Canadian green lentil grading system is based upon the visible colour which is mostly due to the colour of the seed coat. Crops like canola and green pea have grading systems based upon the cotyledon colour. Green lentil also has differences in seed coat colour because of darkening of the seed coat, a major source of quality loss in storage. To reduce darkening, storage should be

short term under conditions of low temperature and humidity. The darkening occurs due to some type of tannin reaction (Matus et al., 1993). Darkening and differences in green lentil colour are two different problems that occur in the seed coat but both reduce final desirability of the sample. These two problems are unique to green lentil as they both occur in the seed coat. Other factors like staining or the presence of disease may occur during field weathering as well. Cotyledon colour may affect the colour perceived by acting as a background. Different hues of green seed coats may allow the yellow cotyledon background to have some effect.

2.5 Colour in soybean (*Glycine max*) tissue

In soybean reproductive and vegetative tissues, green colour retention is associated with the stay-green trait. Guiamét et al. (1991) report that the stay-green trait does not have the same amount of chlorophyll a and chlorophyll b degradation as compared to conventional soybean. Only about ten percent of chlorophyll loss occurs at maturity. The stay-green trait is associated with the presence of thylakoid proteins that are associated with both photosystem I and photosystem II. In soybean, two loci control the trait. One locus controls the trait in leaves while the other controls the stay-green trait in seeds. Lentil does not have the stay-green trait but it is not yet determined if green seed coat colour loss is similar to that of conventional soybean.

2.6 Cotyledon chlorophyll degradation in *Brassica* spp.

It is currently not known in lentil seed coats which factors play major or minor roles in chlorophyll degradation and if it is possible to minimize the degradation. The *Brassica* genus includes species which are very important to the world's vegetable oil supply. To be of maximum value the sample must have few green cotyledons, or few seeds that are still green, because it causes the oil to be green due to elevated levels of chlorophyll. The chlorophyll reduces the shelf life of the oil and thus expensive industrial processes have to be employed to remove it. It has been a major objective of plant breeders to reduce the incidence of green cotyledons in canola samples. The goal of green lentil is to have a seed coat that appears more green while in *Brassica* sp. the goal is to have a cotyledon that appears less green. It is unknown if similar mechanisms that prevent green cotyledon loss in *Brassica* sp. are similar to increased green colour desirability in lentil.

The green colour that is observed in seeds of canola and rapeseed is due to the cotyledon retaining a high level of chlorophyll or pheophytins, which are the initial chlorophyll breakdown product (Daun, 1982). As the plant ripens, chlorophyll degradation in the cotyledon was related to the moisture content of the seed (Figure 2.2). If environmental conditions are 'normal' during ripening there is little chlorophyll left in the seed. The ripening period occurs between physiological maturity and harvest maturity (Elias and Copeland, 2001). There is minor genotype × environment interaction that affects the amount of chlorophyll that is present in the seed at harvest. The production of ethylene, which is known as a ripening hormone in many plant species, has no effect on chlorophyll loss from canola or

rapeseed cotyledons (Ward et al., 1995). This suggests that the degradation of chlorophyll in canola cotyledons is independent of ethylene production and is caused by some other factor. Baardseth and Von Elbe (1989) found that the amount of ethylene was related to the amount of chlorophyll degradation in spinach leaves (*Spinacea oleracea*). This shows that different tissues may have different mechanisms to degrade chlorophyll. The ethylene increased the activity of peroxidase while decreasing the activity of chlorophyllase. In green lentil ethylene probably acts in a similar manner to canola cotyledons because the seed coat is in close proximity to the cotyledons and undergoes similar moisture and metabolic changes while developing.

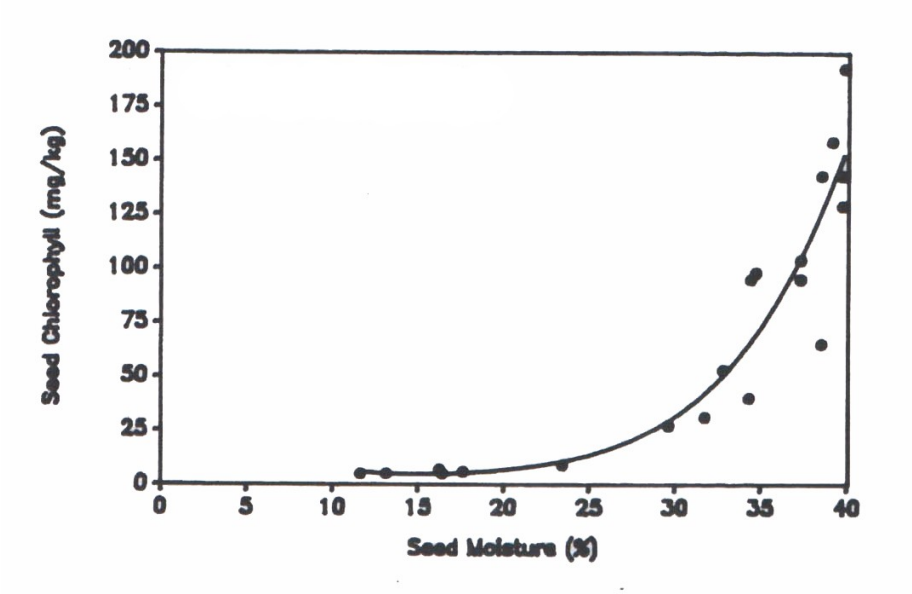

Figure 2.2 Relationship between cotyledon moisture content and cotyledon chlorophyll content in the rapeseed cultivar Stellar during ripening (Ward et al., 1995).

Ward et al. (1995) reported that the rate of chlorophyll breakdown in canola was temperature dependent with a much higher rate of breakdown at warmer

temperatures. The more enzyme activity measured in the canola seed the less chlorophyll remained in the seed at harvest maturity (Tsang et al., 2003). Johnson-Flanagan et al. (1994) reported that the enzymes peroxidase and chlorophyllase break down the chlorophyll in canola seeds. Both lead to chlorophyll degradation and reflect different wavelengths of light causing a different colour being detected.

Another important type of chlorophyll loss occurs from photo bleaching. Environmental factors like frost can inhibit the ability of the plant to degrade chlorophyll. This most likely occurs as the cold temperature prevents the enzymes from degrading chlorophyll during rapid seed desiccation. Several days after a frost event the chlorophyllase and peroxidase activity may return to pre-frost levels as long as the frost was not lethal to the plant. Johnson-Flanagan et al. (1994) also report that the use of humidification on the seed, below the level required for germination, will increase the activity of chlorophyllase and peroxidase after a non lethal frost. In the field, moist weather could possibly increase enzyme activity and further reduce the amount of chlorophyll in the seed. If canola seed is harvested at too high a moisture content before the seed was fully ripened, the chlorophyll content in the cotyledon would be difficult to reduce by environmental means. The frequency of green cotyledons is usually reduced in the field by natural air drying after swathing. If canola seed is dried artificially in commercial grain dryers the chlorophyll would not be reduced even though the moisture content is reduced to a level that is conducive to long term storage (Cenkowski et al., 1989a). In lentil this would translate into beginning harvest prior to optimum moisture content for storage, which could produce a more desirable sample.

2.7 Bleaching of green colour in field pea

Bleaching or weathering that compromises the appearance of green in field pea is very important economically. One major parallel between bleaching in pea cotyledons and green seed coat colour loss in lentil is that both involve a decrease in the desirability of perceived green colour. Similar to green lentil, the value of green field pea is affected by the perceived colour of the seed. In the case of green pea, the cotyledons are green and the seed coat is opaque. Green peas are consumed whole or decorticated and split, instead of eaten whole like green lentil. The more green the sample appears the more it is worth in the market place. Bleached seeds may also exhibit reduced germination and emergence when used for planting (Riehle and Muehlbauer, 1975).

There are several compounds that affect bleaching in field pea but chlorophyll is the most important (Cheng et al., 2004). It has been shown that bleached green peas have a higher content of lutein in the cotyledons compared to the unbleached samples. A low chlorophyll a:b ratio in the seed coat of green peas can cause the seed coat to appear less green (McCallum et al., 1997, Cheng et al., 2004, and Chao et al., 1995). The low ratio is expected because chlorophyll a is less stable than chlorophyll b. Another possible reason is that chlorophyllase has a higher affinity for chlorophyll a than chlorophyll b. This is often observed in senescing leaves (Raven et al., 1999).

When green pea seeds are soaked in water and subsequently exposed to light, the chlorophyllase activity increases significantly compared to unsoaked seeds as the seed leaves the dormant state. This explains why subsequent dehydration of

the seed produces bleached seeds with reduced germination and emergence compared to unbleached seeds. Cheng et al. (2004) found that chlorophyllase, a chlorophyll degrading enzyme, activity was approximately the same in green pea genotypes that bleach versus genotypes that were more resistant to bleaching. Their conclusion was that proteins attached to chlorophyll differ which allowed for bleaching differences that were not explained by chlorophyllase activity, but little is known of this process. Chlorophyll-degrading peroxidase played a much smaller role in bleaching than chlorophyllase and its concentration was correlated to pigment loss but the biochemical pathway of pigment loss was unknown.

Nitrogen level in the soil appears to play a small part in the bleaching resistance of green peas. Browning and George (1981) found that plants that received high levels of applied nitrogen were more susceptible to bleaching than plants grown in a soil with low nitrogen concentration. In practice however this probably has little effect on bleaching because the high nitrogen treatments in the study had much more nitrogen available than would typically be found in soils in western Canada.

The hardness of the seed coat also affects the amount of bleaching that occurs in green pea. Riehle and Muehlbauer (1975) discovered that when their laboratory bleaching technique was used, seeds that did not imbibe water quickly (hard seeds) did not bleach as much as genotypes that imbibed water quickly. They observed high correlation between seed hardness and bleaching resistance in laboratory and field trials. This suggested that the seed coat's physical attributes

may provide some level of bleaching resistance. It was not reported if the green pea genotypes that imbibe water slowly have desirable cooking qualities.

Several studies report environmental factors that affect the incidence of bleaching in green field pea. McCallum et al. (1997) and Gubbels and Ali-Khan (1990) both reported that wet weather or the use of irrigation during maturation will cause green pea to have a greater incidence of bleaching. McCallum et al. (1997) found that green pea canopies that had a more upright stature would have less bleaching because the seeds in the pods were exposed to the wet environment for a shorter period of time. It is also known that the rate of bleaching is higher in the light than the dark. The effect of light could be confounded with the effect of heat because chlorophyllase is a heat activated enzyme (Baardseth and Von Elbe, 1989).

Another factor that increases the rate of bleaching is seed moisture content. The longer the seed is kept above 20 percent moisture content the higher the level of bleaching that occurs (Riehle and Muehlbauer, 1975). Cheng et al. (2004) found that wet conditions followed by sunny conditions produced the largest amount of bleaching in field pea.

The genetic control of seed bleaching in field pea was reported to be controlled by two minor and two major genes that act in a quantitative manner (McCallum et al., 1997). The two major genes were tightly linked in repulsion and followed the dominant gene model of inheritance while the two minor genes acted in an additive manner. Biochemically the genes were hypothesized to affect the photosynthetic pigment concentration in the cotyledon rather than the total amount of photosynthetic pigment present in the cotyledon. This was shown by the lack of

correlation between seed weight in field pea and photosynthetic pigment concentration. It was found that even if a genotype had a higher initial chlorophyll level that it may be the most prone to bleaching, and after bleaching it may have the least amount of chlorophyll present (Cheng et al., 2004).

The green colour that the human eye detects may be affected by certain factors like seed coat characters. A thicker seed coat may affect light reflectance differently than a thin seed coat or less translucency of the seed coat can cause the cotyledon to have less effect on the colour detected by the human eye (McCallum et al., 1997). It is not known if any of the factors that increase or decrease bleaching in green pea is related to differences in green seed coat colour in lentil. It is also not known how closely related green pea bleaching is to differences in green seed coat colour in lentil.

2.8 Desiccation and Swathing in Lentil Production

In lentil desiccation or swathing prior to harvest is a common practice by producers. The swathing or desiccation causes the entire field to have even maturity for combine harvesting. This is important as lentil is an indeterminate crop and depending upon environmental conditions large variations in the field may occur for maturity.

2.8.1 Desiccants

Many green lentil producers in Saskatchewan believe that desiccation produces a lower quality green lentil sample compared to swathing (Vandenberg,

2005). Diquat (9,10-dihydro-8a,10a-diazoniaphenanthrene) is a desiccant commonly used in lentil production. It is used to quickly dry the green tissues of the crop to enhance rapid drying of the seed to moisture levels suitable for combine harvesting and safe storage. In western Canada it is most often used on pulse crops like field pea, lentil, and chickpea. It is also used on other crops like potato (*Solanum tuberosum L.*) that are grown to a limited extent in western Canada. Diquat and glufosinate ammonium (ammonium (2*RS*)-2-amino-4-(methylphosphinato)butyric acid) are the only two registered desiccants for lentils in western Canada. For efficacy reasons and concerns about herbicide rotation, diquat is more commonly used.

Due to the high cost of diquat, other herbicides like glyphosate (N-(phosphonomethyl)glycine) are often used for desiccation even though plant dry down is much slower with glyphosate. Yenish and Young (2000) reported that glyphosate applied at the proper preharvest timing in spring wheat (*Triticum aestivum*) can work well to provide a dry uniform crop at harvest. A significant issue with the use of glyphosate as a preharvest treatment is the potential loss of vigour if the seed is used for planting the following year. Baur et al. (1977) indicated that the seed may have poor germination and seedling vigour because glyphosate is translocated to the developing seed thereby killing the embryo. In their study if glyphosate was applied too early yield was reduced mainly through a loss in kernel weight. Slower dry down occurs with glyphosate because it is a systemic herbicide and diquat is a contact herbicide. The main reason that diquat is

used widely as a desiccant is that it does not affect the germination or seedling vigour of the seed (Kopriva, 1971).

2.8.1.1 Mode of action

Diquat is the only group 22 herbicide available in western Canada. The mode of action for diquat is as a photosystem I inhibitor. It does not actually block the action of photosystem I but rather diverts the excited electrons to a new electron accepting molecule (Hall et al., 1999). Instead of the electron being transferred to the iron rich ferredoxin it is diverted to an oxygen molecule. This oxygen becomes a free radical and is extremely toxic. It destroys lipids and plant membranes. As membranes are destroyed in the chloroplast they are no longer able to function in the chemiosmotic synthesis of ATP (Raven et al., 1999). This leads to the loss of the ability to make ATP and results in cell death. Diquat is a catalyst and is not destroyed in the reaction therefore it is able to form more free radicals and thereby cause quick plant death.

2.8.2 Swathing

Swathing, also known as windrowing, has been a part of western Canadian agriculture from its inception. It is used to promote uniform maturity and seed moisture for harvest and is an alternative to the use of desiccants. All of the foliage will be dry by the time of grain separation from the rest of the material and the grain will have uniform moisture content leading to more stable handling and storage.

2.8.2.1 Proper swathing and desiccation timing for lentil

The proper timing for desiccating and swathing in lentil has been well established. SAFRR (2005) published agronomic guidelines for swathing of lentil crops indicating that the best timing for preharvest treatment is when the bottom one third of the lentil canopy is near harvest maturity. This means that the seeds in the bottom one third of the canopy would rattle inside the pods when shaken. The middle one third of the canopy would be close to the same stage and would be brown in colour but the seeds would not rattle. The top one third will have a majority of the pods either green or in the buckskin stage of maturity, which is described as light brown or buckskin in colour and the seeds inside are soft but changing colour. Care must be taken in checking the maturity by checking the uppermost seeds because in some years the pod walls may remain green while the seed is physiologically mature. The top of the plant may still be flowering at the proper timing for swathing.

It is considered best to swath when there is high humidity in the air or dew on the canopy, for example in early morning or at night. This will reduce the amount of shatter loss that is experienced from the bottom one third of the canopy. It is also suggested that the swath be rolled, a common practice in canola production. Rolling presses the windrow down into the remaining stubble so it is anchored to prevent wind damage. Lentil swaths have a large surface area to weight ratio and they are susceptible to movement by the wind (SAFRR, 2005). To help reduce swath movement by wind it is advised that the swath be laid parallel to

prevailing winds. A problem that occurs when the swath is close to the ground but not supported by the stubble is that air is unable to flow under the swath to dry it from the bottom. To increase the drying potential of the crop, the lentil swath could be laid wider than traditional wheat swaths to increase the drying rate. This technique works well for alfalfa used as forage (Shearer et al., 1992).

2.8.2.2 Windrowing and desiccating in other crops

The effect of windrowing on grain yield and quality has been studied in other crops. In flax (*Linum usitatissmium*), Gubbels et al. (1993) demonstrated that if swathing does not commence at exactly the proper stage, the yield was reduced compared to desiccation with diquat. They also reported that desiccation with diquat should begin at the same time as swathing for optimum yield and quality for the parameters tested in flax. Cenkowski et al. (1989b) found that swathing canola did not reduce chlorophyll levels compared to leaving the plants standing. Swathing is often done at physiological maturity which is before harvest maturity. In pea, fababean, chickpea, lupin, and soybean the proper timing for desiccation is when seed is at 45 to 50 percent moisture content (Ellis et al., 1987).

Much research has been conducted about desiccation of cereal crops. Clarke et al. (1982) found that swathing caused little yield loss in oat (*Avena sativa*) unless it was done at the milk stage or earlier. Quality losses due to green kernels were a downgrading factor when the oats were swathed too early. Bovey and McCarty (1965) found that desiccation with diquat accelerated the onset of grain sorghum maturity and only negatively affected grain yield and quality when applied at

greater than 40 percent moisture in the seed. Bovey et al. (1999) reported that the effect of glufosinate as a desiccant in grain sorghum was similar to the effect of diquat, with only minor effects on quality and yield. There is no literature available indicating yield or quality loss when swathing or desiccation of lentil occurs either earlier or later than currently considered acceptable timing.

2.9 Heritability

Heritability is a measurement of how much that the progeny of a cross is explained by the genotype. There are two types of heritability: broad sense which is a measurement for heterozygous individuals and narrow sense which is used when measuring homozygous individuals. For completely homozygous individuals broad sense heritability is equal to narrow sense heritability. The calculation of heritability has been well established in self pollinated crops like lentil. A cross is made between two individuals that are from the extremes for the trait to be evaluated. The resulting progeny are taken to near homozygousity through single seed descent, where each plant has one seed harvested and planted which becomes the following generation. When the seed is at a desired level of homozygosity the examination of heritability can occur. To study heritability five types of variation must be evaluated. They are genetic variation, variation due to locations, years, interactions, and replications. Proper experimental set up will allow these to be evaluated. At least two locations, replications, and years must be used to evaluate the trait. More should be used until available resources are exhausted. The more locations, years, and replications used the better the estimate of genetic variation

and the higher the heritability estimate (Falconer and MacKay, 1996). Heritability estimates are grouped into three categories. If the heritability is larger than 0.75 the heritability is considered to be high. If the heritability is 0.5 to 0.75 it is considered to be moderate. If it is below 0.5 heritability is low.

3.0 OBJECTIVES

The overall goal of this research into differences in green seed coat colour in lentil genotypes was to ultimately provide a higher value product for agricultural producers to market. The heritability study's main objective was to estimate heritability of differences in weathered green seed coat colour for a green lentil cross. For the desiccation study the objective was to determine which preharvest treatment, desiccation or swathing, produces the highest quality sample for green lentil growers.

4.0 HERITABILITY STUDY

4.1 Heritability Study Objectives

The first objective of the heritability study was to estimate heritability of differences among genotypes for green seed coat colour. The other objectives were estimating the range of colour phenotypes to determine if selection will be effective using existing germplasm.

4.2 Materials and Methods

4.2.1 Experimental Setup

The heritability study started in 2005 and was repeated in 2006. The plant material that was used was a cross between two green lentil lines. Both lines were specifically chosen to be parents. Agronomically they were similar in most characters like maturity, and seed weight and would fit into the medium size green lentil market class. Line 1294M-23 was the parent that had the more desirable green seed coat colour. 1048-8R was the parent that had a less desirable green seed

coat. The cross was made in 2002 using 1294M-23 as the maternal parent. The 224 RILs were produced using single seed descent from individual F_2 plants. Seed was bulked at F_6. Near homozygosity was assumed to be reached at F_7. Due to seed number constraints for some of these RILs, only 173 were used in the study. Seed used in 2006 was $F_{6:8}$.

The heritability study took place at the same three sites over the two years (Table 4.1). There were two sites near Saskatoon, the SPG farm southeast of Saskatoon (Elstow clay loam) and the Sutherland farm east of Saskatoon (clay), and one site at Elrose (clay). Elrose was chosen because it was representative of a region where a large amount of green lentil is grown.

Table 4.1 Location, soil type, and soil zone for each of the three locations used in 2005 and 2006 for the heritability study of differences in green seed coat colour in green lentil.

Site	Location	Soil Type	Soil Zone
Elrose	10 km north of Elrose	clay	Brown
SPG	10 km south east of Saskatoon	clay loam	Dark Brown
Sutherland	2 km east of Saskatoon	clay	Thin Black

A randomized complete block design was used. Each site had two blocks arranged in a long narrow pattern to accommodate space restrictions, and to allow the use of mechanized seeding and spraying equipment. In 2005 there was only enough seed of some genotypes to sow three locations with two blocks at each. To simplify statistical analysis the experimental design and size was kept the same in 2006 even though seed was not limiting.

Plant material for the study included the set of RILs, parents, and a tester set consisting of all cultivars of green lentil registered in Canada up to 2004 (Table 4.2). The tester set was included once in each block while the parents were included three times in each block. Breeder seed was only available for five genotypes. Seed from previous plots was used for genotypes with limited breeder seed availability.

Table 4.2 Market class and seed source for the tester set and RILs used in the heritability study of green seed coat colour differences in green lentil for 2005 and 2006.

Genotype	Market Class	2005 Seed Source	2006 Seed Source
RILs	medium green	greenhouse	plot
CDC Glamis	large green	plot	plot
CDC Grandora	large green	plot	plot
CDC Greenland	large green	plot	plot
CDC Plato	large green	plot	plot
CDC Sedley	large green	plot	plot
CDC Sovereign	large green	breeder seed	plot
Laird	large green	breeder seed	plot
1048-8R	medium green	plot	plot
1294M-23*	medium green	plot	plot
CDC Meteor	medium green	plot	plot
CDC Richlea	medium green	plot	plot
CDC Vantage	medium green	breeder seed	plot
CDC Milestone	small green	breeder seed	plot
CDC Viceroy	small green	plot	plot
Eston	small green	breeder seed	plot

* Denotes a parent of the RILs used in the heritability study

Seed of each genotype was planted in one meter long microplots that were three seed rows wide with 30 cm row spacing. Approximately 100 seeds were planted per microplot with a targeted plant density of 80 plants/m^2. Approximately

50 cm separated each plot. After every 20 plots a plot of faba bean (*Vicia faba*) was planted to allow quick calculation of plot number.

The sites were seeded in early May, during the recommended window for lentil planting. One site in Saskatoon was deliberately seeded later (Table 4.3) to ensure a different environment occurred to increase the power of the test. In 2005 the late site was Sutherland while in 2006 the late site was SPG.

Table 4.3 Seeding, desiccation and harvest dates for the heritability study of green seed coat colour differences in green lentil for the three sites in 2005 and 2006.

Site	Year	Seeding date	Desiccation date	Harvest date
Elrose	2005	May-11	Aug-31	Sep-21
	2006	Apr-28	N/A	Aug-02
Sutherland	2005	May-25	Sep-19	Sep-29
	2006	May-06	Aug-23	Sep-25
SPG	2005	May-12	Sep-19	Sep-29
	2006	May-16	Aug-30	Oct-12

N/A: not applied

Chemical weed control was used in the field research as needed at each site (Table 4.4). When imazethapyr and ethalfluralin were used they were applied in the autumn prior to lentil planting. Glyphosate (Monsanto, Winnipeg, Manitoba) was applied prior to seeding as a pre-seed burnoff where lentil would be seeded. Metribuzin (Bayer, Calgary, Alberta) and clethodim (Bayer, Calgary, Alberta) were both applied in crop. Diquat (Syngenta, Guelph, Ontario) was used as the desiccant at a rate of 1.75 l ha^{-1}. It was applied with a CO_2 powered hand wand sprayer with 170 l ha^{-1} of water and 172 ml ha^{-1} of the surfactant, Agsurf ^{TM} (IPCO, Saskatoon, Saskatchewan). All of the plots were hand weeded after flowering.

Table 4.4 Herbicide and desiccant applications and rates that were used in the heritability study of green seed coat colour differences in green lentil at Elrose, Sutherland, and SPG for 2005 and 2006.

		Herbicide					
Site	Year	Imazethapyr	Ethalfluralin	Glyphosate	Metribuzin	Clethodim	Diquat
Elrose	2004	N/A	N/A	N/A	N/A	N/A	N/A
	2005	N/A	12.5 kg ha^{-1}	2.5 l ha^{-1}	150 g ha^{-1}	N/A	1.75 l ha^{-1}
	2006	N/A	12.5 kg ha^{-1}	2.5 l ha^{-1}	N/A	N/A	1.75 l ha^{-1}
Sutherland	2004	N/A	N/A	N/A	N/A	N/A	N/A
	2005	N/A	N/A	2.5 l ha^{-1}	N/A	N/A	1.75 l ha^{-1}
	2006	N/A	N/A	2.5 l ha^{-1}	N/A	N/A	1.75 l ha^{-1}
SPG	2004	70 ml ha^{-1}	28.25 kg ha^{-1}	N/A	N/A	N/A	N/A
	2005	70 ml ha^{-1}	28.25 kg ha^{-1}	2.5 l ha^{-1}	200 g ha^{-1}	200 ml ha^{-1}	1.75 l ha^{-1}
	2006	N/A	N/A	2.5 l ha^{-1}	N/A	200 ml ha^{-1}	1.75 l ha^{-1}

N/A denotes this herbicide was not applied

To promote seed coat colour deterioration in the field the material was not harvested at proper agronomic timing. The microplots were desiccated with diquat at 1.75 l ha^{-1} when approximately 70 percent of the plant had dried down, deliberately later than recommended timing. The plots were harvested using a Wintersteiger® plot combine. After harvest the seed was dried using warm air dryers at approximately 40°C then placed in storage at -16°C to stop metabolic activity and to minimize further post-harvest seed coat colour change.

The RILs and tester set were also grown in the phytotron in the summer of 2005 using the same experimental design as the field experiments. Each 15 cm pot was considered one experimental unit, comparable to a microplot. Four seeds of a genotype were seeded into a single pot containing a 50/50 blend of Sunshine #4 and Sunshine #5 soilless mixtures (Sun Gro, Vancouver, Canada). The growth room settings were 18 hour days at 21°C and six hour nights at 15°C. The pots were re-randomized near flowering to reduce positional effects within the growth room.

The plants in the pots were also thinned at this time to two plants per pot and they were staked to prevent canopy collapse. At the beginning of flowering a liquid fertilizer regime of 20-20-20 NPK blend was applied with the watering cycle twice weekly for the remaining four weeks of growth corresponding to flowering, pod fill, and until physiological maturity. The cycle of planting to harvest was 84 days. All of the samples were machine threshed with a single plant thresher (Agriculex®, Guelph, Ontario) before analysis and stored in the freezer at -16°C with the samples from the microplots.

All of the seed samples were analyzed for colour using the Acurum® machine. Approximately 500 seeds from each field plot were analyzed. From the material grown in the phytotron, all of the seeds from each of the pots were analyzed. All of the samples were taken out of the freezer just prior to colour analysis and placed back in the freezer after colour analysis to reduce colour deterioration. Once the output was recorded by the Acurum® machine it was manually transferred to a personal. The data were analyzed by first developing an index as the mean of the frequency distribution:

$$\text{Index} = \sum [(\text{seeds per category})/(\text{total seeds per sample}) * (\text{category number})]. \quad (4.1)$$

The data from the study were analyzed using SAS for Windows version 8 (SAS Institute, Cary, North Carolina). A mixed model was used with all factors being considered random. Only the RIL scores were used to determine the heritability of colour differences in green seed coat colour. The significance level

was a=0.05. The heritability was calculated from the ANOVA of the RILs. The heritability estimate is broad sense as the RILs were not at complete homozygosity. They are approximately 99.2 percent homozygous at F_7. The calculation of heritability is described by Falconer and MacKay (1996):

$$H^2 = s^2\text{genetic} / s^2\text{phenotypic}, \quad (4.2)$$

H^2 = broad sense heritability,

$$s^2\text{phenotypic} = s^2_g + s^2_{gl}/l + s^2_{gy}/y + s^2_{gly}/ly + s^2_{residual}/rly,$$

g = genotype,

y = year,

l = location and,

r = block.

4.2.2 Chlorophyll Extractions

Chlorophyll extractions were performed on selected genotypes from a range of index scores and locations from the heritability study. Genotypes with an index score range of 2.40 to 8.62 were used (Table 4.5) to cover a large range from high quality green seed coats to those exhibiting bleaching and oxidation, in each sample used some seeds would have individually had lower index scores and some higher index scores. This range was used so that most of the seed could be sourced from one site.

Table 4.5 Genotype, seed source, and index score for green lentil seed coats subjected to chlorophyll extractions for the heritability of differences in green seed coat colour study.

Genotype	Seed Source	Index Score
RIL-114	Elrose	2.40
1294M-23	SPG	2.97
RIL-163	SPG	3.29
RIL-95	SPG	3.92
1048-8R	SPG	4.62
RIL-218	Sutherland	5.21
RIL-84	SPG	5.28
1294M-23	SPG	5.81
RIL-67	SPG	5.84
RIL-110	SPG	5.88
1294M-23	SPG	6.00
1048-8R	SPG	6.16
1294M-23	SPG	6.55
1048-8R	SPG	6.67
1048-8R	SPG	6.70
1048-8R	SPG	6.76
1294M-23	SPG	6.87
1294M-23	SPG	7.40
CDC Grandora	SPG	7.57
RIL-208	SPG	8.07
RIL-219	SPG	8.62

Chlorophyll was extracted from 30 randomly selected seed coats for each genotype using a method described by Porra (2002). The random selection of seeds was done by thoroughly mixing the subsample and selecting the first 30 seeds that came out of the envelope. Ten seeds constituted an experimental unit with three replications for each genotype. The seeds were soaked in water for 14 hours before the seed coat was removed by hand. After removal the seed coats were allowed to air dry for 24 hours on the bench to remove excess water. It was assumed that

during the time taken to remove the seed coat that little chlorophyll degradation occurred. After the seed coats were dry they were weighed to the nearest 0.1 mg and placed into a test tube with 4 mL of an 80% acetone and 20 % 0.1 M TRIS HCl buffer solution with a pH of 7.0. The samples were placed on a shaker in a dark box for 90 hours for chlorophyll extraction. Then the samples were centrifuged and 2 ml of the supernatant was removed and placed into a quartz cuvette and the absorbance measured with a spectrophotometer (Ocean Optics, Dunedin, Florida) as a percentage. It was calibrated with the quartz cuvette filled with the acetone TRIS buffer solution. The equations used to calculate chlorophyll content yielded chlorophyll amount (mg) / volume of acetone buffer solution (ml). To calculate the amount of chlorophyll a and chlorophyll b in the seed coat, the following equations were used (Porra, 2002):

$$\text{Chlorophyll a } (\mu g/mL) = 12.25\ (A_{663.6}) - 2.55\ (A_{646.6}) \text{ and,} \quad (4.3)$$

$$\text{Chlorophyll b } (\mu g/mL) = 20.31\ (A_{646.6}) - 4.91\ (A_{663.6}), \quad (4.4)$$

where,

$A_{646.6}$ is the percentage of light absorbance measured at 646.6 nm and,

$A_{663.6}$ is the percentage of light absorbance measured at 663.6 nm.

The same chlorophyll extraction method was used on seed coats of a second set of four genotypes: 1294M-23, CDC Plato, CDC Greenland, and CDC Meteor. The seed originated from plants grown in three different phytotron environments when all four genotypes were grown simultaneously. All four genotypes were used

as parents in crossing blocks grown in the fall of 2003, summer of 2004, and winter of 2004. All of the seed was stored at room temperature in the Crop Science field laboratory from harvest until April 2007. Fifteen seeds were used as an experimental unit with no replication.

4.3 Results and Discussion

4.3.1 Heritability Study

After the analysis was completed a labeling error for the plant material was discovered. The parents of the RIL population were screened using amplified fragment length polymorphism (AFLP) markers (Bett, 2007). The seed source used as the less desirable parent was mislabeled. Consequently the data from the less desirable green parent can not be compared to the RILs, only 1294M-23, the more desirable parent can be compared to the RILs. This means that conclusions about transgressive segregation past the least desirable parent can not be made. The material grown as the less green parent 1048-8R was still included in the tester set. It is unknown what the true line number is for the material labeled 1048-8R.

Figure 4.1 depicts the distribution of the mean index scores for the RILs for all sites and years. The mean index score for all of the RILs for 2005 and 2006 at the three sites was 5.72 with a standard deviation of 1.41. This standard deviation was higher than the individual year × location standard deviations. The histogram was skewed to the right, but the data follow a normal distribution according to the Shapiro-Wilk test for normality. The RIL seed coat colour data followed a normal distribution when combined for all sites and years. In 2005 none of the sites had

normally distributed data. In 2006 all of the sites had data that fit the normal distribution when using the Shapiro-Wilk test for normality. Elrose in 2006 had the most desired green mean index score while Elrose in 2005 had the least desired mean green index score (Table 4.6). In 2005 Sutherland had the most desired green mean index score. In 2006 SPG had the least desired mean green index score. The smallest range in phenotype scores was Elrose in 2006 at 3.81. Elrose in 2005 had the largest range in phenotype scores at 6.60. The range in standard deviations of mean index score was 0.73 for Sutherland in 2005 to 1.48 for Elrose in 2005. This could mean that less weathering occurred on the lentil samples in the field compared with sites with a larger standard deviation. All sites in both years had significant differences among the genotypes evaluated.

Table 4.6 Mean, range, test for normality, and dispersion statistic for the RILs that were grown at Elrose, SPG, and Sutherland during 2005 and 2006 for the heritability study of green seed coat colour differences in lentil.

	2005			2006			All Sites
	Elrose	SPG	Sutherland	Elrose	SPG	Sutherland	
Mean	6.86	6.45	5.03	4.31	5.90	5.20	5.72
Minimum	3.52	3.70	3.17	2.40	3.29	2.63	2.40
Maximum	10.44	10.30	8.58	6.21	8.85	8.65	10.44
LSD (0.05)	0.16	0.11	0.08	0.08	0.11	0.11	0.06
Standard deviation	1.48	1.05	0.73	0.73	1.05	1.05	1.41
Shapiro-Wilk statistic	0.99	0.98	0.98	0.99**	0.99**	0.99**	0.99**

** Denotes significant at a=0.05

Differences in green seed coat colour in lentil appear to be a quantitatively inherited trait with some environmental effects. There are no discrete index categories but rather a continuous range of possible desirability scores. Depending upon the site and year the size of the range of index scores differs. As the number of samples measured increases the distribution became more normal. This is seen in

2005 when individual sites do not have a normal distribution but when all samples from all sites and years are combined the distribution is normal.

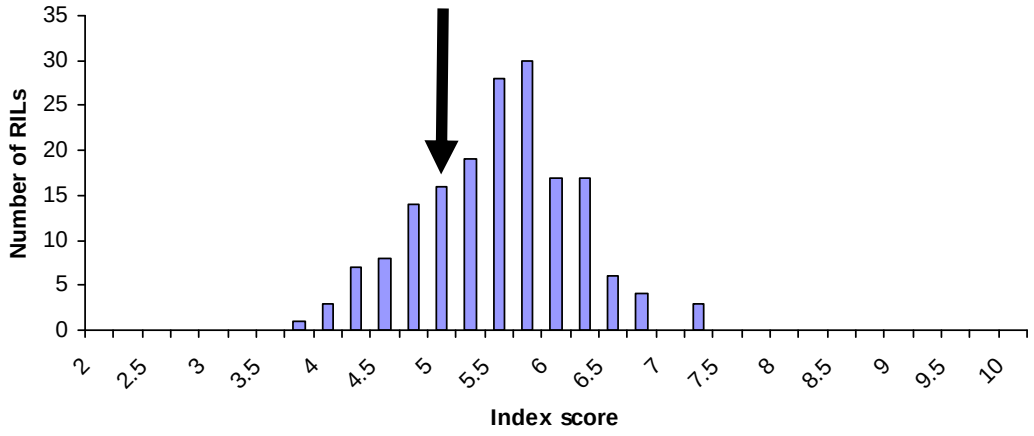

Figure 4.1 Histogram of the mean index scores for the RILs used in the heritability study for green seed coat colour differences in lentil for 2005 and 2006 at Elrose, SPG, and Sutherland. Arrow denotes where the more desirable parent, 1294M-23, would lie in the histogram.

The data were subjected to an ANOVA using a mixed model. When the residual analysis was completed it showed a normal distribution of the residues when using the Shapiro-Wilk test for normality (a=0.05). The Bartlett test for homogeneity of the variances also showed that the inclusion of both years into the analysis was justified. This included all of the data from all three sites over the two year period. The ANOVA showed that the following effects were highly significant: location, year, location × year interaction, block, genotype, genotype × location interaction, genotype × year interaction, and genotype × location × year interaction. Because genotype was significant some of the variability in index scores was explained by the genotype. As expected with most quantitative traits,

location and year explained a large part of the variability of index scores. This is because they explain environmental variation which is high for this trait. The estimate of genetic variance was 0.43 (Table 4.7). Phenotypic variance was 0.52. The phenotypic variance is based on the number of years, locations, and blocks used in the testing. The broad sense heritability was 0.82 (Appendix A). The estimate shows the heritability for differences in green seed coat colour are similar to the heritability of bleaching resistance in split green pea cotyledons which is estimated at 0.86 and a quantitative trait (Ubayasena, 2007). The high heritability of green seed coat colour differences among genotypes should ensure that future selection is effective for producing green lentil lines that have a more desirable green appearance.

Table 4.7 Variance estimates of the sources of variation from the ANOVA of the heritability study of green seed coat colour differences in lentil with their standard error estimate.

Parameter	Variance	Standard error
genotype	0.43	0.05
location	0.00	-
year	0.18	0.68
year x location	0.82	0.59
block(year x location)	0.02	0.01
location x genotype	0.03	0.03
year x genotype	0.00	-
year x location x genotype	0.29	0.03
error	0.44	0.02

RIL 95 was the most desired green RIL from the population based on its average index of 4.00 across the two years and three environments. RIL 95 had a

significantly (a = 0.05) lower index score, thus was more green, than 1294M-23, the desirable green parent, indicating that transgressive segregation occurred. The least green RIL was RIL 81, with a high mean index of 7.45. It was significantly (a = 0.05) less green than most RILs in the study. 1294M-23 was a more desired green than the mean index of 5.72. The ten greenest RILs all had significantly lower index scores than the ten least desired green genotypes (Table 4.8).

Table 4.8 Mean index score and relative rank of 1294M-23, the ten most desirable, and ten least desirable RILs from SPG, Sutherland, and Elrose during 2005 and 2006 for the green seed coat colour differences study in lentil.

Rank	Genotype	Index score
1	RIL-95	4.00
2	RIL-128	4.13
3	RIL-78	4.18
4	RIL-114	4.21
5	RIL-154	4.33
6	RIL-163	4.35
7	RIL-123	4.37
8	RIL-188	4.39
9	RIL-206	4.39
10	RIL-172	4.46
45	1294M-23*	5.21
164	RIL-55	6.67
165	RIL-219	6.72
166	RIL-208	6.73
167	RIL-135	6.79
168	RIL-112	6.81
169	RIL-213	6.81
170	RIL-194	6.91
171	RIL-31	7.26
172	RIL-180	7.42
173	RIL-81	7.45
LSD(0.05)		1.11

* Denotes parent of the RILs

The tester set that was included in the study fell into two groups relative to the distribution of the RILs (Figure 4.1 and Table 4.9). 1294M-23 was the most desired green genotype from the tester set with an index score of 5.21 (Table 4.9). All of the other members of the tester set except CDC Viceroy had index scores of 6.45 or higher. This makes the majority of the tester set similar to the less desirable RILs. The least desired green line was CDC Sedley which had an index score of 7.64 when all sites and years were combined. CDC Viceroy had a mean index score of 5.49. This shows that up until now there has been relatively little genetic diversity for this trait in currently registered Canadian lentils. In the breeding program much more genetic variation for better green seed coat appearance is now available based on the fact that 1294M-23 has been used as a parent for the past five years.

Table 4.9 Mean index score for the tester set of 15 genotypes grown at SPG, Sutherland, and Elrose during 2005 and 2006 for the heritability study of green seed coat colour differences in lentil.

Genotype	Index score
1294M-23*	5.21
CDC Viceroy	5.49
CDC Grandora	6.45
CDC Greenland	6.47
CDC Glamis	6.49
CDC Meteor	6.58
Laird	6.70
CDC Sovereign	6.71
CDC Milestone	6.77
Eston	6.78
CDC Richlea	6.80
1048-8R	6.92
CDC Plato	7.51
CDC Vantage	7.55
CDC Sedley	7.64
LSD(0.05)	1.11

* Denotes parent of the RILs

The regression of the average field index score and the index score from the plants grown in the phytotron is shown in Figure 4.2. The regression had an R^2 = 0.03. A higher positive regression line was expected based on the fact that it was under phytotron conditions the green seed coat colour differences were first observed. This indicates that using the growth rooms will not predict which genotypes will have the most or least desired phenotype in the field. The phytotron will not provide an acceptable selection environment because of the low R^2 value based on this study. Green seed coat colour differences are a quantitative trait and the one environment does not provide enough insight into location effects to provide reliable selection. More replications might increase the relationship

between field and phytotron index scores as this was a trait that had large environmental effects. Another reason the phytotron does not predict the field index score well is the phytotron index score may be an estimate of the most desirable colour of each genotype's seed coat. Where the field index scores included weathering which may have caused a colour change. The field index score could be affected by different retention rates for colour pigments that the phytotron is unable to replicate without some type of artificial weathering.

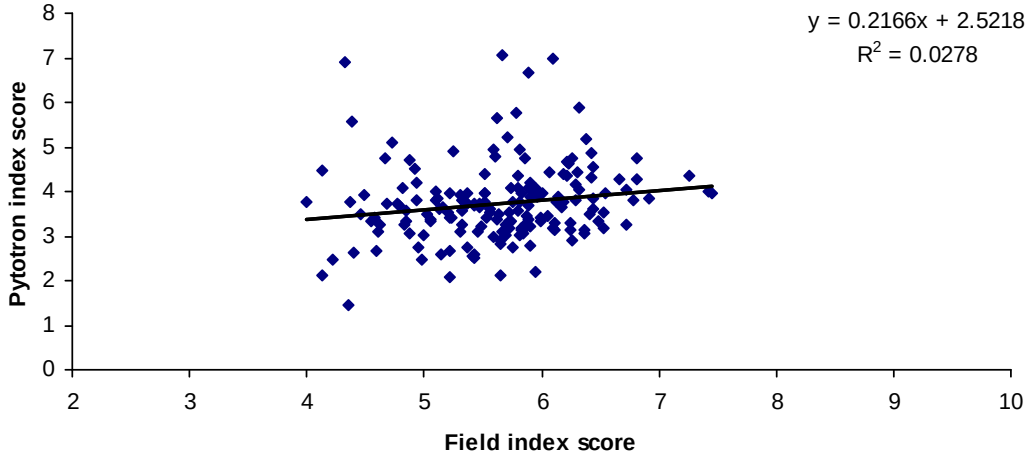

Figure 4.2 Regression between the mean field index score of SPG, Sutherland, and Elrose during 2005 and 2006 and the phytotron index score for all RILs in the green seed coat colour differences study of lentil.

Table 4.10 Mean index scores of the 15 lentil genotypes in the tester set for green seed coat colour differences for all years and locations, all years, all locations, and for each individual year and location.

Genotype	Mean	2005 and 2006			All Sites		2005			2006		
		Elrose	SPG	Sutherland	2005	2006	Elrose	SPG	Sutherland	Elrose	SPG	Sutherland
1294M-23*	5.21	5.01	5.97	4.58	5.75	4.68	6.76	5.62	4.86	3.43	6.32	4.30
CDC Viceroy	5.49	5.50	6.15	4.83	5.84	5.15	6.45	6.41	4.66	4.55	5.89	4.99
CDC Grandora	6.45	7.42	6.99	4.93	6.72	6.18	9.32	6.33	4.50	5.52	7.65	5.35
CDC Greenland	6.47	6.98	6.00	5.92	6.95	5.99	8.85	6.96	5.04	5.11	6.07	6.80
CDC Glamis	6.49	6.93	6.88	5.66	6.88	6.09	9.01	6.16	5.48	4.84	7.60	5.83
CDC Meteor	6.58	7.59	6.74	5.39	6.81	6.35	9.00	6.61	4.81	6.19	6.88	5.97
Laird	6.70	6.97	7.26	5.88	7.02	6.39	8.65	6.70	5.70	5.29	7.82	6.06
CDC Sovereign	6.71	7.34	7.10	5.69	7.23	6.20	8.67	7.81	5.21	6.03	6.40	6.17
CDC Milestone	6.77	7.67	6.87	5.76	7.27	6.26	9.57	7.13	5.12	5.77	6.62	6.39
Eston	6.78	6.16	8.10	6.07	7.47	6.09	7.14	9.34	5.93	5.18	6.87	6.21
CDC Richlea	6.80	7.29	7.66	5.45	7.31	6.29	8.47	7.83	5.64	6.11	7.50	5.25
1048-8R	6.92	7.27	7.11	6.41	7.40	6.46	8.55	7.91	5.75	6.00	6.33	7.07
CDC Plato	7.51	8.59	7.73	6.21	7.41	7.61	10.00	7.40	4.85	7.19	8.07	7.57
CDC Vantage	7.55	8.08	8.01	6.56	7.83	7.28	10.01	7.98	5.47	6.14	8.04	7.65
CDC Sedley	7.64	8.36	7.87	6.59	7.65	7.57	9.99	7.52	5.43	6.74	8.22	7.75
Mean	6.67	7.14	7.10	5.73	7.04	6.31	8.70	7.18	5.23	5.61	7.09	6.22
LSD (0.05)	1.11	3.42	1.29	1.47	2.22	1.14	0.68	1.61	1.06	0.53	1.24	0.95

Table 4.10 shows the mean index scores for the tester set in all locations and years. From this table some genotype × environment interactions are evident. 1294M-23 was expected to provide the most desirable seed from the tester set and it had the most desired green seed coat colour when all years and locations were considered. As with some traits however, several cross over interactions did occur. At least one cross over interaction occurred at each site in each year. A cross over interaction was when a genotype that was expected to have a smaller index score than another genotype actually had an index score that was significantly larger. At Elrose in 2005 CDC Meteor had a significantly larger index than Eston. At SPG and Sutherland in 2005 CDC Plato had a smaller index score than Eston. At Elrose in 2006 CDC Glamis produced a more desirable sample than CDC Grandora. Also at Elrose in 2006 CDC Vantage produced a significantly lower index score than CDC Plato. At SPG in 2006 1048-8R produced a more desirable sample than CDC Sovereign. In 2006 at Sutherland CDC Richlea produced a significantly lower index score than CDC Greenland. At these sites the environment could have produced specific triggers that allowed them to produce samples that were less desirable. This could be due to less rainfall after harvest maturity. Little precipitation occurred on the lentil plots at Elrose in 2006 after harvest maturity. When autumn rainfall occurred at Sutherland in 2005 the lentils were not as close to harvest maturity as the other sites in 2005. This indicates that bleaching is independent of seed coat darkening. In the presence of moisture, chlorophyll degradation leading to seed coat colour differences may be more prevalent but in dry environments the change in green lentil quality could be more dependent on

seed coat darkening due to increased sunlight intensity and temperature. In this study the samples were not allowed to weather in the field for a long enough term to allow darkening. Darkening is a factor that becomes evident when lentil seed has been stored for long periods, for example over winter. After harvest the samples were frozen so minimal darkening was measured in this study.

When comparing all three sites combined for the two years for the tester set (Table 4.10), the site that produced the most desirable green lentils was Sutherland with an average index score of 5.73. Elrose produced the least desirable lentil samples when the two years were combined with an average index score of 7.14. Both sites near Saskatoon had more desirable samples than Elrose when 2005 and 2006 were combined. Sutherland had very desirable samples in 2005 possibly due to later seeding. It was not as near to harvest maturity in 2005 as the other two sites when an unusual amount of fall precipitation occurred. The large amount of rainfall may not have had as much of an effect at Sutherland in 2005 as the other two sites in 2005 (Table 4.11). It appears that as the length of time increases from desiccation until harvest, the greater the chance of having a higher index score.

Table 4.11 Rainfall 30 days prior to harvest, total season long rainfall, and total growing degree days for SPG, Sutherland, and Elrose during 2005 and 2006 for the green seed coat colour differences study in lentil.

Weather Parameter	2005			2006		
	Elrose	SPG	Sutherland	Elrose	SPG	Sutherland
Rainfall 30 Days Prior to Harvest (mm)	91	94	100	35	149	151
Total Season Long Rainfall (mm)	326	338	426	161	368	425
Growing Degree Days	1068	1285	1195	871	1594	1590

Source: The Weather Network and Kernen Field Crew

Sutherland in 2005 produced the most desirable green lentil of any site for all years (Table 4.10). Elrose in 2006 had the second most desirable lentil colour and was harvested prior to any fall precipitation in 2006. Elrose in 2005 had the least desirable green lentil colour. The lentils at this site stayed in the field longer after harvest maturity than any other site and received some fall precipitation. From Table 4.3 the length of time for desiccation until harvest can be calculated. Comparing this time length to mean index scores from each site (Table 4.6) raises the possibility that a longer length of time between desiccation and harvest increased the chance of poorer quality lentil samples. In both 2005 and 2006 the site that had the longest time between desiccation and harvest produced the least desirable set of lentil samples.

Regression analysis was done using available weather data and the index scores from all locations over the two years. They included total rainfall in the growing season, rainfall 30 days prior to harvest, and growing degree days. All of the correlations were not significant with very low R^2 values (data not shown). From the weather data available it was not possible to predict which location would produce the most desired green lentil.

Table 4.12 depicts how changes in the experimental setup will change the estimate of broad sense heritability. Equation 4.2 was used to calculate the heritabilities. The number of locations and years has more effect than the number of replicates. When resources are limiting it appears the best combinations will be several locations, multiple years if possible, and at least two replicates. It also shows how fluid the heritability estimate is depending on the experimental regime

used. This table will be useful in designing plot setup for selections of different green seed coat colours. Depending on the individual breeder's objectives and resources the heritability required for selection will differ. Breeding objectives will also determine the importance of the differences in green seed coat colour. If the relative importance of the trait is reduced an experimental setup with few replications, locations, and years may be adequate because response to selection does not need to be high. If seed coats with high index scores are an important objective the heritability needs to be raised for increased response to selection. It is important to note that other factors like genetic diversity and selection intensity must be monitored to achieve the desired results. The current level of evaluation for green seed coat desirability used in the lentil breeding program at the Crop Development Center at the University of Saskatchewan, results in a high heritability estimate of 0.93.

Table 4.12 Effect of changing the number of locations, years, and replicates used on the broad sense heritability estimate of green seed coat colour differences in lentil based on this study.

Number of locations	Number of years	Number of replicates	Broadsense heritability
1	1	1	0.36
1	1	2	0.45
1	1	3	0.49
1	2	1	0.54
1	2	2	0.63
1	2	3	0.67
1	3	1	0.64
1	3	2	0.73
1	3	3	0.76
2	1	1	0.56
2	1	2	0.66
2	1	3	0.70
2	2	1	0.73
2	2	2	0.80
2	2	3	0.83
2	3	1	0.80
2	3	2	0.86
2	3	3	0.89
3	1	1	0.69
3	1	2	0.78
3	1	3	0.82
3	2	1	0.82
3	2	2	0.88
3	2	3	0.91
3	3	1	0.88
3	3	2	0.92
3	3	3	0.94
4	1	1	0.77
4	1	2	0.86
4	1	3	0.90
4	2	1	0.88
4	2	2	0.93
4	2	3	0.95
4	3	1	0.92
4	3	2	0.96
4	3	3	0.97

The difference in green seed coat colour among genotypes is a quantitative trait characterized by a normal distribution with evidence of transgressive segregation. Transgressive segregation was found to occur for the more desirable

seed coat colour. Because the less desirable parent could not be accurately identified in the study it was unknown if transgressive segregation occurs for the less desirable seed coats. This trait could be similar to green pea bleaching for inheritance. McCallum et al. (1997) reported that green pea bleaching exhibited quantitative inheritance and a small number of genes control the trait. That would be possible in lentil as well due to the high heritability.

One factor that was not tested in this study was germination of bleached seeds compared to unbleached seeds. In green pea, the bleached seeds exhibit a lower percentage germination compared to unbleached seeds (Riehle and Muehlbauer, 1975). In this study, seed in 2006 was used from plot seed in 2005. Approximately the same number of seeds was seeded into each microplot, all microplots had a constant size. Some of the 2005 lentil samples had poor green seed coat colour but no obvious differences in plant population developed among the different lines in the microplots, even from lines that had desirable green colour to lines that did not have desirable green colour. There could have been some level of reduced germination but differences in desirability did not result in a noticeable reduction of germination for green lentil seed.

It is possible that the difference of green seed coat desirability could be related to the physical characteristics of the seed coat. Lentil samples that did not have as desirable a green seed coat colour often had seed coats that were wrinkled or adhered less tightly to the cotyledons. Samples that had improved desirability of green seed coat colour generally had seed coats that tightly adhered to the cotyledon and little wrinkling. The Canadian Grain Commission describes the seeds with little

wrinkling as sound seeds. It is difficult to determine if the Acurum® machine inadvertently measures seed coat wrinkling and gives the seed a higher score if wrinkling is present. In green pea, seed coats that do not imbibe water as readily exhibit reduced cotyledon bleaching (Riehle and Muehlbauer, 1975).

4.3.2 Chlorophyll Extractions

Significant differences were observed among lentil genotypes for content of total chlorophyll, chlorophyll a, chlorophyll b in the seed coat and chlorophyll a:b ratio (Table 4.13). For total chlorophyll levels, several 1294M-23 measurements had more total chlorophyll than CDC Grandora and 1048-8R. RIL 114, the line with the lowest index score (best colour) compared to all other genotypes in the group, also had the most chlorophyll both a and b across all of the locations and years.

Generally, genotypes with lower index scores had more chlorophyll a and b (Table 4.13). Microclimates at each plot reflect differences in environments, resulting in a range of index scores and seed coat chlorophyll content. For example, chlorophyll a content of some samples of 1048-8R was higher than that of 1294M-23. From the heritability study it was known that 1294M-23 had a more desired green colour. It may be necessary to use large numbers of replicated chlorophyll extractions at multiple locations and years to reliably use this as a method of ranking desirability of green lentil genotypes making it less useful than running the samples through the Acurum® machine.

Table 4.13 Acurum index score, total chlorophyll content(mg/g), chlorophyll a content (mg/g), chlorophyll b content (mg/g), and chlorophyll a:b ratio from green lentil seed coats of selected genotypes grown at either SPG, Sutherland, or Elrose in 2006 for the differences green seed coat colour study.

Genotype	Seed Source	Index Score	Total Chlorophyll (mg/g)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	a:b ratio
RIL-114	Elrose	2.40	286.3	173.6	112.7	1.5
1294M-23	SPG	2.97	138.0	79.8	58.2	1.4
RIL-163	SPG	3.29	155.3	91.6	63.7	1.4
RIL-95	SPG	3.92	129.5	78.8	50.7	1.6
1048-8R	SPG	4.62	110.3	66.6	43.7	1.5
RIL-218	Sutherland	5.21	78.0	45.1	32.9	1.4
RIL-84	SPG	5.28	144.2	86.0	58.2	1.5
1294M-23	SPG	5.81	176.3	107.6	68.7	1.6
RIL-67	SPG	5.84	89.6	44.4	45.2	1.2
RIL-110	SPG	5.88	84.8	48.9	35.8	1.4
1294M-23	SPG	6.00	159.2	87.5	71.7	1.3
1048-8R	SPG	6.16	103.0	51.9	51.0	1.2
1294M-23	SPG	6.55	100.8	58.8	42.0	1.4
1048-8R	SPG	6.67	57.4	32.1	25.3	1.3
1048-8R	SPG	6.70	40.3	23.6	16.6	1.4
1048-8R	SPG	6.76	58.6	34.0	24.6	1.4
1294M-23	SPG	6.87	117.4	70.7	46.7	1.5
1294M-23	SPG	7.40	158.1	93.6	64.5	1.5
CDC Grandora	SPG	7.57	66.0	38.5	27.6	1.4
RIL-208	SPG	8.07	78.1	47.2	30.9	1.6
RIL-219	SPG	8.62	87.4	54.0	33.4	1.8
LSD (0.05)			44.2	22.7	22.5	0.3

The regression between the total chlorophyll content in the lentil seed coat and the index score from the Acurum® was significant (a=0.05) (Figure 4.3). The total chlorophyll content in the seed coat represented about 34 percent of the variation of the index scores. The regression between the total chlorophyll content and the index score was low and negative (Figure 4.3). As chlorophyll content increased the index score decreased. The regression between chlorophyll a and the index score presented similar results. The regression was significant but was relatively low with an $R^2 = 0.32$ (Figure 4.4). The regression between chlorophyll b and the index score had a slightly higher $R^2 = 0.37$ (Figure 4.5), with a significant

negative relationship. The regression between the ratio of chlorophyll a:b and index score was low.

Chlorophyll concentration in the seed coat explains some of the variation between different index scores. The Acurum® machine measures all of the different colours while the chlorophyll extractions measure only one aspect in the colour spectrum. Other factors like staining caused by seed borne disease, darkening due to seed coat oxidation, seed coat bleaching, or earth tag (soil adhering to the seed coat) may have contributed to a low R^2 value between chlorophyll content and index score. The poor relationship between chlorophyll content of the seed coat and the index score could be due to the relationship between reflectance measurements (index score) and absorbance measurements (chlorophyll content) which has a non linear relationship. If chlorophyll extractions were to be done again, each individual seed should have had an index score assigned and chlorophyll extracted individually. Then there would be no effect of out lying index scores for individual seeds on the chlorophyll extractions.

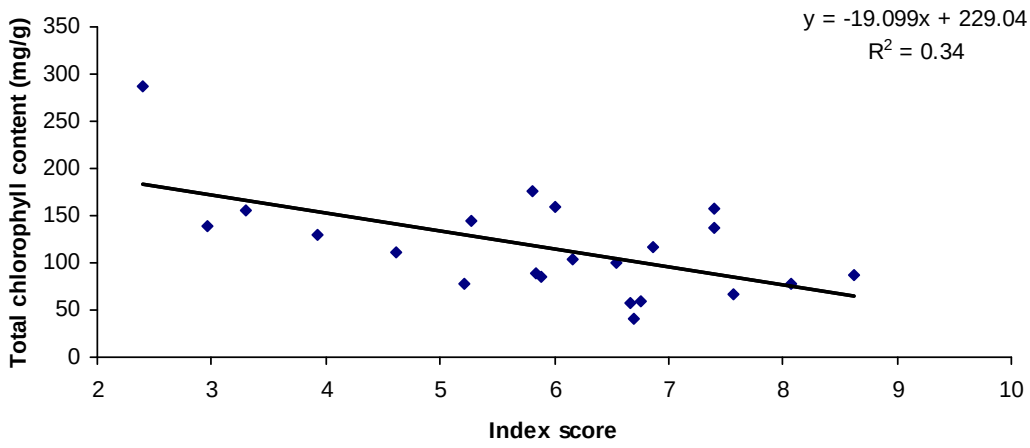

Figure 4.3 Regression between mean green index score and mean total seed coat chlorophyll content (mg/g) for 21 green lentil genotypes grown at either SPG, Sutherland, or Elrose in 2006.

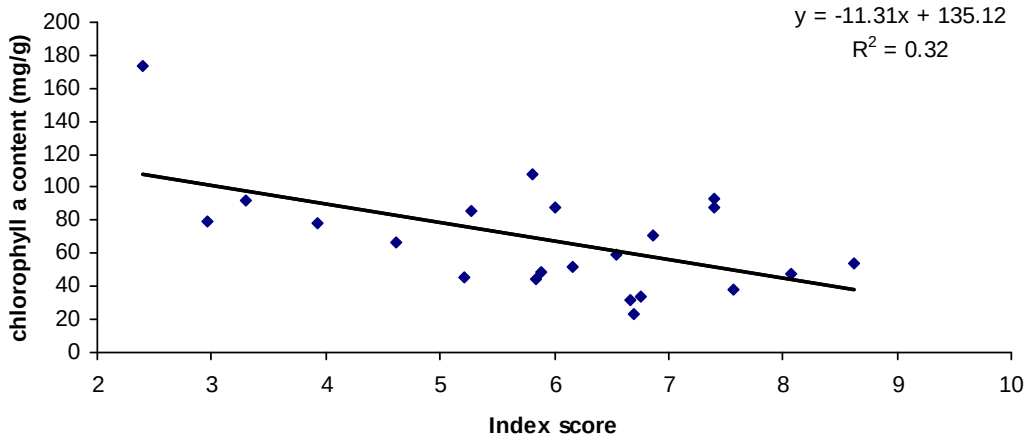

Figure 4.4 Regression between mean green index score and mean seed coat chlorophyll a content (mg/g) for 21 green lentil genotypes grown at either SPG, Sutherland, or Elrose in 2006.

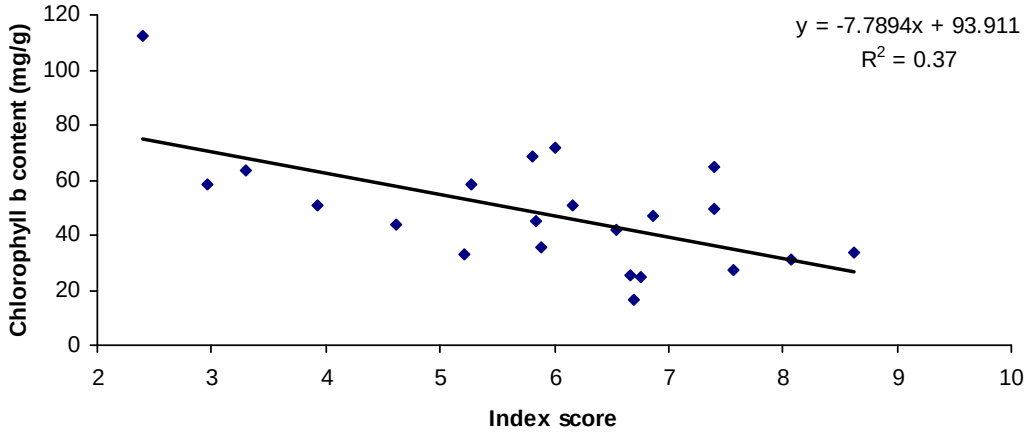

Figure 4.5 Regression between mean green index score and mean seed coat chlorophyll b content (mg/g) for 21 green lentil genotypes grown at either SPG, Sutherland, or Elrose in 2006.

Chlorophyll was extracted from the seed coats of four genotypes grown at the same time in three separate crossing blocks in the phytotron (Table 4.14). The chlorophyll content reported was the mean of the extractions from three different times that all four genotypes were grown simultaneously in the same chamber. Seed coats of 1294M-23 had significantly more chlorophyll a and b than CDC Plato. When total chlorophyll and chlorophyll a content were considered, CDC Plato had significantly less than the other three genotypes. For chlorophyll b content CDC Greenland and CDC Plato were not statistically different. There was no significant difference for the regression of chlorophyll a:b ratio and the index score (data not shown).

Chlorophyll extraction may not work well as an indirect measurement of desired commercial colour because 1294M-23 was not statistically different in chlorophyll content from some of the genotypes that are known to have less

desirable green seed coats. One problem within this preliminary evaluation was the high LSD indicating high variability within genotypic treatments. More replications grown at the same time in the same chamber would probably produce better results with less variation.

Table 4.14 Mean values for content of total chlorophyll (mg/g), chlorophyll a (mg/g), chlorophyll b (mg/g) content, and chlorophyll a:b ratio of the seed coat for four green lentil genotypes grown on three separate occasions in the phytotron.

Genotype	Total chlorophyll (mg/g)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Chlorophyll a:b ratio
1294M-23	226.2	135.1	91.1	1.5
CDC Meteor	155.2	76.8	78.3	1.0
CDC Greenland	124.3	69.7	54.7	1.3
CDC Plato	8.9	4.8	4.1	1.2
LSD(0.05)	113.2	58.9	55.1	0.5

From the literature of other crops it was expected that chlorophyll content could be a good indicator of desirable green seed coat colour after weathering. This study indicated that in green lentil chlorophyll content is not the only factor involved in identifying less desirable samples. For green pea, Cheng et al. (2004) showed that differences in the chlorophyll a:b ratio could determine which lines will have reduced cotyledon bleaching. In this study chlorophyll a:b ratio did not significantly predict more desirable lines, although line 1294M-23 did have a high ratio. In field pea Cheng et al. (2004) reported that chlorophyllase activity was correlated to cotyledon bleaching. Chlorophyllase and peroxidase may be involved in the differences of index scores among lentil samples as they have been found to cause the loss of chlorophyll from cotyledons in canola (Johnson-Flanagan et al.,

1994). It is important to note however, that these comparisons are made between green colour in cotyledons versus seed coats.

Differences in green seed coat colour in lentil appear to be somewhat different than in soybean. In soybean the stay-green trait in seeds was controlled by one gene locus. The biochemical pathway was characterized by changing the chlorophyll a:b ratio (Guiamét et al.,1991). In lentil the chlorophyll a:b ratio explained little of the index score variation.

Some type of colour measurement tool should be used in selecting for green seed coat colour in green lentil. The Acurum® machine would work well because it is programmed to categorize green lentil on current Canadian grading standards. There would be less person-to-person variation when examining the same sample. The use of a machine would consistently categorize the lentil samples. Before machine selection is implemented it would be important to understand the relationship between visual selection and the machine score to understand variability in consistency and accuracy of selections. If the people making the selections are consistent, the use of a machine would not be required. The most consistent way to measure green seed coat colour in lentil may be machine scoring because the distribution of scores within a sample can be easily described.

Several phenomena may cause variation in the green seed coat colour of green lentil. One factor is seed coat darkening due to oxidation of condensed tannins (Matus et al., 1993). This usually occurs in storage. The amount and rate of seed coat darkening is affected by the storage environment. More seed coat darkening will occur in a warm moist storage area with air movement. The most

important factor for seed coat darkening is time. As the time in storage increases the amount of seed coat darkening will increase. To prevent many complications selections for desirability in the field should be made soon after harvest.

4.4 Conclusion

The heritability study proved that selection for improved green seed coat colour in lentil would be successful. There is currently little genetic variation in registered green lentil genotypes in Canada but some breeding material and breeding lines have much more variation for the Acurum® index score. The heritability of the trait was relatively high even though it was a quantitatively expressed trait that had the environment producing some of the variation in phenotypes. To obtain an accurate estimate of the genotype effect from the phenotypic measurements several locations and years should be analyzed. This allows for estimates of interaction variances and raises the heritability estimate.

If releasing green lentil lines with enhanced green seed coat colour desirability is a high priority, more sites, years, and replications should be used during testing. This will maximize the heritability and thus the response to selection assuming selection intensity and genetic variation are constant. Testing in the phytotron does not appear to work efficiently based on the results of this analysis even though the trait was first noticed in the phytotron. Phytotron index scores do not accurately rank the genotypes from most desired to least desired when comparing with field results. It is possible that without some deliberate treatment to reduce the green seed coat colour, that measurements from the phytotron were

estimates of which lines produced the most “green” seed coat at harvest, not the seed coat that withstood weathering the best. The amount of weathering that green lentil seed coats are exposed to increases their index score. In the phytotron no environmental or artificial weathering occurred to these samples and there index scores were different than the field index scores. The ranking of the genotypes grown in the phytotron would probably mimic field ranking if no weathering was allowed and harvest commenced individually for each genotype as it became ready for harvest. The ranking of most desired to least desired seed coat colour from the Acurum® machine included field weathering.

The amount of chlorophyll in the seed at harvest did not appear to be highly associated with the index score. When regression analysis was done on total chlorophyll, chlorophyll a, chlorophyll b, and chlorophyll a:b ratio and the index score, none produced strong relationships.

5.0 DESICCATION STUDY

5.1 Desiccation Study Objectives

The main objective of the desiccation study was to determine which preharvest treatment, swathing or desiccation, produced the highest quality green lentil sample based on seed coat colour index scores. The goal was to identify if certain agronomic practices increase or decrease the chance of producing the highest quality lentil.

5.2 Materials and Methods

The preharvest treatment study was conducted in 2005 and 2006 at both the Sutherland and Goodale farms near Saskatoon. The tester set and parents from the heritability study were used as the genotypes in the preharvest treatment study (Table 4.2). The experiment was a completely randomized design with three replications. All genotypes were seeded into three-row plots that were three meters long with 30-cm row spacing. One site was deliberately seeded later than the other

in both years to produce two different local environments. In 2005 Goodale was planted later while SPG was planted later in 2006 (Table 5.1).

Table 5.1 Seeding date, swathing and desiccation treatment date, and harvest date, at SPG and Goodale during 2005 and 2006 for the preharvest treatment study.

Site	Year	Seeding date	Treatment date	Harvest date
SPG	2005	May-12	Aug-29	Sep-29
	2006	May-16	Aug-15	Sep-15
Goodale	2005	May-29	Aug-31	Sep-29
	2006	May-06	Aug-03	Aug-16

Chemical weed control was employed (Table 5.2). Imazethapyr and ethalfluralin were both applied the autumn before the lentils were planted. Glyphosate (Monsanto, Winnipeg, Manitoba) was used as a preseed burnoff and metribuzin (4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one) and clethodim (Bayer, Calgary, Alberta) ((±)-2-[(*E*)-1-[(*E*)-3-chloroallyloxyimino]propyl]-5-[2-(ethylthio)propyl]-3-hydroxycyclohex-2-enone) were applied in crop. Diquat (Syngenta, Guelph, Ontario) was applied on selected plots as the desiccant with a CO_2 powered hand wand sprayer. The diquat rate was 1.75 l ha^{-1} with 172 ml ha^{-1} of the surfactant Agsurf™ (IPCO, Saskatoon, Saskatchewan) at a water volume of 170 l ha^{-1}.

Table 5.2 Herbicide application rates for the preharvest treatment study at SPG and Sutherland for 2005 and 2006.

Site	Year	Herbicide					
		Imazthapyr	Ethalfluralin	Glyphosate	Metribuzin	Clethodim	Diquat (selected plots)
Goodale	2004	70 ml ha^{-1}	28.25 kg ha^{-1}	N/A	N/A	N/A	N/A
	2005	70 ml ha^{-1}	28.25 kg ha^{-1}	2.5 l ha^{-1}	200 g ha^{-1}	200 ml ha^{-1}	1.75 l ha^{-1}
	2006	N/A	N/A	2.5 l ha^{-1}	N/A	200 ml ha^{-1}	1.75 l ha^{-1}
SPG	2004	70 ml ha^{-1}	28.25 kg ha^{-1}	N/A	N/A	N/A	N/A
	2005	70 ml ha^{-1}	28.25 kg ha^{-1}	2.5 l ha^{-1}	200 g ha^{-1}	200 ml ha^{-1}	1.75 l ha^{-1}
	2006	N/A	N/A	2.5 l ha^{-1}	N/A	200 ml ha^{-1}	1.75 l ha^{-1}

N/A: herbicide not applied

Two pre-harvest treatments were applied to the experimental plots. The first was swathing at recommended timing. The second was desiccation with diquat at the recommended timing. Recommended swathing and desiccation timing was as defined by SAFRR (2005); that is when the bottom third of the plant was dry and the seeds would rattle inside the pod when shaken and the middle third of the plant had brown pods but the seeds inside would not rattle when shaken. The top third had the pods in the buckskin stage of maturity. Recommended timing was considered to occur when most of the plots were at the proper stage. At this time all of the plots had the predetermined treatment applied. Swathing was done with a gas powered sickle mower. The material was bunched together and bird netting was placed over top to prevent the wind from spreading the small swaths around. When all of the plots were close to proper harvest moisture which is 14% they were combined with a Wintersteiger® plot combine. The seed was dried in a warm air dryer at approximately 40°C then placed in storage at -16°C to minimize post harvest colour change.

At the time of desiccation or swathing the maturity of the plants was noted on a 1-9 scale (Table 5.3). A score of one meant that all of the pods on the plant were green. Two represented the buckskin stage of development where the bottom one third of the plant had pods tan-coloured and were leathery to the touch. Four was considered the recommended treatment timing when the bottom third of the pods had seeds that rattled inside the pod when shaken. The middle third of the plant had pods in the buckskin stage. The top third had pods in that were still green. The numbers progress until nine where all of the pods on the plant have seeds that rattle in the pod when shaken and the plant material was completely senesced with some seeds shattering out of the pods.

Table 5.3 Rating scale for maturity based on visual descriptions of lentil plants to assess maturity at pre-harvest treatment application.

Scale	Plant			Treatment
	Bottom Third	Middle Third	Top Third	timing
1	green coloured pods	green coloured pods	green coloured pods	very early
2	buckskin coloured pods	green coloured pods	green coloured pods	early
3	brown coloured pods	green coloured pods	green coloured pods	early
4	seeds rattle when shaken	buckskin coloured pods	green coloured pods	proper
5	seeds rattle when shaken	buckskin coloured pods	buckskin coloured pods	late
6	seeds rattle when shaken	brown coloured pods	buckskin coloured pods	late
7	seeds rattle when shaken	brown coloured pods	brown coloured pods	late
8	seeds rattle when shaken	seeds rattle when shaken	seeds rattle when shaken	very late
9	seeds rattle when shaken	seeds rattle when shaken	seeds rattle when shaken	very late
	plant material completely senesed with pods shattering			

A subsample of seed from each plot was analyzed for seed coat colour using the Acurum® machine and an index value for each plot was calculated. The statistical analysis was done using SAS for Windows version 8 (SAS Institute Inc. Cary, N.C.). A mixed model was used with location, genotype, and genotype x

location as fixed effects with years as random effects. The significance level used was a=0.05.

5.3 Results and Discussion

When the Bartlett test for homogeneity of the variance was conducted it showed that 2005 and 2006 had variances that were not equal and should not be analyzed together, therefore, 2005 and 2006 results are presented separately.

5.3.1 ANOVA for 2005 and 2006

From the 2005 analysis genotype, location, treatment, location x genotype, and location x treatment effects were significant. The genotype x treatment interaction was not significant in 2005 (Table 5.4).

Table 5.4 Main effects and F values for the preharvest treatment study in green lentil grown at SPG and Goodale during 2005.

Effect	df	F Value
Genotype	14	7.21**
Treatment	1	152.42**
Location	1	43.33**
Location x genotype	14	4.96**
Location x treatment	1	25.85**
Genotype x treatment	14	1.68

** Denotes significance of greater than a=0.05

In 2006 all of the main effects and interactions were highly significant. This was similar to 2005 except that the genotype x treatment interaction was also significant (Figure 5.5). A significant genotype x treatment interaction is similar to

a significant genotype x environment interaction, as a treatment is in effect a different environment for each genotype.

Table 5.5 Main effects and F values for the preharvest treatment study in green lentil grown at SPG and Goodale during 2006.

Effect	df	F Value
Genotype	14	27.34**
Treatment	1	135.76**
Location	1	257.70**
Location x genotype	14	3.37**
Location x treatment	1	5.30**
Genotype x treatment	14	2.83**

** Denotes significance of greater than a=0.05

Swathing the lentil plot at proper preharvest timing consistently produced a more desired green lentil sample compared to desiccation with diquat (Table 5.6), although there was some genotype crossover effect between desiccation and swathing. There were significant differences among locations for 2005 and 2006. For both treatments Goodale (later seeding) produced the more desired green samples than SPG in 2005 while SPG (later seeding) produced the more desirable samples in 2006 (Table 5.6).

Table 5.6 Mean green index scores of green lentil seed coats from the preharvest treatment study for 2005 and 2006 for swathing and desiccation treatments at SPG and Goodale.

Treatment or location	Mean green index score	
	2005	2006
Swathing	5.08	4.21
Desiccation	5.97	4.91
SPG	5.76	4.08
Goodale	5.29	5.04
LSD (0.05)	0.22	0.17

Table 5.7 shows the index score for all of the genotypes used in the desiccation study for both 2005 and 2006. The mean index across genotypes for 2005 was 5.53 and 4.56 in 2006. The range of index scores for the two years combined was 3.29. For 2005 the range was 1.29 while in 2006 it was 2.23. 1294M-23, the green parent of the RILs, was the most desired green genotype in both years. A crossover interaction occurred between many of the other genotypes with a change in rank of which lines were a more desired green in 2005 and 2006. CDC Greenland was the most desired commercial line in 2005. In 2006 Laird was the most desired green commercial line. In 2005 CDC Milestone was the least desired line. CDC Meteor was the least desired line in 2006.

Table 5.7 Mean green index score of green lentil seed coats for the 15 genotypes used in the preharvest treatment study at SPG and Goodale for 2005 and 2006.

Genotype	Index Score	
	2005	2006
1294M-23*	4.99	2.99
CDC Greenland	5.08	4.26
CDC Grandora	5.12	4.21
Laird	5.16	4.01
CDC Glamis	5.31	4.25
CDC Sedley	5.36	5.25
CDC Richlea	5.42	4.68
CDC Viceroy	5.44	4.28
CDC Plato	5.54	5.04
CDC Sovereign	5.72	4.54
CDC Meteor	5.73	5.22
Eston	5.83	4.49
CDC Vantage	5.85	4.84
1048-8R	6.05	5.12
CDC Milestone	6.28	5.20
LSD (0.05)	0.30	0.26

* Denotes parent of the RILs used in the heritability study

In almost all situations, swathing produced a more desired sample of green lentil. The only sample that had an equal or better score from the desiccated lentils was Eston in 2006, but this was not a significant difference (Figure 5.1). For 2006 the mean score for swathing was also lower than the mean score for desiccation (Figure 5.2). In many of the other genotypes significant differences were noted in both 2005 and 2006 where swathing was significantly more green than desiccation with diquat (Figures 5.1 and 5.2). The differences between the mean of swathing and desiccation for each genotype were not the same for 2005 and 2006.

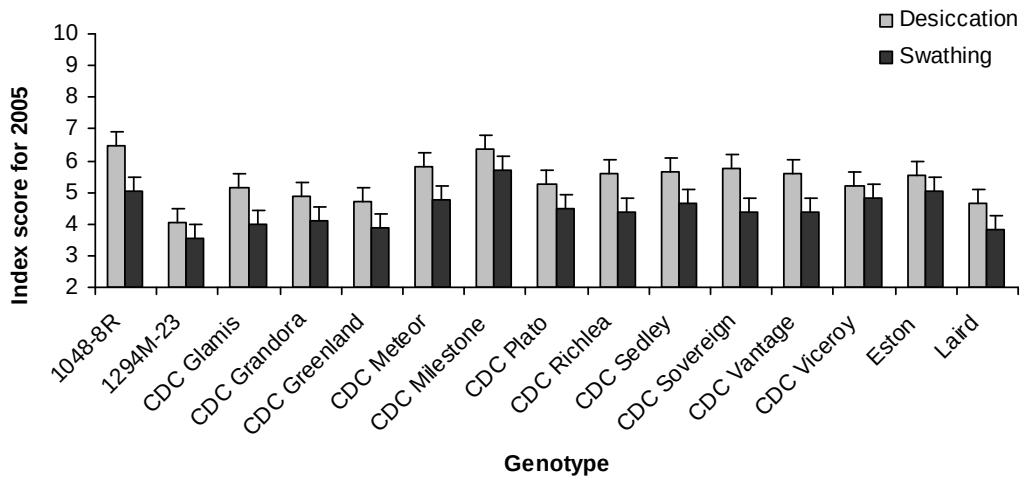

Figure 5.1 Mean green index score of green lentil seed coats for the 15 genotypes used in the preharvest treatment study in 2005. Error bars represent LSD (a = 0.05).

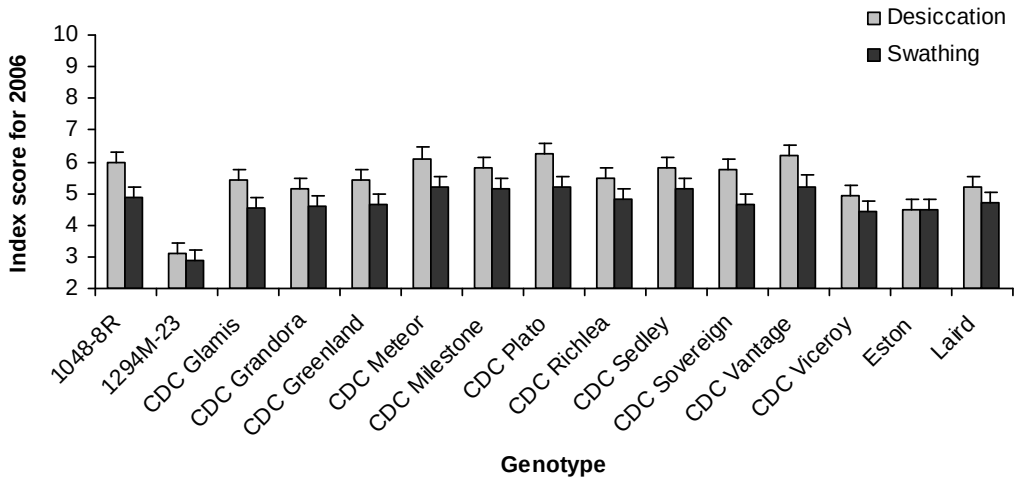

Figure 5.2 Mean green index score of green lentil seed coats for the 15 genotypes used in the preharvest treatment study in 2006. Error bars represent LSD (a = 0.05).

In Tables 5.8 and 5.9 the medium maturity is considered proper preharvest treatment timing. Table 5.8 shows that when the genotypes were classified into three maturity groups, significant differences among them were observed in 2005.

All three groups for both desiccation and swathing had significant genotypic differences within their scores. Table 5.9 shows genotype × treatment interactions for 2006. In 2005 the interaction was not significant. This means that there were few crossover interactions for the genotypes between the treatments. In 2006 the genotype × treatment interaction was significant. In 2006 the swathing treatment usually produced a lower mean index score. The mean index scores for swathing and desiccation of Eston were the same. 1294M-23 was the most desired green sample for desiccation in 2005 and 2006. For swathing it was the most desired green in 2006.

Table 5.8 Mean green index score of green lentil seed coats for the 15 genotypes grouped by maturity for both desiccation and swathing treatments in 2005.

Genotype	Maturity	Desiccation	Swathing
1048-8R	early	6.78	5.31
CDC Milestone	early	6.88	5.67
CDC Viceroy	early	5.78	5.10
Eston	early	6.07	5.59
early LSD(0.05)		0.34	0.34
1294M-23	medium	5.03	4.95
CDC Meteor	medium	6.34	5.12
CDC Richlea	medium	5.91	4.93
CDC Sedley	medium	5.84	4.88
CDC Vantage	medium	6.50	5.20
medium LSD(0.05)		0.20	0.20
CDC Glamis	late	5.76	4.86
CDC Grandora	late	5.39	4.85
CDC Greenland	late	5.48	4.67
CDC Plato	late	5.91	5.17
CDC Sovereign	late	6.35	5.10
Laird	late	5.59	4.73
late LSD(0.05)		0.18	0.18
LSD (0.05) for all genotypes		0.11	0.11

Table 5.9 Mean green index score of green lentil seed coats for the 15 genotypes grouped by maturity for both desiccation and swathing treatments in 2006.

Genotype	Maturity	Desiccation	Swathing
1048-8R	early	5.65	4.59
CDC Milestone	early	5.28	5.11
CDC Viceroy	early	4.37	4.18
Eston	early	4.49	4.49
early LSD(0.05)		0.23	0.23
1294M-23	medium	3.12	2.86
CDC Meteor	medium	5.58	4.86
CDC Richlea	medium	5.15	4.22
CDC Sedley	medium	5.60	4.90
CDC Vantage	medium	5.29	4.38
medium LSD(0.05)		0.20	0.20
CDC Glamis	late	4.83	3.68
CDC Grandora	late	4.63	3.80
CDC Greenland	late	4.67	3.85
CDC Plato	late	5.58	4.51
CDC Sovereign	late	5.13	3.94
Laird	late	4.22	3.79
late LSD(0.05)		0.15	0.15
LSD (0.05) for all genotypes		0.09	0.09

Based on results of the heritability study it was expected that the preharvest treatment would have some effect on the index score because of the large environmental effect. It was not surprising that the swathing treatment produced a more desired green sample as this was consistent with commercial practices. There was little crossover interaction in 2006 even though the genotype x treatment interaction was significant. In 2006, 1294M-23 produced the most desired green sample for both desiccation and swathing treatments. For all of the genotypes, swathing treatments produced a more desired mean index score except for Eston. Eston had identical index scores for swathing and desiccation. The decrease in the

index score was not equal for each genotype. CDC Glamis, CDC Plato, CDC Sovereign, and 1048-8R all had decreases in the mean index score in 2006 of at least 1.0 when desiccation was compared to swathing. CDC Viceroy, CDC Milestone, and 1294M-23 all had decreases in the mean index score of less than 0.3 when desiccation was compared to swathing.

There is little literature on the effects of swathing or desiccation based on the seed coat colour of any crop. In literature for canola it was not expected that a large difference would occur in desirable green seed coat colour from swathing to desiccation, assuming similar mechanisms control canola cotyledon degreening and green seed coat colour differences in lentil. Cenkowski et al. (1989b) reported that swathing in canola does not reduce chlorophyll content in cotyledons compared to leaving the crop standing. The possibility exists that early swathing in lentil may produce a more desired green seed coat.

5.3.2 Maturity

The maturity rating of the plots using the scale described in Table 5.3 was well documented prior to the preharvest treatments being applied. The regression of the mean index score for genotype and maturity at the preharvest timing is shown in Figures 5.3 and 5.4, for 2005 and 2006 respectively. In 2005 the regression was significant with an $R^2 = 0.20$. As maturity rating increased a more desirable green lentil sample was produced (Figure 5.3). The maturity range in 2005 was much smaller than 2006. For 2006 the regression between maturity and index score was not significant (Figure 5.4).

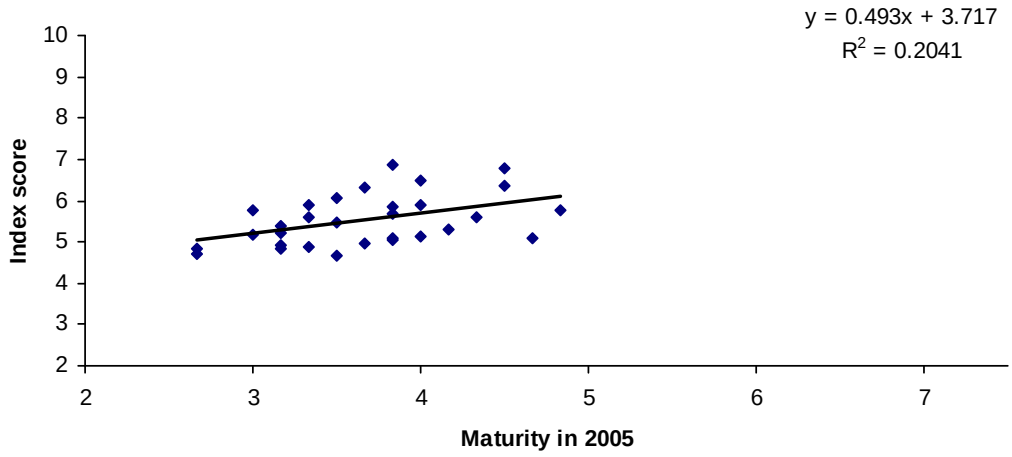

Figure 5.3 Regression of the maturity rating at the time of swathing and desiccation and mean green index score during 2005 for the preharvest treatment study.

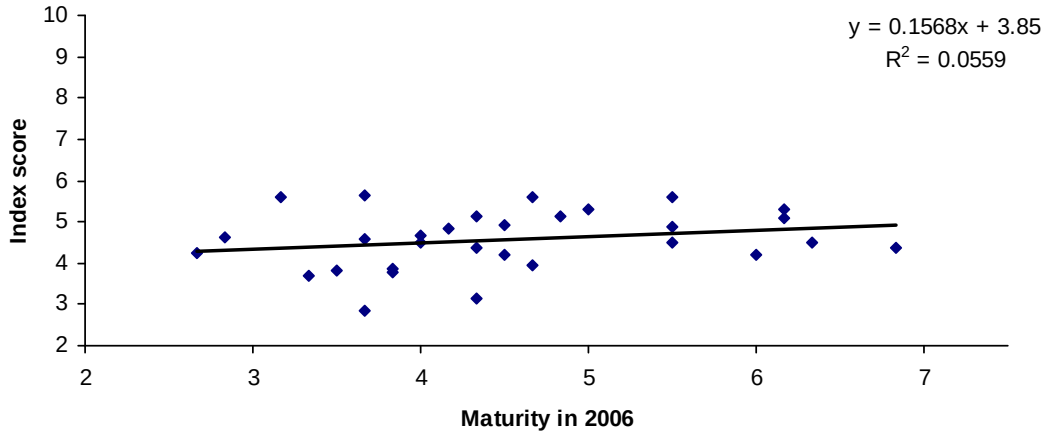

Figure 5.4 Regression of the maturity rating at the time of swathing and desiccation and mean green index score during 2006 for the preharvest treatment study.

It was expected that maturity might have some effect on the green seed coat colour in lentil. Later maturing lentil lines would not be as near to harvest maturity when harvest occurred, if harvest occurred at one time for all genotypes.

Alternatively, the samples had less time to weather after harvest maturity but prior to harvest. Due to the large range in maturity in the set of genotypes used in the desiccation study it is not surprising therefore that there was some maturity effect even though it only occurred in 2005. When canola is swathed before it is physiologically mature the chlorophyll is unable to degrade in the cotyledon (Ransom, 2006). This could be similar to lentil where there was a maturity effect for 2005. Some of the lines were swathed too early. The seed coat appeared more desirable than did the seed coat from a line that was swathed at the proper timing. Lines that were swathed past optimum timing would have experienced more weathering than other lines. The extra weathering after physiological maturity may have explained some of the less desirable index scores for the early maturing genotypes.

To reduce the effect of maturity, it would be better to apply treatments to each genotype separately and harvest separately. This would eliminate the effects of immature samples or samples that remain in the field for a period of time after the recommended preharvest treatment stage. One disadvantage to individual harvest is some later genotypes may be subjected to variable weather conditions like more rainfall which would have occurred in 2005 in this study if individual genotype harvest had been employed. The best way to attempt to eliminate maturity factors would be to group genotypes with similar maturity so they would be exposed to the same environments for the same length of time.

An opportunity exists for green lentil producers who are willing to take extra risk. By choosing to grow a variety that has a long maturity they may be able to produce a lentil sample with improved visual seed coat quality compared to shorter maturing lines in the same environment. There would be more risk from late season frost for the longer maturing lentil varieties. Other options for green lentil producers could be applying the preharvest treatment earlier than currently recommended. It is not known the yield and seed weight effects of the early preharvest treatment but a better visual seed quality could be harvested.

It is not known what the effect of preharvest treatment with glyphosate would have on the quality of the green lentil sample. Although glyphosate is not a true desiccant, its mode of action does not affect any membranes and may allow for the harvest of better green colour of the seed coat in lentil in comparison to diquat. However the use of glyphosate as a preharvest treatment would restrict the use of the lentils for seed.

Another factor that may affect the quality of the sample is the thickness of the swath. Less light would penetrate the dense swath allowing less light to reach the seed coats of the lentil compared to the open canopy of a standing desiccated lentil crop. One problem with swathing occurs when a large amount of precipitation occurs. The pods near the ground remain moist longer than pods in a standing senesced crop and some level of disease may occur. Some of the samples from the swathing preharvest treatment in 2005 had botrytis grey mold (*Botrytis cinerea*) and

sclerotinia white mold (*Sclerotinia sclerotiorum*) on some of the seed coats. Some level of seed cleaning using an apparatus like a gravity table would have been required to increase the grade of the lentil sample.

5.4 Conclusion

During both years of the study, swathing the lentil crop produced a more desired lentil sample as compared to desiccation. This is consistent with the experience of lentil growers who report that swathing produces a higher quality sample compared to desiccation with diquat.

For lentil growers wishing to manage the risk of differing green lentil seed quality at harvest, swathing some of the crop may be advised. Depending on a number of factors like equipment accessibility and time constraints, the appropriate amount of swathing for each producer will vary. Variety choice could also be a risk management tool. 1294M-23, the parental genotype with the more desirable green seed coat from the heritability study, also produced the most desirable green lentil sample in this study. It is possible that an individual lentil producer could save up to $25 per acre if a line like RIL-114 or 1294M-23 is grown instead of a line like CDC Plato for an average Saskatchewan lentil crop (Dutton, 2007).

The preharvest treatment study showed that maturity can have an effect on the green seed coat desirability. Based on the preharvest treatment study, later maturing genotypes have an increased chance of producing a more desirable green

lentil seed coat, if a single harvest date is used for many genotypes. It is important to note that maturity had an effect in only 2005. When the 15 lentil genotypes used were grouped based on maturity significant differences appeared in the desirability of green seed coat colour within each group. The maturity of the plant could be slightly affecting the growing environment of the crop, and may have a confounding effect with green seed coat colour desirability.

6.0 GENERAL DISCUSSION

Knowing which green lentil genotypes produce the most desirable samples in the field will help lentil growers immensely. Some estimates put the increased value of producing lentil one grade better to be $30,000,000 CDN annually (Dutton, 2007). Most of this money would go directly to lentil producers. Therefore it is important to have lentil genotypes that have the potential to produce more desirable green seed coats and to understand the agronomy involved to maximize the genotypic potential. A combined effort to realize the genotypic and agronomic parameters will maximize the economic potential of green lentil producers.

Green seed coat colour in lentil is a complex trait. The genetics as well as the environment work together to produce the phenotype exhibited. In commercial green lentil fields approximately one half of the phenotypic expression of the seed coat colour can be attributed to the environment. It is therefore only part of the package for astute green lentil producers to have improved genotypes. They also need agronomic information to allow them the opportunity to produce the highest quality lentil. Based on the results from the preharvest treatment study, swathing

will provide a better opportunity to harvest a better quality sample. If a green lentil producer was more of a risk taker they could swath and harvest the crop earlier than is currently recommended. The preharvest treatment study illustrated samples may have a more desirable green seed coat colour if the crop is harvested slightly earlier than what is currently considered as proper preharvest timing. It is unknown if there are penalties, for example yield loss due to seed shrinkage, or other factors such as distortion of seed shape.

Green seed coat colour in lentil is a unique quality consideration among pulse crops. Most non pulse crops do not have their value based solely on the appearance of the seed coat, and they seldom have green seed coats. It is also quite challenging for green lentil producers to produce a consistently high quality crop because of other seed coat discolouration factors like seed coat darkening. More agronomic research is required to maximize the quality potential of currently grown cultivars. Also more research is required into the genetic and physiological control of the green seed coat colour differences.

Green lentil appears green due to the green colour of the seed coat. The green in the seed coat is due at least somewhat to the presence of chlorophyll. The seed coat also contains tannins that oxidize causing a darkening of the seed coat over time. This appears to be independent of the green seed coat colour differences studied. The only confirmed agronomic practice that reduces preharvest seed coat weathering is swathing. There are no conditions after harvest that are known to increase the desirability of the seed coat but many environmental factors like field weathering may reduce the quality of the seed coat.

The Acurum® machine has some potential in the commercial market. It is able to accurately and quickly describe a lentil sample. For it to be successful the machine must be used and adopted by importers of Canadian green lentil from around the world. If they become dependent on the Acurum® descriptions of lentil samples they will make an Acurum® description mandatory for all samples prior to purchase. Having consistent descriptions of samples is important because Canadian grade standards change every year. There is no incentive for Canadian pulse buyers and processors to begin using the machine for buying samples from producers unless they are required for describing the export product. Currently pulse buyers and exporters are able to grade samples lower than their actual grade and pay the corresponding value for the sample. The Canadian pulse buyer or processor makes the difference in grades and price as extra profit at the expense of the green lentil producer. The Acurum® machine would decrease the chance of domestic pulse buyers purposely undervaluing a producer's lentil sample.

7.0 OVERALL CONCLUSIONS

7.1 Recommendations for Selection

Based on the heritability study several suggestions for selection improvement can be made. In a breeding program where gains in green seed coat colour desirability are an important objective the selections should be made early in the program. The relatively high broad sense heritability estimate means that many locations and years are not required to find large differences between genotypes. For selection the plants should be grown outdoors with a known tester set included for reference, for example in the F_3 nursery. After-harvest measurements of the desirability of the green seed coat colour will be made. A cutoff should be developed that is similar to a known lentil line from the tester set and all breeding material that is not of more desirable green should be discarded. If resources permit, all measurements and selections could be done in one or all following years that the material is included in the breeding program.

If green seed coats that are more resistant to weathering are not a high priority in the breeding program the selections could be made later in the breeding

process. If small gains in desirability of green seed coat colour are adequate, selections could be made at the time of yield selections with the same amount of testing and measuring of the seed coat. In this case smaller gains would be made but the heritability of the trait would be close to maximum so most phenotypic gains at selection would actually be genotypic gains.

In either case parental material with more desirable green seed coat colour must be found and incorporated into different crosses. Possible sources could include past parental material and breeding lines from the breeding program or green lentil breeding programs around the world. Gene banks could also be a useful source of variability. For colour measurement the Acurum® machine would work well. It is calibrated to work for Canadian grade standards. It also is under going evaluation in the commercial market as a grading tool. The machine would allow for cost reduction of each individual sample measurement.

7.2 Maturity and Index Score

Lentil maturity may confound the index score of samples. In one year of the preharvest treatment study a maturity effect was observed. One way to avoid any maturity effect would be to harvest each plot at its own specific harvest maturity. This would eliminate any maturity effect but would require a large amount of labour. When performing selections a possible way to deal with a maturity effect where longer maturing lines produce a more desirable index score is to estimate the maturity of each line at harvest. Harvest would occur at the harvest timing of the

mean days to maturity of all lines. After harvest the lines could be divided into three maturity levels: early, medium, and late. Each maturity level could be evaluated separately to maintain the same selection intensity for each level. Within each maturity level a known genotype for example CDC Viceroy (early) and 1294M-23 (medium) should be included to use as a standard. Any genotype that does not exhibit a more desirable phenotype would be discarded. This would allow the same proportion of lines to be selected from each maturity range independent of how the index scores for each level compare.

For final selections near the end of the breeding process, harvesting of individual lines at the right stage may be required to decide upon the most desirable green lentil lines. This should be successful as the preharvest treatment study found that even when maturity was affecting the index score, there were still significant differences in index scores for lines with similar maturities. Differing maturity levels did not seem to affect index scores dramatically in the heritability study. The tester set contained a wide range of maturities but still produced seed coat samples that were in the least desirable green colour range of the index score. If maturity ranges are not recognized there could be a shift towards longer maturing lentil lines under the pretense that the lines are producing a more desirable green seed coat.

7.3 Recommendations for future research

This study has asked many questions that future research could answer. The first could be to find quantitative trait loci (QTLs) that are associated with the genetic variation for improved green seed coat colour in lentil. This would allow

for more efficient selection sooner in the program even under phytotron conditions. Investigating QTLs would be relatively easy as the heritability study will act as the phenotyping portion of finding QTLs. This study has already commenced.

Another factor that could be investigated could be the phenomenon of seed coat darkening. This would help answer questions as to whether condensed tannin levels could be reduced in the seed coat without the loss in seed and plant vigour exhibited by zero tannin lentil. Investigations into which specific biochemical pathways affect green seed coat colour loss would provide excellent insight. Future breeding efforts could then be focused on these specific pathways and enzymes. This should allow more effective breeding in less time. More research could be done to determine if there is any possible way to grow green lentil lines in the phytotron combined with some type of artificial bleaching to duplicate field results. If indoor phenotypic selection could accurately predict outdoor phenotypes selection could be made through the winter to reduce time requirements. To better understand the differences between the phenotype developed in the phytotron and the phenotype from the field an indoor study could be undertaken. A protocol could possibly be developed so that material grown indoors would accurately predict the outdoor phenotype.

More research must be done on how to effectively deal with differential maturity. This would allow a better understanding of how maturity relates to more desirable green seed coat colour. The desiccation versus swathing study raised an

issue. Diquat does not necessarily produce the best possible sample of green lentil. Another area that should be addressed is how the desiccant glufosinate ammonium or a product like glyphosate affects green seed coat colour. This could be done in a similar research project to the preharvest treatment study described in section 5.0.

8.0 REFERENCES

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9.0 APPENDIX A

Appendix A contains calculations of broad sense heritability made in this thesis in section 4.3.1.

$H^2 = s^2\text{genetic} / s^2\text{phenotypic}$ (4.2)

where $s^2\text{phenotypic} = s^2_g + s^2_{gl}/l + s^2_{gy}/y + s^2_{gly}/ly + s^2_{residual}/rly$

g = genotype

y = year

l = location

r = block

Therefore $s^2\text{phenotypic} = (0.43) + (0.03/3) + (0/2) + (0.29/(3*2)) + (0.44/(2*3*2))$

$s^2\text{phenotypic} = 0.43 + 0.01 + 0 + 0.0483 + 0.0367$

$s^2\text{phenotypic} = 0.525$

$H^2 = s^2\text{genetic} / s^2\text{phenotypic}$

Therefore $H^2 = 0.43/0.525$

Therefore $H^2 = 0.82$