Implementation of Marker-Assisted Selection For Lodging Resistance in Pea Breeding

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ABSTRACT

Pea populations derived from ten crosses were scored by coupling phase linked sequence characterized amplified region (SCAR) markers A001 and A002, and repulsion phase linked SCAR marker A004 for lodging resistance during the F_2 generation. The objective of this project was to test the efficiency of implementation of these three SCAR markers in marker-assisted selection (MAS) for lodging resistance in pea breeding.

Chi-square tests showed that A001 and A004 followed a two independent gene segregation model in all of the eight populations that segregated for these two markers. In the F_3 field trial, the differences between mean lodging score of A001 (DNA band present) and a001 (DNA band absent) classes varied from -0.5 to -0.9 with an average of -0.6, based on a 1 to 9 lodging scale, across the eight populations surveyed. The differences between mean lodging score of a004 (DNA band absent) and A004 (DNA band present) classes varied from -0.4 to -1.1 with an average of -0.7, across the eight populations surveyed. In comparison, when the combination of two markers (A001; a004 vs. a001; A004) was used, lodging score differences varied from -0.7 to -1.5, with an average of -1.0 across the eight populations. T-test results showed that significant differences ($P<0.05$) in lodging score were observed between A001 and a001 classes in seven out of eight populations, and between A004 and a004 classes in six out of eight populations. Further T-tests showed that significant lodging differences were observed among the four classes of the A001 and A004 marker combination in seven out of eight populations assessed, including differences at $P<0.01$ level in six populations. The

greater differences among marker combination classes than between individual marker classes showed that combining two markers was more effective than use of each marker alone in MAS. The marker combination explained (R^2) 19-57% of lodging and 4-43% of plant height variation in the eight populations surveyed. The high temperature and potential nitrogen leaching in the summer of 2003, reduced plant growth and lodging. Under optimal growth conditions, differences in lodging between resistant and susceptible cultivars could have been greater.

Five new markers generated by simple sequence repeat (SSR) primers SAD134, SAB81 and SAD141 were identified in the recombinant inbred line (RIL) population derived from MP1401 × Carneval. The markers generated from primers SAD134 and SAB81 explained 12% and 13% of lodging variation in the RILs, respectively. Primer SAD141 produced three markers which explained 19%, 11% and 25% of lodging variation in the RILs, respectively. Linkage analysis showed that none of the three markers derived from primer SAD141 were allelic. The combination of the three markers from primer SAD141 explained 28% of lodging variation. However, utilization of any of these new markers with A001 and A004 did not substantially increase the proportion of lodging variation being explained. Thus, the new markers have limited potential to improve the efficiency of MAS for lodging resistance in pea breeding.

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ABBREVIATIONS

AFLP: Amplified Fragment Length Polymorphism

ANOVA: Analysis Of Variance

cM: Centimorgan

CRD: Completely Randomized Design

GLM: General Linear Model

LOD: Logarithm of Odds Ratio

MAS: Marker-Assisted Selection

PCR: Polymerase Chain Reaction

QTL: Quantitative Trait Loci

RIL: Recombinant Inbred Line

r: Correlation Coefficient

RAPD: Random Amplified Polymorphic DNA

SAS: Statistical Analysis System

SCAR: Sequence Characterized Amplified Region

SSR: Simple Sequence Repeat

STS: Sequence Tagged Sites

TABLE OF CONTENTS

LIST OF TABLES

.

LIST OF FIGURES

1 INTRODUCTION

The area of production of pulse crops in Saskatchewan has increased steadily over the past 15 years. Field pea production grew to about one million hectares and 1.5 million tonnes per year in recent years, comprising 70% of the national total (Specialty Crop Report 2000, 2001, 2002, 2003; McVicar 2004). Development of new pea cultivars for Canadian pulse growers is a critical component in the effort to ensure that the cost of production remains competitive and the crop retains its high quality. Lodging is usually referred to as that condition in which the stems of crops bend at or near the surface of the ground, which could lead to the collapse of the canopy. It is a well-known phenomenon in crops. Lodging is a key constraint in field pea production: it enhances the canopy microclimate for fungal disease development, reduces photosynthetic ability of the plants, reduces harvest efficiency, and increases harvest cost (Warkentin et al. 2001a). For these reasons, lodging can cause up to 74% yield loss in some dry pea cultivars (Amelin and Parakhin 2003). Furthermore, one study showed that lodging was correlated with reduced yield in 20 cultivars in Saskatchewan (Hashemi et al. 2003). One of the most important criteria in pea breeding programs is lodging resistance.

Lodging resistance is a quantitative trait in pea, and is related to morphological traits such as stem stiffness, plant height, and leaf type (Sutcliffe and Pate 1977; Amelin et al. 1991). In traditional pea breeding, selections for lodging resistance are focused on greater stem stiffness, shorter plant height, and plants with semi-leafless leaf type. As a quantitative trait, lodging is highly affected by environmental conditions. Since lodging can occur at different stages of plant development, scoring for lodging resistance in the

field can be inconsistent. However, using statistical analyses, the variation of a quantitative trait can be dissected into the effect of individual quantitative trait loci (QTL) linked to markers on a genetic map (Irzykowska et al. 2002).

The presence or absence of DNA fragments or isozymes linked to a specific trait is called the molecular marker of the corresponding trait. Using molecular markers linked to one or more traits associated with lodging as a selection method could be useful in breeding for lodging resistance. Molecular markers for lodging resistance can be identified during mapping of qualitative and quantitative trait loci. DNA markers are stable in any environment and could be selected in the lab. Thus, marker-assisted selection (MAS) may have the potential to improve the efficiency of selection for lodging resistance in breeding programs.

In traditional pea breeding, breeders apply selection for lodging resistance in the F_3 or later generations, since it is difficult to assess on a single plant basis in earlier generations. A large number of early generation lines are discarded because of susceptibility to lodging. Much labor and other costs are spent during traditional selection. Implementation of selection using molecular markers for lodging resistance at earlier generations (three-way cross F_1 or single cross F_2) would significantly enhance the efficiency of the breeding process. It could enrich the F_3 and subsequent nurseries for lodging resistance, and allow more intense selection for other traits of interest.

Several genes controlling the traits associated with lodging in pea have been mapped, and molecular markers linked to these traits have been reported. For example, *Fa* (normal/fasciated stem) (Blixt 1974), *Det* (indeterminate/determinate growth habit)

(Marx 1986), *Tl* (tendrils/tendrilless) (Makasheva and Drozd 1987), *rms2*, *rms3* and *rms4* (ramous genes) (Poole et al. 1993), and a marker locus *p275* linked to QTL for plant height (Dirlewanger et al. 1994), have been mapped on the pea genome. Furthermore, a sequence characterized amplified region (SCAR) marker linked to *rms3* was developed that can be scored as a codominant marker (Rameau et al. 1998).

A linkage map of pea was constructed by Irzykowska et al. (2002). The map, which consisted of 204 morphological, isozyme and DNA markers, was used for interval mapping of the QTL controlling plant height and internode number of pea. Six QTL per trait were identified across five linkage groups.

Three amplified fragment length polymorphism (AFLP) markers, namely A001, A002 and A004, linked to lodging resistance were identified in 88 pea recombinant inbred lines (RILs) derived from a cross between MP1401 (lodging susceptible) and Carneval (lodging resistant) (Tar'an et al. 2003). The A001 and A002 markers were linked in coupling phase, while the A004 marker was linked in repulsion phase with the lodging resistance allele. The A001 marker captured 47% of the variation for lodging in the RILs, while the combination of A001 and A004 markers captured 59% of the variation for lodging in the RILs. These markers were converted into SCAR markers to simplify future analyses. Preliminary analysis indicated that the A001 marker was present in ten pea cultivars with lodging resistance and absent in six out of nine lodging susceptible cultivars (Warkentin et al. 2001a). To date, there has been no report regarding the efficiency of using molecular markers for MAS in pea lodging resistance breeding. The objective of this thesis was to test the efficiency of MAS in pea lodging resistance breeding using the three SCAR markers developed by Tar'an et al. (2003).

In this thesis, the terms A001, A002 and A004 will be used for the corresponding DNA bands present on the gel, while a001, a002 and a004 will be used for the absence of those bands. It is hypothesized that the A001, a004 class will have the best lodging resistance, while the a001, A004 class will have the poorest lodging resistance.

Some molecular markers may not be consistent in different populations. Generally, the larger the distance between a marker and the gene of interest, the less valuable is the marker. The current study focused on evaluation of the efficiency of MAS, using the three SCAR markers, A001, A002 and A004, on ten populations other than the RILs from which they were derived. If the SCAR markers are useful in MAS in most of these populations, they might be widely applicable in selection for lodging resistance in pea breeding.

Since the SCAR markers used in this project are dominant markers, heterozygous plants cannot be distinguished from the plants that are homozygous for the marker loci. Using a codominant DNA marker, this problem could be solved and MAS could be more efficient, especially in early generations. When using a codominant marker, the three classes, which are homozygous resistant, homozygous susceptible and heterozygous, can be distinguished easily by running one gel. Therefore, an attempt to identify codominant markers using simple sequence repeat (SSR) primers was also included in this project.

2 LITERATURE REVIEW

2.1 Pea origin and production

2.1.1 Pea origin

Pea (*Pisum sativum* L) is a member of the cultivated legume crops belonging to the order *Fabale*s, family *Fabaceae* and tribe *Viciae* (Stebbins 1974; Griga and Novak 1990). The chromosome number (2n=14) of pea was established early in the last century (Cannon 1903; Nemec 1904; Casey and Davies 1993). *Vicia* and *Lathyrus* are the genera that are most closely related to *Pisum* (Blixt 1974). Pea was among the earliest of cultivated plants. Archaeological evidence indicates that it was cultivated in Near Eastern and Greek Neolithic settlements as early as 6000 B.C (Zohary and Hopf 1973). The area of distribution of all wild pea sub-species is situated in the Middle East suggesting that the pea originated in northwest Asia. This was also the primary center of distribution of cultivated peas into Europe and into southwest Asia (Makasheva 1984; Tiwari 1998).

2.1.2 Economic and agronomic importance of pea

Pea is a cool season crop and is widely grown in the cooler temperate zones and in the highlands of tropical regions of the world. *Rhizobium leguminosarum* can infect the roots forming nodules which are capable of fixing atmospheric nitrogen.

2.1.2.1 Economic value of pea

The economic value of pea is primarily due to the chemical composition of its seeds. Pea seeds, depending upon the cultivar and growing environments, contain 18-35%

protein, 20-50% starch, 4-10% sugars, 0.6-1.5% fat, 2-10% cellulose, 2-4% minerals and 9-15% water (Sumner et al. 1980). Like other grain legumes, because of symbiosis with nitrogen-fixing bacteria, peas accumulate up to three times more protein than narrow leaf cereals. Seeds of pea cultivars usually contain 22-28% protein. Furthermore, compared to cereal crops, legumes, including peas, have better quality protein. Peas contain all the essential amino acids which are important for the entire living organism, but can be synthesized only by plants, such as lysine, methionine, tryptophan, threonine, valine, phenylanine, leucine and isoleucine. These amino acids are essential for human nutrition as well as in the feed for animals. Although many experiments have shown that methionine and tryptophan content usually limit the biological value of proteins in grain legumes, among grain legumes pea is superior to lupin, lentil and dry bean in methionine content; but inferior to soybean (Pate 1977; Matthews and Arthur 1985).

Since pea is high in protein quantity and quality, it is an important protein resource for human consumption, especially in developing countries. Consumption occurs in many forms, such as split, whole and flour. Pea is also fractionated into starch, protein and fiber fractions. Distinguished by high protein content and balanced amino acid composition, it is also a valuable fodder whose use has increased significantly during the last forty years (Saskatchewan Pulse Grower 2000). Fodder consumption can be reduced when peas are used in animal feed as concentrate, green fodder or silage. Some pea cultivars have long been used as important vegetable crops. Canning of mature grain peas is common in most countries, while the use of freshly frozen green peas, dehydrated green peas and canning of immature pods of sweet cultivars are also popular (Pate 1977). The peas used in these ways are characterized by a high degree of active

lypotropic antisclerotic substances, especially choline, an important nutritional requirement which is usually deficient in daily diets (Matthews and Arthur 1985).

2.1.2.2 Agronomic value of pea

Pea in the crop rotation provides many benefits to the soil and to subsequent crops. As a nitrogen-fixing crop, it can improve soil fertility and reduce fertilizer input. The amount of nitrogen fixed by pea under a range of conditions in the UK was 71-119 kg N ha^{-1} (Davies et al. 1985) and in New Zealand was 17-83 kg N ha^{-1} (Askin et al. 1985). Nitrogen fixation by pea under conditions in Saskatchewan ranges from 50-150 kg N ha^{-1} (Saskatchewan Pulse Grower 2000). In Germany, winter pea fixed more nitrogen (242 Kg ha^{-1}) than clover (178 Kg ha^{-1}) (Karpenstein and Stuelpnagel 2000). Fixed N can provide about 75% of the nitrogen needed by the pea plant, and most of the N taken up by the pea plant is accumulated in the seeds. The N that remains in the plant residues can be released in an available form when the plant residues decompose and can be absorbed by succeeding crops (Saskatchewan Pulse Crop Development Board 1996). Pea also can be used as a green manure crop to improve soil quality.

As a preceding crop, pea can increase the efficiency of utilization of organic matter by subsequent crops (Makasheva 1984). Cereal yields, following pulse crops, are often higher than those following cereals. Studies showed that the yield advantage of growing wheat after pea, compared to wheat after wheat, was 11% in Manitoba, 13% in Alberta and 10-43% in Saskatchewan (Brandt et al. 1996; ADF report 2000). Cereals following pea in the rotation often not only have increased yield, but also have increased protein concentration. In Swift Current, wheat following pea or two other pulse crops, had a

higher protein concentration compared to wheat following wheat (Saskatchewan Pulse Grower 2000). The benefits of pea to the subsequent cereal crops were mainly due to increased available soil nitrogen and decreased disease incidence. Beckie (1997) reported that the increased yield of wheat following pea in a rotation in Saskatchewan was primarily due to the nitrogen contribution of pulse crops, rather than to the non-nitrogen effects. On a nitrogen-deficient soil in New Zealand, wheat yield was 67% greater following pea than following barley (Askin et al. 1985).

Compared to other legume crops, pea requires a relatively short growing season and has a high level of cold tolerance. Therefore, pea can mature in cool regions with a short growing season whereas other legumes, such as soybean and most dry bean cultivars, cannot. This makes pea superior to most other legume crops in cool temperate regions, such as in most of the western prairies of Canada.

2.1.3 Pea production in western Canada

Pea is one of the most important grain legumes, as a source of both human food and animal feed. The world dry pea production in 1988 was 9.8 million hectares and 14.5 million tonnes (Griga and Novak 1990) and in 1998 it grew to 16.5 million hectares and more than 30 million tonnes (Saskatchewan Pulse Grower 2000).

Pea reached North America in the late 15th century, first sown by Columbus on Isabella Island (Makasheva 1984). The first pea seeds were brought to Canada by the early settlers and were grown in eastern Canada by 1535 (Ali-Khan and Zimmer 1989). Until the late 19th century, pea production was concentrated in Ontario and shifted to the Canadian prairies in the late 19th century (Ali-Khan and Zimmer 1989). Pea was grown

in a limited area until the middle to late 20th century. Pea was planted on only about 20 thousand hectares in 1945 which was concentrated in Manitoba. Until 1978, only 26 thousand ha of field pea was grown in Manitoba, 11 thousand ha in Saskatchewan and 3 thousand ha in Alberta (Slinkard and Blain 1988; Slinkard and Vandenberg 1993). Dry pea production has increased dramatically in Canada since 1985. Saskatchewan became the leading province in pea production in 1986 (Slinkard 1993), and pea production in Alberta also increased markedly. In 1988, the dry pea area in Canada was 255 thousand hectares and 465 thousand tonnes and comprised 70% of the dry pea production in North and Central America (Griga and Novak 1990). In 2000, Saskatchewan dry pea production was about 900 thousand hectares and 2.1 million tonnes, comprising 70% of the national total. In the years 2001, 2002, and 2003, the planting area in Saskatchewan was relatively constant, but the yield was decreased due to drought (Specialty Crop Report 2000, 2001, 2002, 2003; McVicar 2004).

The main reasons for the increase in pea production during the last two decades are: 1) the increase in world demand for dry pea, especially for feed with the opening of the European market (Slinkard 1994; Ali and Slinkard 1995); 2) the relatively higher price of dry pea compared to cereal grains (Ali-Khan and Zimmer 1989; Slinkard 1994); and 3) increased emphasis on crop diversification, crop rotation, value-added processing and sustainability of agriculture (Slinkard 1994).

2.1.4 Lodging is a barrier in pea production

Lodging is a well-known phenomenon in crops and is usually referred to as that condition in which the stems in large areas of crops bend at or near the surface of the ground resulting in the collapse of the canopy. It occurs commonly where the growth

has been rapid, as on soils rich in nitrogen and well supplied with moisture. Lodging and mycosphaerella blight are the two problems considered to be the most important causes of reduced biomass production and seed yield in field pea in Saskatchewan (Hashemi et al. 2003). Moreover, lodging and mycosphaerella blight are usually correlated with each other.

Lodging results in enhanced microclimate conditions for fungal disease development (Xue and Warkentin 2001). Lodging increases humidity and decreases light intensity under the crop canopy which are suitable conditions for most fungal diseases. Under moist conditions, mycosphaerella blight lesions enlarge and coalesce faster than under dry conditions, potentially resulting in the complete destruction of the above-ground portions of the plant (Xue and Warkentin 2001). Some fungal diseases including mycosphaerella blight affect stem stiffness and thus increase lodging. Lodging reduces the light availability and thus reduces the photosynthetic ability of the lodged plants.

Lodging decreases harvest efficiency and reduces seed quality. Harvesting difficulty is one of the major problems in pea production (Heath and Hebblethwaite 1985). The seeds from lodged plants often have poor color and are often affected by fungi (Armstrong et al. 1999; Warkentin et al. 2001 b).

Due to the above reasons, lodging reduces crop yield. Yield losses due to lodging in cultivars with long internodes were three times that of losses in cultivars with short internodes (Samarin and Samarina 1981). Kertikov (1998) found that use of oats to reduce lodging could reduce grain loss in forage pea by 54-75% compared to stands of pea only. Amelin and Parakhin (2003) reported that early lodging caused 74% yield loss in dry pea.

2.2 Lodging resistance

2.2.1 Physiological basis of lodging resistance

Lodging is due to the collapse of pea canopies which occurs when the stem and petioles cannot support the weight of the canopy. In pea crops, wind is a secondary cause of lodging (Holland 1990). The tendency to lodging varies greatly in different crops and cultivars and is also related to plant growth stage. However, stem stiffness and plant height are the two main factors affecting the lodging of pea and other crops (Amelin et al. 1991). For example, in a population derived from a cross of wheat × spelt, the culm stiffness and plant height explained 77% of the phenotypic variance of lodging across three environments (Keller et al. 1999). In a soybean population, the *Dt*1 locus which explained 68% of plant height variation, also explained 56% of lodging variation (Lee et al. 1996).

2.2.1.1 Stem stiffness

The main factors conditioning lodging resistance in pea were reported to be the content of structural tissue in the stem, especially at internodes 5-6 (Tsitlenok and Bondar 1977; Samarin and Samarina 1979). The stem cross-section diameter, vascular bundles and xylem content, all of which affect stem stiffness, also affect the lodging performance in pea plants (Tsitlenok and Bondar 1977; Drozd et al. 1979; Amelin et al. 1991). In many crops, cultivars having stiff straw lodge very little even under lush growth conditions, while others having very slender or weak stems lodge easily. In pea, the portion of cross-sectional xylem area at critical internodes affected stem stiffness. Hashemi et al. (2003) reported that the proportion of cross-sectional xylem at internodes

3 and 9 was negatively correlated with both lodging and whole-plant mycosphaerella blight severity in the twenty cultivars assessed. Linear stem density (stem dry weight/unit stem length) has been considered a useful marker in selecting for pea lodging resistance in Russia since the 1980's. The correlation between linear stem density with lodging resistance was r = 0.82 (Obraztsov and Amelin 1990; Amelin et al. 1991). Stem stiffness is quite variable among pea genotypes. McPhee and Muehlbauer (1999) indicated that the crushing and shearing forces (perpendicular to the main axis) for 418 accessions ranged from 3.5 to 36.8 and from 3.6 to 64.9 newtons, respectively. They also reported that stem strength was positively correlated with internode diameter ($r = 0.68$, $p < 0.001$) and internode length ($r = 0.36$, $P<0.001$), and shearing force was positively correlated with grain yield.

The cellulose-xyloglucan network is believed to be the major load-bearing structure in the primary cell wall, and the major factor which affects stem stiffness in annual plants (York et al. 1990; York and Hawkins 2000). Cellulose content is the most important factor for stem stiffness. The cellulose content of a brittle culm mutant in barley, with the maximum culm-bending stress less than one half of non-brittle strains, was lower than the wild type barley plants, while there were no significant differences in the amount of lignin, pectin, and noncellulosic polysaccharides in the cell wall of the mutants compared to the wild-type (Kokubo et al. 1989, 1991). A mutation reducing cellulose content in *Arabidopsis* plants caused the collapse of xylem elements (Taylor et al. 1999). Cellulose also affects cell elongation in plants. An *Arabidopsis* mutant exhibited decreased cell elongation because of a cellulose deficiency in the primary cell wall (Fagard et al. 2000). Thus, within a certain range, stem stiffness may correlate with stem length.

2.2.1.2 Plant height

Pea cultivars and accessions were classified into four groups based on stem length by Makasheva (1984), i.e., dwarf (less than 50 cm), semidwarf (51-80 cm), medium-tall (81-150 cm), and tall (151-300 cm). Plant height itself is a complex trait and is influenced by the environment and cultivation. Pea plant height is dependent on the internode number and internode length. Vasileva et al. (1980) reported that dwarf cultivars had greater lodging resistance than tall cultivars. Dirlewanger et al. (1994) reported a significant positive correlation between plant height and the number of nodes in pea ($r = 0.55$; $P<0.001$).

In many cases, stem length is correlated with the length of the internode at the level of the first or second flower. Short internodes are generally characteristic of dwarf or semi-dwarf pea cultivars, while tall cultivars usually have long internodes. The lower part of the stem from the root collar approximately up to the first flower is the critical part for lodging. The ratio of internode length to internode width increased markedly from the first internode in the cultivars with long internodes, but less markedly in cultivars with short internodes (Samarin 1975). Thus, breeding short plants with short thick internodes while retaining the same internode number, has been recommended for lodging resistance (Obraztsov and Amelin 1990).

Samarin (1976) indicated that when tied to a trellis, cultivars with long internodes had greater increases in yield and leaf area than cultivars with short internodes. This suggests that under conditions without trellis, the long internode cultivars may lose more yield than the short internode cultivars. Samarin and Samarina (1981) reported

differences in yield losses due to lodging between cultivars with long internodes (20-27% loss) and cultivars with short internodes (8-9% loss). Moreover, they found cultivars with short internodes had a greater harvest index. By the end of the growing season, the weight of seeds and pods was 61-73% of the total above-ground organs, while in the cultivars with long internodes the corresponding ratio was 52-56%.

In most cases, lodging resistance is negatively correlated with plant height. However, in some cases, lodging resistance and seed yield were positively correlated with stem length within a certain range. For example, Knyaz'kova (1987) found that some tall plants had better lodging resistance than some short plants in a pea F_{10} population. McPhee and Muehlbauer (1999) also reported that stem strength was positively correlated with internode length ($r = 0.36$, $P<0.001$) among 418 *Pisum* accessions. Obraztsov and Amelin (1990) indicated that the optimum height for lodging resistance in pea cultivars was 60-90 cm. Lukashevich and Kukrash (1994) found that pea seed yield was curvilinearly correlated with stem length with a theoretical maximum at about 120 cm.

2.2.1.3 Semi-leafless leaf type

Normal pea leaves are composed of two large stipules, one or more pairs of leaflets, and several tendrils. In semi-leafless types the leaflets are replaced by tendrils but the stipules are still present, while in leafless types the leaflets are replaced by tendrils and the stipules are stunted (Sutcliffe and Pate 1977). Plants with tendrils are firmly supported by each other through their tendrils and are kept in a vertical position. Therefore, semi-leafless and leafless types are more lodging resistant than plants with normal leaves (Snoad 1975; Davies 1977; Sutcliffe and Pate 1977; McEwen et al. 1979;

Jones 1990; Wang et al. 2002). An experiment using pea near isogenic lines showed that lodging occurred earliest in the leafed peas, followed in turn by the semi-leafless and then leafless (Jones 1990). However, because of reduction in leaf area, the assimilation surface in semi-leafless and leafless types is reduced compared to plants with normal leaf type (Jones 1990). In pea breeding and production, a balance between lodging resistance and assimilation surface needs to be considered. Thus, the semi-leafless type is preferred by most pea producers and has become the dominant leaf type in commercial cultivars. Most cultivars released during the past 20 years have the semi-leafless leaf type.

2.2.1.4 Mycosphaerella blight

Mycosphaerella blight, caused by *Mycosphaerella pinodes*, is a serious fungal disease of pea in western Canada which can infect stems, leaves and pods. Under conditions favorable for disease, large stem lesions develop reducing stem stiffness (Xue and Warkentin 2001), thus inducing lodging. On the other hand, lodging will increase the possibility of mycosphaerella blight infection. Hashemi et al. (2003) reported that lodging was positively correlated with mycosphaerella blight scores in a study of twenty pea cultivars evaluated in three seasons.

2.2.1.5 Other lodging related factors

Lodging is not only related to inherently weak stems and internode length, but also related to cultural conditions, environment, diseases, growth stage and other factors. Nitrogen and potassium fertilizers affect the lodging performance in crops. Biswas et al. (2001) reported that internode circumference increased with increasing potassium rate,

resulting in improved lodging resistance in a rice line. A proper N and K rate was important not only for rice yield, but also important for lodging performance. Reduced rates of nitrogen fertilizer reduced lodging risk in many crops (Kaack and Schwarz 2001; Saskatchewan Pulse Growers 2000). Excessive nitrogen application (>400 Kg N Ha^{-1}) and biomass accumulation caused crop lodging and yield loss in pea (Sophie et al. 2002) and barley (Benare 1996).

Plant population density and light quality also affected lodging in pea plants. At plant populations sufficient to optimize yield, both plant height and lodging score were reduced in peas (Pullan and Hebblethwaite 1990; Sawicki et al. 2000). Further studies showed that light quality affected stem extension. Decreased lodging in low vs. high density populations was associated with a 25% reduction in plant height and a 29% increase in main stem thickness in soybean (Board 2001). These morphological changes were more closely related to differences in red/far red light ratios rather than to changes in blue light irradiance (Board 2001).

Lodging is also affected by growth stage. Lodging usually occurs at the stage between fruiting and maturity in most crops, such as after heading in wheat and barley and after podding in peas. Wheat and barley crops that have lodged because of a storm in the early growth stage often become partly or nearly upright again with the return of drier weather. Because of the weight of heads or pods, crops which lodge after physiological maturity often stay in the lodged position (Fellows 1948; Benare 1996).

Excessive moisture during the vegetative stages can delay or prevent flowering in pea cultivars with indeterminate growth habit. Excess vegetative growth increases disease

potential and lodging which results in decreased yield (Saskatchewan Pulse Growers 2000).

2.2.2 Genetic basis for lodging resistance

2.2.2.1 Genes controlling stem stiffness

The cellulose-xyloglucan network is believed to be the major load-bearing structure in the primary cell wall and the major factor affecting the stem stiffness in annual plants (York et al. 1990; York and Hawkins 2000). Although few cellulose synthesis related genes have been reported in peas, many cellulose synthesis related genes have been characterized in *Arabidopsis* and other crops.

Arioli et al. (1998) found that the *RSW1* locus of *Arabidopsis* encodes the catalytic subunit of cellulose synthase. The *CesA* gene family is believed to encode the catalytic subunit of cellulose synthase and at least nine *CesA* genes have been found in *Arabidopsis*. Mapping studies showed that closely related *CesA* genes are located in different chromosomal locations in both *Arabidopsis* and maize (Holland et al. 2000). Three distinct rice cellulose synthase catalytic subunit genes, named *CesA4, CesA7, CesA9,* were shown recently to be required for cellulose synthesis in the cell wall (Tanaka et al. 2003). Loss of function of any one of these three *CesA* genes led to 8-25% reduction in stem cellulose content, suggesting that these three genes are not functionally redundant to each other.

Xyloglucans are members of a group of polysaccharides typically referred to as hemicelluloses which are also defined chemically as plant cell wall polysaccharides. In *Arabidopsis*, a series of nine genes (*AtFUT2–10*) were identified that relate to

xyloglucan-specific fucosyltransferase biosynthesis, and some of them may also be involved in xyloglucan biosynthesis (Sarria et al. 2001). Faik et al. (2002) found that an *Arabidopsis* gene encoding xylosyltransferase activity was involved in xyloglucan biosynthesis in both *Arabidopsis* and pea.

2.2.2.2 Genes controlling plant height

In pea, stem length is mainly controlled by two groups of genes. One determines the length of the internodes and the other determines the number of internodes. Although the characteristics of length and number of internodes are variable depending upon conditions of growth, it was determined in the early 1900's that cultivars with the gene *Le* are distinguished by their long internodes, while those with the *le* allele have short internodes (Potts et al. 1982). Further, more than ten genes were shown to be related to pea vine length (Sutcliffe and Pate 1977; Makasheva 1984). At least eight of these genes, *Cry, Le, La, Lm* (Ingram et al 1984), *Cot, Coe, Coh* and *Cona* (Makasheva 1984) determine the internode length. Dominant alleles *Cry*-Cry^{c} with the recessive alleles *le* and *la* resulted in the development of dwarf plants with short internodes, whereas, in the recessive state (cry^{s}), very tall plants with long internodes developed (Reid et al. 1983). The dominant allele *La* with the alleles *le* and *cry* determine the development of plants with short internodes, whereas, the recessive allele *la* leads to very tall plants with long internodes. *Lm* gives medium internode length, whereas *lm* results in a 50% reduction in internode length (Reid et al. 1983).

At another locus, the alleles *Dim*-dim^{1}-*dim* influence reduction in some plant parts, particularly leaf and stipule sizes, and also reduce plant height. *Dim* causes normal

development of plants; *dim* causes a reduction in size of plant parts; while *dim^{l}* gives less reduction (Makasheva 1984).

At least three genes, *mie, miu* and *min*, influence the number of internodes (Makasheva 1984). Stem length of pea plants depends on both internode length and internode number. In most cases, long stems are dominant over short stems. One or more of the genes C*ry, Le, La, Lm, Mie, Miu and Min*, could cause the dominance. These seven genes were located in five linkage groups in the pea genome (Reid et al. 1983; McKay et al. 1994).

2.2.2.3 Genes controlling leaf structure

Two loci are associated with leaf structure in pea. Plants with alleles *Af/Af*, have normal leaves (wild type). The recessive *af/af* genotype converts all leaflets to tendrils. This leaf type is commonly called semi-leafless. Plants with alleles *Tl/Tl* have one to three pairs of leaflets per petiole and a distal tendril, while genotype *tl/tl* converts all tendrils to leaflets. Thus, in the genotype *af/af Tl/Tl*, all leaflets are converted into many branched tendrils, while genotype *Af/Af tl/tl,* has no tendrils but has five or six pairs of leaflets. Plants with the homozygous double recessive *af/af* and *tl/tl* have many-branched tendrils and relatively minute leaflets (Harvey 1972; Snoad 1975; Viliani and Demason 2000). Another mutation *st/st*, which is independent of the leaflet mutations, can reduce the normal stipule (*St/St*) to a vestigial form. Usually, the *Af/af* and *St/st* loci work together in controlling the phenotype. In summary, the genotype *AfAf StSt tltl* produces leafed plants, *afaf StSt TlTl* produces semi-leafless plants, and the *afaf stst TlTl*

produces leafless plants (Snoad 1975; Jones 1990). In general, the semi-leafless leaf type is preferred commercially as an aid to lodging resistance.

2.2.3 Breeding for lodging resistance in pea

Since lodging resistance is a quantitative trait and many genes are involved, selection efficiency has been limited in traditional breeding. In general, breeding for lodging resistance has focused on the selection of short plants with short, thick internodes while retaining the same internode number (Samarin and Samarina 1981; Obraztsov and Amelin 1990; Park et al. 1998; Amelin and Parakhin 2003). Linear stem density has been considered a useful marker in selecting for lodging resistance in pea (Samarin 1975; Obraztsov and Amelin 1990; Amelin et al. 1991). The semi-leafless trait has been important in improving lodging resistance over the past 20 years. Studies using F_2 pea plants derived from half-diallel crosses indicated that the variance of general combining ability (GCA) for lodging at the end of flowering and plant height was significant, but the specific combining ability (SCA) was not significant (Boros and Sawicki 2001).

2.3 Marker-assisted selection in breeding for lodging resistance

2.3.1 Marker-assisted selection in breeding

The molecular marker for a trait is the presence or absence of DNA bands or isozymes linked to the specific trait. Using statistical analysis, the variation of a quantitative trait can be dissected into the effect of individual QTL linked to markers on a genetic map (Irzykowska et al 2002). Lodging is a quantitative trait and is highly affected by environmental conditions. In addition, since lodging can occur at different stages of plant development, scoring for lodging resistance in the field can be

inconsistent. It is, therefore, difficult to assess lodging resistance on a phenotypic basis. Molecular markers for lodging resistance can be identified during mapping of qualitative and quantitative trait loci. Molecular markers are stable in any environment and can be selected in the lab. Marker-assisted selection (MAS) may thus have potential to improve the efficiency of selection for lodging resistance in breeding programs. However, no report regarding the use of MAS for pea lodging resistance breeding has been published to date.

2.3.2 QTL mapping for lodging resistance in other crops

QTL mapping for lodging resistance has been applied in several crops and has focused on mapping of genes linked to stem stiffness, plant height or other genes associated with lodging resistance. Many such achievements have been obtained to date, such as in hexaploid wheat (Keller et al. 1999; Börner et al. 2002), rice (Luo et al. 2000), barley (Kjaer et al. 1991; Larson et al. 1997), maize (Guingo et al. 1998; Barriere et al. 2001; Flint et al. 2003) and soybean (Mansur et al. 1993; Mansur et al 1996; Lee et al. 1996). Some genes or QTL associated with lodging resistance have been mapped in these crops. Most of these genes or QTL were for plant height or stem stiffness. Some molecular markers linked to lodging resistance have been used for MAS (Anderson et al.1998; Barr et al. 2000).

2.3.3 Mapping qualitative or QTL markers associated with lodging resistance in pea

Several genes controlling the traits associated with lodging in pea have been mapped, and molecular markers linked to these traits have been reported. For example, *Fa/fa*, which controls normal or fasciated stem type (Blixt 1974); *Det/det,* which controls indeterminate or determinate growth habit (Marx 1986); *Tl/tl,* which controls tendril

type (tendril/tendrilless) (Makasheva and Drozd 1987); *rms2*, *rms3* and *rms4,* which control the amount of branching (ramous) (Poole et al. 1993); and *p275* marker, which was associated with plant height (Dirlewanger et al. 1994), have been mapped in pea. Furthermore, a sequence characterized amplified region (SCAR) marker linked to *rms3* was developed and can be scored as a codominant marker. It mapped 16.7 cM from the *Rm3* locus (Rameau et al. 1998).

A linkage map of pea was constructed by Irzykowska et al. (2002) based on a population of 104 recombinant inbred lines (RILs). The map, which consisted of 204 morphological, isozyme and DNA markers, was used for interval mapping of the QTL controlling plant height and internode number of pea. Six QTL per trait were identified in five linkage groups. One QTL for plant height was identified on each of linkage groups III, IV, V, VII, and two QTL for plant height were identified on linkage group II. One QTL for internode number was identified on linkage group V, and five QTL for internode number were identified on linkage group II. In total, seven of these markers were located on linkage group II. Thus, linkage group II is important in controlling plant height in pea.

Mapping of QTL linked to lodging resistance in pea was reported by Tar'an et al. (2003) using amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD) markers. Three QTL for lodging resistance were identified in a population consisting of 88 RILs derived from a cross between the lodging-resistant cultivar Carneval and the lodging-susceptible cultivar MP1401 evaluated under 11 environments across western Canada during 1998 to 2000. Two of the QTL for lodging resistance explained 58% of the total phenotypic variation in the mean environment. Moreover, two QTL for plant height and two QTL for mycosphaerella blight resistance

were identified in the same population. Most of the QTL were mapped on a linkage map, except one QTL for lodging resistance. Three of the QTL for lodging resistance were converted into SCAR markers, which are easier to use than AFLP markers in MAS.

2.3.4 SSR markers in pea breeding

Simple sequence repeats (SSR) are short repeats of 1 to 5 nucleotides in length that are present in genomes of all higher eukaryotes (Tautz and Renz 1984). SSR are also referred to as microsatellites. Variation in tandem repeat number at a particular locus causes the length of the microsatellites to vary (Zischler et al. 1992). The regions flanking microsatellite repeats are conserved and are sources for the design of locus-specific primers to amplify the internal repeated regions (Pandian et al. 2000). SSR markers have been used in many crops for breeding or genetic analysis, such as in maize (Taramino and Tingey 1996), rice (Wu and Tanksley 1993), and wheat (Gupta and Varshney 2000). Microsatellites are attractive because they have a high level of polymorphism, are widely dispersed throughout the genome, and are usually codominant (Pandian et al. 2000).

Some pea SSR primers have been developed by AGROGENE consortium (www.agrogene.com/SSRdevelopment.htm). Thus far, only primer sequence, melting temperature and limited information regarding the product band size are available for most of the primers. No reports regarding utilization of these primers have been published thus far, due to the proprietary nature of the primers.

3 MATERIALS AND METHODS

3.1 Plant materials

3.1.1 Plant materials used in SCAR marker scoring and lodging evaluation

Parental cultivars were selected based on their lodging reaction and other traits of economic importance. Ten cultivars with good lodging resistance, which had previously tested positive for the presence of the A001 marker (Tar'an et al. 2003), were crossed as males with Carrera, a lodging susceptible cultivar that did not carry the A001 marker (Table 3.1). All ten male cultivars had the A002 marker except Nitouche and Toledo; and none carried the A004 marker except Nitouche. Carrera had both A002 and A004 markers. The F_1 seeds from each cross were planted in a phytotron and selfed to get F_2 seeds in 2001. A001 and A004 markers were expected to segregate in eight of these F_2 populations, except the population derived from cross 4, in which A001 and A002 markers should segregate. In the F_2 population derived from cross 3, segregation for all three markers was expected (Table 3.1).

All ten F_2 families were planted in the Sutherland nursery located 10 km east of Saskatoon under non-irrigated conditions in May 2002. One hundred and twenty seeds from each family were planted in 2-row plots with 2.0 m row length under appropriate management conditions for pea production in Saskatchewan. All F_2 plants (around 1000 in total) were labeled individually before flowering. After labeling, three to five pieces of young leaves were sampled from each individual plant for DNA extraction. Powdery mildew severity, mycosphaerella blight severity, days to flower and days to mature were recorded during the growing season. Seeds from each F_2 plant were harvested separately and kept in an envelope. The $F_{2:3}$ seeds were counted and weighed using a seed counter

and a balance. Those $F_{2:3}$ families with more than 40 seeds were separated from others and used for further lodging evaluation.

Table 3.1. Lodging performance and marker status of parents

Cross No.	Cultivars used as males in crosses	Lodging resistance	Markers		
			A001	A002	A004
1	DS Dominator	G	1	1	0
2	Carneval	G	1	1	0
3	Toledo	G	1	0	0
4	Nitouche	G	1	0	1
5	Swing	G	1	1	0
6	DS Admiral	G	1	1	0
7	Integra	G	1	1	0
8	Miami	G	1	1	0
9	Majoret	G	1	1	0
10	MP1101	G	1	1	0
Female parent	Carrera	F	0	1	1

G= good; F= Fair;
1=DNA band present on gel (A); 0=DNA band absent on gel (a).

Fifty-three pea cultivars available in Canada were also scored for A001, A002 and A004 markers to test the frequency of these markers in the commercial cultivars and to test the possibility of extension of MAS using these markers in other crosses.

3.1.2 Plant materials and DNA used in identification of new markers for lodging resistance

The population derived from the cross MP1401 × Carneval, which was used to develop the A001, A002 and A004 markers originally, as well an AFLP linkage map (Tar'an et al. 2003), was used to map markers derived from SSR primers. Two replications of 88 RILs derived from the MP1401 × Carneval were planted in the Sutherland nursery in 2003 to test lodging performance and to sample leaf tissue. DNA

was extracted from the leaf sample bulked from six plants for each of these RILs. The DNA extracted in 2003 and DNA stocks of the same RILs from 2001 were used in polymerase chain reaction (PCR) amplification. The mean lodging scores collected from 1998-2001 over 11 station-years across western Canada (Tar'an et al. 2003) were used in the analyses of the amount of variation (R^2) for lodging reaction being accounted for by the identified markers.

3.2 DNA extraction, PCR amplification and marker scoring

3.2.1 DNA extraction

DNA was extracted from the F_2 plants and the parental cultivars following the improved CTAB method (Saghai-Maroof et al. 1984) as follows: 1) 0.2-0.3 g leaf tissue from each plant was placed in a 1.5 ml Eppendorf tube and covered by a piece of foil with one or a few tiny holes; 2) the tubes with the leaf tissue were quickly put into liquid nitrogen for 2 minutes or more, and then put into a vacuum-freeze dryer for 24 hours to dry the samples; 3) the leaf tissue was ground into a fine powder using a high-speed shaker with a tungsten steel bead in each tube; 4) 0.4-0.5 ml of 2× CTAB buffer (Doyle and Doyle 1987) with newly mixed 1% mercaptoethanol was added into each tube and adequately mixed with samples; 5) the tubes with the samples were incubated for 30 minutes at 60 °C in a water bath and inverted a few times while in the water bath; 6) 0.4-0.5 ml of chloroform: isoamyl-ethanol (24:1) was added to each tube after the tubes returned to room temperature, and the tubes were shaken continuously for 5 minutes; 7) each tube was centrifuged at 5000 RPM for 10 minutes and the upper (aqueous) phase was removed into a clean tube; 8) steps 6 and 7 were repeated once more; 9) 0.4-0.5 ml

of –20 °C ethanol (95%) was added into the aqueous phase and the tubes were rocked until DNA precipitated; 10) DNA was spun down and ethanol was decanted; 11) 0.7-0.8 ml 76% ethanol/0.2M Na-acetate was added to each tube and left for 20 minutes; 12) ethanol/Na-acetate was decanted and 0.5 ml 76% ethanol/10 mM NH_4-acetate was added to each tube and left for 2 minutes; 13) the tubes were spun for 3 minutes and ethanol/NH_4-acetate was decanted; 14) 0.3 ml of ddH_2O was added into each tube and left overnight to allow the DNA to resuspend; 15) the solution was centrifuged 10 minutes to pellet any undissolved particles and the supernatant was transferred to a fresh tube; 16) 5 µl of the DNA solution was diluted with 95 µl of water and assayed on a spectrophotometer to quantify the DNA concentration.

After DNA quantification, the tubes containing DNA were labeled and stored at -20 °C or -70 °C as stocks. A different amount of stock DNA from each tube was taken and diluted with sterilized distilled water to a final volume of 500 µl at a concentration 25 ng/µl. The diluted solution was stored at 4 °C and used for PCR amplification.

3.2.2 SCAR marker analysis

All of the single F_2 plants, three populations of $F_{2:3}$ single plants and 53 pea cultivars were scored for two or three of the A001, A002 and A004 SCAR markers.

PCR was performed in a total volume of 25 µl containing 20 mM TRIS-HCl (pH 8.4), 50 mM KCl, 3.0 mM $MgCl_2$, 100 µM of each of the four dNTPs, 5 pmol of each forward and reverse primer, 25 ng of pea genomic DNA and 1 U *Taq* polymerase (Life Technology). The primer sequences of the A001, A002 and A004 SCAR markers are listed below:

A001: Forward: 5'-CTT CAC CAT CCA TAG TGT CG-3';

Reverse: 5'-CTT CAC CAT CCA TAG TGT CG-3';

A002: Forward: 5'-CAA ACA ATG AAA CTC CGG TG-3';

Reverse: 5'-GCA GCC AAT CAA ACA CAT C-3';

A004: Forward: 5'-GCG CAT GAA ATC TAG GTT TG-3';

Reverse: 5'-CAC AAG AAC GAA GAA CAT CG-3'.

Amplification was done using a MJ Research PTC-200 Thermo Cycler, which included a 3 min initial denaturation step at 94°C followed by 35 cycles at 94°C for 30 s, a 45 s annealing at 60°C (or 65°C for A002 and A004) and a 2 min elongation at 72°C. A final extension step at 72°C for 5 min was applied. The PCR products were loaded into a 1.4% agarose gel and run in TAE buffer to separate the products. The bands were visualized after ethidium bromide staining and photographed using a FluorChem imaging system (Alpha Innotech Corporation). Presence or absence of the markers was recorded for each individual line.

3.2.3 Identification of new markers for lodging resistance

3.2.3.1 Identification of markers derived from SSR primers

The 88 RILs derived from the cross MP1401 × Carneval were used in identification of markers derived from SSR primers linked to lodging resistance. More than 400 SSR primers (AGROGENE pea SSR consortium //www.agrogene.com/SSRdevelopment.htm) were screened. PCR amplification was performed in a 25 µl volume containing 20 mM TRIS-HCl (pH 8.4), 50 mM KCl, 3.0 mM $MgCl_2$, 100 µM of each of the four dNTPs, 5 pmol of each forward and reverse primer, 1 ng/µl of pea genomic DNA and 1 U *Taq*

polymerase. PCR conditions consisted of an initial denaturation step at 94°C for 4 min, followed by 36 cycles at 94°C for 30 sec, 50°C (if the recommended primer annealing temperature was close to 50°C) or 60°C (if the recommended primer annealing temperature was close to 60°C) for 30 sec, and 72°C for 2 min with a final extension step at 72°C for 5 min. PCR products were run on 2% agarose gels in TAE buffer. Bands were visualized after ethidium bromide staining and photographed using a FluorChem imaging system (Alpha Innotech Corporation).

The 400 SSR primers were first screened using DNA of the two parents (MP1401 and Carneval). Those primers which produced a polymorphism between the two parents were screened on the ten RILs with the best lodging resistance and the ten RILs with the poorest lodging resistance. Finally, the primers which produced a polymorphism between the RILs with the best lodging resistance vs. those with the poorest lodging resistance were screened on the rest of the RILs.

The amount of variation (R^2) for lodging reaction being accounted for by a given marker was analyzed using the statistical analysis system (SAS) general linear model (GLM) procedure (Command PROC GLM), according to the procedure used by Tar'an et al. (2003) and Narvel et al. (2001).

3.2.3.2 Analysis of linkage between markers derived from SSR primers and A001, A002 and A004 markers

Analysis was conducted using MAPMAKER/EXP program version 3.0 (Lander et al. 1987) to test if the lodging-related markers derived from SSR primers were linked to A001, A002 or A004. Linkage groups were determined using the group command of

MAPMAKER/EXP program version 3.0 at a logarithm of odds ratio (LOD) score of 4.0 with a maximum distance between two markers of 25 cM (Haldane map function). The order of the markers within the same linkage group was determined using the COMPARE command at a LOD score of 4.0. The best order of the markers was then verified using the MAP command at a LOD score of 4.0 and the results were compared to the linkage map generated by Tar'an et al. (2003).

3.3 Lodging evaluation

3.3.1 Experimental design for lodging evaluation

$F_{2:3}$ families from eight populations were planted at the Canada-Saskatchewan Irrigation Development Centre in Outlook, Saskatchewan in the summer of 2003. A completely randomized design (CRD) with four treatments was used. Treatments were based on marker classes with each treatment represented by one marker class. Since the A001 and A004 markers segregated independently in the F_2 generation following a typical 9:3:3:1 digenic model, the number of replications in each marker class varied, thus, an unequal number of replications were used in the $F_{2:3}$ lodging evaluation trial (Table 3.2).

Forty seeds harvested from each F_2 plant were planted in three rows in a 1.0 × 0.8 m microplot with 0.25 m between rows. Other families, which had less than 40 seeds, were omitted from the lodging evaluation, since plant stands would have been too thin to effectively assess lodging. Four to eleven microplots of the relevant parents were included for each cross to achieve a relatively square layout in the field. Microplots of the cultivar Carrera were planted as borders surrounding the experiment to reduce edge effects.

Table 3.2. Number of replications for each population

Trt	Marker combination		Replications in populations 1, 2 and 5-10								
			1	2	5	6	7	8	9	10	Total
T1	A001	a004	12	7	13	9	4	10	9	7	
T2	A001	A004	16	27	23	22	25	44	24	40	
T3	a001	a004	0	1	1	0	0	2	0	2	
T4	a001	A004	8	12	13	12	13	17	12	17	
Total			36	47	50	43	42	73	45	66	402
Susceptible parent			4	4	4	4	4	4	4	4	
Resistant parent			8	5	10	9	10	11	6	10	503

Note: Lodging susceptible parent was Carerra; lodging resistant parents were: 1 DS Dominator; 2 Carneval; 5 Swing; 6 DS Admiral; 7 Integra; 8 Miami; 9 Majoret; 10 MP1101.

3.3.2 Management of the field trial for lodging evaluation

The microplots were planted under irrigation to provide a suitable environment for lodging expression. Soil test results (Table 3.3) showed that fertility was sufficient for pea production (Saskatchewan Pulse Grower 2000). The pea seeds were sown on May 22, 2003. The first irrigation (15 mm) was applied on June 2. Subsequent irrigations were carried out ten times between July 17 and August 11 at 2-3 day intervals with approximately 25 mm water each. Total water available for the field was 265 mm irrigation plus 129 mm rainfall during the growing season. The $F_{2:3}$ seeds were treated with fungicides Vita-Flo and Apron FL (Table 3.4) to control seed-borne and soil-borne diseases. Necessary herbicides, fungicides and insecticides were applied (Table 3.4) and a hand weeding was carried out on July 2. To improve the nitrogen fixation ability and vigor of pea plants, a commercial pea/lentil granular inoculant supplied by Becker Underwood was planted with the seeds at a rate of 6 kg/ha.

Table 3.3. Soil test results of the field used to grow pea microplots at Outlook assessed in October 2002

Depth (cm)	Texture	PH (1 soil: 2 water)	Salinity Rating	NO_3-N (kg/ha)	P (kg/ha)	K (kg/ha)	SO_4-S (kg/ha)
0-30	Loam	8.4	Non Saline	39	46	430	>108
30-60	Loam	8.7	Non Saline	87	NA	NA	>108

NA: not available.

Table 3.4. Herbicides, fungicides and insecticides applied before and during the growing season in the lodging evaluation trial

Date/Activities	Materials	Concentration	Target pests
Seed treatment	Vita Flo 280 Apron FL	3.28 ml/kg seeds 0.32 ml/kg seeds	Seed-borne and Soil-borne diseases
May 20 (Applied to soil)	Edge (5% ethalfluralin)	22 kg/ha	Weeds
June 10	Basagan	7.8 L/ha	Broadleaf weeds
July 28	Headline	18.0 L/ha	Mycosphaerella blight and powdery mildew
July 28	Sevin XLR	2.2 L/ha	Aphids
July 31	Orthene	59 g/ha	Aphids

Note: The active ingredients of Vita Flo 280 are 14.9% carbathiin and 13.2% thiram;
The active ingredient of Apron FL is 317 g/L metalaxyl.

3.3.3 Lodging rating scale

Lodging scores of $F_{2:3}$ microplots were assessed twice during the growing season. The first assessment was before physiological maturity on August 7 and the second assessment was at physiological maturity (August 18) using a 1-9 scale where 1 = completely upright and 9 = completely lodged (Wang 1998; Table 3.5). Since the

differences between the lodging ratings of the susceptible and resistant parents were greater at the second assessment than at the first assessment, the lodging scores of the second assessment were used in the data analyses. Data were analyzed separately for each population and compared among the marker classes (Section 3.3.5).

Table 3.5. Lodging rating scale used in the pea $F_{2:3}$ microplots

Lodging assessing	Lodging development
1	Main stems strictly upright
2	Main stems incline slightly
3	Main stems at 60° angle
4	Main stems at 45° angle
5	Main stems at 30° angle
6	1/2 of the main stems flat
7	2/3 of the main stems flat
8	4/5 of the main stems flat
9	All main stems flat

3.3.4 Other agronomic traits recorded

Other agronomic traits, including plant height, days to flower, and days to mature were recorded using the microplot as a unit during the growing season. Plant height was recorded from the ground to the top of the plants at physiological maturity. Days to flower were calculated by determining the days from sowing to when half of the plants in the microplot had started flowering. Days to mature were calculated by determining the days from sowing to when 80% of the pods in the microplot had turned yellow.

3.3.5 Lodging data analysis

Lodging variations among marker classes in each population were tested using analysis of variance (ANOVA) by SAS GLM procedure (Du 1999). Since unequal number of replications was used in the lodging evaluation trial (see 3.3.1 and Table 3.2), a different variance was observed for each marker class. F-tests could not be applied in this analysis to compare the differences among marker classes, thus, the differences between pairs of marker classes were analyzed using SAS T-test (Command TTEST). The amount of variation (R^2) for lodging reaction being accounted for by a single marker or combination of two markers was analyzed using SAS GLM procedure (Command PROC GLM). Correlation coefficient (r) between characters was calculated by using the SAS CORR procedure (Command PROC CORR).

4 RESULTS

4.1 Marker scores for the F_2 populations

A total of 869 F_2 plants (72 -101 plants per population) derived from ten crosses were scored for the A001/a001 SCAR marker (Table 4.1 and Table 4.2). Plants derived from all crosses except cross 4 were scored for the A004/a004 marker. Plants from crosses 3 and 4 were scored using the A002/a002 marker. The polymorphic bands of the A001, A002 and A004 markers were about 300 bp, 140 bp and 180 bp, respectively, in all of the populations scored. Examples of polymorphisms are shown in Figure 4.1.

Chi-square analysis showed that the combination of the A001 and A004 markers followed a two independent gene segregation model (9:3:3:1; P>0.05) in all nine segregating populations (Table 4.1). P-values varied from >0.05 (population 3) to >0.6 (population 8). The P-values were >0.3 in six populations (Table 4.1).

Chi-square analysis showed that in populations 3 and 4, the P-values for the independent segregation of the A001 and A002 markers were 0.3 and 0.1 (Table 4.2), respectively. However, since the A001 and A002 markers were linked at 5.5 cM in the RILs derived from MP1401 × Carneval (Tar'an et al. 2003), further Chi-square tests were applied in population 3 and 4. In these two populations, the A001 marker was from the lodging resistant parents, while the A002 was from the lodging susceptible parent Carrera. Assuming the genetic distance between A001 and A002 was similar to that in the RIL population derived from MP1401 × Carneval; the P-values for the linked gene (5.5 cM) segregation model were 0.7 and 0.5 (Table 4.2), respectively. Thus, the

probability for the linkage of A001 and A002 is greater than probability for their independent segregation in populations 3 and 4. However, the size of the scored populations, especially for population 3, was relatively small.

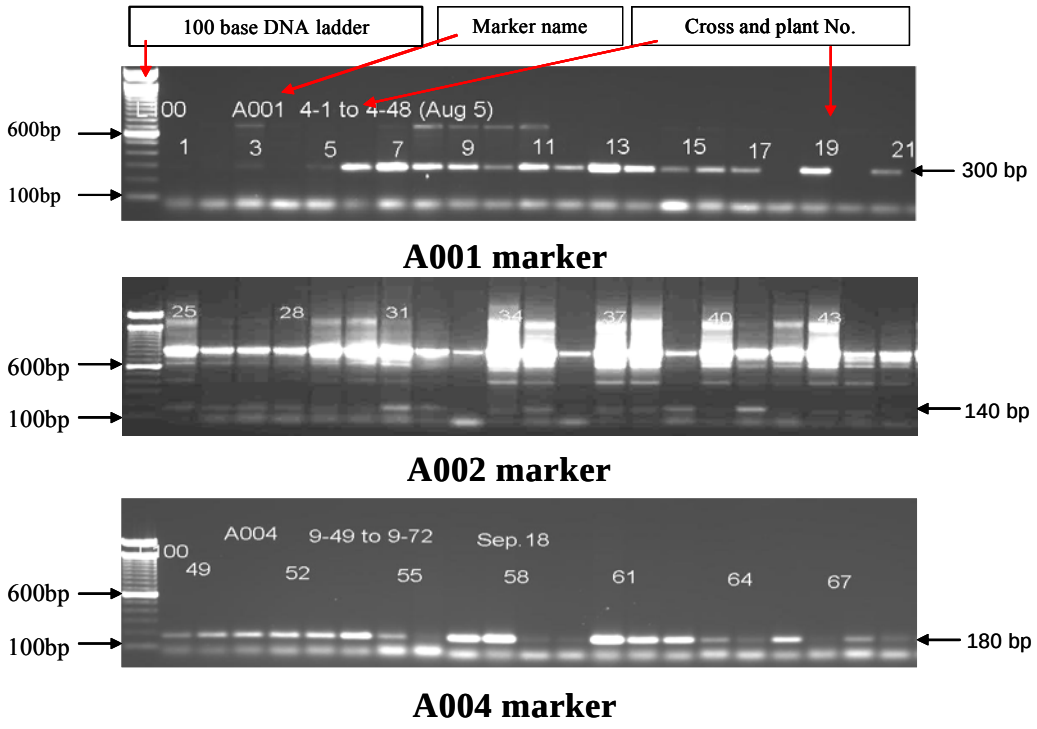

Figure 4.1. Example of the polymorphisms from SCAR markers A001, A002 and A004

Table 4.1. The A001 and A004 marker frequency in the F_2 populations 1-3 and 5-10

	Marker combination		Expected frequency	Observed number in each population								
				1	2	3	5	6	7	8	9	10
For populations 1-3 and 5-10	A001	A004	9/16	46	50	43	47	44	41	54	41	44
	A001	a004	3/16	19	19	25	19	19	15	22	19	13
	a001	A004	3/16	19	23	25	16	19	21	21	11	19
	a001	a004	1/16	2	4	5	2	1	3	4	1	2
Total			16/16	86	96	98	88	83	80	101	72	78
X^2=				3.26	2.41	7.60	2.75	5.05	3.60	1.71	5.43	3.19
P value				>0.3	>0.4	>0.05	>0.4	>0.1	>0.3	>0.6	>0.1	>0.3

*$X^2_{3,\,0.05}$=7.815.

Table 4.2. The A001 and A002 marker frequency in F_2 populations 3 and 4

	Marker combination			Expected as independent genes	Observed Number	X^2 and P value (Indep.)	*Expected as linked loci	X^2 and P value (Linked)
Pop 3	A001	A002	/	0.563 (9/16)	13	X^2= 2.96 P>0.3	0.500	X^2= 0.93 P>0.7
	A001	a002	/	0.188 (3/16)	7		0.249	
	a001	A002	/	0.188 (3/16)	4		0.249	
	a001	a002	/	0.063 (1/16)	0		6.3×10^{-4}	
Total				1.000 (16/16)	24		1.000	
Pop 4	A001	A002	/	0.563 (9/16)	44	X^2= 5.09 P>0.1	0.500	X^2= 2.33 P>0.5
	A001	a002	/	0.188 (3/16)	16		0.249	
	a001	A002	/	0.188 (3/16)	15		0.249	
	a001	a002	/	0.063 (1/16)	0		6.3×10^{-4}	
Total				1.000 (16/16)	75		1.000	

* Assuming the linkage between the A001 and A002 marker is the same as in the RIL populations, e.g. crossover rate ≈ 5.5% (Tar'an et al. 2003);.
Pop= population; Indep = independent;
* $X^2_{3,\,0.05}$ = 7.815 (Du 1999).

4.2 Marker scores for 53 pea cultivars

A survey of 53 pea cultivars recommended for production in Saskatchewan was conducted using the three markers linked to lodging resistance (Table 4.3). This analysis indicated that the A001 marker was present in 15 out of 16 pea cultivars with 'good' lodging resistance, and absent (a001) in 20 out of 37 cultivars rated as 'fair' or 'poor' for lodging resistance. The A004 marker was absent (a004) in 12 out of 16 cultivars with 'good' lodging resistance, and present (A004) in 18 out of 37 cultivars with 'fair' or 'poor' lodging resistance. The A002 marker was present (A002) in 12 out of 16 cultivars with 'good' lodging resistance and absent (a002) in 12 out of 37 cultivars with 'fair' or 'poor' lodging resistance. In total, each of the A001/a001, A004/a004 and A002/a002 marker classes were consistent with lodging performances in 66%, 57% and 45% of assessed cultivars, respectively. In other words, the A002 marker was present more often in cultivars with fair or poor lodging resistance than in cultivars with good lodging resistance, although it was linked to lodging resistance in the RILs derived from MP1401× Carneval.

The A001 and A002 markers were only present or absent simultaneously in 34 out of 53 cultivars, equaling 64% of the total number of cultivars. In the other 19 cultivars, one marker was present, while the other was absent.

Table 4.3. Lodging score and the A001, A002 and A004 marker score of 53 pea cultivars recommended for production in Saskatchewan

Cultivar	Lodging	A 001	A 002	A 004	Cultivar	Lodging	A 001	A 002	A 004
AC Melfort	F	0	1	1	Grande	F	0	0	1
Alfetta	F	0	1	1	SW Parade	F	1	1	0
Atomic	F	1	0	0	Venture	F	1	1	0
Baccara	F	0	0	1	AC Advantage	P	0	1	1
Badminton	F	0	0	1	Keoma	P	1	0	1
Carrera	**F**	**0**	**1**	**1**	Olivin	P	1	1	0
Cascade	F	1	1	0	Princess	P	1	1	0
CDC April	F	0	0	0	Trapper	P	0	1	0
CDC Handel	F	1	1	1	Victoria	P	0	0	0
CDC Minuet	F	0	1	0	Whero	P	0	1	1
CDC Montero	F	1	1	0	**Carneval**	**G**	**1**	**1**	**0**
CDC Mozart	F	1	1	0	DS 4931	G	1	1	0
CDC Verdi	F	1	1	0	**DS Admiral**	**G**	**1**	**1**	**0**
CDC Vienna	F	0	0	0	**DS Dominator**	**G**	**1**	**1**	**0**
CDC Winfield	F	0	1	0	Eclipse	G	0	0	1
Cobra	F	1	1	1	Eiffel	G	1	0	0
Cresta	F	0	0	1	Espace	G	1	1	1
Croma	F	0	1	1	**Integra**	**G**	**1**	**1**	**0**
Delta	F	1	1	1	Logan	G	1	1	0
DS Stalwarth	F	1	1	0	**Majoret**	**G**	**1**	**1**	**0**
Highlight	F	1	1	0	**Miami**	**G**	**1**	**1**	**0**
Millenium	F	0	1	1	**MP1101**	**G**	**1**	**1**	**0**
Nicole	F	0	1	1	**Nitouche**	**G**	**1**	**0**	**1**
Passat	F	0	1	1	SW Bravo	G	1	1	1
Pekisko	F	1	0	0	**Swing**	**G**	**1**	**1**	**0**
Radley	F	1	0	0	**Toledo**	**G**	**1**	**0**	**0**
Scuba	F	1	1	1					

*Lodging resistance ratings: G=good, F=fair, P=poor;
1= DNA band present (A), 0= DNA band absent (a);
*Bold writing indicates the cultivars used as parents in this project.

4.3 Lodging evaluation

4.3.1 Mean lodging score differences among marker classes

4.3.1.1 Mean lodging score of the A001 or A004 marker classes

Lodging scores of the $F_{2:3}$ families ranged from 1 to 8 in the eight populations using a 1 to 9 lodging scale (Table 3.5), and most of the scores ranged between the two parental values. Examples of lodging variation in pea microplots are shown in Figure 4.2. Lodging scores of marker classes in $F_{2:3}$ families were analyzed (Table 4.4; Figure 4.3). The mean lodging score of the A001 class varied from 3.8 to 5.4 with an average of 4.9 across the eight populations surveyed, while that of the a001 class varied from 4.6 to 5.9 with an average of 5.5 across the eight populations. The difference between the A001 and a001 classes varied from -0.5 to -0.9 in the eight populations. The mean difference between the two classes was -0.6 across the eight populations surveyed. The T-test results showed that significant differences ($P<0.05$) between A001 and a001 classes were observed in seven out of eight populations surveyed. The mean lodging score of A001 class across the eight populations was also significantly different ($P<0.05$) than that of the a001 class.

The mean lodging score of the A004 class varied from 4.3 to 5.6 with an average 5.2 across the eight populations (Table 4.4; Figure 4.3), while the mean lodging score of the a004 class varied from 3.4 to 5.1 with an average 4.5 across the eight populations. The differences between the A004 and a004 classes varied from -0.4 to -1.1 in the eight populations surveyed. The mean difference between the two classes was -0.7 across all the populations surveyed. The T-test results showed that significant differences ($P<0.05$)

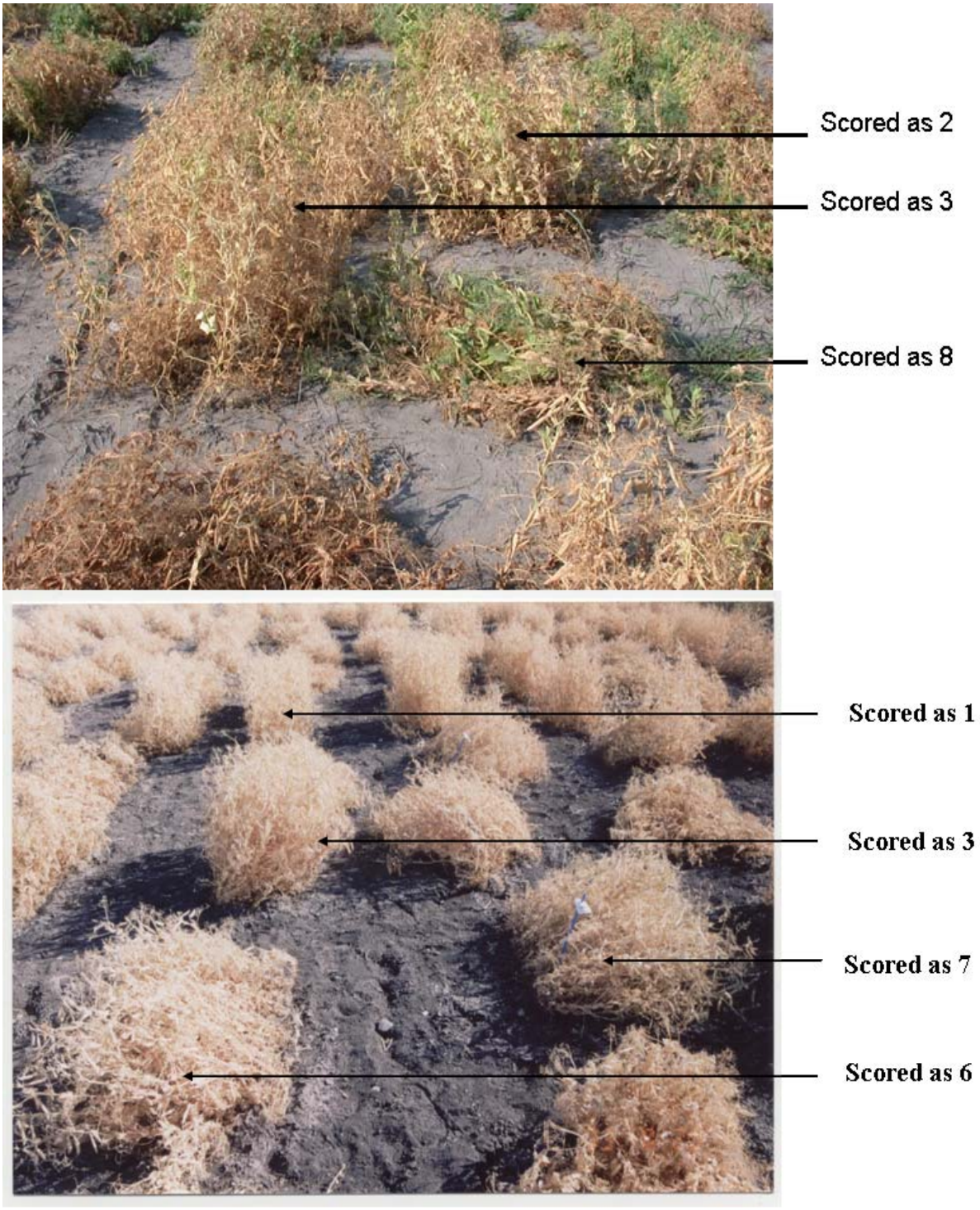

Figure 4.2. Examples of lodging variation in pea $F_{2:3}$ microplots

(1 = Main stems completely upright; 9 = All main stems flat)

between A004 and a004 classes were observed in six out of eight populations. The mean lodging score of A004 class across the eight populations was also significantly different ($P<0.05$) than that of the a004 class.

Comparing the A001/a001 and the A004/a004 markers revealed minor differences in their effectiveness in MAS in the trial in 2003 (Table 4.4). The mean lodging scores of A001 and a004 classes were generally close to each other in the same population, greater than that of the lodging resistant parents but lower than that of the mid-parent value. The average lodging difference between these two classes was 0.4 across all eight populations. The mean lodging score of the a001 and the A004 classes was generally close to each other in the same population and lower than that of the lodging-susceptible parents but greater than the mid-parent value. Only in population 7 was the mean lodging score of the a001 class (5.9) greater than that of the lodging-susceptible parent (5.4), and in population 8, in which the mean lodging score of a001 class was equal to that of the lodging-susceptible parent. The average lodging difference between the a001 and the A004 classes was 0.3 across all eight populations.

The lodging score differences between A001 and a001 classes were also similar to the differences between A004 and a004 classes within the same population. The mean difference between the two classes was only 0.1 across all eight populations. T-test results showed that the level of significance in lodging differences between the A001 and a001 classes was similar to that of the A004 and a004 classes in seven populations. However, in population 8, a significant difference was observed between the A001 and a001 marker classes, which was not observed between the A004 and a004 marker classes.

Table 4.4. Mean lodging score of marker classes in $F_{2:3}$ families and parents and T-test results between marker classes

Marker combinations		Mean lodging score in eight populations								
		1	2	5	6	7	8	9	10	Ave.
A001		3.8	4.4	4.9	5.0	5.0	5.3	5.0	5.4	4.9
a001		4.6	5.2	5.6	5.5	5.9	5.8	5.5	5.9	5.5
Differences[a]		-0.8	-0.8	-0.7	-0.5	-0.9	-0.5	-0.5	-0.5	-0.6
T-test difference level		***	***	***	*	***	*	ns	*	*
A004		4.3	4.8	5.3	5.3	5.4	5.5	5.3	5.6	5.2
a004		3.4	3.9	4.6	4.7	4.3	5.1	4.9	4.9	4.5
Differences[b]		-0.9	-0.9	-0.7	-0.6	-1.1	-0.4	-0.4	-0.7	-0.7
T-test difference level		***	***	***	*	***	ns	ns	*	*
T1: A001; a004		3.4	3.8	4.8	4.7	4.3	5.1	4.7	4.9	4.5
T2: A001; A004		4.1	4.6	5.1	5.1	5.1	5.4	5.2	5.5	5.0
T3: a001; a004		na	4.5	5.0	na	na	5.3	na	5.3	5.2
T4: a001; A004		4.6	5.3	5.7	5.5	5.9	5.9	5.4	5.9	5.5
Differences[c]		-1.2	-1.5	-0.9	-0.8	-1.6	-0.8	-0.7	-1.0	-1.0
Population's mean		4.0	4.6	5.1	5.1	5.3	5.5	5.2	5.5	5.0
Susceptible parent		5.4	5.6	5.9	5.6	5.4	5.8	5.9	6.0	5.7
Resistant parent		2.9	3.2	4.3	4.0	4.1	4.3	4.6	4.3	4.0
Parental differences		2.5	2.4	1.6	1.6	1.3	1.5	1.3	1.7	1.7
Mid-parental value		4.2	4.4	5.1	4.8	4.8	5.1	5.3	5.2	4.9
T-test Difference level	T1 – T4	***	***	***	***	***	*	ns	***	***
	T1 – T2	***	*	*	ns	*	ns	ns	ns	ns
	T1 – T3	na	ns	ns	na	na	ns	na	ns	ns
	T2 – T4	ns	*	*	ns	***	*	ns	*	*
	T2 – T3	na	ns	ns	na	na	ns	na	ns	ns
	T3 – T4	na	ns	ns	na	na	ns	na	ns	ns

a: Differences calculated from A001– a001;
b: Differences calculated from a004 – A004;
c: Differences calculated by T1-T4, i.e., from (A001, a004) – (a001, A004);
*: Significant at P<=0.05 level; ***: Significant at P<=0.01 level;
Ave.: average; ns: not significant;
na: not applicable due to lack of $F_{2:3}$ families in those T3 treatments (see Table 3.2).

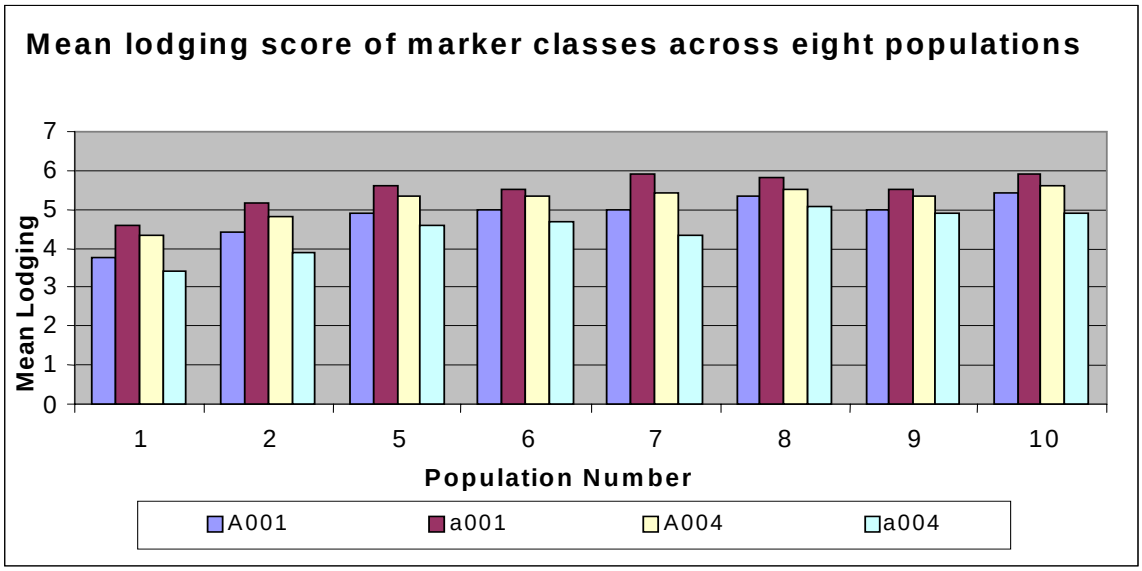

Figure 4.3. Comparison of mean lodging score of marker classes for individual markers, across eight populations

4.3.1.2 Mean lodging score from the combination of two markers

The mean lodging score of parents and marker classes in $F_{2:3}$ families for the combination of the A001/a001 and the A004/a004 markers displayed a similar trend in all eight populations (Table 4.4; Figure 4.4); i.e., susceptible parent > T4 > T3 ≅ T2> T1 > resistant parent, where average lodging scores from all the surveyed populations were 5.7, 5.5, 5.2, 5.0, 4.5, 4.0, respectively. An exception to this trend occurred in populations 7 and 8, in which the mean lodging scores of T4 were slightly greater than the susceptible parent. The mean lodging score of T1 (A001; a004) class varied from 3.4 to 5.1, while the mean lodging score of T4 (a001; A004) class varied from 4.6 to 5.9 across the eight populations. T-tests showed significant differences ($P<0.05$) in lodging score between the T1 and T4 classes in seven out of eight populations assessed, six of them were at $P<0.01$ level. The mean lodging score of the T1 class across the eight

populations was also significantly different from that of the T4 class (P<0.01). Significant differences (P<0.05) were also observed between classes T2 and T4 in five populations, as well as between classes T1 and T2 in four populations. Mid-parental values were close to lodging scores of the T2 and T3 classes.

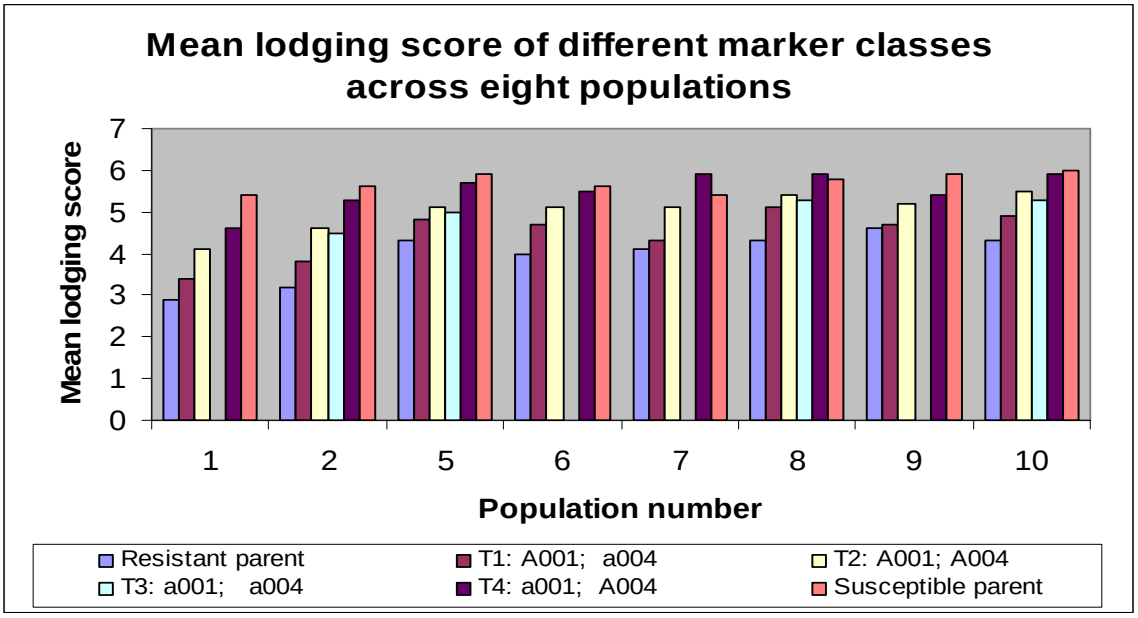

Figure 4.4. Comparison of mean lodging score of marker combination classes across eight populations

Note: T3: (a001; a004) was omitted in populations 1, 6, 7 and 9 due to a lack of $F_{2:3}$ families in those populations (See Table 3.2).

When comparing the efficiency of marker combinations with that of each individual marker, obvious differences in MAS were observed (Table 4.4). In all the populations assessed, the mean lodging scores from class T1 (A001; a004), was lower or equal to the lodging scores of the corresponding A001 or a004 class alone, except in population 5, in which the lodging score of the class a004 was lower than that of T1. On the other hand, the mean lodging scores from class T4 (a001; A004) were greater or equal to the lodging scores of the corresponding class a001 or A004 alone, except in population 9. The

differences between T1 and T4 varied from -0.7 to -1.6 with an average -1.0, across the populations assessed. Greater lodging score differences between T1 and T4 were attained from the combination of two markers than from individual markers in all of the populations assessed. On average, the difference in lodging score (T1 vs. T4) from the combination of two markers (-1.0) was greater than that of the individual A001 marker (-0.6) or the a004 marker (-0.7) by 0.3 and 0.4, respectively. Thus, using the combination of both markers was more effective than using the corresponding individual markers in MAS.

4.3.2 Amount of variation (R^2) for lodging reaction accounted for by markers

The amount of variation (R^2) for lodging reaction being accounted for by the markers in the $F_{2:3}$ lodging trial was analyzed using the SAS GLM procedure (Command PROC GLM) (Du 1999). The R^2 values in Table 4.5 represent the proportion of the lodging variation that could be explained by the variation from marker classes in the populations assessed. The R^2 from A001/a001 marker classes ranged from 0.16 to 0.49 in the eight populations assessed. Five of these were greater than 0.3. The R^2 from A004/a004 marker classes ranged from 0.14 to 0.55 in the eight populations. Six of these were greater than 0.20. The average of R^2 generated from A001/a001 marker was 0.29 and the average R^2 from A004/a004 was 0.28 across the eight populations assessed. There were no obvious differences observed between the R^2 generated from the A001/a001 marker and that from the A004/a004 marker. The R^2 for the marker combination class was generally greater than that from either the A001/a001 or A004/a004 marker alone.

Table 4.5. The amount of variation (R^2) for lodging reaction being accounted for by markers in $F_{2:3}$ families

Marker classes	The amount of variation for lodging reaction being accounted for by markers in each population								
	1	2	5	6	7	8	9	10	Average
A001/a001	0.49	0.31	0.31	0.31	0.34	0.16	0.16	0.21	0.29
A004/a004	0.55	0.29	0.31	0.31	0.27	0.14	0.15	0.21	0.28
A001/a001 & A004/a004	0.57	0.38	0.37	0.35	0.41	0.19	0.21	0.26	0.34

The marker combination explained 19% to 57% of lodging variation in the eight populations surveyed (Table 4.5). In population 1, the marker combination explained the greatest proportion of lodging variation (57%) of all populations assessed. In four other populations, this combination explained more than 30% of lodging variation, and only in one population did it explain less than 20% of lodging variation. On average, the A001/a001 marker alone, the A004/a004 marker alone and the combination of the two markers explained 29%, 28% and 34% of lodging variation respectively, across the eight populations. These results showed that using the two marker combination in MAS would be more effective than using either marker alone. Also, these markers had a different effectiveness in the different populations in MAS. For example, in population 1, a high R^2 was generated from the each of the marker classes, while in population 8 and 9 a relatively low R^2 was generated by the same marker classes.

The amount of variation (R^2) for plant height being accounted for by markers was also analyzed in each population in the lodging trial. The marker classes explained a small proportion of plant height variation in these populations (Table 4.6). The R^2 generated from the A001/a001 marker for plant height ranged from 0.04 to 0.39, and the

R^2 generated from the A004/a004 marker ranged from 0.04 to 0.19. The combination of the two markers generally explained a similar or a slightly greater proportion of plant height and ranged from 0.04 to 0.43. The R^2 generated from the A001/a001 marker classes was greater than 0.2 in four out of eight populations. However, it appeared that neither individual markers, nor the marker combinations, explained plant height in population 1, in which the R^2 was close to zero. The R^2 generated from the A004/a004 marker classes was relatively lower or the same as that from the A001/a001 marker classes, except for population 9. The R^2 generated from the marker combination was 0.18 or greater in seven out of eight populations. The A001/a001 marker explained a greater proportion of the variation of plant height than the A004 marker in five of the eight populations. In populations 2, 5, 6, 7 and 8, the R^2 generated from the A001/a001 marker alone was the same, or very similar to, the R^2 generated from the marker combination. On average the A001/a001, A004/a004, and the combination of the two markers explained 19%, 12% and 22% of plant height, respectively, across the eight populations.

Table 4.6. The amount of variation (R^2) for plant height being accounted for by markers in $F_{2:3}$ families

Marker classes	The amount of variation for plant height being accounted for by markers in each population								
	1	2	5	6	7	8	9	10	Average
A001/a001	0.04	0.27	0.17	0.30	0.20	0.39	0.11	0.06	0.19
A004/a004	0.04	0.11	0.10	0.19	0.06	0.17	0.18	0.07	0.12
A001/a001 & A004/a004	0.04	0.27	0.18	0.30	0.20	0.43	0.21	0.18	0.22

4.3.3 Correlation coefficient (r) between lodging, plant height and other traits

Plant height, days to flower, and days to mature for the $F_{2:3}$ families were recorded during the growing season. The population mean of each of these traits is shown in Table 4.7. The mean plant height of the lodging resistant parents ranged from 50 to 59 cm across the eight populations. The mean plant height of the lodging susceptible parent Carrera was shorter than that of any of the corresponding resistant parents and ranged from 45 to 51 cm across the eight populations. The plant height variations across populations showed that there was environmental variation among the experimental populations (See discussion section 5.3). However, due to the use of the relatively square field layout for each population assessed, the variations within each population were much lower than that among the eight populations. For example, the standard deviation of height of Carrera within each population varied from ± 1 cm to ± 7 cm, with only three of these variations greater than ± 5 cm. However, variation across all surveyed Carrera microplots was ± 9 cm (data not shown), equivalent to ± 18% of mean plant height.

Table 4.7. Correlation coefficients (r) among lodging, plant height, days to flower and days to mature in $F_{2:3}$ families

Traits	Trait mean value in each population								
	1	2	5	6	7	8	9	10	Ave.
R parent PH (cm) Std. Dev.	52 ±10	55 ± 6	53 ± 2	59 ± 4	57 ± 5	58 ± 3	50 ± 5	50 ± 4	54 ± 5
S parent PH (cm) Std. Dev.	50 ± 3	45 ± 5	47 ± 3	50 ± 7	51 ± 3	49 ± 3	47 ± 1	45 ± 5	48 ± 4
R parent days to flower Std. Dev.	52 ± 1.9	49 ± 0.5	47 ± 0.8	48 ± 0	47 ± 1.9	49 ± 1.0	49 ±1.3	51 ± 1.0	49 ± 1.1
S parent days to flower Std. Dev.	48 ± 0.7	48 ± 0.7	48 ± 0.4	48 ± 0.5	48 ± 0.7	48 ± 0.5	48 ± 0.4	48 ± 0	48 ± 0.5
R parent days to mature Std. Dev.	83 ± 0.8	76 ± 0.8	78 ± 1.3	79 ± 0.6	78 ± 1.2	79 ± 1.0	79 ± 1.0	79 ± 2.9	79 ± 1.2
S parent days to mature Std. Dev.	78 ± 1.4	78 ± 1.6	78 ± 0.9	79 ± 0.5	79 ± 0.7	78 ± 1.3	79 ± 1.2	78 ± 1.2	78 ± 1.1
	Correlation coefficient (r) with lodging in each population								
Plant height	0.11	-0.28	0.00	0.10	-0.17	-0.09	0.00	-0.24	-0.07
Significance level		*						*	
Days to flower	-0.52	-0.31	0.17	0.23	0.26	0.09	0.05	-0.13	-0.02
Significance level	***	*			*				
Days to mature	-0.36	-0.06	0.03	0.21	0.21	0.03	0.05	-0.22	-0.01
Significance level	***							*	
	Correlation coefficient (r) with plant height in each population								
Days to flower	0.10	0.18	0.14	-0.07	-0.20	0.04	0.31	0.26	0.10
Significance level							*	*	
Days to mature	0.25	0.25	0.13	0.01	0.25	0.21	0.20	0.50	0.23
Significance level								***	

Std. Dev.: Standard deviation;
R parent PH: Resistant parent plant height;
S parent PH: Susceptible parent plant height;
*: Significant at P<=0.05 level;
***: Significant at P<=0.01 level.

Mean days to flower among the different lodging-resistant parents ranged from 47 to 52 days. The lodging susceptible parent Carrera was quite uniform in days to flower, which was 48 days, with no differences, among the eight populations. Similar to days to flower, the trait of days to mature ranged from 76 to 83 days among lodging-resistant parents, and from 78 to 79 days for Carrera among the eight populations (Table 4.7).

Correlation analysis showed that lodging score was significantly ($P<0.05$) negatively correlated with plant height in two (populations 2 and 10) out of eight populations (Table 4.7). Results from these two populations were consistent with the RIL population derived from MP1401 × Carneval, from which these QTL markers were originally identified. In the RIL populations, lodging score was also significantly negatively correlated with plant height. A significant negative correlation was also observed between lodging and days to flower in two populations (populations 1 and 2), and lodging with days to mature in two populations (populations 1 and 10). However, a significant positive correlation between days to flower and lodging was also observed in one population (population 7). Further analysis showed that plant height was significantly correlated with days to flower in two populations, and plant height was significantly correlated with days to mature in one population.

4.4 Differences in plant height, days to flower, days to mature among marker classes

4.4.1 Differences in plant height among marker classes

The mean plant height varied from 48.4 to 57.4 cm across the eight populations (Table 4.8). The population mean plant height was generally within the range of the two

corresponding parents and close to the mid-parent value. In six populations significant differences in plant height were observed between the A001 and a001 marker classes. The mean plant height difference between A001 and a001 marker classes varied from 0.9 cm to 9.7 cm with an average 5.2 cm across the eight populations; the mean difference was significant. In two populations significant differences in plant height were observed between the A004 and a004 marker classes. The mean plant height difference between A004 and a004 marker classes varied from 0 cm to 7.2 cm with an average 2.9 cm across the eight populations; the mean difference was not significant. Regarding the use of the combination of markers, in five populations significant differences in plant height were observed between T1 and T4; the mean difference was significant.

Table 4.8. Mean plant height of marker classes in $F_{2:3}$ families and parents and T-test results between marker classes

Marker combinations		Mean plant height (cm) in the eight populations								
		1	2	5	6	7	8	9	10	Ave.
A001		54.4	54.1	52.9	60.6	56.8	60.4	53.2	49.5	55.2
a001		53.5	46.8	48.7	53.8	50.7	50.7	48.4	47.1	50.0
Differences[a]		0.9	7.3	4.2	6.8	6.1	9.7	2.8	2.4	5.2
T-test difference level		ns	***	*	***	***	***	ns	*	***
A004		54.0	51.8	51.8	58.1	54.7	57.2	50.4	48.3	53.3
a004		54.8	53.4	51.8	61.1	57.3	61.6	57.6	52.1	56.2
Differences[b]		0.8	1.6	0	3.0	2.6	4.4	7.2	3.8	2.9
T-test difference level		ns	ns	ns	ns	ns	*	***	ns	ns
T1: A001; a004		54.8	54.6	52.2	61.1	57.3	64.1	57.8	55.1	57.1
T2: A001; A004		54.2	54.0	53.4	60.4	56.8	59.6	51.5	48.6	54.8
T3: a001; a004		na	45.0	47.0	na	na	49.0	na	41.5	45.6
T4: a001; A004		53.5	46.9	48.8	53.8	50.7	50.9	48.3	47.7	50.1
Differences[c]		1.3	7.7	3.4	7.3	6.6	13.2	9.5	7.4	7.0
Population mean		53.4	51.6	51.0	57.4	54.6	56.8	51.3	48.4	53.1
Susceptible parent		50.9	44.0	46.5	50.1	51.9	49.5	46.5	45.5	48.1
Resistant parent		51.5	55.0	53.0	59.3	57.3	57.8	50.3	49.5	54.2
Parental differences		-0.6	-11.0	-6.5	-9.2	-5.4	-8.3	-3.8	-4.0	-6.1
Mid-Parental value		51.2	49.5	49.8	54.7	54.6	53.7	48.4	47.5	51.2
T-test Difference level	T1 – T4	ns	*	ns	*	ns	***	***	*	***
	T1 – T2	ns	ns	ns	ns	ns	*	*	*	ns
	T1 – T3	na	ns	ns	ns	na	***	na	*	*
	T2 – T4	ns	*	*	***	***	***	ns	ns	*
	T2 – T3	na	ns	ns	ns	na	*	na	ns	ns

a: Differences calculated from A001 – a001;
b: Differences calculated from a004 – A004;
c: Differences calculated by T1-T4, i.e., from (A001, a004) – (a001, A004);
*: Significant at P<=0.05 level; ***: Significant at P<=0.01 level;
Ave.: average;
na: not applicable due to lack of $F_{2:3}$ families in those T3 treatments (see Table 3.2).

4.4.2 Differences in days to flower and days to mature among marker classes

The mean number of days to flower varied from 46.5 to 49.5 days across the eight populations (Table 4.9). The population mean was generally within the range of the two corresponding parents' values. Significant differences in days to flower between the A001 and a001 marker classes were observed in only two populations (5 and 7), and there was no significant difference between the A004 and a004 marker classes across eight populations.

The mean number of days to mature varied from 76.4 to 80.0 days across the eight populations (Table 4.10). Similar to plant height and days to flower, the population mean was generally within the range of the two corresponding parents' value. Significant differences in days to mature were observed between the A004 and a004 marker classes in only two populations (7 and 9), but not between the A001 and the a001 marker classes in any population.

Table 4.9. Mean days to flower of each marker class in $F_{2:3}$ families and parents and T-test results between marker classes

Marker combinations		Mean days to flower (day) in the eight populations								
		1	2	5	6	7	8	9	10	Ave.
A001		49.7	48.0	46.6	47.7	45.8	47.2	47.7	49.0	47.7
a001		49.3	47.9	47.5	47.8	47.2	47.6	47.8	48.4	47.9
T-test difference level		ns	ns	*	ns	***	ns	ns	ns	ns
A004		49.4	47.9	46.9	47.7	46.3	47.3	47.6	48.8	47.7
a004		50.0	48.3	46.6	47.9	45.3	47.2	48.4	49.3	47.9
T-test difference level		ns	ns	ns	ns	ns	ns	ns	ns	ns
T1: A001; a004		50.0	48.3	46.6	47.9	45.3	47.3	48.6	49.6	48.0
T2: A001; A004		49.4	47.9	46.6	47.6	45.9	47.1	47.4	48.9	47.6
T3: a001; a004		na	48.0	47.0	na	na	46.5	na	48.5	47.5
T4: a001; A004		49.3	47.9	47.5	47.8	47.2	47.7	47.8	48.4	48.0
Population's mean		49.5	48.1	47.0	47.8	46.5	47.4	47.9	48.8	47.9
Susceptible parent		47.8	48.0	47.8	47.7	47.4	47.6	47.8	48.0	47.8
Resistant parent		52.3	49.3	47.0	48.0	46.8	48.8	49.5	50.8	49.1
Parental differences		-4.5	-1.3	+0.8	-0.3	+0.6	-1.2	-1.7	-2.8	-1.5
T-test Difference level	T1 – T4	ns	ns	*	ns	*	ns	ns	ns	ns
	T1 – T2	ns	ns	ns	ns	ns	ns	*	ns	ns
	T1 – T3	na	ns	ns	ns	na	ns	na	ns	ns
	T2 – T4	ns	ns	*	ns	*	ns	ns	ns	ns
	T2 – T3	na	ns	ns	ns	na	ns	na	ns	ns

*: Significant at P<=0.05 level; ***: Significant at P<=0.01 level;
Ave.: average;
ns: not significant;
na: not applicable due to lack of $F_{2:3}$ families in those T3 treatments (see Table 3.2).

Table 4.10. Mean days to mature of each marker class in $F_{2:3}$ families and parents and T-test results between marker classes

Marker combinations		Mean days to mature in the eight populations								
		1	2	5	6	7	8	9	10	Ave.
A001		79.7	76.4	77.7	78.6	77.8	79.1	78.9	79.0	78.4
a001		80.9	76.0	78.1	78.8	78.4	79.6	78.2	78.6	78.6
T-test difference level		ns	ns	ns	ns	ns	ns	ns	ns	ns
A004		80.0	76.3	77.9	78.7	78.2	79.1	78.5	79.0	78.5
a004		79.8	76.3	77.7	78.4	76.3	79.5	79.6	78.7	78.3
T-test difference level		ns	ns	ns	ns	***	ns	*	ns	ns
T1: A001; a004		79.8	76.6	77.7	78.4	76.3	79.4	79.7	79.1	78.4
T2: A001; A004		79.6	76.3	77.7	78.6	78.0	79.0	78.7	79.0	78.4
T3: a001; a004		na	74.0	78.0	na	na	80.0	na	77.0	77.3
T4: a001; A004		80.9	76.2	78.1	78.8	78.4	79.5	78.2	78.8	78.6
Population's mean		80.0	76.4	77.8	78.6	78.1	79.1	78.8	78.8	78.5
Susceptible parent		78.4	77.6	78.0	78.6	78.7	78.3	78.2	78.1	78.2
Resistant parent		83.0	76.0	77.5	78.5	78.0	79.3	79.3	79.3	78.9
Parental differences		-4.6	+1.6	+0.5	+0.1	+0.7	-1.0	-0.9	-1.2	-0.96
T-test Difference level	T1 – T4	ns	ns	ns	ns	***	ns	*	ns	ns
	T1 – T2	ns	ns	ns	ns	***	ns	ns	ns	ns
	T1 – T3	na	ns	ns	ns	na	ns	na	ns	ns
	T2 – T4	ns	ns	ns	ns	ns	ns	ns	ns	ns
	T2 – T3	na	ns	ns	ns	na	ns	na	ns	ns

*: Significant at P<=0.05 level; ***: Significant at P<=0.01 level;
Ave.: average;
ns: not significant;
na: not applicable due to lack of $F_{2:3}$ families in those T3 treatments (see Table 3.2).

4.5 New markers linked to lodging resistance

4.5.1 Development of markers derived from SSR primers linked to lodging resistance

The RILs derived from MP1401 × Carneval, from which the A001 and A004 markers were identified, were used to identify new markers derived from SSR primers. More than 400 SSR primers were screened on the two parents, Carneval and MP1401. Then 102 primers that produced polymorphisms on the parents were screened on the ten RILs with the best lodging resistance and the ten RILs with the poorest lodging resistance. Finally, the 13 primers which produced a polymorphism between the RILs with the best lodging resistance vs. those with the poorest lodging resistance were screened on all 88 RILs.

Three primers were identified that were related to lodging resistance. Using 60°C annealing temperature (AGROGENE recommended 64°C), primer SAB81 produced a single band in most lodging resistant RILs and some lodging susceptible RILs (Figure 4.5); the band size was similar to that designated by AGROGENE (304 bp). Since agarose gels were used in these experiments instead of polyacrylamide gels (see Discussion section 5.6), band size differences less than 20 bp could not be conclusively determined, thus band size was referred to as 'similar' to that designated by AGROGENE.

Using 60°C annealing temperature, (AGROGENE recommended 61°C), primer SAD134 produced two bands in most lodging resistant RILs and only the longer band in most lodging susceptible RILs (Figure 4.6); the size of the shorter band was similar to that designated by AGROGENE (284 bp).

The amount of variation (R^2) for lodging reaction being accounted for by markers was analyzed using SAS GLM procedure. The band produced by primer SAB 81 and the shorter band produced by SAD134 explained 13% and 12% of lodging variation, respectively, in the RILs, and could be considered as dominant markers (Table 4.11).

The primer SAD141 did not produce any bands at 60°C annealing temperature (AGROGENE recommended 63°C). However, it produced several bands at 50°C annealing temperature (Figure 4.7). The size of band-a was similar to that designated by AGROGENE (335 bp). In total, three markers were identified from primer SAD141. Band-a and band-b (approximately 220 bp) originated from the lodging-resistant parent Carneval and explained 19% and 11% of lodging variation in the RILs, respectively. Band-c (approximately 260 bp) originated from the lodging susceptible parent MP1401 and explained 25% of the lodging variation in the RILs. The combination of these three markers explained 28% of the lodging variation. Band-a was present in 57 RILs and band-c was present in 37 RILs across the 88 RILs (Table 4.11). Six of these RILs had both band-a and band-c and no RIL was missing both bands. Chi-square test showed that band-a and band-c were not allelic (Chi-square test result for 1:1 segregation ratio of band-a and band-c loci is $X^2 = 5.3$; while the threshold is $X^2_{1,\ 0.05} = 3.9$). Band-b was present in 41 RILs. Twelve RILs had both band-b and band-c, and 23 RILs were missing both bands across the 88 RILs. Four RILs had all three bands. Based on the above information, none of the three markers derived from the primer SAD 141 were allelic.

Further analysis showed that the combination of SAD141a, SAD141b, SAD141c, SAD134, and SAB81 markers explained 40% of lodging variation in the RILs derived

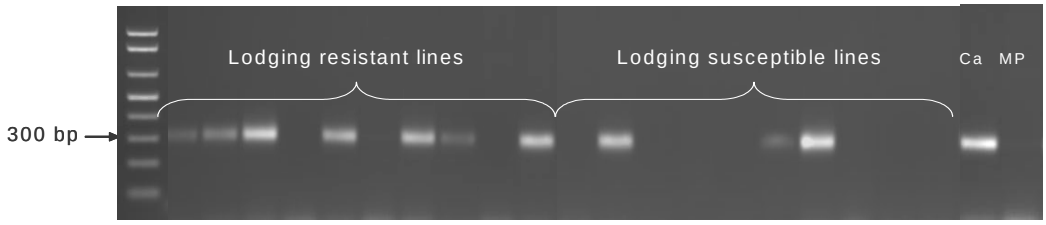

Figure 4.5. Polymorphisms produced from SSR primer SAB 81 in RILs derived from MP1401 × Carneval

Note: parents were Ca: Carneval; MP: MP1401.

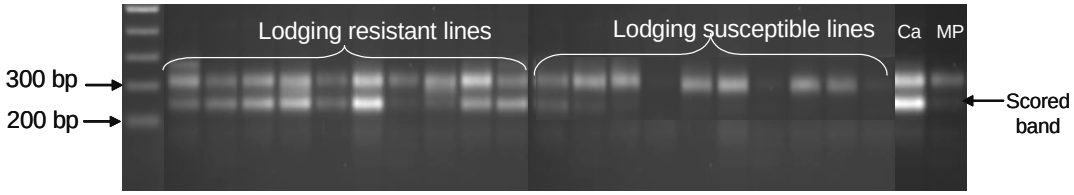

Figure 4.6. Polymorphisms produced from SSR primer SAD134 in RILs derived from MP1401 × Carneval

Note: parents were Ca: Carneval; MP: MP1401.

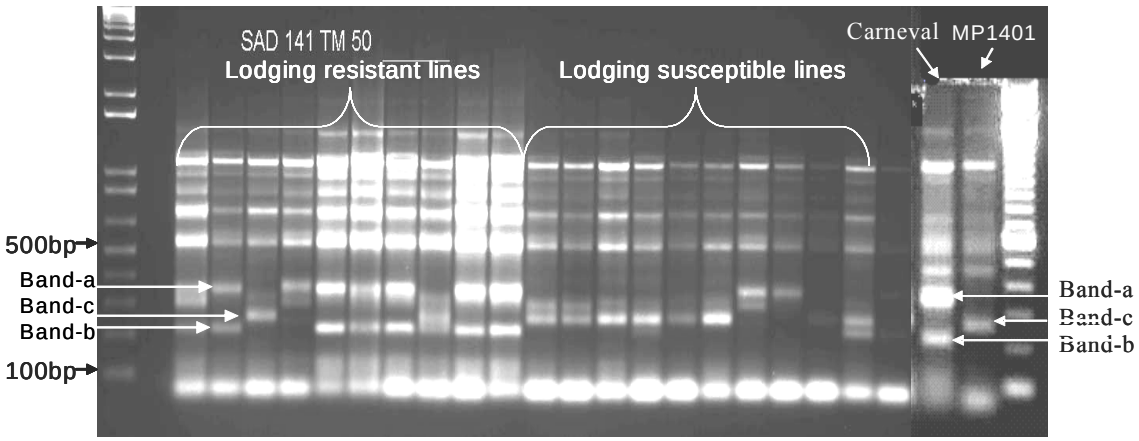

Figure 4.7. Polymorphisms produced from SSR primer SAD 141 in RILs derived from MP1401 × Carneval

Note: parents were Ca: Carneval; MP: MP1401.

Table 4.11. Lodging score of RILs and score of markers SAD141, SAD134 and SAB81 on the RILs

RIL No.	Lodging Score	SAD 141a	SAD 141b	SAD 141c	SAD 134	SAB 81	RIL No.	Lodging score	SAD 141a	SAD 141b	SAD 141c	SAD 134	SAB 81
10	3.0	1	1	0	1	1	47	4.6	0	1	1	1	0
15	3.0	1	1	0	1	1	52	4.6	1	1	1	0	1
22	3.2	1	1	0	1	1	64	4.6	1	0	0	1	0
30	3.2	1	0	0	2	2	72	4.6	0	0	1	1	0
24	3.3	1	1	0	1	1	14	4.7	1	1	0	2	1
27	3.3	0	0	1	1	1	33	4.7	0	1	1	1	1
19	3.4	1	0	0	1	1	35	4.7	1	0	0	1	1
70	3.4	1	0	0	2	2	42	4.7	1	1	0	1	1
2	3.5	1	1	0	1	1	66	4.7	0	0	1	1	1
5	3.5	1	1	0	1	1	84	4.7	0	1	1	1	1
41	3.5	1	1	0	1	1	88	4.7	0	0	1	0	0
69	3.5	0	1	1	1	0	11	4.8	1	0	1	1	0
3	3.6	1	1	0	1	1	54	4.8	1	0	0	2	2
39	3.7	1	1	0	1	1	65	4.8	0	1	1	1	0
46	3.7	1	0	0	1	1	73	4.8	0	0	1	1	1
60	3.7	1	1	0	1	1	79	4.8	0	0	1	0	0
74	3.7	1	1	0	0	0	29	4.9	1	1	0	0	1
58	3.8	1	1	0	1	1	61	4.9	1	1	1	0	1
59	3.8	1	0	0	2	2	13	5.0	0	0	1	0	0
81	3.9	1	1	0	0	1	40	5.0	0	0	1	2	2
67	4.0	1	0	0	0	1	48	5.0	0	0	1	0	1
85	4.0	1	0	0	1	1	53	5.0	1	1	1	1	0
26	4.1	1	0	0	1	0	68	5.0	1	1	0	0	1
34	4.1	0	0	1	1	1	78	5.0	0	0	1	1	0
43	4.1	1	0	0	0	1	9	5.1	0	1	1	1	0
44	4.1	1	1	0	1	1	23	5.1	1	1	1	1	1
56	4.1	0	1	1	1	0	82	5.1	0	0	1	0	1
87	4.1	1	0	0	1	0	7	5.3	1	1	0	0	1
1	4.2	1	1	0	1	1	18	5.3	0	0	1	0	0
20	4.2	1	1	0	1	1	17	5.4	0	0	1	0	0
55	4.2	1	1	0	1	0	25	5.4	1	1	0	0	0
62	4.2	1	0	0	1	0	45	5.4	0	0	1	1	1
71	4.2	1	1	0	1	1	49	5.4	0	0	1	0	0
4	4.3	0	0	1	1	0	51	5.4	1	0	0	2	2
83	4.3	1	1	0	1	1	12	5.5	0	0	1	0	0
21	4.4	1	1	0	1	1	37	5.5	0	0	1	1	1
50	4.4	1	0	1	1	0	38	5.5	0	0	1	0	0
57	4.4	1	0	0	0	1	76	5.5	1	0	0	0	0
75	4.4	1	0	0	1	0	80	5.6	0	0	1	1	1
6	4.5	1	1	0	1	0	36	5.8	1	0	0	1	0
28	4.5	1	0	0	1	1	86	5.8	0	0	1	0	0
32	4.5	1	1	0	0	0	16	6.0	0	1	1	1	0
63	4.5	1	1	0	1	0	8	6.3	0	0	1	0	1
77	4.5	1	0	0	1	0	Ca	3.0	1	1	0	1	1
31	4.6	1	0	0	2	2	MP	6.1	0	0	1	0	0

Note: Lodging scores were from Tar'an et al. (2003). 0= absent; 1= present; 2= data missing; a, b, c: represent the three bands produced by primer SAD141; Ca: Carneval; MP: MP1401.

from MP1401 × Carneval. Combining any one of SAD141a, SAD141b and SAD141c markers with A001 did not increase the proportion of lodging variation explained compared to A001 alone. Combining any one of SAD141a, SAD141b and SAD141c markers with A004 increased the proportion of lodging variation explained by 8-15% compared to A004 alone. However, none of these combinations explained more lodging variation than the combination of the A001 and A004 markers (59%). The combination of SAD141a, SAD141b, SAD141c markers with A001 and A004, only increased the proportion of lodging variation explained by 4% beyond that of the combination of A001 and A004.

4.5.2 Linkage between markers derived from SSR primers and A001, A002 and A004 markers

To test whether the markers derived from the SSR primers were linked to the SCAR markers A001, A002 or A004, a linkage analysis was conducted using MAPMAKER/EXP. The results showed that band-a derived from the primer SAD141 was linked to the SCAR marker A001 at a distance of 13.0 cM, and was linked to A002 at a distance 7.5 cM on pea linkage group III (Figure 4.8). Band-b derived from the primer SAD141 was located in linkage group C, 21 cM from the peak of a QTL for plant height. The marker developed from primer SAD134 was located on linkage group VI, 18 cM from the peak of a QTL associated with lodging resistance, and 18 cM from the peak of a QTL associated with mycosphaerella blight resistance. These markers were added into the linkage map generated by Tar'an et al. (2003). Band-c derived from the primer SAD141 and the marker derived from SAB81 could not be added to any of the existing linkage groups. None of these new markers derived from SSR primers were linked to A004.

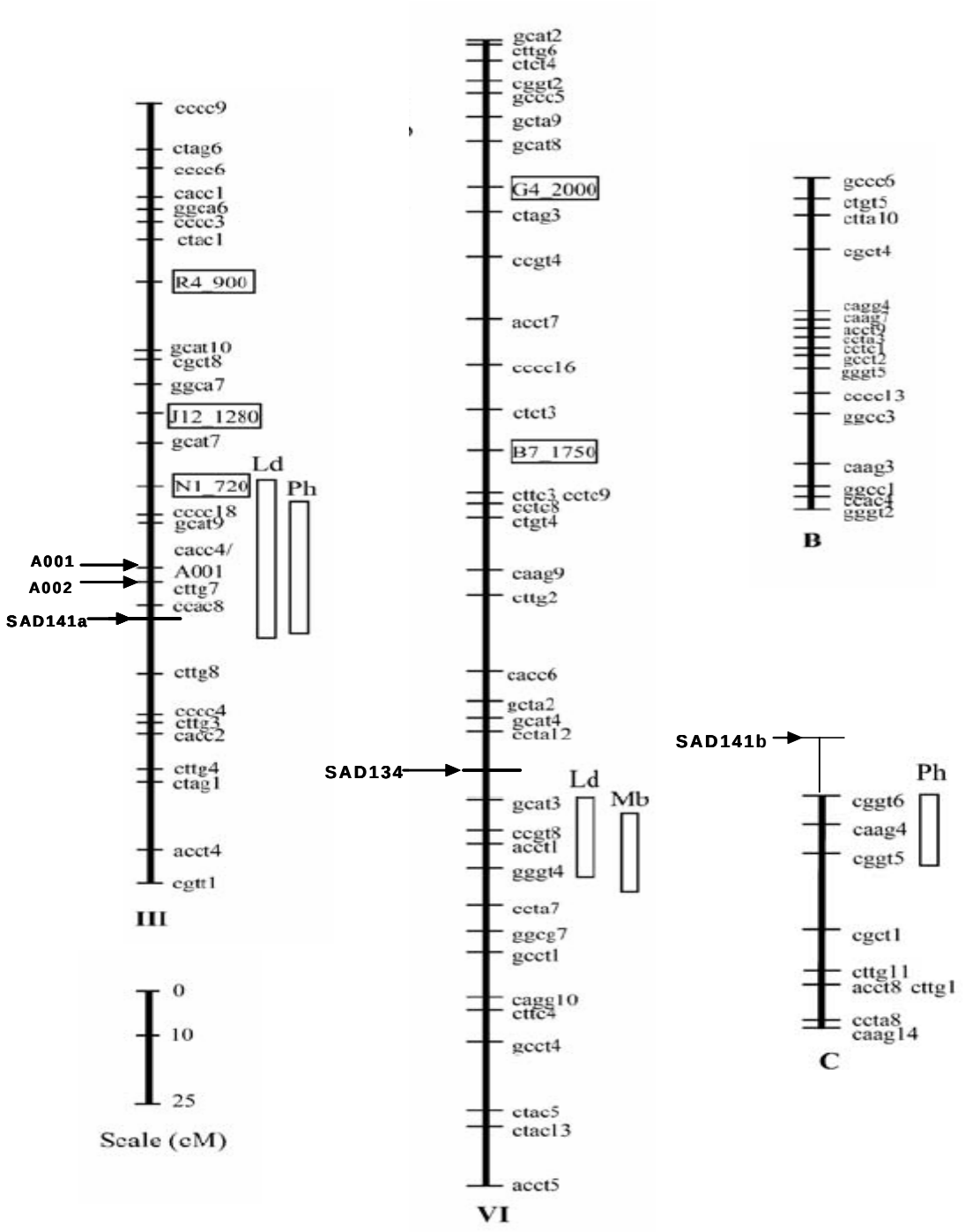

Figure 4.8. Lodging resistance markers SAD141a, SAD141b, SAD134, SAD135 and SAD126 added into the linkage map generated by Tar'an et al. (2003)

Note: The vertical rectangles show the QTL for lodging resistance (Ld), plant height (Ph) and mycosphaerella blight resistance (Mb); The horizontal rectangles are the markers used as anchors in the map development.

4.5.3 SAD 141 marker score for 53 pea cultivars

A survey of 53 pea cultivars recommended for production in Saskatchewan was conducted using the markers derived from primer SAD141 (Table 4.12). At least one of the three markers (Figure 4.7) could be found in each of the 53 cultivars. Band-a and band-b had been identified in lodging resistant cultivar Carneval, and band-c had been identified in the lodging susceptible cultivar MP1401. The results showed that band-a was present in 9 out of 16 cultivars with good lodging resistance and absent in 27 out of 37 cultivars with fair or poor lodging resistance (Table 4.12). Band-b was present in 9 out of 16 cultivars with good lodging resistance and absent in 28 out of 37 cultivars with fair or poor lodging resistance. Band-c was present in 28 out of 37 cultivars with fair or poor lodging resistance and absent in 9 out of 16 cultivars with good lodging resistance. Two of the 53 cultivars had both band-a and band-c, and seven cultivars had both band-b and band-c. No cultivars had all three bands.

Table 4. 12. Lodging score and the SAD141 marker score of 53 pea cultivars recommended for production in Saskatchewan

Cultivar	Lodging	SAD 141a	SAD 141b	SAD 141c	Cultivar	Lodging	SAD 141a	SAD 141b	SAD 141c
AC Melfort	F	0	0	1	Scuba	F	1	0	0
Alfetta	F	0	0	1	SW Parade	F	0	0	1
Atomic	F	0	0	1	Venture	F	0	0	1
Baccara	F	0	0	1	**Carneval**	**G**	1	1	0
Badminton	F	0	0	1	DS 4931	G	1	0	0
Carrera	**F**	0	0	1	**DS Admiral**	**G**	1	1	0
Cascade	F	0	0	1	**DS Dominator**	**G**	1	1	0
CDC April	F	0	0	1	Eclipse	G	0	1	1
CDC Handel	F	0	0	1	Eiffel	G	1	0	0
CDC Minuet	F	1	1	0	Espace	G	1	1	0
CDC Montero	F	0	1	1	**Integra**	**G**	0	0	1
CDC Mozart	F	0	0	1	Logan	G	1	1	0
CDC Verdi	F	0	1	1	**Majoret**	**G**	0	1	1
CDC Vienna	F	0	1	1	**Miami**	**G**	1	1	0
CDC Winfield	F	0	0	1	**MP1101**	**G**	0	0	1
Cobra	F	0	0	1	**Nitouche**	**G**	1	0	1
Cresta	F	0	0	1	SW Bravo	G	1	1	0
Croma	F	0	0	1	**Swing**	**G**	0	0	1
Delta	F	1	0	0	**Toledo**	**G**	0	0	1
DS Stalwarth	F	1	1	0	AC Advantage	P	0	0	1
Grande	F	0	0	1	Keoma	P	0	1	1
Highlight	F	0	1	1	Olivin	P	1	0	0
Millenium	F	0	0	1	Princess	P	1	0	0
Nicole	F	1	0	0	Trapper	P	0	0	1
Passat	F	1	0	0	Victoria	P	1	1	0
Pekisko	F	0	0	1	Whero	P	0	0	1
Radley	F	1	0	1					

*Lodging resistance ratings: G=good, F=fair, P=poor;
a, b, c: represent the three bands produced from SAD141;
1= DNA band present (A), 0= DNA band absent (a);
*Bold writing indicates the cultivars used as parents in this project.

5 DISCUSSION

5.1 Effectiveness of the A001 and A004 markers in MAS

The effectiveness of the A001/a001 and A004/a004 SCAR markers for MAS in pea lodging resistance breeding was confirmed by the significant differences in lodging score among marker classes in the $F_{2:3}$ lodging evaluation trial. The results demonstrated that the $F_{2:3}$ families with the A001 marker had improved lodging resistance in seven out of eight populations surveyed, and the $F_{2:3}$ families with the a004 marker had improved lodging resistance in six out of eight populations surveyed (Table 4.4). The amount of lodging variation (R^2) accounted for by A001 and A004 markers also showed that the markers were effective in selection for lodging resistance (Table 4.5). Although the individual A001/a001 or A004/a004 markers were effective in most populations, using the combination of two markers was more effective. Using two markers, the most lodging resistant class was T1 (A001; a004). Since the expected frequency of T1 is 3/16 (19%) (Table 4.1), up to 81% of plants without the desired marker combination could be discarded in F_2 populations segregating for these two markers.

The mean lodging differences between the A001 and a001 classes, between A004 and a004 classes and between the T1 and T4 classes were -0.6, -0.7 and -1.0 (1-9 scale), respectively, across the eight population assessed. The 1-9 ordinal scale has been used for the past >20 years in pea breeding assessments in Canada and Europe. No important cultivars remain in production which have mean lodging scores (as assessed over >10 station-years) at physiological maturity of 8 or 9; they are commercially unacceptable (T. Warkentin, personal communication). Conversely, no important cultivars exist with

mean lodging scores at physiological maturity of 1 or 2. Thus, in a practical plant breeding sense, the 1-9 scale is reduced to a 3-7 scale, i.e., a 5 point ordinal scale. Plant breeding efforts, with or without the use of markers, which reduce the long-term mean lodging score by one unit on the de facto 5 point ordinal scale are valuable contributions indeed. All other traits being equal, resulting varieties will gain market share and deliver commercial value to the breeding institution from which they were derived and to the industry that uses them.

The effectiveness of these SCAR markers for MAS was affected by the lodging performance of the parental cultivars. The greater the difference in lodging score between the two parental cultivars, the more effective were the markers in MAS. For instance, in the eight populations assessed, the lodging differences between the two parents were greater than 1.6 in populations 1, 2, 5, 6 and 10. In populations 1, 2 and 5, the lodging differences of the single marker scored classes were significant at $P<0.01$ level. In populations 8 and 9, in which the parental lodging differences were 1.5 and 1.3, respectively, the lodging differences of the single marker scored classes were generally not significant ($P>0.05$). Population 7 (Carrera × Integra) was an exception to the above trend in that the parental lodging difference was relatively small (1.3), but the lodging differences of the single marker scored classes were significant at $P<0.01$ level (Table 4.4). This suggests that a greater usefulness could be obtained in MAS for populations in which the parents have large differences in lodging resistance. This rule might also be useful in other QTL marker analyses.

In the current research, a similar trend in the lodging performance of the parental cultivars and marker or marker combination classes was observed. This trend was:

Susceptible parent > T4 (a001; A004) >T2 (A001, A004) ≅ T3 (a001, a004) > T1 (A001; a004) > Resistant parent (Table 4.4 and Figure 4.4). This trend supported the initial hypothesis.

There could be two reasons why the lodging performance of the marker/marker combination classes did not exceed the lodging performance of the corresponding parental cultivars. Firstly, the R^2 between lodging performance and the markers was limited. For example, the highest R^2 in the populations assessed was 0.57 (Table 4.5). In the RIL population derived from MP1401 × Carneval, the R^2 was 0.59. These results suggest that some other unknown factors related to lodging exist which could not be explained by these SCAR markers. Secondly, there were some heterozygous F_2 individual plants in the dominant marker/marker combination classes. This could have also reduced the efficiency of MAS. This fact was demonstrated by additional analyses of marker scores of individual plants selected from three $F_{2:3}$ populations (2, 7, 8), i.e. there was segregation in the A001 and the A004 classes but no segregation in the a001 and the a004 classes (data not shown).

5.2 Effect of environment and control of variation in the lodging trial

The lodging trial was performed at Outlook, Saskatchewan under irrigation to provide favorable conditions for the expression of lodging. However, lodging differences between susceptible and resistant parents were not as great as expected. A potential explanation could be that the plots were lacking nitrogen at the pod-filling stage of the season. Although a soil test had indicated that N was sufficient at the time of planting, it may have been leached out during 11 irrigations, and no top-dressed N was added. As a result, the

pea plots were not as vigorous as they could have been, and lodging was not expressed as obviously as expected under ideal conditions. For instance, under similarly irrigated conditions, the plant height of Carrera was 65 to 70 cm and its lodging score was 6-7 in 2001 (Canada-Saskatchewan Irrigation Diversification Centre 2001a), but in the current evaluation trial, the average plant height of Carrera was 48 cm (Table 4.7) and the average lodging score was 5.7 (Table 4.4). Another abiotic stress affecting plant growth and lodging expression was the high temperature during the stage from flowering to pod filling. Lodging is usually correlated with plant height and the weight of pods at physiological maturity and the growing conditions at the pod-filling stage are critical to lodging expression (Samarin 1975; Obraztsov and Amelin 1990). Due to sub-optimal physiological growth, the plant yield (1540 kg/ha) was also lower than average (approximately 4000 kg/ha) under irrigated conditions at Outlook, Saskatchewan (Canada-Saskatchewan Irrigation Diversification Centre 2001b). In the current trial, the reduced plant height and below average yield were probably the major reasons for the sub-optimal expression of lodging.

Some environmental variation affected the lodging performance and plant height in the $F_{2:3}$ lodging trial. For example, the mean lodging score of the susceptible parent Carrera varied from 5.4 to 6.0 (Table 4.4), and the mean plant height varied from 45 cm to 51 cm (Table 4.7) across the eight populations. However, a square layout for each population was used in the lodging trial to decrease the environmental variation within populations. This method was effective in reducing environmental effects. For instance, the standard deviations of mean plant height of Carrera within each population varied from ± 1.2 cm to ± 7.1 cm (Table 4.7). The average within-population variation was ±

3.7 cm (± 8% of mean plant height). The within-population variation was much lower than inter-population variation, which was ± 8.7 cm, (± 18% of mean plant height). These results showed that using a square layout for each population effectively decreased the effects from environmental variation. The mean lodging score of the eight resistant parents varied from 2.9 to 4.6. Large variation occurred among the different lodging-resistant parents and the major portion of this variation could be deduced to come from genetic variation.

In the current study, herbicides, fungicides and insecticides were applied to reduce the effects from weeds, diseases and insects. In addition, a few weeds which escaped herbicidal control were removed by hand weeding. With the fungicidal control, the maximal rating was only 2 (1-9 scale) for both mycosphaerella blight and powdery mildew. Thus, no serious damage from diseases was observed in the $F_{2:3}$ lodging evaluation, and no significant differences occurred among marker classes for reaction to these diseases. Therefore, the effect from mycosphaerella blight on lodging was not considered in the current experiments. Only minor damage was detected from aphids, before they were controlled by insecticides.

5.3 Relationships between lodging, plant height and other traits

In the current study, a significant negative correlation coefficient (r) between lodging score and plant height was observed in two out of eight populations. This result differs from the general understanding that taller lines tend to lodge more. However, this result is comparable to that reported previously for pea and other crops. For example, this result was consistent with the RIL population derived from MP1401 × Carneval, in

which lodging score was negatively correlated with plant height ($r = -0.59$; $P<0.001$; Tar'an et al. 2003). Knyaz'kova (1987) also found that some taller lines had better lodging resistance than some shorter lines in a pea F_{10} population.

There might be a balance between the factors of plant height, stem stiffness and lodging performance. In crop production and breeding, some studies have suggested existence of this balance: for example, Obraztsov and Amelin (1990) indicated that the optimum height for lodging resistant pea plants is 60-90 cm. Taller or shorter plants were inferior for lodging resistance and yield. McPhee and Muehlbauer (1999) reported that stem strength was positively correlated with internode length ($r = 0.36$, $P<0.001$) among 418 *Pisum* accessions. In the current study, the lodging susceptible parent was shorter than most lodging resistant parents (Table 4.8). In populations 2 and 10, in which a significant correlation was observed between lodging and plant height (Table 4.7), the mean plant height of the lodging susceptible parent Carrera was 44 cm, while the mean plant height of the lodging resistant parents Carneval and MP1101 was 55 cm and 50 cm, respectively.

In the current study, lodging was correlated with days to flower and days to mature in a few populations, but clear trends were not evident (Table 4.7).

5.4 Segregation of the A001, A002 and A004 markers

The marker analysis across ten F_2 populations confirmed the association of A001 and A004 markers with lodging resistance in these pea populations. The A001 and A004 markers followed a two independent gene segregation model in the population assessed.

Similar to the linkage between A001 and A002 in RILs derived from the cross MP1401 × Carneval (Tar'an et al. 2003), these two markers appeared to be linked in population 3 (Carrera × Toledo) and population 4 (Carrera × Nitouche) (Table 4.2). The P-values for segregation of A001 and A002 markers as linked loci (0.7 and 0.5, respectively) were greater than those for segregation as independent loci (0.3 and 0.1, respectively) (Table 4.2). However, it is dangerous to draw a conclusion on this point, because of the small population size which was scored, especially for population 3. Although A001 and A002 markers were linked (5.5 cM) in the RILs derived from MP1401 × Carneval and in populations 3 and 4, they were only present simultaneously in 34 out of 53 pea cultivars in the current study (Table 4.3).

5.5 Potential of the A001, A002 and A004 markers for MAS

The presence at a high frequency (25 out of 37; Table 4.3) of the A002 marker in cultivars with fair or poor lodging resistance suggests that A002 could not be used broadly in MAS in pea lodging resistance breeding. The A001 marker was present in 15 out of 16 cultivars with good lodging resistance and absent in 20 out of 37 cultivars with fair or poor lodging resistance. The A004 marker was absent (a004) in 12 out of 16 cultivars with good lodging resistance, and present (A004) in 18 out of 37 cultivars with fair or poor lodging resistance. The A001, A004 and A002 markers were consistent with lodging performance in 66%, 57% and 45% of assessed cultivars, respectively. Therefore, the A001 and A004 markers have a higher potential than A002 to be used in MAS in pea lodging resistance breeding. These results also suggest that pre-screening of the parents using the markers is important in MAS for lodging resistance in pea breeding programs.

The consumable cost of conducting SCAR marker analysis in MAS is approximately $2 per sample. Approximately 200 samples could be analysed in one day by one technician at a cost of approximately $160 in labour. Thus, the total cost per sample is $2.8. The cost of evaluating one $F_{2:3}$ microplot is approximately $10 (T. Warkentin, personal communication). Thus, if MAS allows for the discarding of undesirable marker classes in F_2, an overall saving to the breeding program should be realized.

Since the A002 marker was linked to A001 marker at a distance of 5.5 cM in the RILs derived from MP1401 × Carneval and they were also linked in populations 3 and 4 in current study (Table 4.2), it is not necessary to use both markers together. It is also difficult to score the A002 marker since the A002 SCAR band was much weaker than the other major band on the gels (Figure 4.1). The A002 primer should have been designed more specifically, for example, extending the primer by one or more base pairs in length, or improving the extent of matching with the binding sites, such that only one band appeared on the gels. These results suggested that A002 is not a good SCAR marker for MAS in lodging resistance. The only case in which A002 should be used is if the lodging resistant parent lacks the A001 marker but carries the A002 marker. However, in the 53 pea cultivars recommended for production in Saskatchewan, no cultivar with good lodging resistance carried the A002 marker but lacked the A001 marker (Table 4.3).

5.6 Lodging resistance markers derived from SSR primers and their potential implementation for MAS

SSR markers are useful because they have a high level of polymorphism, are widely dispersed throughout the genome, and are usually codominant (Pandian et al. 2000).

Typically, SSR markers are assessed on polyacrylamide gels in order to detect minor differences in band lengths (Choumane et al. 2000; Pandian et al. 2000). In the current study, agarose gels were used to separate the PCR products. Agarose gels were used instead of polyacrylamide gels because they are less expensive and easier to run. Agarose gels should allow for the identification of polymorphic bands with large size differences (>50 bp) that segregate in a codominant manner. Markers of this nature could have been utilized for MAS on agarose gels, thus saving time and expenses. Initial analyses of a few pea SSR primers using polyacrylamide gel electrophoresis followed by silver staining generated similar band sizes as the agarose gels. However, for other SSR primers evaluated using agraose gels, the polymorphisms may be underscored.

In retrospect, the decision to use agarose gels instead of polyacrylamide gels was probably unwise. Many polymorphisms derived from SSR primers are less than 50 bp (Choumane et al. 2000). These small band size differences are not detected on agarose gels. Furthermore, with increasing band size, it becomes more difficult to detect small band size differences. For instance, it is easy to detect the difference between 100 bp and 150 bp DNA bands, but it is difficult to detect the difference between 900 bp and 1000 bp bands on the same agarose gel. Thus, many SSR primers that could have produced polymorphisms were ignored during the marker identification on agarose gels.

All five new markers identified in this study behaved as dominant markers. Dominant markers are occasionally found using SSR primers (Pandian et al. 2000; Bezawada et al. 2003), but in most cases SSR markers are codominant (Choumane et al. 2000). The presence of dominant polymorphisms could be due to loss of primer binding sites in one

parent, or use of inappropriate annealing temperatures (Pandian et al. 2000). Information regarding the performance of the majority of the SSR primers from the AGROGENE consortium has not yet been published. In fact, these primers have not yet been shown to be definitively associated with SSRs and may also identify other repeated regions of the genome.

Polymorphisms associated with lodging resistance developed from primer SAD141 could be considered as three independent dominant markers. SAD141c could not be mapped to any of the linkage groups described by Tar'an et al. (2003) suggesting that there might be another QTL associated with lodging which was not identified in the RILs. SAD141b was located on linkage group C which has a QTL for plant height. However, when SAD141c or SAD141b was combined with the SCAR markers A001 and A004, they did not explain a higher proportion of lodging variation than the combination of A001 and A004 alone. The markers SAD134 and SAB81 each explained less than 15% of lodging variation. Thus, all of the five markers derived from SSR primers are less useful than the SCAR markers A001 and A004.

5.7 Improvement of MAS for lodging resistance in pea breeding

DNA-based MAS has been applied in plant breeding for more than a decade. However, some problems still need to be addressed during MAS. The most important problem is inconsistency of molecular markers because of breakdown of linkage due to the genetic distance between the marker and the target gene (Lonnig and Saedler 1997; Fedoroff 1999; Bennetzen 2000). Breakdown of linkage means the marker can be applied only in the crosses derived from a limited number of parents, but not broadly.

In a pea genetic map developed Irzykowska (2002), the QTL linked to plant height were not collinear to the QTL identified by Tar'an et al. (2003). In Irzykowska's work, twelve QTL for plant height were identified and seven of them were located in linkage group II (chromosome No. 6) in the RILs population derived from cross Wt10245 × Wt11238. This suggested that linkage group II is the most important group in controlling plant height. However, the three QTL for plant height in RIL population derived from MP1401 × Carneval were located in three other linkage groups and one of the most important QTL was located on linkage group III. In the two studies, no QTL for plant height from one population was coincident with any QTL from the other population.

The best marker in MAS for a qualitative trait is the gene itself, and the trend in MAS research is towards using the gene itself or part of the gene as a genetic marker. When the gene sequence is known, a pair of PCR primers can be designed based on the flanking regions or some part in the middle of the gene (Yan et al 2002). When using the gene itself as a marker no crossover between the marker and the gene in the offspring can occur.

Considering complicated quantitative traits, such as yield and lodging resistance, identifying QTL markers accounting for a large amount of variation (R^2) for the target trait is important. Since quantitative traits are unstable under varying conditions, replications at multiple environments and multiple years are needed to reduce the effects from environmental variation during QTL marker identification. Furthermore, the amount of variation (R^2) for the target trait being accounted for by a given marker is usually different in different populations. In the current study, the amount of lodging variation (R^2) being accounted for by the A001 marker varied from 0.16 to 0.49, from 0.14 to 0.55 for the A004 marker, and from 0.19 to 0.57 for the combination of the two

markers, across the eight populations assessed (Table 4.5). No R^2 was higher than that in the RIL population used for developing these QTL markers.

Lodging resistance is a complicated quantitative trait and many genes might be involved in the trait expression. QTL markers have different efficiency in different breeding populations, and may not be efficient in some populations. For instance, in the current study, the lodging difference between A001 and a001 classes was only significant in seven out of eight populations, and that between A004 and a004 classes was only significant in six out of eight populations assessed. Furthermore, these markers are only effective in the populations derived from the cultivars in which lodging performance is consistent with these markers (Table 4.3). One QTL can only partially explain lodging variation in any breeding population. Thus, combining two or more QTL markers in MAS is important to get maximal response. Moreover, phenotypic selection should be utilized when QTL(s) only explain a low percentage of phenotypic variation. Keller et al. (1999) indicated that the most efficient way to improve lodging resistance would be by a combination of indirect selection on plant height and stem stiffness, together with MAS on the QTL for lodging resistance that did not coincide with QTL for other morphological traits.

For some complicated quantitative traits, it may be difficult to identify QTL markers especially if many minor genes are involved. Under such circumstances, phenotypic selection is still the only choice. MAS could speed up the breeding process when markers are tightly linked to the target traits, and when they explain a high proportion of the phenotypic variation.

6 SUMMARY AND CONCLUSIONS

1. The effectiveness of SCAR markers A001 and A004 for MAS in pea lodging resistance breeding was demonstrated by the significant differences in lodging score among marker classes in the lodging evaluation trial of $F_{2:3}$ families.

2. The mean lodging differences between the A001 and a001 classes, between A004 and a004 classes and between the T1 and T4 classes were -0.6, -0.7 and -1.0 (1-9 scale), respectively, across the eight population assessed. A one unit improvement in lodging resistance is valuable in pea breeding.

3. The amount of variation (R^2) for lodging reaction accounted for by A001, A004 or the combination of the two markers also showed that the markers were effective in selection for lodging resistance. The A001 and A004 marker combination explained 19% to 57% of lodging variation over eight different pedigrees.

4. Although individual A001 and A004 markers were effective in most populations, using the combination of two markers was more effective. By using the combination of two markers, up to 81% (13/16) of individual plants without desired markers could be discarded in F_2 populations segregating for both markers.

5. A001 and A004 markers followed an independent gene segregation model in the eight populations assessed. A001 and A002 appeared to be linked in populations 3 and 4. These results were consistent with the result in the original RIL population, i.e., that A001 and A004 were independent loci, while A001 and A002 were linked at a distance of 5.5 cM.

6. The amount of variation (R^2) for plant height accounted for by the A001 marker was consistent with the fact that A001 was linked to plant height and lodging resistance in the original RIL population from which these QTLs were identified. The A001 marker explained 4-39% of plant height variation over eight pedigrees.

7. Five new dominant markers were identified from SSR primers SAD134, SAB81 and SAD141, using the RIL population derived from MP1401 × Carneval. These markers explained from 12% to 25% of lodging variation. Combining any of these markers with A001 and A004 did not substantially improve the amount of lodging variation explained.

8. Combining all of the information above, the A001 and A004 markers should be useful in MAS for lodging resistance in pea breeding, while A002 is not as useful. The markers derived from SSR primers identified in this project may not have great potential to be used in the future, except in parents that cannot be differentiated by A001 and A004, but can be differentiated by SAD141c, which explained 25% of lodging variation in the RILs.

9. The A001 and A004 SCAR markers could be used in MAS for lodging resistance in pea breeding, if the marker scores are consistent with lodging performance in the parents, i.e., A001 associated with lodging resistance and A004 associated with lodging susceptibility. Evaluation of lodging performance and marker scores of parents are necessary before using these markers in MAS. The F_1 from three-way or double crosses, or the F_2 from single crosses segregating for A001, A004 or both markers are the best generations to start MAS. Since the A001 class is a mixed population for homozygous (A001; A001) and heterozygous (A001; a001) individuals in F_2 generation, assessment

of individual F_3 plants using the A001 marker plus progeny testing is necessary to obtain homozygous (A001; A001) populations. The a004 class is a pure population, i.e., homozygous (a004; a004), thus further selection in F_3 is not required.

7 REFERENCES

Agriculture Development Fund (ADF) Final Report (2000) Saskatchewan Agriculture Food and Rural Revitalization

AGROGENE pea SSR consortium (2004) //www.agrogene.com/SSRdevelopment.htm

Ali-Khan ST, Zimmer RC (1989) Production of field peas in Canada. Agriculture Canada Publication 1710E

Ali SM, Slinkard AE (1995) Field pea. In: Slinkard AE, Knott DR (ed) *Harvest of Gold: the history of field crop breeding in Canada*. University Extension Press. University of Saskatchewan, pp 181-190

Amelin AV, Obraztsov AS, Lakhanov AP, Uvarov VN (1991) Possibility of increasing the resistance of pea to lodging. *Selektsiya-i-Semenovodstvo-Moskva* 2: 21-23. Abstract from *CAB Abstract*, AN: 911698886

Amelin AV, Parakhin NV (2003) Scientific grounds for pea selection for fodder production. *Kormoproizvodstvo* 2: 20-25. Abstract from *CAB Abstract* AN: 20033049804

Anderson JA, Waldron BL, Moreno-Se-villa B, Stack RW, Frohberg RC (1998) Detection of *Fusarium* head blight resistance QTL in wheat using AFLPs and RFLPs. In Slinkard AE (ed) *Proc. 9th Int. Wheat Genetics Symp*., Saskatoon, Saskatchewan, Canada. 2-7 Aug, vol. 1, p.135-137

Anderson J (1998) Marker assisted selection of disease resistance genes in wheat. In Kolhi M, Estanzuela IL (ed) *International Workshop on the Application of Biotechnologies to Wheat Breeding*

Arioli T, Peng LC, Betzner AS, Burn J, Wittke W, Herth W, Camilleri C, Höfte H, Plazinski J, Birch R, Cork A, Glover H Redmond J, Williamson RE (1998) Molecular analysis of cellulose biosynthesis in *Arabidopsis*. *Science* 279: 714-720

Armstrong E, Matthews P, Carpenter D, Cassells J (1999) Desiccation and harvest-monitor moisture and maturity for quality seed. *Farming Ahead* No. 93

Askin DC, White JGH, Rhodes PJ (1985) Nitrogen fixation by pea and their effect on soil fertility. In: Hebblewaite PD, Heath MC, Dawkins TCK (ed) *The pea crop: A basis for improvement* Butterworths, London, pp 421-430

Barr AR, Jefferies SP, Warner P, Moody DB, Chalmers KJ, Langridge L (2000) Marker assisted selection in theory and practice. In: *VIII International Barley Genetics Symposium,* Adelaide, Australia: Department of plant science, Adelaide University, 2000: 167-178

Barriere Y, Gibelin C, Argillier O, Mechin V (2001) Genetic analysis in recombinant inbred lines of early dent forage maize. I-QTL mapping for yield, earliness, starch and crude protein contents from *per se* value and top cross experiments. *Maydica* 46: 253-266

Beckie H (1997) Field pea magic! *Research letter* #97-01. Agriculture and Agri-Food Canada, Saskatoon, SK

Benare DA (1996) Barley field diagnostic information release #9- Some strategies for handling lodging. Release by the Soil and Crop Diagnostic Centre, Alberta Agriculture, Food and Rural Development

Bennetzen JL (2000) Transposable element contributions to plant gene and genome evolution. *Plant. Mol. Biol.* 42: 251-169

Bezawada C, Saha S, Jenkins JN, Greech RG, McCarty JC (2003) SSR marker(s) associated with root knot nematode resistance gene(s) in cotton. *The J. of Cotton Sci.* 7: 179-184

Biswas JC, Ahmed MT, Islam MR (2001) Lodging vs. non-lodging in BRRI dhan32, *Bang. J. of Training and Development* 14: 107-113

Blixt S (1974) The pea. In: King RC (ed) *Handbook of genetics, Plenum Press*, New York, Vol 2, pp 181–221

Board J (2001) Reduced lodging for soybean in low plant population is related to light quality. *Crop Sci.* 41: 379-384

Börner A, Schumann E, Furste A, Coster H, Leithold B, Roder MS, Weber WE (2002) Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum L.*). *Theor. Appl. Genet.* 105: 921-936

Boros L, Sawicki J (2001) Combining ability analysis of seed yields and its components in pea. *Instytut Hodowli i Aklimatyzacji Roslin, Radzikowie* 216: 417-423. Abstract from *CAB Abstracts* AN: 20013088927

Brandt SA, Miller PR, Johnston AM, Entz MH (1996) Pulses in rotation—Current knowledge and future needs. In: proc. *Pulse Crop Research. Workshop*. University of Saskatchewan, Saskatoon, SK, pp 35-36

Canada-Saskatchewan Irrigation Diversification Centre (2001a) Irrigated field pea research highlights

Canada-Saskatchewan Irrigation Diversification Centre (2001b) Field pea optimum seeding rates for irrigated pea production research highlights

Cannon, WA (1903) Studies in plant hybrids: the spermatogenesis of hybrid peas. *Bull Torrey Bot. Club* 30: 519-543

Casey R, Davies DR (1993) Peas: genetics, molecular biology and biotechnology, *Biotechnol. Agric. Ser.* No. 10, CAB international

Choumane W, Winter P, Weigand F and Kahl G (2000) Conservation and variability of sequence-tagged microsatellite sites (STMSs) from chickpea (*Cicer arietinum* L.) within the genus *Cicer. Theor. Appl. Genet.* 101: 269-278

Davies DR (1977) Restructuring the pea plant. *Sci. Prog.* 64: 201-214

Davies DR, Berry GJ, Heath MC, Dawkin TCK (1985) Pea (*Pisum sativum* L.). In: Summerfield RJ, Roberts EH (ed) *Grain Legume Crops*, Collins, London, pp 147-199

Dirlewanger E, Isaac PG, Ranade S, Belajouza M, Cousin B, de Vienne D (1994) Restriction fragment length polymorphism analysis of loci associated with disease resistance genes and developmental traits in *Pisum sativum* L. *Theor. Appl. Genet.* 88: 17-27

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bulletin* 19: 11-15

Drozd AM, Ershova EV, Volchkov YA (1979) Preliminary results of using mutant forms of pea in breeding garden varieties. *Tr. po Prikl. Bot. Genet. I Sele.* 65: 48-51. Abstract from: *CAB Abstract*, AN: 821612029

Du RQ (1999) Biostastistics, China Higher Education Press Beijing and Springer-Verlag Heidelberg, pp 103-107

Faik A, Price NJ, Raikhel NV, Keegstra K (2002) An *Arabidopsis* gene encoding an α-xylosyltransferase involved in xyloglucan biosynthesis. *Proc. Natl. Acad. Sci.* 99: 7797-7802

Fagard M, Desnos T, Desprez T, Goubet F, Refregier G, Mouille G, McCann M, Rayon C, Vernhettes S (2000) PROCUSTE1 encodes a cellulose synthase required for normal cell elongation specifically in roots and dark-grown hypocotyls of *Arabidopsis*. *Plant C.* 12: 2409-2423

Fedoroff NV (1999) Transposable elements as a molecular evolutionary force. *Ann. N. Y. Acad. Sci.* 870: 251-64

Fellows H (1948) Falling of wheat culms due to lodging, buckling, and breaking. *Circ 767*, *U.S. Dep. of Agric.*

Flint GSA, McMullen MD, Darrah LL (2003) Genetic relationship of stalk strength and ear height in maize. *Crop Sci.* 43: 23-31

Griga M, Novak FJ (1990) Pea (*Pisum sativum* L.). In: Bajaj YPS (ed) Biotechnology in Agriculture and Forestry 10, *Legumes and Oilseed Crops I* pp 65-99

Guingo E, Hebert Y, Charcosset A (1998) Genetic analysis of root traits in maize. *Agronomie* 18: 225-235

Gupta PK, Balyan HS, Sharma PC and Ramesh B (1996) Microsatellites in plants: a new class of markers. *Curr. Sci.* 70: 45-54

Gupta PK, Varshney RK (2000) The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica* 113: 163-185

Harvey DM (1972) Carbon dioxide photoassimilation in normal-leaved and mutant forms of *Pinus sativum* L. *Ann. Bot.* 36: 981-991

Hashemi P, Banniza S, Davis A, Warkentin T, Vandenberg A (2003) Relationships between lodging, stem anatomy, and reaction to mycosphaerella blight in field pea. *In: Saskatchewan Pulse Growers, Pulse Days*, Saskatoon, Canada, pp 64

Heath MC, Hebblethwaite PD (1985) Agronomic problems associated with the pea crop. In: Hebblethwaite PD, Heath MC, Dawkins TCK (ed) *The Pea Crop*: a basis for improvement. Butterworth, London, pp 19-30

Holland MR (1990) Canopy collapse of dried pea crops. *Diss. Abstr. Int. –B Sci. Eng.* 50: (10) 4284B

Holland N, Holland D, Helentjaris T, Dhugga KS, Xoconostle-Cazares B, Delmer DP (2000) A comparative analysis of the plant cellulose synthase (*CesA*) gene family. *Plant Physiol.* 123: 1313-1324

Ingram TJ, Reid JB, Murfet IC, Gaskin P, Willis CL, MacMillan J (1984) Internode length in *Pisum*. The *Le* gene controls the 3β-hydroxylation of gibberellin A_{20} to gibberellin A_1. *Planta* 160: 455-463

Irzykowska L, Wolko B, Seiecicki W (2002) Interval mapping of QTLs controlling some morphological traits in pea. *Cell. Mol. Biol. Lett.* 7: (2A) 417-422

Jones C (1990) Agronomic and evapotranspiration studies in peas of differing leaf morphology. *Diss. Abstr. Int., –B Sci. Eng.* 51: (1) 5B

Kaack K, Schwarz KU (2001) Morphological and mechanical properties of Miscanthus in relation to harvesting, lodging, and growth conditions. Ind. *Crop Prod*. Amsterdam, New York: Elsevier, 14: 145-154

Karpenstein MM, Stuelpnagel R (2000) Biomass yield and nitrogen fixation of legumes monocropped and intercropped with rye and rotation effects on a subsequent maize crop. *Plant and Soil* 218: 215-232

Keller M, Karutz Ch, Schmid JE, Stamp P, Wainzeler M, Keller B, Messmer MM (1999) Quantitative trait loci for lodging resistance in a segregating wheat×spelt population. *Theor. Appl. Genet.* 98: 1171-1182

Kertikov T (1998) Possibilities for lowering the extent of lodging in winter and spring forage pea, grown for grain. *Rasteniev'dni-Nauki* 35: 536-539. Abstract from: *CAB Abstract*, AN: 990701221

Kjaer B, Haahr V, Jensen J (1991) Associations between 23 quantitative traits and 10 genetic markers in a barley cross. *Plant Breeding* 106: 261-274

Knyaz'kova SR (1987) A breeding and genetic analysis of pea forms with different types of leaf and stem. *Vestn. Sel'skokhoz. Nauk*i, Moscow, USSR 1: 115-118

Kokubo A, Kruaishi S, Sakurai N (1989) Culm strength of barley: correlation among maximum bending stress, cell wall dimensions, and cellulose content. *Plant Physiol.* 911: 876-882

Kokubo A, Sakurai N, Kuraishi S, Takeda K (1991) Culm brittleness of barley (*Hordeum vulgare* L.) mutants I caused by small number of cellulose molecules in cell wall. *Plant Physiol.* 97: 509-514

Lander ES, Green P, Abrahamson J, Barlow A, Daley M, Lincoln S, Newburg L (1987) MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1: 174-181

Larson SR, Habernicht DK, Blake TK, Adamson M (1997) Backcross gains for six-rowed grain and malt qualities with introgression of a feed barley yield QTL. *J. of the Amer. Soci. of Brewing Chem.* 55: 52-57

Lee SH, Bailey MA, Mian MAR, Shipe ER, Ashley DA, Parrott WA, Hussey RS, Boerma HR (1996) Identification of quantitative trait loci for plant height, lodging, and maturity in a soybean population segregating for growth habit. *Theor. Appl. Genet.* 92: 516-523

Lonnig WE, Saedler H (1997) Plant transposons: contributors to evolution? *Gene* 205: (1-2) 245-53

Lukashevich NP, Kukrash LV (1994) The dynamics of growth of pea stems. *Vestsi Akad. Agrar. Navuk Belarusi* 1: 71-75. Abstract from: *CAB Abstract*, AN: 970700689

Luo LJ, Zhong DB, Mei HW, Guo LB, Wang YP, Xu JL, Y SB, Ying CS, Li ZK (2000) QTLs for stem thickness and strength in rice. *Chin. Rice Res. Newslett.* 8: (4)

Makasheva RK (1984) The Pea. *Russian translation series*, Gorokh, Kolos Publishers, Leningrad

Makasheva RK, Drozd AM (1987) Determinate growth habit (*det*) in peas: isolation, symbolization and linkage. *Pisum Newslett.* 19: 31–32

Mansur LM, Orf J, Lark KG (1993) Determining the linkage of quantitative trait loci to RFLP markers using extreme phenotypes of recombinant inbreds of soybean. *Theor. Appl. Genet.* 86: 914-918

Mansur LM, Orf JH, Chase K, Jarvik T, Cregan PB, Lark KG (1996) Genetic mapping of agronomic traits using recombinant inbred lines of soybean. *Crop Sci.* 36: 1327-1336

Marx GA (1986) Linkage relations of *Curl*, *Orc* and ‘‘*Det*’’ with markers on chromosome 7. *Pisum Newslett.* 18: 45–48

Matthews P, Arthur E (1985) Genetic and environmental components of variation in protein content of peas. In: Hebblethwaite PD, Heath MC, Dawkins TCK (ed) *The Pea Crop*. Butterwroths

McEwen J, Cockbain AJ, Fletcher KE, Salt GA, Wall C, Whitehead AG, Yeoman DP (1979) The effects of aldicarb triazophes and benomyl plus zineb on the incidence of pests and pathogens and on the yields and nitrogen uptakes of leafless peas (*Pisum sativum* L.). *J. Agri. Sci.* 93: 687-692

McPhee KE, Muehlbauer FJ (1999) Stem strength in the score collection of *Pisum* germplasm. *Pisum Genet.* 31: 21-23

McKay MJ, Ross JJ, Lawrence NL, Cramp RE, Beveridge CA, Reid JB (1994) Control of internode length in *Pisum sativum*: further evidence for the involvement of indole-3-acetic acid. *Plant Physiol.* 106: 1521-1526

McVicar R, Hartley S, Brenzil C, Panchuk K, Pearse P, Warkentin T, Vandenberg B, Hogg T (2004) Dry pea in Saskatchewan, Publication of Saskatchewan Agriculture, Food and Rural Revitalization

Narvel JM, Walker DR, Rector BG, All JN, Parrott WA, Boerma HR (2001) A retrospective DNA marker assessment of the development of insect resistant soybean. *Crop Sci.* 41: 1931-1939

Nemec B (1904) Uber die Wahrnehm ung des Schwerkraftreizes bei den Pflanzen. *J.B. Wiss. Bot.* 39: 645-730. Abstract in English

Obraztsov AS, Amelin AV (1990) Ideotype of pea plants in relation to their resistance to lodging in the south of the Non-chernozem zone of the RSFSR. *Sel'skok hozyaistvennaya-Bologiy* No.1: 83-89. Abstract from: *CAB Abstract*, AN: 901696951

Pandian A, Ford R, Taylor PW (2000) Transferability of sequence tagged microsatellite site (STMS) primers across four major pulses. *Plant Mol. Bio. Reporter* 18: 395a-395h.

Park CK, Jung CS, Baek IY, Shin DC, Kwack YH, Suh HS, Lee SK, Oh YJ, Son CK, Choi JK (1998). A new high yielding, resistant to disease and lodging, good seed quality and dwarf green pea variety "Sachulwandu" (*Pisum sat*ivum L.) RDA. *J. of Crop Sci.* 39: 136-140

Pate JS (1977) The pea as a crop plant. In: Sutcliffe JF, Pate JS (ed) *The Physiology of the Garden Pea.* A subsidiary of Harcourt Barace Jovanovich, Publishers

Poole AT, Murfet IC, Vaillancourt RE (1993) Ramosus loci *rms-3* and *rms-4* are in pea linkage groups 1 and 7 respectively. *Pisum Genet.* 25: 40–42

Potts WC, Reid JB, Murfet IC (1982) Internode length in *Pisum*. I. The effect of the *Le/le* gene difference on endogenous gibberellin-like substances. *Physiologia Plant.* 55: 323–328

Pullan MR, Hebblethwaite PD (1990) The interaction between lodging and plant population in combining peas. *Ann. of Appl. Biol.* 117: 119-127

Rameau C, Denou D, Farval F, Haurogne KM Josserand J, Laucou V, Batge S, Murget IC (1998) Genetic mapping in pea. 2. Identification of RAPD and SCAR markers linked to genes affecting plant architecture. *Theor. Appl. Genet.* 97: 916-928

Reid JB, Murfet IC, Potts WC (1983) Internode length in *Pisum*. II Additional information on the relationship and action of loci *Le*, *La*, *Cry*, *Na* and *Lm*. *J. Exp. Bot.* 34: 349–364

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci.* USA 81: 8014 - 8019

Samarin NA (1975) Methodology of selecting pea for resistance to lodging. *Byull. Vses. Ordena Lenina-i-Ordena-Druzhby-Narodov-Instituta-Rastenievodstva-Imeni-N.-I.-Vavilova* 53: 51-55. Abstract from: *CAB Abstract* AN: 761646566

Samarin NA (1976) Photosynthetic productivity and yield in pea in relation to the length of the internodes and the position of the stem. *Tr. Prikl. Bot. Genet. Sel.* 56: 90-93. Abstract from: *CAB Abstract* AN: 771648965

Samarin NA, Samarina LN (1979) Anatomical characteristics of the stem in *Pisum* L. species in relation to lodging resistance. *Tr. Prikl. Bot. Genet. Sel.* 65: 52-55. Abstract from: *CAB Abstract* AN: 821612030

Samarin NA, Samarina LN (1981) Effect of lodging and stem length in pea plants on yield and quality. *Tr. Prikl. Bot. Genet. Sel.* 70: 36-39. Abstract from: *CAB Abstract* AN: 841634852

Sarria R, Wagner T, O'Neill M, Faik A, Wilkerson C, Keegstra K, Raikhel N (2001) Characterization of a family of *Arabidopsis* genes related to xyloglucan fucosyltransferase1. *Plant Physiol.* 127: 1595–1606

Saskatchewan Pulse Crop Development Board (1996) Pulse production manual, Saskatoon, SK, Canada

Saskatchewan Pulse Grower (2000) Pulse production manual, Saskatoon, SK, Canada

Sawicki J, Boros L, Wawer A (2000) The effect of plant densities on agronomic traits of three semi-leafless pea (*Pisum sativum* L.) genotypes. *Biul. Inst. Hodowli I Aklimatyzacjj Roslin* No.214: 253-261

Slinkard AE, Blain HL (1988) Production of pea, lentil, faba bean and chickpea in North America. In: Summerfield RJ (ed) *World Crops*: *Cool season food legumes*. Kluwer, Dordrecht, Netherlands, pp 1059-1063

Slinkard AE, Vandenberg A (1993) Introduction of new crops in Canada: Emerging success stories. In: *International Crop Science I.* Crop Sci. Soci. Am, Madison, WI. pp 473-477

Slinkard AE (1993) Breeding methods for stress tolerance in self-pollinated crops. In: Singh KB, Saxena MC (ed) Breeding for stress tolerance in cool season food legumes. ICARDA-Wiley-Sayce Co-publication. New York, pp 429-538

Slinkard AE (1994) Pea production in Canada. In: Canadian Peas Feed Industry Guide. The Canadian Special Crops Association and the Western Canada Pulse Growers Association. Winnipeg, MB, pp 5-9

Snoad B (1975) Alteration of plant architecture and the development of the leafless pea. Austria, International atomic energy: Induced mutations for the processing of grain legumes in South East Asia, pp 123-132

Sophie VA, Christophe S, Nathalie MJ, Bertrand N (2002) Effect of mineral nitrogen of nitrogen nutrition and biomass partitioning between the shoot and roots of pea (*Pisum sativum* L). *Plant and Soil* 242: 251-262

Specialty Crop Report (2000), Saskatchewan Agriculture and Food, pp 11-13

Specialty Crop Report (2001), Saskatchewan Agriculture and Food, pp 11-13

Specialty Crop Report (2002), Saskatchewan Agriculture and Food, pp 11-13

Specialty Crop Report (2003), Saskatchewan Agriculture and Food, pp 11-13

Stebbins GL (1974) Flowering plants: Evolution above the species level. Belknap Press of Harvard University Press, Cambridge, Massachusetts

Sumner AK, Nielsen MA, Youngs CG (1980) Production and evaluation of pea protein isolate. Presented at the 40th annual meeting of the Institute of Food Technologists, New Orleans, La, June 8-11

Sutcliffe JF, Pate JS (1977) The physiology of the garden pea. Academic Press, London, New York San Francisco, A subsidiary of Harcourt Brace Jovanovich, Publishers

Tanaka K, Murata K, Yamazaki M, Onosato K, Miyao A, Hirochike H (2003) Three distinct rice cellulose synthase catalytic subunit genes required for cellulose synthesis in the secondary wall. *Plant Physiol.* 133: 73-78

Tar'an B, Warkentin T, Somers D, Miranda D, Vandenberg A, Blade S, Woods S, Bing D, Xue A, DeKoeyer D, Penner G (2003) Identification of quantitative trait loci for plant height, lodging resistance and reaction to mycosphaerella blight in pea (*Pisum sativum* L.) using an AFLP-based linkage map. *Theor. Appl. Genet.* 107: 1482-1491

Taramino G, Tingey S (1996) Simple sequence repeats for germplasm analysis and mapping in maize. *Genome* 39: 277-287

Tautz D and Renz M (1984) Simple sequences are ubiquitous repetitive components of eukaryotic genomes. *Nucleic Acid. Res.* 12: 4127-4138

Taylor NG, Scheiblew R, Cutler S, Sommerville CR, Turner SR (1999) The irregular xylem-3 locus of *Arabidopsis* encodes a cellulose synthase required to secondary cell wall synthesis. *Plant C.* 11: 769-779

Tiwari KR (1998) Pea and Powdery Mildew: Genetics of host-pathogen interaction and identification of molecular markers for host resistance. *Ph. D. Thesis* of University of Manitoba

Tsitlenok SI, Bondar LM (1977) Characteristics of the stem structure in a compact mutant of pea. *Referativnyl Zhurnal* 7.55.88

Vasileva M, Ancheva M, Vassileva M (1980) Genetical and biochemical analysis of stem mutants of pea. *Genet. Sel.* 13: (1) 3-18

Viliani PJ, Demason DA (2000) Roles of the *Af* and *Tl* genes in pea leaf morphogenesis: shoot ontogeny and leaf development in the heterozygotes. *Ann. Bot.* 85: 123-135

Wang FB, Fu JF, Dong LF (2002) Study on tendril inheritance of semi-leafless pea and its application to pea breeding. *J. of Hebei vocation technical teachers college* 16: 6-8

Wang TF (1998) Evaluation of mycosphaerella blight resistance in pea. M.Sc. thesis of University of Saskatchewan.

Warkentin T, Tar'an B, Vandenberg A (2001a) Implementation of marker-assisted selection for lodging resistance in pea breeding. Agriculture Development Fund Project Application

Warkentin T, Vandenberg A, Banniza S, Chongo G, Tullu A, Tar'an B, Lulsdorf M (2001b) Pulse crop variety development strategies in Saskatchewan, in Pulse Days 2002, Saskatoon

Wu KS, Tanksley SD (1993) Genetic and physical mapping of telomeres and macrosatellites of rice. *Plant Mol. Biol*. 22: (5) 861-872

Xue AG, Warkentin TD (2001) Partial resistance to *Mycosphaerella pinodes* in field pea. *Can. J. Plant Sci.* 81: 535–540

Yan L, Loukoianov A, Tranquilli G, Helguera M, Gfahima T, Dubcovsky J (2002) Positional cloning of the wheat vernalization gene *VRN1*. *Proc. Natl. Acad. Sci.* 100: 6263-6268

York S, Halbeek H, Darvill A, Albersheim P (1990) The structure of xyloglucan oligosaccharide fragments from rape seed hull, tamarind gum and sycamore extracellular polysaccharides. *Carbohydr. Res.* 200: 9-31

York W, Hawkins R (2000) Preparation of oligomeric *β*–glycosides from cellulose and hemicellulosic polysaccharide in the glycosyl transferase activity of a *Trichoderma reesei* cellulose. *Glycobiology* 10: 193-201

Zischler H, Kammerbauer C, Studer R, Grzeschik KH and Epplen JT (1992) Dissecting $(CAC)_5/(GTG)_5$ multilocus fingerprints from man into individual locus-specific, hyper-variable components. *Genomics* 13: 938-990

Zohary D, Hopf M (1973) Domestication of pulses in the Old World. *Science* 182: 887-894

Appendix 1. ANOVA tables for lodging variation analyses in $F_{2:3}$ populations

Table A5.1. ANOVA table of the lodging evaluation trial for $F_{2:3}$ population 1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	27.03	6.76	14.46	<.0001
Error	43	20.09	0.47		
Corrected Total	47	47.12			

Table A5.2. ANOVA table of the lodging evaluation trial for $F_{2:3}$ population 2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	22.72	4.54	6.01	0.0002
Error	50	37.77	0.76		
Corrected Total	55	60.50			

Table A5.3. ANOVA table of the lodging evaluation trial for $F_{2:3}$ population 5

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	15.81	3.16	6.86	<.0001
Error	58	26.72	0.46		
Corrected Total	63	42.53			

Table A5.4. ANOVA table of the lodging evaluation trial for $F_{2:3}$ population 6

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	10.77	2.69	6.75	0.0002
Error	51	20.35	0.40		
Corrected Total	55	31.13			

Table A5.5. ANOVA table of the lodging evaluation trial for $F_{2:3}$ population 7

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	14.80	3.70	8.97	<.0001
Error	51	21.04	0.41		
Corrected Total	55	35.84			

Table A5.6. ANOVA table of the lodging evaluation trial for $F_{2:3}$ population 8

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	11.49	2.30	3.75	0.004
Error	82	50.23	0.61		
Corrected Total	87	61.72			

Table A5.7. ANOVA table of the lodging evaluation trial for $F_{2:3}$ population 9

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	7.69	1.92	3.26	0.020
Error	50	29.70	0.59		
Corrected Total	54	37.39			

Table A5.8. ANOVA table of the lodging evaluation trial for $F_{2:3}$ population 10

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	15.12	3.02	5.16	0.0004
Error	74	43.37	0.59		
Corrected Total	79	58.49			