

MORPHOLOGICAL, AGRONOMIC,  
AND GENETIC  
CHARACTERIZATION OF S9240  
TETRAPLOID CRESTED  
WHEATGRASS  
(*Agropyron cristatum*)

A Thesis Submitted to the College of  
Graduate Studies and Research  
in Partial Fulfilment of the Requirements  
for the Degree of Master of Science  
in the Department of Plant Sciences  
University of Saskatchewan  
Saskatoon

By

Angus Edward Mellish

Spring 2001

### Permission to Use

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence by the Head of the Department or Dean of the college in which my thesis was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other use of material in this thesis in whole or in part should be addressed to:

Head of the Department of Plant Sciences

University of Saskatchewan

Saskatoon, Saskatchewan S7N 5A8

## Abstract

Crested wheatgrasses (*Agropyron spp.*) are forage grasses of importance on the great plains of North America. S9240 crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.) was produced by using colchicine to double the chromosome number of individuals selected from the diploid, *A. cristatum* cv. Parkway, followed by several cycles of selection for height, seed size, floret fertility, and forage yield. Some introgression of tetraploid *A. cristatum* cv. Kirk germplasm occurred during the selection cycles. The goals of this study were to compare seed characteristics, emergence, height, crown width, tiller density, tiller weight and tiller angle of S9240 with those of other crested wheatgrass cultivars, both diploid and tetraploid, and to estimate the heritability of plant height and crown diameter of two S9240 populations. In addition, an analysis of the relationships among selected species and populations from the genus *Agropyron* was carried out using AFLP markers. Morphological data were collected from a number of sward-seeded and spaced-planted trials during the summers of 1999 and 2000. Emergence trials were conducted in the greenhouse. AFLP analysis was conducted on bulks and individuals from selected *Agropyron* lines to determine relationships and levels of variance within and among populations. The mean seed weight and total seed yield of S9240 were significantly ( $p < 0.05$ ) greater than those of other tested crested wheatgrass cultivars. Heavier seeds had higher emergence relative to light seeds within cultivars, but bulked S9240, which had the highest mean seed weight, did not have superior emergence levels compared to other cultivars. In general S9240 was taller, narrower crowned and had fewer, heavier, more upright tillers than other crested wheatgrass cultivars. Plant height and crown diameter were moderately to highly heritable characteristics in a

population of S9240. Even though the majority of molecular variance occurred within populations it was possible to differentiate among all tested populations using AFLP markers. Most populations were grouped as expected from previous taxonomic analyses, but some populations may require reclassification based on the results of this study.

## ACKNOWLEDGEMENT

I would first and foremost like to acknowledge the guidance and direction of my supervisor, Dr. Bruce Coulman. I feel that Bruce is truly an exceptional advisor whose honesty, patience and support must be commended.

I would also like to thank my advisory committee, Dr. Graham Scoles, Dr. Stephen Fox and Dr. Pierre Hucl, for providing an outside perspective and guidance to the project. I would also like to thank Dr. Paul Jefferson for acting as my external examiner.

I would particularly like to thank fellow graduate students Y asas Fernandez and Marcelo Araujo not just for their help in conducting my project, but also for being my friends. I would also like to thank the technical staff in the forages section at AAFC, Cheryl, Nancy, Bruce, Tim, Don and the summer staff.

On a more personal note I would like to express my deep gratitude to my fiancé, Shauna Humble, for the sacrifices of the last three years. I thank my parents, Ken and Teresa Mellish and my sister Martha Mellish whose lifetime of support and encouragement have brought me to where I am today. Daniel, Lisa, and Cerah must also be thanked as they are fine set of friends and roommates whom I am very fortunate to have known and spent time with. I also extend my thanks to all of my other friends in the department and at AAFC whom have made my time in Saskatoon a memorable one.

## Table of Contents

Permission to Use .....	i
Acknowledgement .....	iv
Table of Contents .....	v
List of Tables .....	x
List of Figures .....	xiii
1. INTRODUCTION .....	1
2. LITERATURE REVIEW .....	3
2.1 Description and distribution of crested wheatgrass .....	3
2.2 Establishment of crested wheatgrass .....	5
2.3 Tillering in a grass sward .....	9
2.3.1 Role of tillering in a grass sward .....	10
2.3.2 Conditions which stimulate tiller production .....	11
2.3.3 Tillering control .....	11
2.3.4 Relationship between tiller number and tiller size .....	13
2.4 Phylogeny of the genus <i>Agropyron</i> : .....	14
2.4.1 Taxonomical history of <i>Agropyron</i> .....	14
2.4.2 Use of molecular markers in phylogenetic studies: .....	16
2.5 Forage breeding .....	18
2.5.1 History and principles of forage breeding .....	18
2.5.2 Forage cultivar development .....	19
2.5.3 Quantitative genetics .....	21

3. SEED WEIGHT, SEED YIELD AND SEEDLING VIGOUR OF S9240 CRESTED WHEATGRASS COMPARED TO OTHER CRESTED WHEATGRASS CULTIVARS OF VARIABLE ORIGIN .....	23
3.1 Abstract .....	23
3.2 Introduction .....	24
3.3 Materials and methods .....	26
3.3.1 Plant material .....	26
3.3.2 Data collected .....	28
3.3.3 Statistical analysis .....	29
3.4 Results and discussion .....	30
3.4.1 Seed weight .....	30
3.4.2 Seed yield .....	33
3.4.3 Bulk density .....	34
3.4.4 Emergence and seedling weight .....	35
3.5 Conclusions .....	38
4. PLANT HEIGHT, CROWN DIAMETER AND TILLER CHARACTERISTICS OF S9240 CRESTED WHEATGRASS COMPARED TO OTHER CRESTED WHEATGRASS CULTIVARS OF VARIED ORIGIN .....	39
4.1 Abstract .....	39
4.2 Introduction .....	40
4.3 Materials and methods .....	43
4.3.1 Experimental design .....	43
4.3.2 Plant material .....	43

4.3.3 Data collected .....	43
4.3.4 Statistical analysis .....	45
4.4 Results .....	46
4.4.1 Row width .....	46
4.4.2 Height .....	49
4.4.3 Tiller weights .....	52
4.4.4 Tiller density .....	54
4.4.5 Tiller angle .....	55
4.5 Discussion .....	56
4.6 Conclusions .....	58
5. HERITABILITY OF CROWN DIAMETER AND PLANT HEIGHT IN S9240	
CRESTED WHEATGRASS .....	59
5.1 Abstract .....	59
5.2 Introduction .....	60
5.3 Materials and methods .....	62
5.3.1 Experimental design and plant material .....	62
5.3.2 Data collection .....	63
5.3.3 Statistical analysis .....	63
5.4 Results and discussion .....	64
5.4.1 ANOVA results .....	64
5.4.2 Heritability estimates .....	68
5.5 Conclusions .....	71

6. THE GENETIC RELATIONSHIP OF SELECTED CRESTED WHEATGRASS ( <i>AGROPYRON</i> SPP) LINES AND SPECIES BASED ON AFLP MARKERS	72
6.1 Abstract	72
6.2 Introduction	74
6.3 Materials and methods	77
6.3.1 Plant material	77
6.3.2 Tissue Collection and DNA Extraction	79
6.3.3 AFLP analysis	79
6.3.4 Analysis	80
6.4 Results and discussion	81
6.4.1 Line bulks	81
6.4.2 AMOVA analysis	85
6.4.3 Comparison of AMOVA and bulk clustering	88
6.5 Conclusions	90
7. GENERAL DISCUSSION	91
7.1 Relationship between tillering and seed production	91
7.2 Further selection in S9240	93
7.4 Similarity of S9240 to parental populations	94
8. CONCLUSIONS	96
9. REFERENCES	98

## List of Tables

Table	Page
3.1: Description of plant material included in the emergence and seed size study . . . . .	27
3.2: Minimum, maximum and mean weights and distribution in five classes of seed weight of five crested wheatgrass cultivars harvested from the sward-seeded stand 1994A . . . . .	31
3.3: Minimum, maximum and mean weights and distribution in six classes of seed weight of six lines of crested wheatgrass harvested from a spaced-planted nursery . . . . .	31
3.4: Seed yield, from crested wheatgrass cultivars and lines, in a sward-seeded stand (1994A) and spaced-planted nursery (1996A) in 1999 . . . . .	34
3.5: Average seed bulk density, of crested wheatgrass lines and cultivars, from a sward-seeded stand (1994A) and spaced-planted nursery (1996A) in 1999 . . . . .	35
3.6: Percent emergence 28 days after seeding of crested wheatgrass seeds of two weight ranges (5.0 - 5.9 mg and 3.0 - 3.9 mg) . . . . .	36
3.7: Mean seedling weight 28 days after seeding of crested wheatgrass seeds of two weight ranges (5.0 - 5.9 mg and 3.0 - 3.9 mg) . . . . .	36
3.8: Mean seed weight, percent emergence and mean seedling weight after 28 days of four crested wheatgrass populations . . . . .	38
4.1: Description of plant material included in the plant morphology study . . . . .	44
4.2: Spring and summer row widths in three sward-seeded trials of crested wheatgrass in 1999 and 2000 . . . . .	47
4.3: Crown diameter of four S9240 crested wheatgrass populations from a spaced-planted	

nursery of crested wheatgrass in 1998, 1999 and 2000 .....	48
4.4: Estimate of the difference in crown width between four S9240 crested wheatgrass populations and CD-II and Kirk based on data taken from a spaced-planted nursery in 1998, 1999 and 2000 .....	48
4.5: Plant height of crested wheatgrass in three sward-seeded trials in 1999 and 2000 .....	50
4.6: Plant height of four S9240 crested wheatgrass populations from a space planted nursery of crested wheatgrass in 1998, 1999 and 2000 .....	51
4.7: Estimate of the difference in plant height between four S9240 populations and CD-II and Kirk based on data taken from a spaced-planted nursery in 1998, 1999 and 2000 .....	52
4.8: Average tiller weights of crested wheatgrass lines grown in a solid seeded stand (1994A) and spaced-planted nursery (1996A) in 1999 and 2000 .....	53
4.9: Estimate of the difference in mean tiller weight between four S9240 crested wheatgrass populations and CD-II and Kirk based on data taken from a spaced-planted nursery in 1998, 1999 and 2000 .....	53
4.10: Tiller density in three sward-seeded trials of crested wheatgrass in 1999 and 2000 .....	54
4.11: Interior and perimeter tiller angle (from horizontal) of S9240 crested wheatgrass grown in a spaced-planted nursery in 1999 and 2000. ....	55
4.12: Estimate of the difference in mean tiller angle between four S9240 crested wheatgrass populations and CD-II and Kirk based on data taken from a spaced-planted nursery in 1998, 1999 and 2000 .....	55

5.1: Results of analysis of variance; expected mean squares, mean squares, and significance levels for 1999 and 2000 data from trial 1996B .....	65
5.2: Results of analysis of variance; expected mean squares, mean squares, and significance levels for 1999 and 2000 data from trial 1998 .....	67
5.3: Narrow-sense heritability estimates ( $h^2$ ) of spring and summer crown diameter and plant height based on two years data from trial 1998 .....	70
6.1: Plant material included in bulk and individual AFLP analysis .....	78
6.2: Analysis of molecular variance (AMOVA) from 90 crested wheatgrass individuals from six different lines, represented by 15 individuals per line, based on 67 AFLP markers .....	86
6.3: Summary of pairwise comparisons (pairwise genetic distance - $\Phi_{st}$ ) of six crested wheatgrass lines, represented by 15 individuals per line. All distances are highly significant ( $P < 0.01$ ) .....	86
6.5: Within line sum of squares for six crested wheatgrass lines, with 15 individuals per line .....	88

## List of Figures

Figure	Page
4.1: Monthly precipitation at Kernan Farm, Saskatoon, SK. ....	47
6.1: Dendrogram of twelve populations from the genus <i>Agropyron</i> based on Euclidian distances for 114 AFLPs .....	83
6.2 Cluster analysis of level of inter population distance ( $\Phi_{st}$ ) between six <i>Agropyron</i> populations, based on 15 individuals per population, calculated using AMOVA .....	89
6.3: A dendrogram of six <i>Agropyron</i> populations, represented by 16 bulked individuals, based on Euclidian distances from 114 AFLP markers .....	89

## 1. INTRODUCTION

The crested wheatgrass complex (*Agropyron spp.*) consists of persistent, drought resistant tussock grasses which originate from the steppes of central Asia (Rogler and Lorenz, 1983). Crested wheatgrass was first cultivated in the Volga region of Russia (Asay and Jensen 1996) and was first evaluated and increased in North America in 1906 (Rogler and Lorenz 1983). It has subsequently become an important forage grass in the central and northern great plains and inter-mountain regions of North America, which receive between 200 and 450 mm of annual precipitation (Mayland et al. 1992).

Three *Agropyron* species are cultivated in North America; 1) fairway crested wheatgrass (*Agropyron cristatum* (L.) Gaertner); 2) standard crested wheatgrass (*Agropyron desertorum* (Fisch. ex Link) Schult); and 3) Siberian crested wheatgrass (*Agropyron fragile* (Roth) P. Candargy). Smoliak and Dormar (1985) estimated that there were approximately 1 million ha of *A. cristatum* in use as rangeland in western Canada while Mayland et al. (1992) estimated that there were over 6 million ha of *Agropyron spp.* in production in North America.

The *Agropyron* genus consists of a polyploid series with a base chromosome number of seven. *A. cristatum* ( $2n=2x=14$ ) and *A. mongolicum* Keng. are the only two diploid species that are present in the genus. Other species within the complex are predominantly tetraploid ( $2n=4x=28$ ), but there are also some hexaploid ( $2n=6x=42$ ) populations. There has been extensive debate as to the origin of the tetraploid members,

in particular *A. desertorum*, with both autopolyploidy and allopolyploidy having been suggested to have played roles.

In 1978, a population of selected colchicine induced autotetraploid *A. cristatum*, which was based on individuals selected from the diploid *A. cristatum* cv. Parkway, was formed and named S9075. This population was established in the field and evaluated until the early 1980s, when a series of selections were made out of S9075 and another experimental population (S7137) related to the natural tetraploid *A. cristatum* cv. Kirk. Crosses between the populations were made in the field and in the greenhouse between 1983 and 1985. The population which was formed from these selections was subsequently named S9240. Since the mid 1980's, S9240 has been through several cycles of selection with the primary traits of interest being height, seed size, floret fertility and forage yield. Observations on the field performance of S9240 indicate that it is taller than other crested wheatgrass cultivars, and may have narrower crowns.

The objectives of this study were; 1) To compare the seed weight of S9240 to that of other crested wheatgrass cultivars and determine the effect of seed weight on seedling emergence and vigour, 2) To compare the tiller height, crown diameter and other morphological traits of S9240 to those of other crested wheatgrass cultivars and examine the relationship between these traits, 3) Estimate the heritability of crown width and tiller height in a S9240 crested wheatgrass population and, 4) Examine the relationship among, and diversity within, selected *Agropyron spp.* using AFLP markers.

## 2. LITERATURE REVIEW

### 2.1 Description and distribution of crested wheatgrass

The genus *Agropyron* consists of 10 to 15 species referred to as the crested wheatgrass complex (Asay and Jensen 1996). The genus consists of persistent, drought resistant tussock grasses which originate from the steppes of central Asia (Rogler and Lorenz 1983). The members of the genus are identified by their solitary, imbricate and usually pectinate spikelets (Barkworth and Dewey 1985). The complex contains a polyploid series of diploid ( $2n = 2x = 14$ ), tetraploid ( $2n = 4x = 28$ ) and hexaploid ( $2n = 6x = 42$ ) species with the type species being *A. cristatum* (Asay and Jensen 1996).

*A. cristatum* is characterized by Looman and Best (1987) as a densely tufted plant with culms 30 to 50 cm tall. The flat spikes are 2-7 cm in length with 3-5 flowered densely crowded spikelets, which are 8-15 mm long. *A. cristatum* and *A. desertorum* can be differentiated by inflorescence, with *A. cristatum* having a wider ( $>1.5$ cm), pectinate spike (Barkworth and Dewey 1985). *A. desertorum* also tends to be more upright in growth habit than *A. cristatum*. *A. fragile* can be differentiated by its long, awnless spikes (Asay 1992).

Crested wheatgrass was first cultivated in the Volga region of Russia (Asay and Jensen 1996). It was first introduced to North America in 1899 from Russia as a result of a plant exploration trip by N.E. Hansen of the United States Department of Agriculture, but no increase of this seed was performed (Rogler and Lorenz 1983). The second

introductions were received from the Moscow Botanical Gardens, labelled as *A. cristatum* and *A. desertorum* and were increased by Dillman in 1906. It was at this point that the potential of crested wheatgrass was recognized. In 1910, due to an editorial misprint, Dillman's report referred to both species as *A. cristatum*; after this point all crested wheatgrass were referred to as *A. cristatum* until the 1950's when the separation of the two species was suggested.

The first improved population was the diploid ( $2n = 14$ ) *A. cristatum* cv. Fairway, which was released by L. E. Kirk at Saskatoon, SK, in 1932 (Elliot and Bolton 1970). Subsequently, a number of tetraploid ( $2n = 28$ ) cultivars, such as *A. fragile* cv P-27, *A. desertorum* cv. Nordan, and *A. cristatum* cv. Kirk, were released in 1953, 1953 and 1987, respectively. In 1995, the hexaploid ( $2n = 42$ ) *A. cristatum* cv Douglas was released (Asay et al. 1995b). Breeding in crested wheatgrass has traditionally been focussed within ploidy levels (Asay 1992). In the recent past USDA-ARS has placed significant resources into inter-ploidy breeding and released two cultivars, cv. Hycrest and cv. CD-II, in 1987 and 1997, respectively. These were derived from crosses between colchicine doubled *A. cristatum* and the natural tetraploid *A. desertorum* (Asay et al. 1986).

Crested wheatgrass played an important role in stabilizing large sections of depleted rangeland which were being eroded by wind during the "dustbowl" period of the 1930's (Rumbaugh et al. 1982). Crested wheatgrass is important as a forage species today due to its hardiness under adverse conditions, its ability to withstand heavy grazing and its early spring forage production. This makes it quite compatible in a rotational grazing system with native grasses that should not be grazed early in the spring. Despite these valuable contributions, crested wheatgrass has developed a poor reputation from an

ecological perspective (Asay and Jensen, 1996), due mainly to the fact that large areas were seeded to monoculture. These species have the ability to persist in such a stand, if correctly managed, for decades, to the detriment of a more diverse ecosystem.

Smoliak and Dormar (1985) estimated that there were approximately 1 million ha of *A. cristatum* in use as rangeland in western Canada. Mayland et al. (1992) estimated that there were over 6 million ha of *Agropyron spp.* in production in North America. The fairway type is predominant in Canada while the standard type is more commonly grown in the United States.

## **2.2 Establishment of crested wheatgrass**

To ensure successful establishment, crested wheatgrass should to be planted at a rate of about 100 seeds per linear metre, as seedling loss can be as high as 80% (Anonymous 1998). This low survival rate is due to factors such as failure of the seedling to emerge, either due to improper seed placement or soil crusting; seedling death after emergence due to dry conditions (Rogler 1954); or damage to the primary leaf during emergence (Hyder et al. 1971). Environmental factors which can affect the successful establishment of a forage stand include weed competition, and soil moisture and temperature (Berhdal and Barker 1984).

Management practices, especially proper preparation of the seed bed, can aid seedling establishment, through proper seed placement and improved seed to soil contact (Anonymous 1998). It is recommended that crested wheatgrass be planted no more than 2 cm deep, as seeding beyond this depth reduces emergence and/or increases the time to emergence. Planting at greater depths decreases moisture stress and temperature fluctuations (Berdahl and Ries 1996); Rogler (1954) however, stated that crested

wheatgrass does not have adequate vigour to emerge from field planting depths of greater than 1 to 1.5 inches (2.5 to 3.75 cm).

The primary factor affecting the depth from which a seedling can emerge is the length of the coleoptile and the subcoleoptile internode (mesocotyl) (Newman and Moser 1988). Hoshikawa (1969) studied the underground organs of 218 members of the family *Gramineae* (*Poaceae*). He observed that in some genera of the *Triticeae* tribe (*Agropyron*, *Triticum*, *Aegilops*, *Secale* and *Hordeum*) there was very little or no elongation of the subcoleoptile internode. In this case emergence of the seedling is dependent on the elongation of the first and second internode and the extension of the coleoptile. Hyder et al. (1971) found that if the coleoptile did not reach the soil surface, emergence was greatly reduced due to the first true leaf not being strong enough to push through to the soil surface. When true leaves encounter resistance, the meristematic tissue kinks and results in the formation of loop structures. Andrews et al. (1997) also found that coleoptile width was positively correlated with plant emergence, as the increased width imparts a greater strength to the coleoptile.

Hunt and Miller (1965) studied the effect of seed size on coleoptile length in intermediate wheatgrass (*Thinopyrum intermedium*). They found that seeds from clones with a high mean seed weight had longer coleoptiles. They also found when seeds from individual clones were sorted into “large and small separates”, that coleoptiles from the “large separates” were longer. They felt that the relationship between the two traits was not linear though, as one clone which had very large seeds had short coleoptiles.

Berdahl and Barker (1984) examined the effects of seed size on seedling vigour in Russian wildrye (*Psathyrostachys juncea* (Fischer) Nevski), and found that once seed

weight surpassed 3.0 mg, increases in emergence were not linear. They found that increases in seed weight did not have a large impact on coleoptile length.

Peterson et al. (1989) sorted winter wheat (*Triticum aestivum* L.) seeds into three weight ranges and removed one-half and three-fourths of the endosperm to contrast the effect of embryo and endosperm size. They found that removal of the endosperm had a much larger effect on emergence than increased embryo size in the larger seeds. Hunt and Miller (1965) found there was a strong maternal influence on the length of the coleoptile in intermediate wheatgrass, also indicating that the endosperm has a large role to play. The role of the endosperm is critical as it provides the energy for early seedling growth.

In a study of the effect of genotype and seed size on the emergence of spring wheat, Lafond and Baker (1986), stated that seed size had an important effect on emergence, development and shoot weight of wheat seedlings. They also reported finding no seed size x cultivar interaction, indicating that the effect of seed size was independent of cultivar.

In a study which examined the interaction of seed size and water stress on emergence of wheat seedlings, Mian and Nafziger (1994), found that the size differential between seedlings from large and small seeds was greatest when under water stress. They attributed this to the production of a larger root system by large seeds.

Rogler (1954) studied the effects of seed size on the depth of emergence of *A. desertorum* from various depths of sowing. He found that there were significant differences in emergence rates between different weight classes at differing depths. The heavier seeded lots had much better emergence from greater depths. He also found that there was no correlation between seed size and emergence until a planting depth of 5 cm

(2 inches) was reached. He concluded that plants from large seeds would have a greater chance of establishing when planted at 2.5 cm (1 inch). Andrews et al. (1997) conducted a similar study on timothy (*Phleum pratense* L.), and found that mean seed weight was positively correlated with percent emergence. They attributed the increased emergence of the larger seeded timothy lines to the increased mesocotyl and coleoptile width which they felt imparted greater strength and as a result improved the cotyledon's ability to penetrate the soil substrate.

Knowles (unpublished data) found no difference in the emergence of crested wheatgrass lines which exhibited varying seed weights. He observed 100% emergence for all lines even at planting depths as great as 2 inches in the greenhouse. He felt that this was due to the soil mixture being too porous to prevent the smaller seeded lines from emerging.

There have been efforts in several cool-season forage grasses, such as crested wheatgrass (Rogler 1954; and Schaaf and Rogler 1962), smooth brome (*Bromus inermis* Leyss) (Trupp and Carlson 1971; and Jessen and Carlson 1985), and Russian wildrye (Asay and Johnson 1980; Berdahl and Barker 1984; Jefferson 1993) to increase mean seed weight with the aim of improving the ease of stand establishment. This has been a particularly important objective in Russian wildrye breeding programs as the species is difficult to establish due to poor seedling vigour (Berdahl and Reis 1996). Lawrence (1963) examined the relationship between seed size and seedling vigour and concluded that seedling emergence from 3.8 cm in the greenhouse was the best indicator of field emergence. Berdahl and Barker (1984) estimated heritabilities of 37 to 85% for seed weight and 52 to 85 % for coleoptile length from 30 open pollinated lines. They

found little increase in coleoptile length with increasing seed weight and felt that discarding low seed weight lines would be more productive than selecting high seed weight lines. Induced autotetraploids were also used in Russian wildrye breeding programs as a method of improving seedling vigour (Lawrence et al. 1990). The cultivar Tetracan, released in 1990, was selected from a population of colchicine induced tetraploids and had seed weight and emergence which were 40% and 14% greater, respectively, than the diploid cultivar Swift. Relative to the increase in seed weight, the small increase in emergence may be due to the low correlation between seed size and coleoptile length in Russian wildrye (Berdahl and Barker 1984).

### **2.3 Tillering in a grass sward**

Grass plants are made up of a group of genetically identical tillers, which are in turn made up of a series of phytomers. A phytomer consists of a leaf blade, leaf sheath, internode, node and an axillary bud (Moore and Moser 1995). The axillary bud is a rudimentary apical meristem with the ability to develop into a distinct phytomer given the correct conditions.

Perennial grasses can be grouped into three “architectural types”: 1) rhizomatous grasses, such as smooth brome grass, which have a creeping stem system below the surface allowing them to spread rapidly; 2) stoloniferous grasses, such as Bermuda grass, which spread by producing modified stems which grow horizontally above the soil surface and produce secondary structures from axillary buds at the nodes; and 3) caespitose, or tussock forming grasses which include most members of the genus *Agropyron* (Asay and Jensen 1996). Tussock grasses are characterized by dense formations of erect culms with successive generations of tillers arising from parental

tillers (Welker and Briske 1991).

Olson and Richards (1988a) suggested that, in an undisturbed plant community, there is a fixed tiller carrying capacity such that tillering occurs at a maintenance rate, ie. one surviving daughter tiller produced by an existing tiller each year. In early spring, leaves are produced and a culm begins to form in May. Essentially all tiller elongation has occurred by early June, when flowering occurs on reproductive tillers. By late July, tillers have senesced and seeds are mostly mature after which fall precipitation stimulates tiller induction for the following year. This results in a tiller life span of about 12 months. Tillers which are produced during the growing season often do not overwinter but, can produce daughter tillers in the fall which do overwinter (Olsen and Richards 1988a).

### **2.3.1 Role of tillering in a grass sward**

The ability to tiller and continually renew photosynthetic area is essential for establishment, perennation and maintenance of a grass plant's position within a competitive sward (Murphy and Briske 1992). Tillering allows a young grass plant to expand rapidly during establishment (Jewiss 1972). Rapid expansion is important as it produces photosynthetic area and improves the plant's competitiveness in its environment. If there is a fixed tiller carrying capacity, as suggested by Olson and Richards (1988a), the individual which produces the largest number of tillers during establishment may be able to maintain a superior position in the community.

When patch disturbance of the sward occurs, due to death, removal or grazing, it provides the opportunity for less competitive and less valuable species to colonize the open area, so rapid vegetative compensation by grasses into these spaces is important in stand maintenance (Marriot et al. 1997). This compensation can occur either by

establishment of new seedlings or by increased tiller production into the vacant area. In mature stands, establishment of new seedlings is rare, so compensation usually occurs in the form of increased tiller production (Olson and Richards 1989). Whenever the ratio of daughter tillers to parental tillers exceeds one, a tussock will expand. This expansion can take advantage of under utilized resources, due to an open area or a less competitive neighbour.

### **2.3.2 Conditions which stimulate tiller production**

Environmental conditions play a strong role in the regulation of tiller production. Resources such as water, nutrients, and light all influence the production of new tillers. In a mature grass stand, where plant mortality is rare, reaction to changing levels of resources is reflected in tiller growth and replacement. Olson and Richards (1989) conducted a study on the effect of fertilization, tiller thinning and neighbour removal in a competitive stand, on tiller initiation in crested wheatgrass. It was found that neighbour removal had the greatest effect on tiller initiation and was likely due to increased nutrient and water availability. The application of fertilizer had a lesser effect on long term tillering in that the nitrogen applied was rapidly used or immobilized within the system. In addition neighbouring plants were also fertilized, resulting in a similar increase in competitive ability to both plants.

Grazing and mechanical harvesting both stimulate the production of axillary tillers. Olson and Richards (1988b) found that axillary tiller development was stimulated most by grazing when it occurred during culm elongation.

### **2.3.3 Tillering control**

Conditions such as water and nutrient availability (McIntyre 1997) have been

shown to have a strong influence on the production of tillers, but McIntyre (1987;1997) felt that they also play a direct role in the control of tiller production. McIntyre (1987) postulated that competition for water by the apical meristem, which is a major sink for water and nutrients, was responsible for the lack of development in the axillary buds. He further expanded the postulate (McIntyre 1997) by including the partitioning of nitrogen to the apical meristem as another factor which limited axillary bud development. Thus, the apical meristem is a sink for both water and nitrogen at the expense of other meristems within the plant. Axillary bud development would be initiated by an increase in nitrogen or water supply to the axillary meristems by such factors as, removal of the apical meristem (grazing or mechanical harvest), an external increase of water or nitrogen (fertilization or rain), or tiller senescence.

Mueller and Richards (1986) looked for anatomical characteristics which differed between *Pseudoroegneria spicata* (Pursh) A. Love, a species which does not tiller after grazing, and *A. desertorum*, which does recover after grazing. They looked at characteristics such as tiller morphology, number and size of axillary buds, apical meristem structure and vascular development to axillary buds. They found no connection between any of these traits and the rate of tillering.

Light quality is also known to modify tiller production in grasses (Casal et al. 1985). The phytochrome system, which is probably the best known and most studied photoreceptor, is sensitive to the ratio of red (660 nm) to far-red (720 nm) light. When light penetrates a canopy, or is reflected off neighbouring plants, the ratio of red to far-red light (r:fr) is altered, increasing far-red. As a result, the r:fr ratio is a signal of competition between plants and tillers. Frank and Hofmann (1994) stated that since

*Agropyron spp.* are shade avoiders, they have a very sensitive phytochrome system and they will produce fewer, taller tillers when exposed to low r:fr ratios.

Increases in ploidy also have an effect on tillering. Tai and Dewey (1966) found that colchicine doubled *A. cristatum* had fewer and larger stems than diploids. Jefferson (1993) found tetraploid Russian wildrye had a smaller number of larger tillers when compared to diploid lines. Similar results were observed when diploid orchard grass (*Dactylis glomerata* L.) was compared to tetraploid lines (Bretagnolle and Lumaret 1995). Sicher et al. (1984) found that induced tetraploid barley lines produced fewer, heavier tillers. They attributed this to a genetically imposed slower development rate which in turn they felt was due to a shift in the metabolic balance between source and sink organs.

#### **2.3.4 Relationship between tiller number and tiller size**

During vegetative growth, plants can partition assimilates to the formation of daughter tillers, to enlargement of existing tillers, to enhancement of root growth or to storage. Sugiyama (1995) found that tall fescue plants (*Festuca arundinacea* Schreb.) could be separated into clones with a greater number of smaller tillers or with fewer, larger tillers, with greater carbon reserves. Zarrouh and Nelson (1980) found that a tall fescue population, which had been selected for high individual tiller yield, had fewer tillers per plant, greater yield per tiller, root weight, culm base weight, and water soluble carbohydrate concentration. This population was better able to restore concentration of soluble carbohydrates after harvest.

Conclusions on the agronomic merits of large tillered populations have been mixed. Casler and Hovin (1985) found that tiller number in reed canarygrass (*Phalaris*

*arundinacea* L.) was positively correlated to yield, but tiller size had a negative or no correlation to yield. However, that study was conducted in a spaced-planted situation, and the situation in a sward-seeded stand may be different (Zarroug et al. 1983a). Zarroug and Nelson (1980) concluded that tall fescue populations with high individual tiller yield had higher total yield and were superior to low individual tiller yield populations in systems where long intervals (more than six weeks) were left between harvests. They felt this was due to the population with high tiller yield using greater amounts of water soluble carbohydrates to re-establish leaf area after defoliation while the population with low tiller yield relied more on photosynthetic energy from remaining basal leaves. The use of stored energy resulted in a quicker re-establishment of photosynthetic area but left the plant vulnerable to frequent defoliation where there is not adequate time to replenish depleted energy reserves.

## **2.4 Phylogeny of the genus *Agropyron*:**

### **2.4.1 Taxonomical history of *Agropyron***

The taxonomy of *Agropyron spp.* is described as complex and sometimes as confusing (Dewey 1969), and has been subject to “a multitude of taxonomic binomials” (Dewey and Pendse 1967). This is mostly due to a continuum of spike morphology, which is the main morphological feature used to differentiate species in this genus. Spike morphology varies continuously, from broad, pubescent, pectinate spikes to narrow, linear, glabrous spikes (Dewey and Asay 1982). Taxonomy is further confounded by geographical overlap and a high level of cross-compatibility between the species.

The relationship between *A. cristatum* and *A. desertorum* has been subject to extensive discussion. Knowles (1955) felt that *A. desertorum* was either an autopoloid of

*A. cristatum*, or an amphiploid of *A. cristatum* and some other closely related diploid. A number of papers written in the 1960's (Dewey 1961, Tai and Dewey 1966, Dewey and Pendse 1967, Dewey and Pendse 1969 and Dewey 1969) concluded, based on meiotic chromosome pairing, that *A. desertorum* was an autotetraploid of *A. cristatum*, or a segmental allotetraploid. Schulz-Schaeffer et al. (1963) felt that Dewey's (1961) interpretation of polyhaploid chromosome pairing was incorrect and that *A. desertorum* is a segmental allotetraploid, and not an autotetraploid. Dewey and Pendse (1967) stated that all crested wheatgrasses had the same basic genome which they called the "C" genome, but this was subsequently changed to the "P" genome (Dewey 1984).

Taylor and McCoy (1973), using chromatographic and karyotypic analysis, concluded that *A. desertorum* had formed through a hybridization of *Agropyron imbricatum* (M. B.) Roem et Schult. and *Agropyron pectiniforme* Roem et Schult, followed by a chromosome doubling. *A. imbricatum* and *A. pectiniforme* are very similar morphologically to each other and to *A. cristatum* (differentiated by level of pubescence on, and spacing between the spikelets (Nevski 1934)) and both have been reclassified as subspecies of *A. cristatum* (Tzvelev 1976).

Dewey (1981) reported on the existence of a second diploid species, *Agropyron mongolicum* Keng., which originated from China. *A. cristatum* has broad pectinate spikes while *A. mongolicum* has narrow linear spikes. All other diploid accessions had broad spikes, which is characteristic of *A. cristatum*. Hsiao et al. (1986) reported that *A. cristatum* and *A. mongolicum* have similar genome length and differ only by small structural rearrangements on some chromosomes. Hsiao et al. (1989) noted that interspecific hybrids of *A. cristatum* and *A. mongolicum* had a similar spike morphology

to *A. desertorum*. Asay et al. (1992) concluded, through a multivariate morphological analysis, that *A. desertorum* was the result of a hybrid between *A. cristatum* and *A. mongolicum*, followed by a chromosome doubling. It was also postulated that *A. fragile* was an autopoloid of *A. mongolicum*.

Recently Vogel et al. (1999) conducted a study of genome length in the perennial Triticeae using flow cytometry. They concluded that the genome length of *A. desertorum* was more consistent with an autopoloid of *A. cristatum* than that of an allotetraploid between *A. cristatum* and *A. mongolicum*.

To further complicate the matter there are variations in ploidy levels within species, and interspecific hybrids are common. The cultivars Kirk (tetraploid) and Douglas (hexaploid) are both described as *A. cristatum*. Hybrids between ploidy levels have been used for genetic improvement of crested wheatgrass (Asay et al. 1986). The cultivar Hycrest was developed from crossing colchicine doubled *A. cristatum* with *A. desertorum*, with successive generations backcrossed to both parental lines (Asay et al. 1985).

#### **2.4.2 Use of molecular markers in phylogenetic studies:**

Schut et al. (1997) stated that knowledge of relationships between genotypes can come from three general areas; geographic origin, pedigree, and plant characteristics. Geographical information can be useful if no other types of information are available. Pedigree data is useful but is often incomplete or nonexistent in the case of open pollinated forages (Roland-Ruiz et al. 2000). There are several methods of determining phylogenetic relationships based on agronomic characters, morphology, cytogenetic studies, isozymes, and molecular (or DNA) markers (Paul et al. 1997). Molecular

markers are a good method for determining genetic relationships as they are not affected by the environmental or epistatic interactions that may affect morphological traits (Schut et al. 1997).

There are two general types of molecular marker techniques, ones which involve hybridization of probes to homologous DNA sequences such as RFLP (restriction fragment length polymorphism) and others which involve exponential amplification of genome segments between priming sites such as RAPD (random amplified polymorphic DNA) (Barrett and Kidwell 1998). The RFLP technique requires large amounts of pure DNA and are relatively labourious and costly to perform on large number of samples (Paul et al. 1997). RAPDs on the other hand are relatively easy and quick to perform, however there is a certain lack of robustness to the results (Barrett and Kidwell 1998).

Amplified fragment length polymorphism (AFLP) is a polymerase chain reaction based fingerprinting technique which is based on the selective amplification of restriction fragments (Vos et al. 1995). It has the ability to detect large numbers of polymorphisms with single primer pairs, without any prior knowledge of the target genome. Results are highly repeatable as there are very stringent reaction conditions for primer annealing. The cost of AFLP analysis is higher than that associated with RAPDs, but there are a larger number of polymorphisms detected with AFLP (Yee et al. 1999).

Sharma et al. (1996) compared the use of AFLPs and RAPDs in examining the phylogeny of *Lens spp.* They found that the results obtained from AFLPs were similar to those for RAPDs but were of much higher resolution. AFLPs have been used to determine diversity within species such as *Vigna anularis* (Willd.) (Yee et al. 1999) and *Camellia sinensis* (L.) O. Kuntze (Paul et al. 1997).

## 2.5 Forage breeding

### 2.5.1 History and principles of forage breeding

Humans have been indirectly selecting for desirable traits in all cultivated crops, including forages, for thousands of years. Formal breeding efforts in forages started as early as the late 19<sup>th</sup> century; however, serious improvements were not made until the 1950's (Vogel and Sleper 1994). Characteristics that have been subject to selection through breeding programs include yield, (both seed and forage), nutritional value, maturity, stress tolerance, pest resistance, range of adaptation, and compatibility with companion crops.

Of the forage grasses in the perennial *Triticeae*, *Agropyron* spp. have received the most attention from plant breeders (Asay 1992). Crested wheatgrass populations have been selected for forage yield, seed yield, forage quality (digestibility, grass tetany potential, and protein concentration), and stand establishment.

Breeding of forage crops is based on the same principles as other crops, but there are certain additional challenges. Most perennial forage grasses are cross-pollinated, highly self sterile, with some notable exceptions such as Kentucky blue grass (*Poa pratensis* L) which is apomictic, or slender wheatgrass (*Elymus trachycaulus* (Link) Gould ex Shinn.) which is self-fertile (Vogel and Pedersen 1993). The high level of self-incompatibility and the associated lack of vigour in inbred plants makes their maintenance as inbred lines unfeasible; however it does facilitate crossing and emasculation is often unnecessary. Polyploidy is also commonly found in many forage species complicating the inheritance of traits. As a result, breeding has focussed on the production of improved heterogeneous populations (Nguyen and Sleper 1983b).

### 2.5.2 Forage cultivar development

In situations where no previous improvement has been applied on a particular species, the initial cultivar is often developed through eco-type selection (Casler et al. 1996). This is conducted by selecting individuals from the wild within the target region for the cultivar, or in areas where the environment is similar to the one where the cultivar will be released (Vogel and Pedersen 1993). These ecotypes are then evaluated and selections are made, further evaluated and an initial cultivar is released.

Forage improvement programs are most commonly based on recurrent selection using mass and phenotypic selection methods (Casler et al. 1996). Mass selection involves collecting open pollinated seed from superior clones, selected based on phenotypic performance, and bulking the seed for production of the following generation (Poehlman and Sleper 1995). Phenotypic selection is differentiated from mass selection in that superior clones are inter-mated in isolation, increasing the level of pollen control (Casler et al. 1996).

Genotypic selection involves choosing individuals on the basis of the performance of their progenies. Genotypic selection is advantageous over phenotypic selection in cases where traits of low heritability are being selected for the general combining ability is evaluated (Casler et al. 1996). Genotypic selection generally requires a longer breeding cycle as it requires the evaluation of progeny.

There are three types of families which are generated for progeny evaluations in forages: 1) selfed (or  $S_1$ ); 2) full-sib; and 3) half-sib.  $S_1$  families are rarely used in forage breeding due to the high level of self-incompatibility and severe inbreeding depression observed in most forage species. Full-sib families are used to some extent, but are limited

due to the increased labour requirement compared to half-sib matings. Evaluations of  $S_1$  and full-sib families can also result in inflated estimates of heritability due to the influence of dominance genetic variance (Ray et al. 1994). The most common type of progeny tests are half-sib families. Half-sibs are advantageous over other crosses, as they allow for a larger number of parents to be tested, require less labour, produce an adequate quantity of seed and information regarding additive genetic variance can be obtained from them (Nguyen and Sleper 1983b). There are three common mating designs used to produce half-sib families: 1) open pollination; 2) polycross; and 3) topcross. Open pollination, involves growing clones in close proximity to each other and often allowing both selected and unselected clones to inter-pollinate (Poehlman and Sleper 1995). A polycross involves replicating and randomizing selected clones in such a manner that random pollination is more likely to occur. Polycross matings require more labour and planning than open pollinated crossing but improve the distribution of pollen and limit inter-pollination to selected clones, resulting in a better evaluation of offspring (Knowles 1950). Topcrosses involve the use of a common parent (usually another synthetic population) as the pollen parent in the cross with selected clones as the maternal parent.

The end result of most forage breeding programs is the production of a synthetic cultivar or open pollinated cultivar (Poehlman and Sleper 1995). Both populations are similar in that they are derived from an open pollination of selected clones, strains, or inbred lines. They differ in that a synthetic must be occasionally reconstituted by inter-pollinating components to produce additional basic seed. Open pollinated cultivars are not reconstituted from the original components.

Synthetic cultivars are popular in forage breeding as they take advantage of the

self-incompatible, out crossing nature of most forage species, and produce an adequate amount of seeds in early generations ( Poehlman and Sleper 1995). Parents for synthetic cultivars are chosen based on their particular combining ability which is determined through progeny tests (Knowles 1959). The aim of a synthetic cultivar is to maximise the combination of favourable alleles while reducing the effects of inbreeding depression (Casler et al. 1996). This is accomplished by choosing an optimum number of plants to form the synthetic. A smaller number of superior combining clones results in higher yield in the first synthetic generation but increases the possibility of inbreeding depression in further generations.

### **2.5.3 Quantitative genetics**

The efficiency of selecting for a particular trait is determined by the level of influence that genetic and non-genetic effects have on the phenotypic expression of a given genotype (Fehr 1987). This concept is often referred to as heritability.

A simple definition of heritability is the ratio of genotypic variance to phenotypic variance. That is to say, the ratio of variance which is accounted for by genetic factors to the variance which is accounted for by genetic and environmental factors (Fehr 1987). Some traits which have very high heritabilities (such as seed coat colour in peas), and are controlled by few genes for which the expression is not affected by the environment (Poehlman and Sleper 1995), are referred to as qualitative traits. When many genes control a trait and environmental factors influence the expression of the trait (such as yield), the heritability is often lower and the traits are referred to as quantitative traits.

Heritability estimates are important in a plant breeding program as the degree of inheritance of a given trait will influence the selection method which is used (Fehr 1987).

Traits with a very high heritability may be selected for using single plants. As heritability of a trait decreases, the need for replication and testing over many environments increases.

There are two types of heritability estimates, broad sense and narrow sense. Broad sense heritability takes into account total genotypic effect including additive, dominance and epistatic effects, while narrow sense only includes additive genetic effects. Narrow sense heritability is often a more important estimate, as additive genetic variation is passed on to the subsequent generation. Additive genetic variance predominates in forage grasses due to the heterogeneous nature of the population and heterozygous individuals (Casler et al. 1996). Phenotypic recurrent selection, the method which predominates in forage breeding programs, is not effective at maximizing other types of genetic variance, thus, further increasing the relative importance of additive genetic variance (Nguyen and Sleper 1983b).

Heritability can be estimated from variance components from the analysis of progeny tests, which is one of the more flexible manners of estimating heritability (Fehr 1987). The progeny tests are best conducted with half-sib families (one parent in common) as they minimize the inflation of heritability estimates characteristic of full-sib families (both parents in common) (Ray et al. 1994). Linear functions of mean squares are calculated, as determined by the expected mean squares, to estimate the appropriate variance components (Nguyen and Sleper 1983b). Within half-sib family variance is considered equal to the covariance among half-sib families which in turn is equal to one-quarter of the additive genetic variance. Additive genetic variance is then divided by the total phenotypic variance to determine heritability (Nyquist 1991). Phenotypic variance includes total genetic variance, the environmental variance along with the interactions of genotype and environment, which can include genotype x location, genotype x year, genotype x replication.

### **3. SEED WEIGHT, SEED YIELD AND SEEDLING VIGOUR OF S9240 CRESTED WHEATGRASS COMPARED TO OTHER CRESTED WHEATGRASS CULTIVARS OF VARIABLE ORIGIN**

#### **3.1 Abstract**

S9240 crested wheatgrass was produced by colchicine-doubling the chromosome number of diploid *A. cristatum* cv. Parkway, followed by several cycles of selection for height, seed weight, floret fertility, and forage yield. Some outcrossing to tetraploid *A. cristatum* cv. Kirk germplasm occurred during the selection cycles. The goal of this study was to compare mean seed weight, seed yield, bulk density and the ability to emerge from deep seeding, of S9240 with other crested wheatgrass cultivars. Seed was collected from a sward-seeded stand and a spaced-planted nursery in 1999. The mean seed weight and total seed yield of S9240 were significantly ( $p < 0.05$ ) greater than the other cultivars, but there were no differences in bulk density among the tetraploid cultivars. Four cultivars (S9240H, S9240J, Kirk and CD-II) were seeded in a greenhouse soil flat, at a depth of 7.5 cm. Seeds which weighed between 5.0 and 5.9 mg had significantly greater ( $p < 0.05$ ) emergence and heavier seedlings than seeds which weighed between 3.0 and 3.9 mg. When a random sample of seeds taken from the same four cultivars was assayed, S9240, even though it had greater mean seed weight than the other cultivars, did not have superior emergence or seedling weight.

### 3.2 Introduction

Members of the crested wheatgrass complex (*Agropyron spp.*) are important forage grasses in the central and northern Great Plains and Intermountain regions, which receive between 200 and 450 mm of annual precipitation (Mayland et al. 1992). Smoliak and Dormar (1985) estimated that there were approximately 1 million ha of *Agropyron cristatum* (L.) in use in rangelands in western Canada, while Mayland et al. (1992) estimated that there were over 6 million ha of *Agropyron spp.* in production in North America.

Three species from this genus are of importance in North American rangelands. Fairway crested wheatgrass (*A. cristatum*) occurs in diploid ( $2n = 2x = 14$ ), tetraploid ( $2n = 4x = 28$ ) and hexaploid ( $2n = 6x = 42$ ) form. Standard crested wheatgrass (*Agropyron desertorum* (Fisch. ex Link) Schlut) and Siberian crested wheatgrass (*Agropyron fragile* (Roth) Candargy) are both tetraploid ( $2n = 4x = 28$ ) species. Diploid and tetraploid *A. cristatum* are grown primarily in Canada, as they are more persistent under moist conditions, while *A. desertorum* is grown predominantly in the United States. *A. fragile* is the least commonly grown of the three but most drought tolerant and is well adapted to light sandy soils (Asay et al. 1995a).

Seed characteristics have been selected for in forage grasses with the objective of improving yield, handling, processing, and stand establishment (Schaaf and Rogler 1963). In crested wheatgrass, and range grasses in general, seed weight and seed yield are usually the two most important seed traits. There have been efforts in several cool-season forage grasses, such as crested wheatgrass (Schaaf and Rogler 1963; and Rogler 1954), smooth brome grass (*Bromus inermis* Leyss) (Trupp and Carlson 1971; and Jessen and Carlson

1985), and Russian wildrye (*Psathyrostachys juncea* (Fischer) Nevski)(Asay and Johnson 1980; Berdahl and Barker 1984; Jefferson 1993) to increase mean seed weight with the aim of improving stand establishment.

Environmental factors which affect the successful establishment of a forage stand include depth of planting, weed competition and soil moisture and temperature (Berdahl and Barker 1984). Planting at greater depths would decrease moisture stress and temperature fluctuations; however, Rogler (1954) stated that crested wheatgrass does not have adequate vigour to emerge from field planting depths of greater than 1 to 1.5 inches (2.5 to 3.75 cm).

The combined length of the coleoptile and the subcoleoptile internode (mesocotyl) determines the depth from which a grass seedling can emerge (Newman and Moser 1988). *Agropyron* species produce a very short or no subcoleoptile internode (Hoshikawa 1969) and, as a result, depend on coleoptile elongation for emergence. Hunt and Miller (1965) conducted a study on the effect of seed weight on coleoptile length in intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkw. & D.R. Dewey), and found that seeds from clones with a high mean seed weight had longer coleoptiles. Berdahl and Barker (1984) found similar results with Russian wildrye, but stated that once seed weight surpassed 3.0 mg, increases in emergence and coleoptile length diminished. In a study of the effect of genotype and seed size on the emergence of spring wheat, Lafond and Baker (1986), found that seed size accounted for more seedling emergence variance than genotype.

S9240 is an experimental population of crested wheatgrass which was developed at the Saskatoon Research Centre of Agriculture and Agri-Food Canada. It is based

mainly on colchicine-doubled individuals of diploid *A. cristatum* cv. Parkway with some inclusion of natural tetraploid *A. cristatum* cv. Kirk. Since 1984 this population has been through several cycles of selection for height, seed yield, seed weight, floret fertility, and forage yield. S9240 shows potential as a new cultivar for hay and pasture production, but little is known about its seed and seedling characteristics relative to other cultivars.

The objectives of this study were: 1) To determine the seed weight of S9240 in relation to other common crested wheatgrass cultivars; and 2) To determine percent emergence and seedling weight, from a deep seeding, of S9240 seeds in relation to other common crested wheatgrass cultivars.

### **3.3 Materials and methods**

#### **3.3.1 Plant material**

Plant material used in this study is described in Table 3.1. There were two field trials used as seed sources for this study, one was a sward-seeded trial with four replications that was established in 1994 (will be referred to as 1994A). The plots were 1.25m x 6 m and were seeded at a rate of 100 seeds/m with rows spaced 30 cm apart. The second was a spaced-planted nursery established in 1996 (will be referred to as 1996A), with three replications of plots of 13 plants, on 1 metre spacings.

Table 3.1: Description of plant material included in the emergence and seed size study

Line	Species	Ploidy	Description	Trials
Parkway	<i>A. cristatum</i>	2x	A 16 clone, synthetic cultivar derived from selections from <i>A. cristatum</i> cv. Fairway (Elliott and Bolton 1970)	1994A
S9240 F, H, I, J and K	<i>A. cristatum</i>	C4x	An experimental line based on colchicine doubled individuals selected from the cultivar Parkway. Each letter represents a different, selected population	1994A and 1996A
Kirk	<i>A. cristatum</i>	4x	A cultivar formed from mass selected individuals from accessions introduced from Finland (Knowles 1990)	1994A, and 1996A
Nordan	<i>A. desertorum</i>	4x	Standard wheatgrass cultivar (Elliott and Bolton 1970)	1994A
CD-II	Hybrid	4x	A 10 clone synthetic, selected from the cultivar Hycrest, which is a population of <i>A. cristatum</i> x <i>A. desertorum</i> interspecific hybrids (Asay et al. 1997)	1994A, and 1996A

### 3.3.2 Data collected

Seed was harvested from trials 1994A and 1996A in 1999. The seed was cleaned and weighed and a sub-sample was collected from each replication and placed in a -20 °C freezer for two months to break dormancy. The seed was then removed from cold storage and tested for percent germination.

One hundred individual seeds from each replication were weighed and maximum, minimum and mean weights were calculated and the distribution of seed weight determined. Bulk density was determined by weighing three 50 cm<sup>3</sup> samples from each plot.

Two greenhouse trials were conducted to examine the effect of mean seed weight on seedling emergence and seedling weight. Seeds from four lines (Kirk, CD-II and S9240H and J), harvested from trial 1996A, were used to determine the effect of cultivar within a specific weight range and cultivar x seed weight interactions. Seventy-five seeds from each line, weighing between 3.0 to 3.9 mg and an equal number weighing between 5.0 to 5.9 mg, were selected to be assayed. The seeds were planted at 1 cm spacings, in 75 cm long rows, at a depth of 7.5 cm in a flat containing sandy-loam soil in a greenhouse. After seeding was completed the soil was packed lightly by hand. Plant counts were performed at two day intervals with the final count twenty eight days after seeding, at which time the seedlings were harvested and weighed. Percent emergence was corrected for germination rates. This experiment was repeated four times to provide replication.

The second trial consisted of randomly selected samples of seventy-five seeds from each of the four lines; CD-II, Kirk and S9240 H and J. Mean seed weight was determined for each sample and then the trial was carried out in the same manner as the

first trial.

### 3.3.3 Statistical analysis

To determine the treatment effect of cultivar on seed weight, total yield, bulk density, emergence and seedling weight, an analysis of variance was performed using the SAS General Linear Means (GLM) procedure, as a randomized complete block design (SAS Institute, Inc. 1985). To determine the effect of seed weight on emergence and seedling weight, an analysis of variance was performed using the SAS General Linear Means (GLM) as a 2 x 4 factorial (two seed weights, and four cultivars), randomized complete block design. Log and arc-sin transformations were performed on emergence data in an attempt to reduce heterogeneity among replication but no improvements were found.

If a treatment was determined to have a significant effect ( $p < 0.05$ ) least significant differences (LSD) were calculated for comparisons of means.

### 3.4 Results and discussion

#### 3.4.1 Seed weight

S9240 had the highest mean seed weight in trial 1994A, the sward-seeded stand (Table 3.2). The majority of Parkway seeds were in the 1 to 1.9 mg weight range which resulted in it having the lowest mean seed weight. All of the tetraploid lines had the majority of their seeds in the 2 to 2.9 mg range, ranging from 62.3% for Kirk to 46.8% for CD-II. There were few seeds in the 4 to 4.9 mg range with only S9240 and CD-II being represented in this category.

The higher proportion of seeds in the 3 to 3.9 mg range and relatively low number in the 1 to 1.9 mg range is the reason that S9240 has a higher mean weight than other crested wheatgrass lines that were grown in this trial.

S9240 H had a higher mean seed weight than S9240 K, Kirk and CD-II in the spaced-planted nursery (Table 3.3). S9240 I and J had larger mean seed weights than CD-II. There were no differences between the lines for minimum or maximum seed weights. CD-II, S9240 K, and Kirk all had the largest portion of their seeds in the 3-3.9 mg range while S9240 H, I and J had the majority of their seeds in the 4-4.9 mg and 5-5.9 mg weight ranges.

**Table 3.2: Minimum, maximum and mean weights and distribution in five classes of seed weight of five crested wheatgrass cultivars harvested from the sward-seeded stand 1994A**

Cultivar	Species	Ploidy	Min	Max	Mean	Weight distribution				
						mg	mg	mg	mg	mg
Parkway	<i>A. cristatum</i>	2x	0.6	2.7	1.3	17.5	76.3	5.8	0.5	0.0
CD-II	Hybrid	4x	0.9	4.2	2.4	0.5	25.5	46.8	24.5	2.8
Kirk	<i>A. cristatum</i>	4x	0.9	3.9	2.3	0.8	19.0	65.3	14.3	0.8
Nordan	<i>A. desertorum</i>	4x	1.1	3.9	2.5	0.0	18.3	59.8	21.3	0.8
S9240 F	<i>A. cristatum</i>	4x	1.2	4.3	2.6	0.0	10.3	56.8	30.5	2.5
LSD(0.05)					0.1	4.8	8.5	10.6	8.3	1.9

**Table 3.3: Minimum, maximum and mean weights and distribution in six classes of seed weight of six lines of crested wheatgrass harvested from a spaced-planted nursery 1996A**

Cultivar	Species	Ploidy	Min	Max	Mean	Weight distribution					
						mg	mg	mg	mg	mg	mg
CD - II	Hybrid	4x	1.7	6.3	3.6	2.6	16.7	48.7	26.7	4.0	1.3
Kirk	<i>A. cristatum</i>	4x	1.8	6.1	3.9	3.0	14.7	39.0	28.3	13.3	1.7
S9240 H	<i>A. cristatum</i>	4x	2.2	6.9	4.8	0.3	2.3	11.7	44.3	33.3	8.0
S9240 I	<i>A. cristatum</i>	4x	2.3	6.5	4.5	0.3	5.3	22.7	41.0	27.0	3.7
S9240 J	<i>A. cristatum</i>	4x	1.8	6.7	4.4	0.7	5.0	26.7	36.0	24.0	7.7
S9240 K	<i>A. cristatum</i>	4x	1.8	6.7	3.9	1.3	17.0	38.0	26.7	13.3	3.3
LSD(0.05)					0.6	4.1	15.0	13.0	21.0	13.0	13.0

The overall mean seed weight from the spaced-planted nursery (4.1 mg) was almost double that of the sward-seeded trial (2.2 mg) (Table 3.2 and 3.3). This would be due to the lower level of inter-plant competition for resources encountered in a spaced-planted nursery. The close row spacing and subsequent inter-plant competition of the sward-seeded stand may have minimized the differences in seed size among cultivars. The recommended row spacing for crested wheatgrass stands that will be harvested for seed for more than four seasons is 60 cm (Anonymous 1998) while the spacing used in the sward-seeded trial was only 30 cm.

Seed from S9240 was heavier than that of other lines (Tables 3.3 and 3.2) and all the tetraploid cultivars were heavier than the diploid cultivar Parkway (Table 3.2). It has previously been found that selection for seed weight in forage grasses such as smooth brome grass (Jessen and Carlson 1985; and Trupp and Carlson 1971), Russian wildrye (Berdahl and Reis 1996) and crested wheatgrass (Schaaf and Rogler 1963) resulted in an actual increase in seed weight. The difference in mean seed weight between tetraploid *A. desertorum* and diploid *A. cristatum* has long been recognised (Rogler 1954). There are also reports of both somatic and sexual autopolyploids of crested wheatgrass (Tai and Dewey 1966), Russian wildrye (Berdahl and Reis 1996) and orchard grass (*Dactylis glomerata* L.) (Bretagnolle and Lumaret 1995) having greater seed mass than their diploid progenitors. Both Tai and Dewey (1966) and Bretagnolle and Lumaret (1995) felt that the increase in seed weight of the autopolyploid progeny was not a direct result of polyploidization, but rather a reflection of less competition between seeds due to the reduced seed set which they observed.

### 3.4.2 Seed yield

S9240 had superior seed yield in the sward-seeded trial compared to the other tetraploid cultivars, which were not different from each other (Table 3.4). The diploid cultivar Parkway had the lowest seed yield. In the spaced-planted nursery, S9240 I had a higher seed yield than Kirk and CD-II. The other S9240 lines were intermediate between S9240 I and Kirk but superior to CD-II.

There are reports that selection for increased seed weight resulted in decreased seed yield and floret fertility in crested wheatgrass (Schaaf and Rogler 1963). The greater seed yield of S9240, over other cultivars (Table 3.4), indicates that selection for increased seed weight has not negatively affected total seed yield or fertility in this population. Trupp and Carlson (1971) found that selection for seed weight would not necessarily reduce yield and fertility as long as simultaneous selection for floret fertility was carried out. This disagrees with Jessen and Carlson (1985) who found that there was a negative correlation between seed weight and seed yield in bromegrass; however, this work was not carried out on selected populations. Coulman (personal communication) has found that the fertility of S9240 is as high or higher than other crested wheatgrass cultivars, indicating that selection for fertility after a polyploidization event has occurred, can restore floret fertility without compromising individual seed weight.

**Table 3.4:** Seed yield, from crested wheatgrass cultivars and lines, in a sward-seeded stand (1994A) and spaced-planted nursery (1996A) in 1999

1994A		1996A	
Line	1999	Line	1999
	— kg/ha —		— kg/ha —
S9240 F	757	S9240 H	577
Kirk	604	S9240 I	610
CD-II	600	S9240 J	519
Nordan	566	S9240 K	491
Parkway	217	Kirk	458
		CD-II	317
Mean	549		495
LSD(0.05)	107		149

### 3.4.3 Bulk density

In both trials there were no differences in bulk density among the tetraploid cultivars but in the sward-seeded stand the diploid cultivar Parkway had a lower density than the tetraploid cultivars (Table 3.5). There is the possibility that increases in individual seed weight are due not to increases in actual caryopsis weight but rather an increase in hull (ie. lemma and palea) weight. Ronald et al. (1999) found, that the proportion of an oats (*Avena sativa* L.) seed which is hull can range from 21 to 41%. One indicator of increased hull percentage can be reduced bulk density, as the hull weighs less than the caryopsis. The lack of a significant difference in bulk density among the tetraploid lines (Table 3.5), irrespective of difference in seed weight, indicates that hull percentage is similar among lines. The difference between the diploid Parkway and the tetraploids was not large and indicates that increases in ploidy level did not have a large effect on bulk density.

**Table 3.5:** Average seed bulk density, of crested wheatgrass lines and cultivars, from a sward-seeded stand (1994A) and spaced-planted nursery (1996A) in 1999

1994A		1996A	
Line	1999	Line	1999
	— kg/hL —		— kg/hL —
S9240	29.1	S9240 H	25.8
Kirk	29.7	S9240 I	27.7
CD-II	28.8	S9240 J	27.8
Nordan	29.0	S9240 K	27.2
Parkway	27.6	Kirk	24.9
		CD-II	27.3
LSD(0.05)	1.1		NS

#### 3.4.4 Emergence and seedling weight

Seeds in the 5.0-5.9 mg weight range had a higher percentage emergence and mean seedling weight 28 days after planting than those in the 3.0 to 3.9 mg weight range (Tables 3.6 and 3.7). There were no differences between cultivars, and there were no significant cultivar x seed weight interactions. This indicates that increased seed weight has a positive effect on seedling emergence and establishment and that there is little difference among the lines within a weight range. These results are in agreement with the results of Lafond and Baker (1986) where they found that seed weight accounted for 50% of the variation in shoot dry weight in spring wheat and that there were no seed weight x cultivar interactions.

**Table 3.6:** Percent emergence 28 days after seeding of crested wheatgrass seeds of two weight ranges (5.0 - 5.9 mg and 3.0 - 3.9 mg)

Seed Weight	Cultivars				Mean
	CD-II	Kirk	S9240 H	S9240 J	
	%				
5.0 - 5.9 mg	34.8	37.3	48.7	41.2	40.5
3.0 - 3.9 mg	21.7	13.8	17.5	16.9	17.5
Mean	28.2	25.5	33.1	29.1	27.7
LSD (0.05) for comparing means of weight classes				7.6	
LSD (0.05) for comparing means of population				ns	
LSD (0.05) for comparing populations within a weight class				ns	

**Table 3.7:** Mean seedling weight 28 days after seeding of crested wheatgrass seeds of two weight ranges (5.0 - 5.9 mg and 3.0 - 3.9 mg)

Seed Weight	Cultivars				Mean
	CD-II	Kirk	S9240 H	S9240 J	
	mg				
5.0 - 5.9 mg	25.2	24.2	31	22	25.7
3.0 - 3.9 mg	11	10	17	21.3	14.8
Mean	18.1	17.1	24	21.8	21.6
LSD (0.05) for comparing means of weight classes				6.4	
LSD (0.05) for comparing means of population				ns	
LSD (0.05) for comparing populations within a weight class				ns	

There were no significant differences in percent emergence or mean seedling weight among lines when random seed samples were assayed (Table 3.8), even though there were significant differences in mean seed weight among populations. These results are in disagreement with a number of trials (Andrews et al. 1997; Berdahl and Barker 1984; Berdahl and Reis 1996; Hunt and Miller 1964; Lafond and Baker 1986; Rogler

1954; and Trupp and Carlson 1971) conducted on several different grass species, which found positive relationships between seed size/weight and emergence and/or seedling vigour. It is possible in the present study that there was not an adequate difference in seed weight between the cultivars for a differentiation in emergence; however, there was a 1.5 mg difference in the mean seed weight of S9240H and CD-II (Table 3.8). A mean difference of 2.0 mg produced a significant difference in emergence in the previous trial (Tables 3.6). Rogler (1954) found significant differences between seed lots which differed in mean weight by only 0.3 mg when planted at 7.5 cm depths in the greenhouse.

Hunt and Miller (1964) and Berdahl and Barker (1984) felt that there was not a linear relationship between seed weight and coleoptile elongation and emergence in intermediate wheatgrass and Russian wildrye, respectively. This also is not likely the cause of the lack of significant difference in this trial as the mean seed weights did not fall outside the range compared in the first trial (Table 3.6). The only way to determine for certain if these factors influenced emergence in this study would be to conduct coleoptile length studies and to include more weight classes in the emergence study.

The most likely the cause of lack of difference in emergence was the difficulty in establishing a uniform growing environment in the soil flat used in this study. Soil moisture, soil crusting and degree of soil compaction varied throughout the soil flat, resulting in inconsistent emergence, even within rows. The high coefficients of variation (Table 3.8) reflect the variations in growing conditions.

**Table 3.8:** Mean seed weight, percent emergence and mean seedling weight after 28 days of four crested wheatgrass populations

Line	Mean seed weight	Mean seedling weight	Emergence
	mg		%
CD-II	3.3	22.5	28.5
Kirk	3.6	31.5	30.2
S9240 H	4.8	30	32.4
S9240 J	4	30	30.4
Mean	4	28.5	30.4
LSD(0.05)	0.3	ns	ns
CV%	2.8	31.8	30.9

### 3.5 Conclusions

S9240 crested wheatgrass produced seeds with a higher mean weight than other common crested wheatgrass cultivars and there were differences in mean seed weights among different selected S9240 populations. Combined selection for seed weight and floret fertility appears to have negated any effect of increased seed weight or polyploidization on floret fertility and total seed yield, as the seed yield of S9240 is higher than the other cultivars. The lack of differences in bulk density indicated that there may have been little difference in hull percentage among the cultivars. There was no significant seed weight x cultivar interaction on seedling emergence or seedling weight, while there was a significant effect of seed weight on seedling vigour, indicating that seed weight was more influential than other factors controlling emergence. The lack of significant differences among random samples in seedling emergence and seedling weight is most likely a reflection variation among replications and within the soil flat.

#### **4. PLANT HEIGHT, CROWN DIAMETER AND TILLER CHARACTERISTICS OF S9240 CRESTED WHEATGRASS COMPARED TO OTHER CRESTED WHEATGRASS CULTIVARS OF VARIED ORIGIN**

##### **4.1 Abstract**

S9240 crested wheatgrass was produced by colchicine-doubling the chromosome number of diploid *Agropyron cristatum* cv. Parkway, followed by several cycles of selection for height, seed size, floret fertility, and forage yield. Some inclusion of tetraploid *A. cristatum* cv. Kirk germplasm occurred during the selection cycles. The goal of this study was to compare the height, crown width, tiller density, tiller weight and tiller angle of S9240 with that of other crested wheatgrass cultivars (Parkway, Kirk, Nordan and CD-II). Data were collected in both 1999 and 2000 from a number of sward-seeded and spaced-planted trials. S9240 was significantly ( $p < 0.05$ ) taller and had fewer tillers than all other lines. S9240 was significantly ( $p < 0.05$ ) narrower crowned than the diploid *A. cristatum* cv Parkway. Differences in row width between S9240 and *A. cristatum* cv Kirk, *Agropyron desertorum* cv Nordan, and *A. desertorum* x *A. cristatum* cv CD-II varied, but generally S9240 produced a narrower crown. S9240 had significantly ( $p < 0.05$ ) greater tiller weight than Nordan, CD-II and Parkway. Tillers on the perimeter of S9240 tussocks were more upright than those of CD-II. In general S9240 is taller, narrower crowned and has fewer, heavier tillers than other crested wheatgrass cultivars.

## 4.2 Introduction

Members of the crested wheatgrass complex (*Agropyron spp.*) are important forage grasses in the central and northern great plains and intermountain regions of North America, which receive between 200 and 450 mm of annual precipitation (Mayland et al. 1992). Smoliak and Dormar (1985) estimated that there were approximately 1 million ha of *Agropyron cristatum* (L.) in use in rangelands in western Canada, while Mayland et al. (1992) estimated that there were over 6 million ha of *Agropyron spp.* in production in North America.

Three species from this genus are of importance in North American rangelands. Fairway crested wheatgrass (*A. cristatum*) occurs in diploid ( $2n = 2x = 14$ ), tetraploid ( $2n = 4x = 28$ ) and hexaploid ( $2n = 6x = 42$ ) form. Standard crested wheatgrass (*Agropyron desertorum* (Fisch. ex Link) Schlut) and Siberian crested wheatgrass (*Agropyron fragile* (Roth) Candargy) are both tetraploid ( $2n = 28$ ) species. Diploid and tetraploid *A. cristatum* are grown primarily in Canada, as they are more persistent under moist conditions, while *A. desertorum* is grown predominantly in the United States. *A. fragile* is the least commonly grown of the three, but is the most drought tolerant and is well adapted to light sandy soils (Asay et al. 1995a). The tetraploid lines tend to be more upright when compared to the finer more decumbent tillers of the diploid *A. cristatum*.

Most members of *Agropyron*, with the exception of some rhizomatous members, are caespitose, or tussock forming grasses (Asay and Jensen, 1996). Caespitose graminoids are characterized by dense formations of erect culms with successive generations of tillers arising from parental tillers (Welker and Briske 1991). A tiller is made up of a number of associated phytomers, which consist of a leaf blade, leaf sheath,

node, internode and axillary bud (Nelson 1996).

During vegetative growth plants can partition assimilates into the formation of daughter tillers, enlargement of existing tillers, enhancing root growth or storage (Sugiyama 1995). Sugiyama (1995) found that tall fescue plants (*Festuca arundinacea* Schreb.) could be generalized into clones with a greater number of smaller tillers or with fewer, larger tillers, with greater carbon reserves. Zarrouh and Nelson (1980) found that a tall fescue population, which had been selected for high individual tiller yield, had fewer tillers per plant, greater yield per tiller, root weight, culm base weight, and water soluble carbohydrate concentration and was better able to restore concentration of soluble carbohydrates after harvest had occurred.

Mueller and Richards (1986) looked for anatomical characteristics which differed between *Pseudoroegneria spicata* (Pursh) A. Love, a species which does not tolerate grazing, and *A. desertorum*, which does recover after grazing. They investigated characteristics such as tiller morphology, number and size of axillary buds, apical meristem structure and vascular development to axillary buds. They found no connection between these traits and the rate of tillering and suggested that rate of tillering is more likely a physiologically controlled trait than a morphologically determined one. Frank and Hofmann (1994) stated that since *Agropyron spp.* are shade avoiding species and have a very sensitive phytochrome system, they will produce fewer, taller tillers when exposed to low red/far red ratios.

Conclusions on the merits of large tillered populations have been mixed. Casler and Hovin (1985) found in reed canarygrass (*Phalaris arundinacea* L.) that tiller number was positively correlated with plant yield but that tiller height had a negative or no

correlation to plant yield. However that study was conducted in a spaced-planted situation, and the situation in a sward-seeded stand may be different (Zarroug et al. 1983a). Zarroug and Nelson (1980) concluded that tall fescue populations, with high individual tiller yield, had higher total yield and were superior to low individual tiller yield populations, in systems where long intervals (more than six weeks) were left between harvests. This is due to the high yield per tiller population containing greater amounts of water soluble carbohydrates to re-establish leaf area after defoliation. If grazing intervals are too closely spaced, the large tiller plants do not have enough time to rebuild their energy stores.

S9240 is an experimental population of crested wheatgrass, which was developed at the Saskatoon Research Centre of Agriculture and Agri-Food Canada. It is based mainly on colchicine-doubled individuals of diploid *A. cristatum* cv. Parkway, with some inclusion of natural tetraploid *A. cristatum* cv. Kirk. Since 1984 this population has been through several cycles of selection for traits such as height, seed weight, floret fertility, and forage yield. S9240 shows potential as a new high yielding hay-type cultivar of crested wheatgrass. Observations made in the field indicate that S9240 is taller than other crested wheatgrass cultivars but also appears to have a narrower crown.

There were three objectives to this study; 1) to determine the plant height and crown diameter of S9240 in relation to other common crested wheatgrass cultivars; 2) to determine if there is a relationship between the narrow crown diameter and increased tiller height of S9240; 3) to determine the tiller density, tiller size and tiller angle in crested wheatgrass cultivars of varying crown diameters.

## **4.3 Materials and methods**

### **4.3.1 Experimental design**

Trials were conducted at the research farm of Agriculture and Agri-Food Canada Saskatoon Research Centre. Three sward-seeded trials were used for compiling data; two were established in 1994 (referred to as trials 1994A and 1994B), and the other was established in 1997 (referred to as trial 1997). These trials had four, three and four replications, respectively. The plots were 1.25m x 6 m and were seeded at a rate of 100 seeds/m of row with rows spaced 30 cm apart.

A spaced-planted nursery with 1 m plant spacings was also sampled. It was established in 1996 (referred to as trial 1996A ) and had three replications, with thirteen plants per replication.

### **4.3.2 Plant material**

Plant material used in trials 1994A, 1994B, 1996A and 1997 are described in Table 4.1.

### **4.3.3 Data collected**

When measuring row width and plant height in the sward-seeded trials, four random points were selected within each plot, while in the spaced-planted nursery measurements were taken on individual plants. Plant height was determined, after anthesis, by measuring the distance from soil level to the tip of the spike on the tallest tiller. The distance between the outermost tillers was measured to determine the row or crown width before tiller elongation in the spring and after the plots had been harvested. Trials 1994B and 1997 were harvested for forage, while trials 1994A and 1996A were harvested for seed.

**Table 4.1:** Description of plant material included in the plant morphology study

Line	Species	Ploidy	Description	Trials
Parkway	<i>A. cristatum</i>	2x	A 16 clone, synthetic cultivar derived from selections from <i>A. cristatum</i> cv. Fairway (Elliott and Bolton 1970)	1994A, 1994B
S9240 F, H, I, J and K	<i>A. cristatum</i>	C4x	An experimental line based on colchicine doubled individuals selected from the cultivar Parkway. Each letter represents a different, selected population	1994A, 1994B, 1996A, and 1997
Kirk	<i>A. cristatum</i>	4x	A cultivar formed from mass selected individuals from accessions introduced from Finland (Knowles 1990)	1994A, 1994B, 1996A and 1997
Nordan	<i>A. desertorum</i>	4x	Standard wheatgrass cultivar (Elliott and Bolton 1970)	1994A and 1994B
CD-II	Hybrid	4x	A 10 clone synthetic, selected from the cultivar Hycrest, which is a population of <i>A. cristatum</i> x <i>A. desertorum</i> interspecific hybrids (Asay et al. 1997)	1994A, 1996A and 1997

To determine tiller density, three random locations were chosen and the number of tillers in a 10 cm section of the row counted and then converted to tillers per square metre. This measurement was taken after harvest in the sward-seeded trials.

To determine tiller weight, 20 tillers were collected from each plot in a sward-seeded trial (1994A), and three tillers were collected from each plant in the spaced-planted trial (1996A). The seed heads were removed, and the remainder of each tiller was bulked on a plot basis and weighed.

In the spaced-planted nursery five plants were randomly selected per plot after harvest had occurred, and the tussocks were split in half so that a cross section of the tussock at soil level was visible. The angle from the horizontal of five tillers on the perimeter and the interior of the tussock was recorded.

#### **4.3.4 Statistical analysis**

To determine the treatment effect of cultivar on plant height, crown diameter, tiller density, tiller weight and tiller angle, an analysis of variance was performed using the SAS General Linear Means procedure (GLM) in a randomized complete block design (SAS Institute, Inc. 1985). Years were analysed separately. Differences were considered significant at  $\alpha \leq 0.05$ . If a treatment was determined to have a significant effect ( $p < 0.05$ ), least significant differences (LSD) were calculated for comparisons of means.

Due to unequal numbers of observations per plot, resulting from establishment failure or plant mortality, the data for plant height and crown diameter from trial 1996A were unbalanced. To compensate for this, treatment means were calculated as least-squares means, using the LSMEANS statement under the GLM procedure of SAS (SAS Institute, Inc. 1985). To determine if differences between treatments were significant, t-

tests were conducted comparing all least-square means to each other, using the PDIF option in the LSMEANS statement in the GLM procedure of SAS.

Since trial 1996A contained four S9240 populations (S9240 H, I, J and K), and two cultivars (CD-II and Kirk), linear contrasts were performed to compare the mean of the four S9240 populations with the individual cultivars. This was performed using the ESTIMATE statement under the GLM procedure of SAS (SAS Institute, Inc. 1985).

#### **4.4 Results**

##### **4.4.1 Row width**

###### **4.4.1.1 Sward-seeded trials**

In trials 1994A and 1994B, S9240 was significantly ( $p < 0.05$ ) narrower than Parkway (Table 4.2). S9240 was almost always numerically narrower than Kirk, but the differences were not significant. In trial 1994A, S9240 was also significantly ( $p < 0.05$ ) narrower than CD-II and Nordan in both years, except the summer sampling in 2000. In 1999, S9240 was significantly ( $p < 0.05$ ) narrower than Nordan in trial 1994B, but was not significantly ( $p < 0.05$ ) different in 2000. The lack of significant difference between S9240 and Nordan in 2000 could be due to the lower level of precipitation in the fall of 1999 (Figure 4.1) when tiller initiation occurs.

There were no significant ( $p < 0.05$ ) differences among Kirk, S9240 and CD-II in trial 1997 in 1999 (Table 4.2); however, the ranking of row widths was consistent with the older trials. In 2000 there were significant ( $p < 0.05$ ) differences between S9240 and CD-II. The lack of significant differences in 1999 could be due to the age of the stand as the rows had not yet spread as much.

**Table 4.2: Spring and summer row widths in three sward-seeded trials of crested wheatgrass in 1999 and 2000**

Year of seeding	1994A				1994B				1997			
	Spring		Summer		Spring		Summer		Spring		Summer	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Line	10.0	11.1	10.5	11.0	13.1	12.1	13.2	11.5	10.6	10.2	9.9	9.7
S9240*	10.4	11.8	11.8	12.1	14.4	12.1	14.3	11.3	10.2	11.3	11.1	10.8
Kirk	11.9	13.5	14.4	12.4	15.7	14.6	14.1	12.3	9.5	12.5	11.5	12.4
CD-II	12.5	11.3	13.5	12.8	16.3	16.2	17.2	16.2				
Nordan	14.1	16.0	15.3	15.2								
Parkway	1.8	1.7	2.0	1.7	2.1	3.0	3.8	2.1	ns	1.9	ns	1.7

LSD(0.05)

\* The S9240 population used in trials 1994A and 1994B is S9240F and the population used in trial 1997 is S9240J.

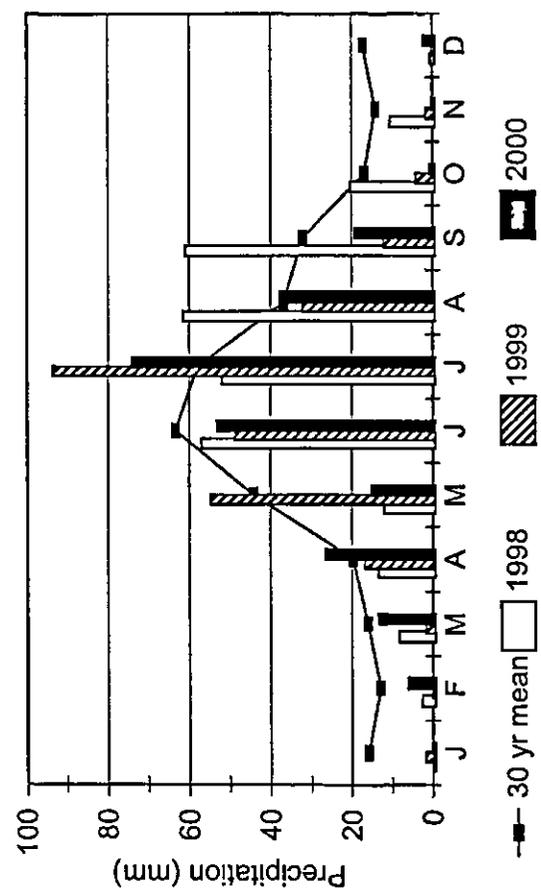


Figure 4.1: Monthly precipitation at Keman Farm, Saskatoon, SK.

#### 4.4.1.2 Spaced-planted nursery

There were no significant ( $p < 0.05$ ) differences in crown diameter among the four S9240 populations, in any of the three sampling years (Table 4.3). The S9240 populations were significantly ( $p < 0.05$ ) narrower than CD-II in all three years, but there were no significant ( $p < 0.05$ ) differences between the S9240 populations and Kirk (Table 4.4).

**Table 4.3:** Crown diameter of four S9240 crested wheatgrass populations from a spaced-planted nursery of crested wheatgrass in 1998, 1999 and 2000

Line	cm					
	1998		1999		2000	
S9240 H	17.4	a	29.1	a	26.5	a
S9240 I	18.7	a	27.1	a	27.6	a
S9240 J	16.9	a	28.0	a	24.9	a
S9240 K	19.1	a	27.9	a	28.6	a
Std. Err. *	1.3		1.6		2.1	

Means within a column with the same letter do not differ

\* Standard errors may vary by approx. 5% between cultivars due to unbalanced data

**Table 4.4:** Estimate of the difference in crown width between four S9240 crested wheatgrass populations and CD-II and Kirk based on data taken from a spaced-planted nursery in 1998, 1999 and 2000

Contrast	Estimated difference					
	1998		1999		2000	
	cm					
S9240/CD-II	-4.4	***	-5.5	***	-4.3	***
S9240/Kirk	-1.0	ns	-0.7	ns	0.1	ns

\*\*\* = significant at  $p = 0.001$ , ns = no significant difference

## **4.4.2 Height**

### **4.4.2.1 Sward-seeded stands**

S9240 was significantly ( $p < 0.05$ ) taller than other lines in all three sward-seeded trials, in both years, except for trial 1994A in 2000 (Table 4.5) . Parkway was shorter than all other lines in trial 1994A and 1994B. There were no significant ( $p < 0.05$ ) differences between Kirk, Nordan, and CD-II in any of the trials, in either year. The mean heights in all trials were greater in 1999 than they were in 2000, this is likely due to a higher level of precipitation in the spring of 1999 than in the spring of 2000 (Figure 4.1).

**Table 4.5: Plant height of crested wheatgrass in three sward-seeded trials in 1999 and 2000**

Year of seeding	1994A		1994B		1997	
	1999	2000	1999	2000	1999	2000
Line	cm		cm		cm	
S9240*	100	70	104	63	106	69
Kirk	84	62	88	58	94	62
CD-II	85	62			92	63
Nordan	85	65	87	54		
Parkway	80	56	76	42		
Mean	87	63	89	54	97	65
LSD(0.05)	4	10	9	5	6	6

\* The S9240 population used in trials 1994A and 1994B is S9240F and the population used in trial 1997 is S9240J.

#### 4.4.2.2 Spaced-planted nursery

The only significant ( $P < 0.05$ ) difference among S9240 lines, was S9240I being significantly ( $p < 0.05$ ) taller than S9240K in 1998 in trial 1996A (Table 4.6). Differences in mean height between years in this trial are also likely due to differences in precipitation (Figure 4.1). The S9240 populations are significantly ( $p < 0.001$ ) taller than CD-II in all three years (Table 4.7), but only significantly ( $p < 0.01$ ) taller than Kirk in 1999.

**Table 4.6:** Plant height of four S9240 crested wheatgrass populations from a space planted nursery of crested wheatgrass in 1998, 1999 and 2000

Line	1998		1999		2000	
	Mean	SE	Mean	SE	Mean	SE
S9240 H	89	ab	109	a	68	a
S9240 I	94	a	111	a	76	a
S9240 J	90	ab	112	a	74	a
S9240 K	86	b	104	a	67	a
Std. err.*	3		3		3	

Means within a column with the same letter do not differ

\* Standard errors may vary by approx. 5% between cultivars due to unbalanced data

**Table 4.7** Estimate of the difference in plant height between four S9240 populations and CD-II and Kirk based on data taken from a spaced-planted nursery in 1998, 1999 and 2000

Contrast	Estimated difference					
	1998		1999		2000	
	cm					
S9240/CD-II	15.1	***	21.7	***	13.1	***
S9240/Kirk	2.2	ns	6.9	**	2.5	ns

\*\*\* = significant at  $p=0.001$ , \*\* = significant at  $p=0.01$ , ns = no significant difference

#### 4.4.3 Tiller weights

S9240 had significantly ( $p<0.05$ ) heavier tillers than Nordan, CD-II and Parkway in both 1999 and 2000 in trial 1994A (Table 4.8). S9240 tillers were also significantly ( $p<0.05$ ) heavier than Kirk in 2000 but not in 1999. Parkway had the lowest tiller weight; these tillers were visibly finer than other lines. The differences in mean tiller weight, between years, are likely a reflection of lower precipitation levels in 2000 (Figure 4.1).

S9240I had significantly ( $p<0.05$ ) heavier tillers than S9240 H and K in 1999 (Table 4.8). There were no differences between S9240 lines in 2000. The differences in mean tiller weight between years are likely due to lower precipitation levels and a heavy infestation of redroot pigweed (*Amaranthus retroflexus* L.) in 2000. The S9240 populations had significantly ( $p<0.05$ ) heavier tillers than CD-II in both 1999 and 2000, but were significantly ( $p<0.05$ ) heavier than Kirk only in 2000 (Table 4.9).

**Table 4.8:** Average tiller weights of crested wheatgrass lines grown in a solid seeded stand (1994A) and spaced-planted nursery (1996A) in 1999 and 2000

Year of establishment	1994A		1996A			
	Line	1999	2000	Line	1999	2000
		— g tiller <sup>-1</sup> —			— g tiller <sup>-1</sup> —	
	S9240 F	1.21	1.01	S9240 H	3.36	1.19
	Kirk	1.06	0.65	S9240 I	4.13	1.34
	CD-II	0.97	0.66	S9240 J	3.77	1.28
	Nordan	0.98	0.65	S9240 K	2.88	1.16
	Parkway	0.55	0.44			
	Mean	0.96	0.68		3.22	1.16
	LSD(0.05)	0.19	0.2		0.69	ns

**Table 4.9:** Estimate of the difference in mean tiller weight between four S9240 crested wheatgrass populations and CD-II and Kirk based on data taken from a spaced-planted nursery in 1998, 1999 and 2000

Contrast	Estimate of difference			
	1999		2000	
	— g tiller <sup>-1</sup> —		— g tiller <sup>-1</sup> —	
S9240/CD-II	0.39	**	1.22	***
S9240/Kirk	0.09	ns	0.63	*

\*\*\* = significant at p=0.001, \*\* = significant at p=0.01, \* = significant at p=0.05 ns = no significant difference

#### 4.4.4 Tiller density

In trial 1994A, S9240 had a significantly ( $p < 0.05$ ) lower number of tillers than all other lines in both 1999 and 2000 (Table 4.10). In 1999, S9240 had significantly ( $p < 0.05$ ) fewer tillers in trial 1994B, but in 2000, there were no differences between S9240, Kirk and Nordan. Parkway had a significantly ( $p < 0.05$ ) greater number of tillers in 1999 and 2000, in both trials 1994A and 1994B (Table 4.10). In trial 1997, S9240 had significantly ( $p < 0.05$ ) fewer tillers than CD-II in 1999 but there were no significant ( $p < 0.05$ ) differences among lines in 2000. The lower number of tillers in 2000 is likely a reflection of the low level of precipitation which occurred in the fall of 1999 (Figure 4.1), when tiller initiation for the following growing season occurs.

**Table 4.10:** Tiller density in three sward-seeded trials of crested wheatgrass in 1999 and 2000

Year of planting	1994A		1994B		1997	
Line	1999	2000	1999	2000	1999	2000
	— Tiller m <sup>-2</sup> —		— Tiller m <sup>-2</sup> —		— Tiller m <sup>-2</sup> —	
S9240*	1570	1130	1160	980	1340	1110
Kirk	2040	1410	1720	1310	1450	1300
CD-II	1950	1490			1570	1320
Nordan	2100	1420	1630	1260		
Parkway	3380	2350	2130	1950		
Mean	2210	1500	1660	1370	1450	1240
LSD(0.05)	240	290	140	520	210	ns

\* The S9240 population used in trials 1994A and 1994B is S9240F and the population used in trial 1997 is S9240J.

#### 4.4.5 Tiller angle

There were no significant ( $P < 0.05$ ) differences among the S9240 lines for either interior or perimeter tiller angle in trial 1996A (Table 4.11). There were no significant ( $p < 0.05$ ) differences between the four S9240 populations and, CD-II and Kirk for interior tiller angle (Table 4.12). The perimeter tiller angle of Kirk was not significantly ( $p < 0.05$ ) different than the four S9240 populations, but CD-II perimeter tillers were significantly ( $P < 0.05$ ) less upright than those of the S9240 populations.

**Table 4.11:** Interior and perimeter tiller angle (from horizontal) of S9240 crested wheatgrass grown in a spaced-planted nursery in 1999 and 2000

Line	Interior Tiller Angle (°)		Perimeter Tiller Angle (°)	
	1999	2000	1999	2000
S9240 H	80.5	82.1	62.7	66.3
S9240 I	80.1	78.5	61.8	63.3
S9240 J	76.4	78.8	66.1	59.8
S9240 K	78.3	76.7	59.7	59.1
LSD(0.05)	ns	ns	ns	ns

**Table 4.12:** Estimate of the difference in mean tiller angle between four S9240 crested wheatgrass populations and CD-II and Kirk based on data taken from a spaced-planted nursery in 1998, 1999 and 2000

Contrast	Estimate of difference							
	Interior				Perimeter			
	1999		2000		1999		2000	
	Degrees							
S9240/CD-II	2.9	ns	1.4	ns	9.6	*	7.8	**
S9240/Kirk	-2.1	ns	0.2	ns	5.1	ns	4.3	ns

\* = significant at  $p = 0.05$  \*\* = significant at  $p = 0.01$  ns = no significant difference

## 4.5 Discussion

S9240 was consistently taller and had fewer, heavier tillers than all other lines in both sward-seeded stands (Tables 4.5 and 4.10) and spaced-planted nurseries (Table 4.7). S9240 also had narrower crowns (Tables 4.2 and 4.4), and heavier, more upright tillers (Table 4.9 and 4.12) than other crested wheatgrass lines, except Kirk. The similarities, in some morphological characteristics, between Kirk and S9240 are not unexpected, as both are tetraploid *A. cristatum* types, and a certain amount of Kirk germplasm has been included in the development of the S9240 populations. The diploid cultivar Parkway was shorter, wider crowned and had a greater number of finer tillers than the tetraploid lines.

An inverse relationship between tiller size (ie: height and weight) and tiller number has been documented in a number of grass species, particularly in tall fescue (Sugiyama 1995; Zarrouh et al. 1983a; Zarrouh et al. 1983b; Zarrouh and Nelson 1980). The S9240 population was selected extensively for height. This could potentially have altered other tiller traits, such as tiller weight, tiller density and tiller angle. Zarrouh et al. (1983b) found that when a tall fescue population was divergently selected for individual tiller yield, genotypes selected for high individual yield had fewer, taller tillers than those selected for low individual tiller yield. This is in agreement with Sugiyama (1995) who concluded that there was a negative correlation between the growth of existing tillers and the production of new tillers.

Ploidy increases are also known to affect tiller characteristics (Tai and Dewey 1966). Studies on the effect of increased ploidy on crested wheatgrass (Tai and Dewey 1966), Russian wildrye (Berdahl and Reis 1996), orchardgrass (*Dactylis glomerata* L.) (Bretagnolle and Lumaret 1995), and barley (*Hordeum vulgare* L.) (Sicher et al. 1984),

have all found that increasing ploidy level reduced the number of tillers. This is consistent with the differences observed between S9240 and Parkway. In the forage grass studies an increase in tiller height in tetraploids was consistently observed, but the study on barley found that the tillers of tetraploid offspring were shorter than the diploids. The results of the current study also demonstrate an increase in tiller height in tetraploid lines over their diploid progenitors.

Bretagnolle and Lumaret (1995) and Berdahl and Reis (1996) found that tetraploid populations of Russian wildrye and orchardgrass, respectively, had improved vigour over their diploid source populations. In contrast to this, Tai and Dewey (1966) concluded that there was no agronomic merit to colchicine-induced tetraploid crested wheatgrass. Sicher et al. (1984) stated that autopolyploidization of outcrossing species may result in inbreeding depression in the first polyploid generation while Bretagnolle and Lumaret (1995) concluded that the immediate results of polyploidization could be changed by successive generations of selection. Both inbreeding depression and lack of selection could explain the negative effects of doubling *A. cristatum* found by Tai and Dewey (1966).

Since *Agropyron spp.* are tussock grasses, the only manner in which they can spread is by tillering. If all tillers emerged perfectly perpendicular to the soil surface then, assuming a mean tiller width of 3 mm, it would take ten years for a tussock to spread 30 cm (Olson and Richards 1988a). This is not the case in the present study, as some cultivars, such as CD-II in the spaced-planted nursery, have spread over 30 cm in only five years (Table 4.4). Thus the angle at which tillers depart from the crown affects the degree of spread. The increased perimeter tiller angle of S9240 populations in trial

1996A, likely contributed to the reduced crown diameter of S9240 populations compared to CD-II.

The reduced degree of spread of S9240 may also result in improved compatibility with legumes in mixed stands. Zarroug et al. (1983a) found that high individual tiller yield populations of tall fescue, which had a lower tiller density, were less competitive with birds foot trefoil (*Lotus corniculatus* L.) when the two crops were inter-seeded. This is of significance, as crested wheatgrass stands benefit greatly from the inclusion of a legume (usually alfalfa) into the crop mixture, but often out-compete it (Berdahl et al. 1989). If the more upright S9240 is less competitive, as was the lower tiller density tall fescue (Zarroug et al. 1983a), then perhaps the legume component of a forage stand could be maintained for a longer period of time, therefore improving the quality and yield. To verify this, planting density and row spacing trials would have to be carried out on mixed stands of S9240 and a legume.

#### **4.6 Conclusions**

S9240 has a narrower crown and is taller than diploid *A. cristatum* cv Parkway, tetraploid *A. desertorum* cv Nordan, and tetraploid *A. desertorum* x *A. cristatum* cv CD-II, and is taller but not significantly narrower than tetraploid *A. cristatum* cv Kirk. The lack of difference between Kirk and S9240 for some characters is not surprising as both are tetraploid *A. cristatum*, as there has been some Kirk germplasm included the development of S9240. The increased height and reduced crown diameter of S9240 appears to be related to a reduced number of tillers which were heavier and had a more upright growth pattern, when compared to other cultivars.

## 5. HERITABILITY OF CROWN DIAMETER AND PLANT HEIGHT IN S9240 CRESTED WHEATGRASS

### 5.1 Abstract

S9240 crested wheatgrass (*Agropyron cristatum* (L.) Gaertn) was produced by colchicine-doubling the chromosome number of selected, diploid, *A. cristatum* cv. Parkway plants, followed by several cycles of selection for height, seed size, floret fertility, and forage yield. Some inclusion of tetraploid *A. cristatum* cv. Kirk germplasm occurred during the selection cycles. The goal of this study was to estimate the heritability of plant height and crown diameter of two S9240 populations. Heritabilities were determined from measurements taken in 1999 and 2000 from two spaced-planted nurseries; one contained polycross half-sib families and the other contained topcross derived half-sib families. There were no significant variances among half-sib families, for either trait in the polycross trial. There were significant variances among the topcross half-sib families for both plant height and crown diameter. There were no significant family x year interactions indicating that selection based one years data may be effective. Heritability estimates from the topcross derived half-sib families were: plant height 67%; spring crown diameter 61%; and summer crown diameter 81%. These moderate to high heritabilities indicate that in a genetically variable population these traits could be altered through selection.

## 5.2 Introduction

Members of the crested wheatgrass complex (*Agropyron spp.*) are important forage grasses in the central and northern great plains and intermountain regions of North America, which receive between 200 and 450 mm of annual precipitation (Mayland et al. 1992). Smoliak and Dormar (1985) estimated that there were approximately 1 million ha of *Agropyron cristatum* (L.) Gaertn. in use in rangelands in western Canada, while Mayland et al. (1992) estimated that there were over 6 million ha of *Agropyron spp.* in production in North America.

Of the forage grasses in the perennial *Triticeae*, *Agropyron spp.* have received the most attention from plant breeders (Asay 1992). Traits which have been the subject of improvement programs include forage yield, seed yield, forage quality (digestibility, grass tetany potential, and protein concentration), and stand establishment. The first improved population was the diploid ( $2n = 2x = 14$ ) *A. cristatum* cv. Fairway, which was released in 1932. Subsequently a number of tetraploid ( $2n = 4x = 28$ ) cultivars, such as *Agropyron fragile* cv P-27 (Roth) Candargy, *Agropyron desertorum* cv. Nordan (Fisch. ex Link) Schlut, and *A. cristatum* cv. Kirk, were released in 1953, 1953 and 1987, respectively. In 1995 the hexaploid ( $2n = 42$ ) *A. cristatum* cv Douglas was released (Asay et al. 1995b). Breeding in crested wheatgrass has traditionally been focussed within ploidy levels (Asay 1992). USDA-ARS has, in the recent past, placed significant emphasis on inter-ploidy breeding and has released two cultivars (cv. Hycrest and cv. CD-II) in 1987 and 1997, respectively. These were derived from crosses between colchicine doubled *A. cristatum* and natural tetraploid *A. desertorum* (Asay et al. 1986).

Knowles (1950), conducted a study of combining ability in crested wheatgrass and

concluded that progeny evaluation was the best manner to evaluate plant material, due to a low correlation between parent and progeny values. He also compared different levels of pollination control, from selfing to open pollination, and concluded that polycross derived half-sib families were superior as they were less labour intensive than diallel crosses, but provided better pollen control than open pollination. Ray et al. (1994) stated that the use of half-sib families was superior to full-sib or S1 families, as these latter types of offspring may inflate heritability estimates due to confounding of dominance and additive x dominance variance.

Heritability estimates are important in breeding programs, as they can be used to determine the degree of genetic control over a trait, the most appropriate selection method for that trait, and for predicting gain from selection (Poehlman and Sleper 1995). Calculated heritabilities often only apply to the population from which they are estimated, unless the estimate is consistent between years, environments and trials. This is the result of differing allele frequencies among species, populations within a species, and selection cycles within a population. In addition, the expression of these alleles may vary due to environment (Ray et al. 1994).

Ray et al. (1994) conducted a study on the genetic variance of a number of traits in tetraploid crested wheatgrass, grown under competitive conditions. They found moderate to high heritabilities for forage yield, tiller height, first cut vigour, regrowth vigour, but found low or no heritability for tiller density and proline content. In a study on diploid crested wheatgrass grown in competitive conditions, Ray et al. (1997) found similar results to the tetraploid population except that estimates were slightly lower and proline content was moderately heritable. They did not estimate the heritability of tiller density.

Lamb et al. (1984) examined the effect of genotype x environment interaction on yield components in crested wheatgrass grown in two very diverse environments. They found that the genotype x environment interaction was only significant for first cut yield and protein.

S9240 is an experimental population of crested wheatgrass, which was developed at the Saskatoon Research Centre of Agriculture and Agri-Food Canada. It is based mainly on colchicine-doubled individuals of diploid *A. cristatum* cv. Parkway, with some inclusion, during the selection cycles, of germplasm from the natural tetraploid *A. cristatum* cv. Kirk. Since 1984 this population has been through several cycles of selection for traits such as, height, seed weight, seed yield, floret fertility, and forage yield. Observations made in the field indicate that S9240 is taller than other crested wheatgrass cultivars and appears to have a narrower crown.

The objective of this experiment was to determine the heritability of plant height and crown diameter in two S9240 populations.

### **5.3 Materials and methods**

#### **5.3.1 Experimental design and plant material**

Trials were conducted at the research farm of Agriculture and Agri-Food Canada's Saskatoon Research Centre. Spaced-planted (1 m centres) nurseries were established in 1996 and 1998 (will be referred to as trial 1996B and 1998) with three replications of eight, and ten plants per replication, respectively. Trial 1996B consisted of 12 polycross derived half-sib families. The polycrosses, made among selected clones of S9240 G and S9240 H, were made in the field in 1995, seed was harvested and seedlings were established indoors and transplanted to the field in the spring of 1996.

Trial 1998 consisted of 32 topcross derived half-sib families. These crosses were made in 1997 by applying bulk pollen, from approximately 80 plants of Kirk, to bagged selected clones of S9240F.

### 5.3.2 Data collection

Data were collected in 1999 and 2000. Plant height was determined after anthesis by measuring the tallest tiller on each plant. The distance between the outermost tillers was measured to determine the crown diameter before tiller elongation in the spring, and in the summer after the plots had been harvested.

### 5.3.3 Statistical analysis

For determining heritabilities, an analysis of variance was conducted on data from both trials (1996B and 1998) as a split-block in time, on a plot mean basis (Steel and Torrie 1960). The analysis of variance was performed using the general linear means (GLM) procedure in SAS (SAS Institute, Inc. 1985). All factors (family, rep and year) were considered random. Tests of significance were conducted based on the expected mean squares. Variances were calculated from expected mean squares, with negative variances being considered to be equal to zero. The heritability was calculated as a ratio of additive genetic variance to total variance (Nguyen and Sleper 1983b):

$$h^2 = \sigma_f^2 / (\sigma_f^2 + \sigma_{fr}^2/r + \sigma_{fy}^2/y + \sigma_e^2/yrf) \quad (5.1)$$

Where  $\sigma_f^2$ ,  $\sigma_{fr}^2$ ,  $\sigma_{fy}^2$ , and  $\sigma_e^2$  are variance among half-sib families, variance due to family x rep interaction, variance due to family x year interaction, and experimental error respectively. Standard errors were calculated by estimating variances of mean squares. (Pesek and Baker 1971)

## **5.4 Results and discussion**

### **5.4.1 ANOVA results**

In trial 1996B there were no significant ( $p < 0.05$ ) variances among half-sib families for either height or crown width (Table 5.1) indicating a lack of additive genetic variation of these two traits in this population. This is in disagreement with the findings of Ray et al. (1994; and 1997), who reported significant variance among half-sib families for height in diploid and tetraploid crested wheatgrass. Lamb et al. (1984) reported significant variance among half-sib families for first year spread and tiller height in tetraploid crested wheatgrass.

There were significant ( $p < 0.05$ ) year effects on both plant height and crown width (Table 5.1). These significant effects on plant height may be due to the variable amounts of precipitation in the spring of 1999 and 2000, resulting in much shorter plants in 2000. The increase in mean crown diameter between years would account for the significant year effects on this trait.

**Table 5.1:** Results of analysis of variance; expected mean squares, mean squares, and significance levels for 1999 and 2000 data from trial 1996B

Source of Variation	df	Expected Mean Squares	Mean Squares	
			Crown Width	Plant Height
Family	11	$\sigma_e^2 + 2.36\sigma_{yf}^2 + 2\sigma_{fr}^2 + 4.73\sigma_f^2$	9.03	24.8
Replication	2	$\sigma_e^2 + 8.5\sigma_{yr}^2 + 2\sigma_{fr}^2 + 17\sigma_r^2$	15.05	114.92
F x R	15	$\sigma_e^2 + 2\sigma_{fr}^2$	8.03	51.01
Year	1	$\sigma_e^2 + 1.98\sigma_{yf}^2 + 7.94\sigma_{yr}^2 + 23.82\sigma_y^2$	304.12 *	4490.98 *
Y x R	2	$\sigma_e^2 + 8.50 \sigma_{yr}^2$	8.27	16.23
Y x F	11	$\sigma_e^2 + 2.36\sigma_{yf}^2$	2.11	54.17
Error	15	$\sigma_e^2$	46.65	49.33

e, f, r, y are error, number of half-sib families, number of replications, and number of years.

\* Indicate significance at the 0.05 level.

In trial 1998, there were significant variances ( $p < 0.05$ ) among half-sib families for all three traits measured, and these were consistent over years, as there were no significant family x year interactions (Table 5.2). This indicates that crown diameter and plant height are under additive genetic control in this population which is consistent with the results of Ray et al. (1994; and 1997), and Lamb et al. (1984).

Years and family x replication interactions were also significant ( $p < 0.05$ ) sources of variations for all three traits. The year effect is likely significant in trial 1998 for the same reasons given previously for trial 1996B (ie precipitation and crown spread). Year x replication ( $p < 0.05$ ) interactions were significant for spring crown diameter and plant height but not for summer crown diameter. The lack of significant ( $p < 0.05$ ) year x family interactions indicates that selection for height and crown diameter could be made from

only a single year's data. Ray et al. (1997) also found no family x year interactions for tiller height in diploid crested wheatgrass, but Ray et al. (1994) found a significant family x year interaction in tetraploid crested wheatgrass; however, it was small compared to the family variance.

**Table 5.2:** Results of analysis of variance; expected mean squares, mean squares, and significance levels for 1999 and 2000 data from trial 1998

Source of Variation	df	Expected Mean Squares	Crown Diameter		Plant Height
			Spring	Summer	
Family	31	$\sigma_e^2 + 2.55\sigma_{yr}^2 + 2\sigma_{fr}^2 + 5.10\sigma_f^2$	9.66 *	11.35 *	108.92 **
Replication	2	$\sigma_e^2 + 25\sigma_{yr}^2 + 2\sigma_{fr}^2 + 50\sigma_f^2$	42.48	1.59	183.14
F x R	48	$\sigma_e^2 + 2\sigma_{fr}^2$	4.45 **	3.70 *	41.45 **
Year	1	$\sigma_e^2 + 2.16\sigma_{yr}^2 + 23.04\sigma_{yr}^2 + 69.12\sigma_y^2$	1575.94 *	1840.86 *	25396.35 **
Y x R	2	$\sigma_e^2 + 25\sigma_{yr}^2$	25.15 ***	4.56	211.50 **
Y x F	31	$\sigma_e^2 + 2.54\sigma_{yr}^2$	1.96	1.1	20.26
Error	48	$\sigma_e^2$	1.87	2.22	17.61

e, f, r, y are error, number of half-sib families, number of replications, and number of years.  
 \*, \*\*, \*\*\* Indicate significance at the 0.05, 0.01 and 0.005 level respectively

There are two possible explanations for the different family effects between trials 1996B and 1998. Trial 1998 contained 32 half-sib lines, while trial 1996B had only 12 half-sib lines. The smaller sample size may have not allowed the difference between lines to be evident and resulted in a lower number of degrees of freedom to test for significance. Ray et al. (1994; and 1997) and Ray and Harms (1994) found that, when they contrasted the heritability estimates obtained from 30 and 300 half-sib families of crested wheatgrass and western wheatgrass (*Pascopyrum smithii* (Rydb) Love), the heritability estimates were quite similar, but that the precision of the estimates was lower for the estimate from only 30 half-sibfamilies.

Trial 1996B had been harvested for seed in years previous to this trial and, there is the possibility that there were volunteer plants established within the trial, which could affect measurements for crown width and possibly plant height. In addition, inter-plant cultivation, which is practised for weed control, can alter the crown width dimensions of the plants, effectively imposing a maximum crown diameter. This would have masked differences among half-sib families in trial 1996B, as the older plants may have approached this maximum diameter.

#### **5.4.2 Heritability estimates**

Heritability estimates of crown diameter and plant height were only calculated for trial 1998 as there was no significant variation among families in trial 1996B.

Heritability estimates for all three traits exceeded their standard errors (Table 5.3).

Ray et al. (1994; and 1997) estimated heritability of tiller height to be 63% and 68% for diploid and tetraploid crested wheatgrass, respectively, which is similar to the heritability estimate of 67% which was estimated from trial 1998 (Table 5.3) . There are

no estimates in the literature for the heritability of crown diameter of crested wheatgrass, although Lamb et al. (1984) reports significant differences among families of crested wheatgrass for amount of first year spread. However Ray et al. (1994) reported 0% heritability for tiller density in tetraploid crested wheatgrass stands. This is of significance, as the number of tillers that a tussock contains is a major component in determining its diameter (Nguyen and Sleper 1983a). The 0% heritability that Ray et al. (1994) reported could be due to the age of the stand, as the measurements were taken in the two years after establishment, and the number of tillers may not have stabilized. Another consideration could be that the sample size (which was three 5 cm row sections per plot) which they looked at was not large enough to accurately determine the tiller density.

The heritability of crown diameter was higher when estimated on the summer sampling data, than it was from the spring sampling. This is due to the mean square of summer crown diameter being larger and the family x replication mean square being larger for spring crown diameter. One potential explanation could be that measurements of summer crown diameter were taken shortly after forage harvest, resulting in the margins of the tussock being more defined and diameter could be more accurately measured.

**Table 5.3:** Narrow-sense heritability estimates ( $h^2$ ) of spring and summer crown diameter and plant height based on two years data from trial 1998

Character	Timing	$h^2$	S.E.
Plant Height	Summer	0.67	0.12
Crown Diameter	Spring	0.61	0.15
	Summer	0.82	0.07

There are biases which must be taken into account when interpreting the heritability estimates obtained in this study. The heritabilities were estimated using data which was collected in only a single location, and as a result genotypic estimates are biased by an amount equal to the genotype x location interaction and the genotype x year x location interaction (Nguyen and Sleper 1983b). This may, or may not have a large effect on these estimates of heritability. Lamb et al. (1984), in a study on the effect of location and family x location, found that there was little family x location effect on height, even though there was a large location effect. If this is the case then the ranking of height may be stable across environments, and the estimates provided here may be relatively unbiased.

Trial 1998 consisted of topcross derived half-sib families. Nguyen and Sleper (1983b) outline the difficulties which are associated with using this type of cross in quantitative genetic studies, specifically that it provides no estimation of the additive genetic variance in the parent population. Waldron et al. (1998), in a study of heritability of winter hardiness in a topcross population of perennial ryegrass, stated that although there were limitations to making heritability estimates with a topcross population, they were not of great concern, as long as the estimate of heritability was applied only to the

population from which it was determined. Knowles (1950) also expressed some reservations as to the use of topcrosses, as he found that there was a great deal of difference between individual pollen donors. This may have been of limited significance in the present study as bulk pollen from a large number of plants was used as the pollen donor, therefore reducing potential impact of any one male parent.

S9240 is an autotetraploid population, which also biases the estimate of heritability. Nguyen and Sleper (1983b), stated that heritability estimates are based on an assumption of diploid and Mendelian inheritance. When these assumptions are met the covariance among half-sib families is a linear function of additive and additive x additive epistasis effects. When a tetraploid population is considered the heritability may be over estimated as tetraploids may transmit dominance effects (Ray et al. 1997). Ray et al. (1997) found heritability estimates for traits such as forage yield, tiller height, first cut vigour, and regrowth vigour in diploid crested wheatgrass were lower than those heritability estimates for the same traits in tetraploid crested wheatgrass, except for tiller height.

## **5.5 Conclusions**

There was significant additive genetic variance for crown diameter among half-sib families in a topcross nursery, but not in a polycross nursery. This may be a reflection of the smaller sample size, inter-plant cultivation and the possible presence of volunteer plants in trial 1996B. The heritability estimates of 67% for height and 61% and 82% for spring and fall crown diameter, respectively, indicate that in a genetically variable population these traits could be modified through selection.

## 6. THE GENETIC RELATIONSHIP OF SELECTED CRESTED WHEATGRASS (*AGROPYRON* SPP) LINES AND SPECIES BASED ON AFLP MARKERS

### 6.1 Abstract

The crested wheatgrass complex (*Agropyron* spp. Gaertn) consists of persistent, drought tolerant, tussock grasses which originate from the steppes of central Asia and is of importance as range grasses on the great plains of North America. Twelve lines from four *Agropyron* spp. and two interspecific hybrid lines were compared using amplified fragment length polymorphism (AFLP) markers to examine the inter line relationships and to compare variance within and among lines. Six were classified as *A. cristatum* and included diploid, natural tetraploid, induced tetraploid, and hexaploid lines. Both *A. desertorum* and *A. fragile* were represented by tetraploid lines. The interspecific lines were synthetic lines selected from an interspecific hybrid (*A. cristatum* X *A. desertorum*) line. Also included was an accession of the wild diploid species *A. mongolicum*. Euclidian distances were calculated based on marker polymorphism and a cluster analysis was performed. Fairway, Parkway and S9240 grouped closely and it is likely that these are the only true *A. cristatum* lines included in this study. Hycrest, CD-II were less dissimilar to the *A. cristatum* cluster than Kirk. This may indicate that Kirk contains some *A. desertorum* germplasm. *A. desertorum* cv. Nordan clustered farthest from the *A. cristatum* with the exception of *A. mongolicum*. This is in agreement with the suggestion that *A. desertorum* is an allopolyploid of *A. cristatum* and *A. mongolicum*. The lines Vavilov,

Douglas and Ephraim clustered with *A. desertorum* even though they are not classified as *A. desertorum*.

An analysis of molecular variance (AMOVA) was conducted on fifteen individuals from six of the lines (Fairway, Parkway, S9240, Kirk, Hycrest and Nordan). The majority (88%) of the molecular variance was accounted for by within line variance, which was expected in these outcrossing lines; however, significant differences were still detected between all six lines. A cluster analysis of  $\Phi_{st}$  (phi-statistic) produced a dendrogram very similar to the one produced using line bulks. The value of AMOVA analysis in this type of study is discussed.

## 6.2 Introduction

The genus *Agropyron* Gaertn. consists of 10 to 15 species, all of which have the P genome, and is referred to as the crested wheatgrass complex (Asay and Jensen, 1996). The genus is characterized by persistent, drought resistant, caespitose grasses which originate from the steppes of central Asia (Rogler and Lorenz, 1983). The complex is made up of a polyploid series of diploid ( $2n = 2x = 14$ ), tetraploid ( $2n = 4x = 28$ ) and hexaploid ( $2n = 6x = 42$ ) species with the type species being *Agropyron cristatum* (L.) Gaertn.

There are three crested wheatgrass species of commercial importance in North American rangelands. Fairway crested wheatgrass (*A. cristatum*) occurs in diploid, tetraploid and hexaploid form. Standard crested wheatgrass (*Agropyron desertorum* (Fisch. ex Link) Schlut) and Siberian crested wheatgrass (*Agropyron fragile* (Roth) Candargy) are both tetraploid.

The taxonomy of *Agropyron* spp. is described as complex, and sometimes as confusing (Dewey, 1969), and has been subject to “a multitude of taxonomic binomials” (Dewey and Pendse, 1967). This is mostly due to a continuum of spike morphology, which is the main morphological feature used to differentiate species in this genus. Spike morphology varies, in a continuous fashion, from broad, pubescent, pectinate spikes to narrow, linear, glabrous spikes (Dewey and Asay 1982). Taxonomy is further confounded by geographical overlap and a high level of cross-compatibility between the species.

The relationship between *A. cristatum* and *A. desertorum* has been subject to extensive study. Knowles (1955) felt that *A. desertorum* was either an autopolyploid of *A. cristatum*, or an amphiploid of *A. cristatum* and some other closely related diploid. A

number of papers written in the 1960's (Dewey 1961, Tai and Dewey 1966, Dewey and Pendse 1967, Dewey and Pendse 1969 and Dewey 1969) concluded, based on meiotic chromosome pairing, that *A. desertorum* was an autotetraploid of *A. cristatum*, or a segmental allotetraploid. Schulz-Schaeffer et al. (1963) felt that Dewey's (1961) interpretation of polyhaploid chromosome pairing was incorrect and that *A. desertorum* is a segmental allotetraploid, and not an autotetraploid. Dewey and Pendse (1967) stated that all crested wheatgrasses had the same basic genome which they called the "C" genome, but this was subsequently changed to the "P" genome (Dewey 1984).

Taylor and McCoy (1973), using chromatographic and karyotypic analysis, concluded that *A. desertorum* had formed through a hybridization of *Agropyron imbricatum* (M. B.) Roem et Schult. and *Agropyron pectiniforme* Roem et Schult, followed by a chromosome doubling. *A. imbricatum* and *A. pectiniforme* are very similar morphologically to each other and to *A. cristatum* (differentiated by level of pubescence on, and spacing between the spikelets (Nevski 1934)) and both have been reclassified as subspecies of *A. cristatum* (Tzvelev 1976).

Dewey (1981) reported on the existence of a second diploid species, *Agropyron mongolicum* Keng., which originated from China. *A. mongolicum* is differentiated from *A. cristatum* in that it has narrow, linear spikes. All other diploid accessions that had been collected at that point had broad spikes, which is characteristic of *A. cristatum*. Hsiao et al. (1986) reported that *A. cristatum* and *A. mongolicum* have similar genome length and differ only by small structural rearrangements on some chromosomes. Hsiao et al. (1989) noted that interspecific hybrids of *A. cristatum* and *A. mongolicum* had a similar spike morphology to *A. desertorum*. Asay et al. (1992) concluded, through a multivariate

morphological analysis, that *A. desertorum* was the result of a hybrid between *A. cristatum* and *A. mongolicum*, followed by a chromosome doubling. It was also postulated that *A. fragile* was an autopoloid of *A. mongolicum*.

Recently Vogel et al. (1999) conducted a study of genome length in the perennial Triticeae. They concluded that the genome length of *A. desertorum* was more consistent with an autopoloid of *A. cristatum* than that of an allotetraploid between *A. cristatum* and *A. mongolicum*.

To further complicate the matter there are variations in ploidy levels within species and interspecific hybrids are common. The lines Kirk (tetraploid) and Douglas (hexaploid) are both described as *A. cristatum*. Hybrids between species of different ploidy levels have been used for genetic improvement of crested wheatgrass (Asay et al. 1986). The line Hycrest was developed from crossing colchicine doubled *A. cristatum* with *A. desertorum*, with successive generations backcrossed to both parental lines (Asay et al. 1985).

Molecular markers are an efficient method for determining genetic relationships as they are not affected by the environmental or epistatic interactions that may affect morphological traits (Schut et al. 1997). Amplified fragment length polymorphism (AFLP) is a polymerase chain reaction based fingerprinting technique which relies on the selective amplification of restriction fragments (Vos et al. 1995) and it has the ability to detect large numbers of polymorphisms with single primer pairs, without any prior knowledge of the target genome. Results are highly repeatable, as there are very stringent reaction conditions for primer annealing. AFLP's have been used to determine diversity within species such as *Vigna anularis* (Willd.) (Yee et al. 1999), and *Camellia sinensis*

(L.) O. Kuntze (Paul et al. 1997). Sharma et al. (1996) compared the use of AFLPs and RAPDs in examining the phylogeny among *Lens spp.* They found that the results obtained from AFLPs were similar to those found using RAPDs but were of much higher resolution.

Analysis of molecular variance (AMOVA) is a statistical analysis, developed by Excoffier et al. (1992), which apportions genetic variance into within and among line variance. It functions by converting inter-individual distance matrices into an analysis of variance (Huff et al. 1993). This tool allows for the separation of lines even when the majority of genetic variance is expressed within lines, such as in outcrossing species. The  $\Phi_{st}$  (phi-statistic) calculated by AMOVA is proportional to the amount of the total variation accounted for by the variation between two lines and is an indication of the inter-line distance between the two lines (Huff 1997).

The objectives of this study were: 1) to examine the phylogeny of selected lines from the genus *Agropyron* using AFLPs; 2) to examine the partitioning of within and among line variances of selected common crested wheatgrass lines and; 3) To compare cluster analyses using  $\Phi_{st}$  and Euclidian distance from line bulks.

### **6.3 Materials and methods**

#### **6.3.1 Plant material**

Twelve lines were examined in this trial; six from *A. cristatum*, one each from *A. desertorum*, *A. mongolicum* and *A. fragile*, two interspecific hybrid lines and a line of unknown classification (Table 6.1). Individual plants were grown in root trainers in the greenhouse until they reached the three to four leaf stage, when tissue samples were collected.

Table 6.1: Plant material included in bulk and individual AFLP analysis

Species	Line	Description	Included in AMOVA
<i>A. cristatum</i>	Fairway	Diploid <i>A. cristatum</i> cultivar released in 1932 (Elliott and Bolton 1970)	Yes
	Parkway	A diploid, 16 clone, synthetic cultivar derived from selections from Fairway (Elliott and Bolton 1970)	Yes
	Kirk	Natural tetraploid cultivar selected from accessions introduced from Finland (Knowles 1990)	Yes
	S9240	Induced tetraploid experimental line based on colchicine doubled individuals selected from the cultivar Parkway	Yes
	Ephraim	Tetraploid rhizomatous cultivar based on an accession from Ankara, Turkey	
	Douglas	Hexaploid cultivar derived from crosses made between natural hexaploid accessions from the former USSR, Turkey and Iran (Asay et al. 1995)	
<i>A. desertorum</i>	Nordan	Tetraploid standard wheatgrass cultivar (Elliott and Bolton 1970)	Yes
<i>A. fragile</i>	Vavilov	Tetraploid 16 clone synthetic Siberian crested wheatgrass cultivar (Asay et al. 1995)	
		Diploid wild Species from China	
Hybrid	Hycrest	An 18 clone tetraploid synthetic cultivar. Clones were selected from a line of <i>A. cristatum</i> x <i>A. desertorum</i> interspecific hybrids (Asay et al. 1985)	Yes
	CD-II	A 10 clone tetraploid synthetic, selected from the cultivar Hycrest (Asay et al. 1997)	
Unknown	S-8959	Unknown tetraploid crested wheatgrass line originating from the former USSR	

### 6.3.2 Tissue Collection and DNA Extraction

Fifteen individuals from each of the twelve lines were sampled. The tissue was stored at  $-80^{\circ}\text{C}$  for two days and then freeze dried. Plant tissue was disrupted in a bead-mill for 10 minutes. DNA was extracted using Qiagen columns, stained using Hoechst 33258 and then quantified on a fluorimeter. DNA concentration was standardized to 25 ng/ $\mu\text{l}$ . To form line bulks equal amounts of DNA from all individuals within a line were combined.

### 6.3.3 AFLP analysis

The procedure followed was explained in detail by Vos et al. (1995). To summarise, a 250 ng of sample DNA was digested using *EcoRI* and *MseI* restriction enzymes. Adapters were ligated to the digested DNA with T4 DNA ligase.

The adapter sequences were:

*EcoRI*:

5-CTCGTAGACTGCGTACC  
CATCTGACGCATGGTTAA-5

*MseI*:

5-GACGATGAGTCCTGAG  
TACTCAGGACTCAT-5

Preamplification reaction included one *EcoRI* primer (5-GACTGCGTACCAATTCA) and one *MseI* primer (5-GATGAGTCCTGAGTAAC) with one selective nucleotide (underlined), 1.5 mM  $\text{MgCl}_2$ , *Taq* DNA polymerase and 230  $\mu\text{M}$  of each dNTP. Twenty cycles were performed at  $94^{\circ}\text{C}$  for 30 s,  $56^{\circ}\text{C}$  for 60 s and then  $72^{\circ}\text{C}$  for 60 s.

Four *EcoRI* primers (5-GACTGCGTACCAATTC+ AGG, ACG, AAG and ACT )

were end labelled with [ $\gamma$ -P<sup>33</sup>] (2000 ci/mmol) in the presence of T4 DNA kinase. The *EcoRI* primers were each combined separately with eight *MseI* primers (5-GATGAGTCCTGAGTAA+ CAG, CAT, CTC, CTT, CTG, CTA, CGC, CAC) in selective amplification, which included 3 selective nucleotides. The reaction included *Taq* DNA polymerase, 1.5 mMol MgCl<sub>2</sub> and 230  $\mu$ M of each dNTP. Twenty three cycles were performed at 94 °C for 30 s, 56 °C for 30 s and then 72 °C for 60 s. After the reaction, formamide dye was added at 0.5 volume to the samples. The samples were then separated by electrophoresis on a 5% polyacrylamide gel for 2.5 hrs at 90 w. The gel was dried onto Wattman 3mm paper and exposed to Kodak Biomax™ film for 3 days.

#### **6.3.4 Analysis**

Primer pairs were chosen based on number and clarity of polymorphic bands. Markers were selected on the basis of definition and intensity of the bands, with intense bands being chosen. A binary matrix was formed where a band of a particular molecular weight was scored as present (1) or absent (0).

Of the thirty two primer pair combinations screened, eight were selected to be scored on the bulked line DNA and five were chosen for the individual analysis. There were between eleven and twenty nine markers derived from each primer pair for a total of 114 scored markers used in the analysis of the bulked lines. A total of 67 polymorphic AFLP markers were scored and used in the AMOVA analysis of the individuals. A smaller number of primer combinations and markers were scored in the individual analysis due to increased difficulty of scoring a large number of individuals on a gel and due to the increased resources required to analyse a large number of individuals.

##### **6.3.4.1 Line bulks**

Pairwise comparisons were conducted using Euclidian distances to calculate dissimilarity between the lines with the following formula:

$$e_{jk} = [\sum(x_{ij} - x_{ik})^2]^{1/2} \quad (6.1)$$

where  $x_{ik}$  is the value assigned to the  $i^{\text{th}}$  attribute of the  $k^{\text{th}}$  object. For example if particular band is present (1) in Parkway but not (0) in Kirk the equation would look like;  $e_{jk} = [\sum(1 - 0)^2]^{1/2}$ . NTSYSpc 2.01(Rohlf 1998) was used to calculate and form a matrix of Euclidian distances between the lines of crested wheatgrass. A cluster analysis based on the unweighed paired group analysis (UPGMA) using arithmetic averages (NTSYSpc 2.01) was performed.

#### 6.3.4.2 AMOVA analysis

Fifteen individuals from six lines were assayed (Table 6.1). Pairwise comparisons were conducted using the Euclidian metric of Excoffier et al. (1992):

$$e_{jk} = n[1 - 2n_{jk}/2n] \quad (6.2)$$

Arlequin (v 2.00; Schnieder et al. 2000) was used to calculate the distance matrix. AMOVA (v 1.55; Excoffier et al. 1992) estimated variance components for AFLP phenotypes. The variance was partitioned into variance between individuals within lines and variance among lines. Homogeneity of the variances between pairs of lines was tested using a Bartlett's test. AMOVA was also used to calculate  $\Phi_{st}$  (phi-statistic). A cluster analysis of the  $\Phi_{st}$ , based on UPGMA using arithmetic averages (NTSYSpc 2.01) was performed.

### 6.4 Results and discussion

#### 6.4.1 Line bulks

Of the six lines which were classified as *A. cristatum*, based on spike morphology,

Parkway, Fairway and S9240 was to be expected. The line Parkway was directly selected from Fairway while S9240 is made up predominantly of colchicine doubled individuals from Parkway. S9240 and Parkway being further apart may be due to the fact that some Kirk germplasm was used in the development of the former. Kirk, Ephraim and Douglas did not cluster with these three *A. cristatum*. It should be noted at this point that S9240, due to its tetraploid chromosome number, is morphologically much more similar to Kirk than to the diploid Parkway.

*A. mongolicum* clustered separately from all other lines. All crested wheatgrass species share the same genome (Dewey and Pendse 1967), but Hsiao et al. (1986) stated that there were some structural rearrangements between the P genome of *A. cristatum* and *A. mongolicum*. In the interests of clarity, in the following discussion, the P genome of *A. cristatum* will be referred to as "P<sub>c</sub>" and the P genome found in *A. mongolicum* as "P<sub>m</sub>".

Nordan, an *A. desertorum* line, clustered closer to *A. cristatum* than to *A. mongolicum*, but the relationship to either species was not close. These molecular data corroborate morphological and cytological evidence that *A. desertorum* is an allopolyploid of *A. cristatum* and *A. mongolicum* (Hsiao et al. 1986, Hsiao et al. 1989, and Asay et al. 1992). This would indicate that it has a genome make up of "P<sub>c</sub> P<sub>c</sub> P<sub>m</sub> P<sub>m</sub>".

Douglas, which is classified as *A. cristatum*, clustered closest to Nordan. A possible explanation for this is presented by Shultz-Schaeffer et al. (1963), who compared karyotypes from diploid and hexaploid *A. cristatum* and tetraploid *A. desertorum*. They found that hexaploids had two genomes which were similar to the one present in *A. cristatum*, and that the third genome was similar to the second genome of *A. desertorum*.

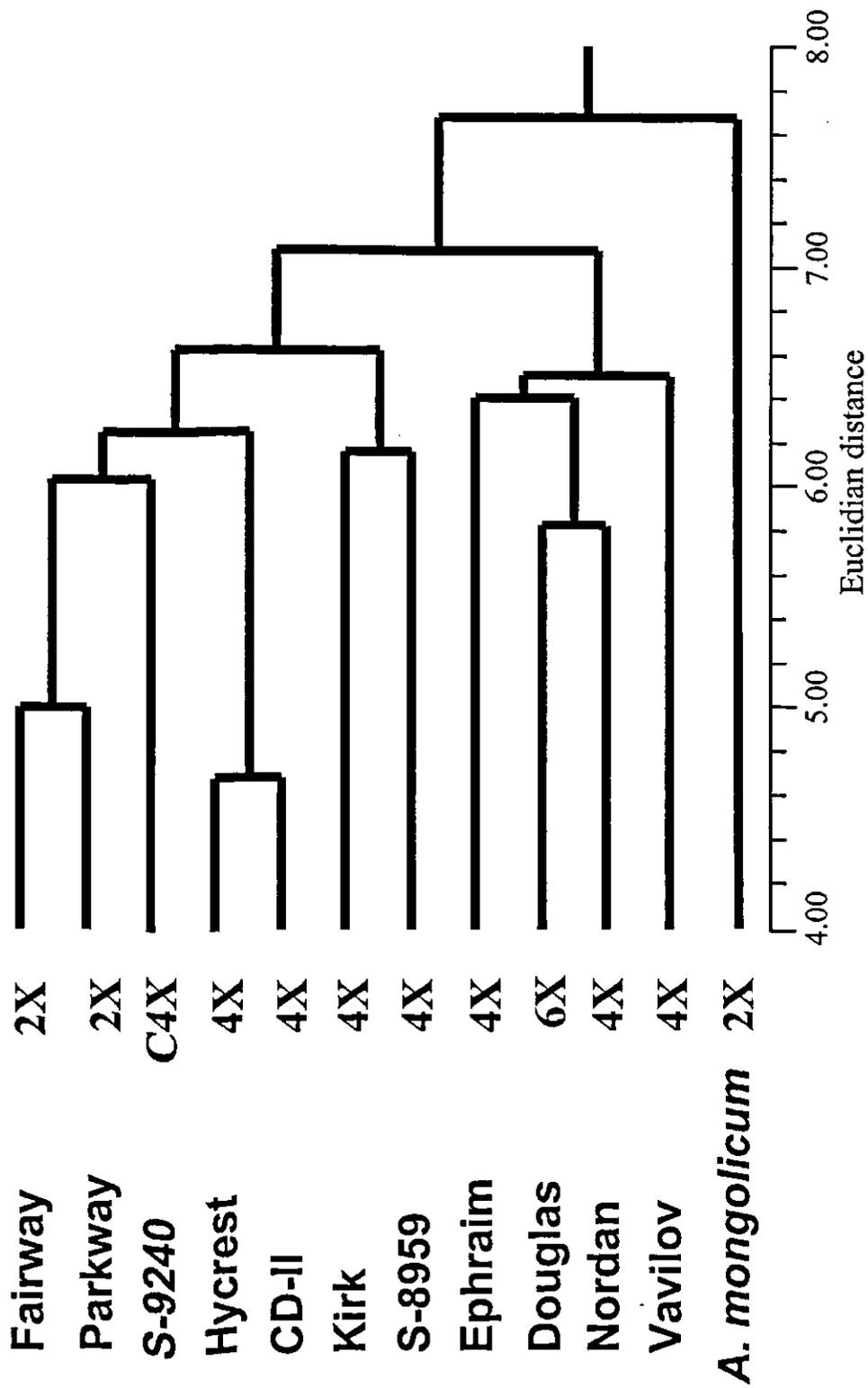


Figure 6.1: Dendrogram of twelve populations from the genus *Agropyron* based on Euclidian distances for 114 AFLPs

Using the genome labels indicated above Douglas would have a genome makeup of " $P_c P_c P_c P_m P_m$ ". This would result in a phenotype which was similar to *A. cristatum*, but would have a similar AFLP profile to *A. desertorum*, due to the presence of both genomes. Svitashv et al. (1996) was unable to differentiate between hexaploid and tetraploid *Elymus* L. species with similar genomic constitutions, using repetitive DNA sequences.

Hycrest, CD-II, Kirk and S-8959 clustered somewhat closer to *A. cristatum* than to *A. desertorum*. Hycrest and CD-II clustered the closest together of all lines, likely a reflection of CD-II being a synthetic line of individuals selected from Hycrest (Asay et al. 1997). The cross between a colchicine doubled *A. cristatum* ( $P_c P_c P_c P_c$ ) with *A. desertorum* ( $P_c P_c P_m P_m$ ) would result in progeny with a genome makeup of " $P_c P_c P_c P_m$ ". After successive generations this genome makeup could be altered by factors such as degree of homology between the  $P_c$  and  $P_m$  genomes, and selection. If the genome had remained as " $P_c P_c P_c P_m$ ", then it would be more likely that the two hybrid lines would have clustered with Nordan, for the same reason that Douglas clustered with the latter. Asay et al. (1986) examined 20  $F_5$  individuals from the hybrid line, during meiosis and stated that they were behaving as autotetraploids. The clustering of Hycrest and CD-II with *A. cristatum* could indicate that the genome of these lines is " $P_c P_c P_c P_c$ ".

Kirk and S-8959 clustered together. Kirk, which is classified as *A. cristatum*, was more distantly grouped to the *A. cristatum* cluster than two hybrid lines, Hycrest and CD-II. The original accession from which Kirk was selected was grown in close proximity to *A. desertorum* accessions, potentially allowing for outcrossing and incorporation of germplasm from the latter (Knowles unpublished). S-8959, a line of unknown species

also appears to be a hybrid line.

Ephraim grouped loosely with Nordan and Douglas even though it is classified as *A. cristatum*. Ephraim is the only rhizomatous line represented here, although Fairway individuals will sometimes produce short rhizomes. Tzvelev (1976) described, *Agropyron cimmericum*, which the main differentiating feature is the production of long branched rhizomes. The inclusion of a known *A. cimmericum* line would be required to confirm if Ephraim was a line of this species.

The line Vavilov was included as a representative of *A. fragile*, to determine if it is an autopoloid of *A. mongolicum* as has been suggested (Asay *et al.* 1992). In the varietal description of Vavilov it is indicated that extensive outcrossing has occurred between the *A. fragile* line used to form this line and *A. desertorum*, resulting in a mixed or hybrid line (Asay *et al.* 1995). This is demonstrated by the clustering of Vavilov with the Nordan cluster. For more conclusive results, on the phylogeny of *A. fragile*, a pure line would have to be used.

#### 6.4.2 AMOVA analysis

The majority of the variance (88%) was accounted for by within line variance (Table 6.2). The 12 % of the variance that was attributed to among line variance is relatively small, but is statistically significant ( $p < 0.001$ ). The higher percentage of within line variance is to be expected in *Agropyron spp.* as they are all obligate outcrossing species.

All pairwise tests of inter-line distances were significant ( $P < 0.01$ ) (Table 6.3), meaning that each line was differentiated from all other lines and the  $\Phi_{st}$  observed were not random. This is an indication of the ability of the AMOVA to detect differences

between closely related lines, even in the face of high within line. variance. Huff et al. (1993) found similar results using RAPD markers to apportion variance in buffalo grass (*Buchloë dactyloides* (Nutt.) Engelm.) into among individuals/within lines, among lines/within regions and among regions. They were able to differentiate both among lines/within regions and among regions , even though there was significant within line and within region variation

**Table 6.2:** Analysis of molecular variance (AMOVA) from 90 crested wheatgrass individuals from six different lines, represented by 15 individuals per line, based on 67 AFLP markers.

Source	df	Sum of Squares	Mean Squares	Variance components <sup>1</sup>	P-value <sup>2</sup>
Among Lines	5	187	37	1.70 (12%)	<0.001
Within Lines	89	1016	12	12.1 (88%)	<0.001

<sup>1</sup> Value in bracket indicates the percentage of the total

<sup>2</sup> Probability computed by non parametric procedures from 1000 data permutations

**Table 6.3:** Summary of pairwise comparisons (pairwise genetic distance -  $\Phi_{st}$ ) of six crested wheatgrass lines, represented by 15 individuals per line. All distances are highly significant (P<0.01)

	Fairway	Parkway	S9240	Kirk	Hycrest	Nordan
Fairway	-					
Parkway	0.03	-				
S9240	0.07	0.07	-			
Kirk	0.14	0.18	0.17	-		
Hycrest	0.08	0.12	0.12	0.11	-	
Nordan	0.15	0.19	0.22	0.06	0.12	-

All pairwise Bartlett tests for heteroscedasticity of variance proved to be significant ( $P < 0.01$ ) indicating that the level of molecular variance expressed within each line was different from the other five lines. S9240 had the largest within line sum of squares (Table 6.5) This may be a reflection of the fact that S9240 is an experimental line which has not been subjected to intense selection pressure.

Both Parkway and Hycrest are synthetic lines based on 16 and 18 individuals respectively (Elliot and Bolton 1970; Asay et al. 1985) which may explain the similar within line sum of squares. Fairway and Kirk are both mass selected lines (Elliot and Bolton 1970; Knowles 1990) but the sum of squares is larger for Fairway than for Kirk, which may be due to differences in the heterogeneity of the genetic base from which the lines were selected. Nordan had the smallest within line sum of squares, which may be a reflection of the line being based on only seven individuals selected out of an open-pollinated line (Elliot and Bolton, 1970).

It is worth noting that the within line sum of squares for the tetraploid lines were not greater than those of the diploid lines. Considering that the tetraploids have twice as many chromosomes it may have been expected that there would be a larger number of polymorphism. Stammers et al. (1995), in a phylogenic study of *Lolium* and *Festuca spp.*, also found that increased ploidy level and nuclear DNA content did not increase the numbers of RAPD polymorphisms detected, but presented no explanation for this phenomena. The lack of increased polymorphism in the tetraploid line may be due to the similarity of the genomes.

**Table 6.5:** Within line sum of squares for six crested wheatgrass lines, with 15 individuals per line

Line	Sum of Squares
Fairway	184
Parkway	181
S9240	195
Kirk	158
Hycrest	164
Nordan	133

#### 6.4.3 Comparison of AMOVA and bulk clustering

The dendrogram formed from a cluster analysis of  $\Phi_{st}$  values, from the analysis of individual plants within six lines, (fig. 6.2) is quite similar to a dendrogram of Euclidian distances among the same six lines using data from line bulks (fig 6.3). Although the respective distances between lines are proportionally different, the clustering is identical. This corroborates the data and conclusions which were drawn from the analysis of the line bulks.

Huff (1997) states that one of the great strengths of the AMOVA analysis is its ability to separate related lines even when obscured by extensive within line polymorphism. AMOVA also has the added ability to provide a test of significance to the estimate of genetic distance between lines. However, AMOVA requires the analysis of individual genotypes from within each population, which is more time and labour consuming than analysing population bulks. In the case of trying to differentiate closely related populations the use of AMOVA may be appropriate, but if the populations are relatively distinct then the increased time and labour required to analyse individual genotypes may not be justified.

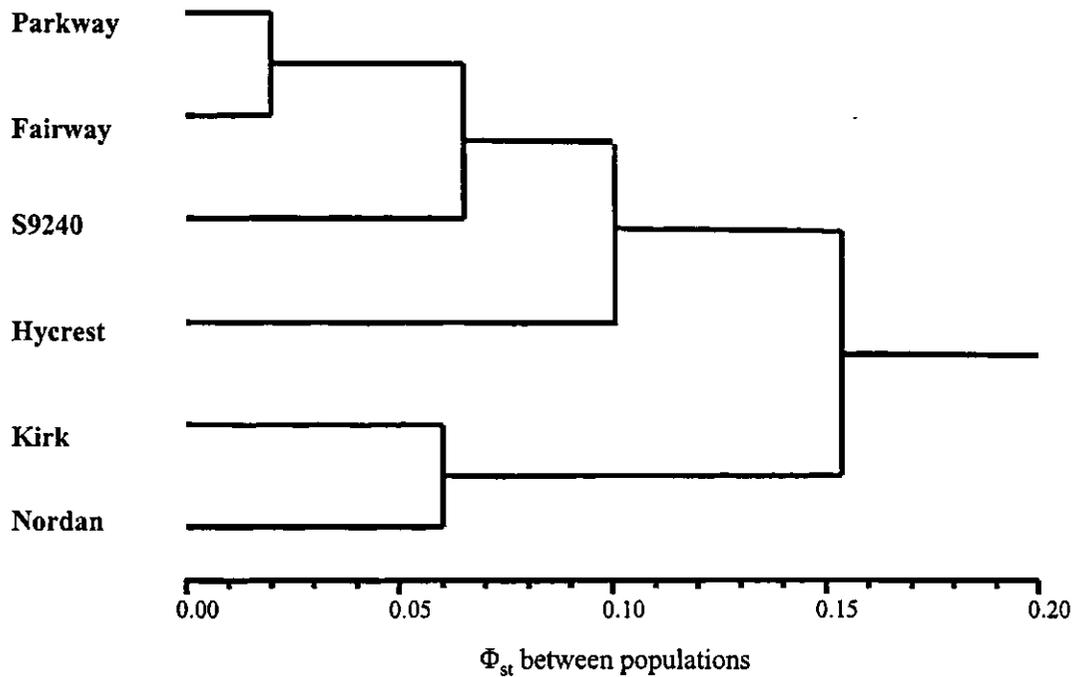


Figure 6.2 Cluster analysis of level of inter population distance ( $\Phi_{st}$ ) between six *Agropyron* populations, based on 15 individuals per population, calculated using AMOVA

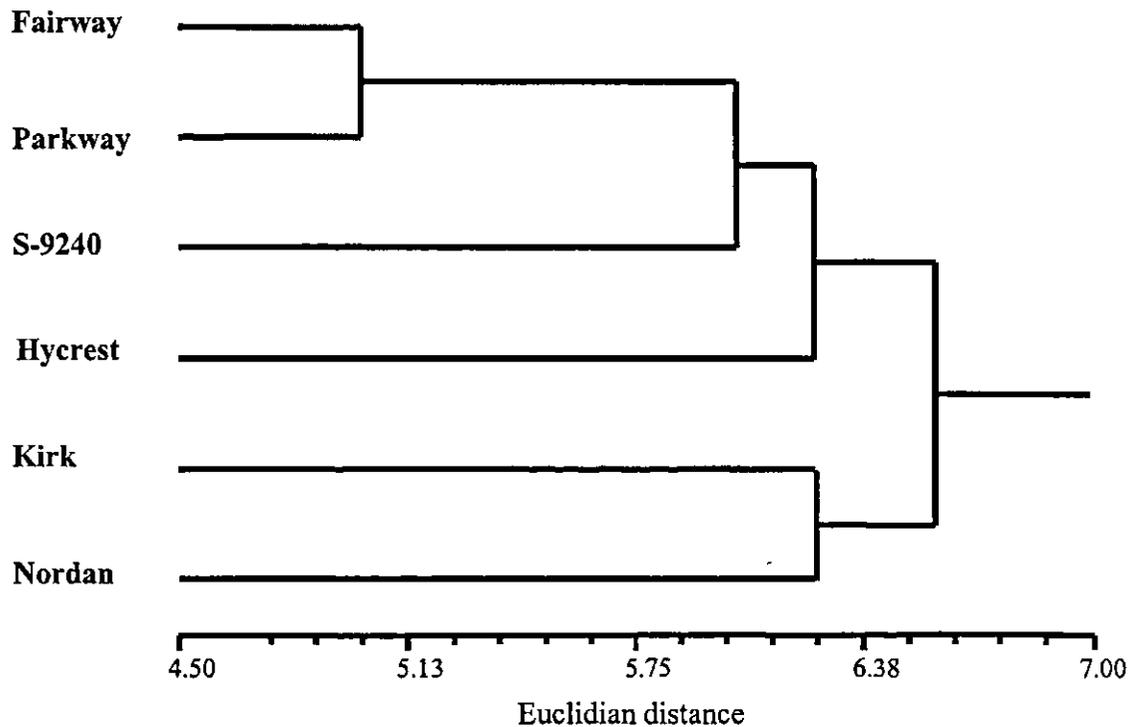


Figure 6.3: A dendrogram of six *Agropyron* populations, represented by 16 bulked individuals, based on Euclidian distances from 114 AFLP markers.

## 6.5 Conclusions

Based on an analysis of AFLP markers Fairway, Parkway, and S9240 lines appear to be true *A. cristatum*. The clustering of Nordan agrees with other evidence suggesting that it is an allotetraploid of *A. cristatum* and *A. mongolicum*. The unique genome constitution of the hexaploid line “Douglas” may justify classification as a separate species. Ephraim may be some hybrid of *A. cristatum* and *A. desertorum* or may be a line of the species *A. cimmericum*. The hybrid nature of Hycrest and CD-II was confirmed. Kirk and S8959 also appear to be hybrids of *A. cristatum* and *A. desertorum*. Due to introgression of *A. desertorum* into the line “Vavilov” it is difficult to draw any conclusions on the relationship of *A. fragile* to *A. mongolicum* from the data obtained.

Within-line variance accounted for 88% of the total molecular variation in the six *Agropyron* lines assayed, while among line variance accounted for only 12% (Table 6.2). There were significant differences between all six lines assayed (Table 6.3).

The phylogenic conclusions drawn from the individual genotype (AMOVA) analysis are the same as those drawn from the bulk analysis. Considering this similarity of results, the increased resources required to assay the large number of individuals needed to perform the AMOVA analysis appeared not to be justified.

## **7. GENERAL DISCUSSION**

### **7.1 Relationship between tillering and seed production**

S9240 plants produce heavier, taller and fewer tillers and also have higher individual seed weights and total seed yield than plants of other crested wheatgrass cultivars. The diploid Parkway, which produces more, smaller and shorter tillers, in particular had a much lower seed yield and produced smaller seeds than S9240. There have been several studies looking at the relationship between vegetative growth and seed production in perennial forage grasses. Schaff et al. (1962) found no correlation between forage yield and seed yield in *A. desertorum*, but did find a relationship between seed weight and forage yield. Casler et al. (1997) found that they were able to simultaneously increase forage yield and seed yield in orchard grass, which indicates that there is at least not a negative relationship between forage yield and seed yield. In neither of these studies was the relationship between tiller components (such as tiller number, height or weight) and seed yield or size examined.

Dewey and Lu (1959) conducted principle component analysis on the interrelationships between plant size, spikelets per spike, fertility and seed size on total seed production in *A. desertorum*. They found that fertility (number of seeds per spikelet) and plant size (total plant mass) were both correlated with total seed yield, but were negatively correlated with each other. They concluded from this that any effort to increase total plant size would have little effect on increasing seed yield as it would have a

negative effect on fertility. Dewey and Lu (1959) did not distinguish between increases in plant size due to increases in tiller number or size, so it is difficult to conclude whether increases in either or both characters have a detrimental effect on fertility. From the results of the current study it would appear that selection for large tillered plants, when combined with selection for fertility as well, permitted increase in total seed yield and seed size.

Nguyen and Sleper (1983a) stated that selection for increased girth (which they felt would increase the number of tillers per plant) would increase seed yield in tall fescue. In contrast Diz et al. (1994) found that in pearl millet x elephantgrass hybrids (*Pennisetum glaucum* (L.) R.Br. X *P. purpureum* Schum.) tiller number had very little effect on the total seed yield of the plant. Rather, seeds per panicle and seed weight had the largest influence on total seed yield. Nguyen and Sleper (1983a) also stated that seeds per panicle and seed weight play a role in determining seed yield. The results of the present study, where S9240 had the lowest tiller density and had the highest total seed yield, also indicates that lower tiller number may be compensated for by other components. S9240 produces larger seeds, and visual assessment indicates that it also produces larger spikes, which would contain more spikelets.

Canode and Law (1978) looked at the relationship between tiller size (diameter of culm at the base) and seed head production in smooth bromegrass and *A. desertorum* and found that a greater proportion of large tillers (>2.5 mm and >2.0mm at the base for bromegrass and *A. desertorum* respectively) produced a seed head than did medium or small tillers. Thus, although not measured, there may have been a larger proportion of reproductive tillers in S9240. This would help compensate for a lower number of total

tillers.

## 7.2 Further selection in S9240

S9240 was selected primarily for plant height, seed yield and size and floret fertility. The high heritability of plant height and crown diameter in the topcross S9240 population, coupled with the significant variation among half-sib families for these traits, indicates that further selection should be effective for these traits. Selection for height may result in further increases in tiller weight, and reduction in tiller number and crown diameter as Zarrough et al. (1983a; 1983b) found in tall fescue. As a result of the negative relationship between the plant height and crown diameter, any selection program aimed at increasing both traits would require that independent culling or a selection index should be used, as tandem selection would be ineffective (Poehlman and Sleper 1995). It may also be possible to increase crown diameter though selection for decreased tiller angle (from the horizontal) which may increase the rate of spread, but studies would have to be done to examine the heritability and usefulness of this trait.

Selection for seed traits has resulted in larger seeds and high fertility in S9240. Before further effort is placed on selection for increased seed size it should be determined whether there is a linear relationship between seed weight and seedling emergence and at what point does the linear relationship end. If seed size is determined to be a trait for which selection should be continued, then it should continue to be accompanied with selection for floret fertility as increases in seed size have been demonstrated to decrease fertility (Schaaf and Rogler 1963), which may compromise total seed yield.

Increased digestibility, protein and mineral concentration play a significant role in improving animal performance for any given forage (Casler and Vogel 1999). It is of

particular concern in crested wheatgrass for as it matures quickly, there is a subsequent rapid decline in quality (Olson and Malechek 1988). There have been a number of studies which have demonstrated that digestibility, and concentration of crude protein, fibre, hemicellulose, and minerals are heritable traits in crested wheatgrass (Asay et al. 1991; Coulman and Knowles 1974). Yet there is very little difference among crested wheatgrass cultivars for digestibility or protein concentration (Unpublished data). Coulman and Knowles (1974) stated that there was no correlation between plant height or yield with *in vitro* digestibility of crested wheatgrass, suggesting that selection for digestibility would not compromise gains in yield or plant height in S9240.

Grass-legume mixtures are often used for forage stands due to yield and quality benefits. Maintenance of both species in the stand over time is desirable. Asay and Mayland (1991) found that there was significant variation among 50 lines, selected from the cultivar Hycrest, for their yield response in mixtures with alfalfa. If S9240 is less competitive with alfalfa due to its more upright growth habit, as Zarrouh et al. (1983b) found with a low tiller number tall fescue, then legume compatibility may be further enhanced by continued selection for plant height.

#### **7.4 Similarity of S9240 to parental populations**

S9240 is based primarily on selected clones from a population of colchicine doubled individuals from the diploid cultivar Parkway, named S9075, and a natural tetraploid population related to the cultivar Kirk, named S7137. The AMOVA analysis indicated that there was less genetic distance between S9240 and Parkway ( $\Phi_{st} = 0.07$ ) than between S9240 and Kirk ( $\Phi_{st} = 0.17$ ). Indicating that S9240 shares more in common genetically with Parkway than with Kirk. This was to be expected because a larger

number of clones from S9075 than S7317 were included in the initial synthesis of S9240. It is evident that Kirk germplasm is present in the S9240 line, as the distance between Parkway and S9240 is twice that of the distance between Parkway and Fairway ( $\Phi_{st} = 0.03$ ), two closely related diploid *A. cristatum*.

In contrast, S9240 is much more morphologically similar to Kirk than to Parkway. For all traits examined (seed weight, tiller height, row width, tiller weight, tiller density and tiller angle), S9240 was always closer to Kirk than Parkway and in some cases there were no significant differences between the two (eg. row width and tiller angle). This discrepancy between morphological and molecular data is likely a reflection of the effects of autopolyploidy on plant morphology. Increases in ploidy have been shown to decrease tiller number and increase tiller size and height, all of which would make S9240 more similar to the tetraploid Kirk. This demonstrates the advantage of using molecular markers in this type of study, as morphologically S9240 would be considered much closer to Kirk and even *A. desertorum* than to Parkway.

## 8. CONCLUSIONS

The history of polyploidization and selection for plant height, seed size, floret fertility and forage yield in the S9240 population has resulted in a number of distinguishing characteristics which make the population unique. The mean seed weight of S9240 is heavier than that of other cultivars, both diploid and tetraploid. The higher mean seed weight of S9240, coupled with the results indicating that increased seed weight increases emergence, would imply that the emergence of S9240 should be superior to other cultivars; however, there was no significant advantage in this character over other tetraploid cultivars.

Selection for height has resulted in a taller, narrower crowned population when compared to other crested wheatgrass cultivars. This seems to be related to a reduced number of tillers which are more upright and heavier than those of other crested wheatgrass cultivars. The presence of significant additive variance in the topcross nursery and in medium to high heritability estimates for crown diameter and plant height, indicate that further gains in plant height could be obtained. From the results of the present study, and associated yield trials, S9240 shows potential as a productive hay-type cultivar of crested wheatgrass with superior seed yield.

The results of the molecular study further demonstrate the complexity and difficulty of taxonomical studies in the *Agropyron* complex, as several populations which were classified as *A. cristatum* may in fact be hybrids, or other species. They also

demonstrate the usefulness of molecular markers for examining relationships between populations of this nature, where morphological characteristics are continuous, interspecific hybrids occur readily and among population variance accounts for a small proportion of total variance.

## 9. REFERENCES

- Aastveit, A. H. and Aastveit, K. H. 1990.** Theory and application of open pollination and polycross in forage grass breeding. *Theor. Appl. Genet.* **79**: 618-624.
- Andrews, M., Douglas, A., Jones, A. V., Millburn, C. E., Porter, D. and McKenzie, B. A. 1997.** Emergence of temperate pasture grasses from different sowing depths: importance of seed weight, coleoptile plus mesocotyl length and shoot strength. *Ann. Appl. Biol.* **130**: 549-560.
- Anonymous. 1998.** Seed production of crested wheatgrass. Saskatchewan Forage Council Publication.
- Asay, K. H. 1992.** Breeding potential in perennial Triticeae grasses. *Hereditas* **116**: 167-173.
- Asay, K. H. and Jensen K. B. 1996.** Wheatgrasses. Pages 691-724 in Moser L. E., Buxton, D. R. and M. D. Casler, eds. *Cool Season Forage Grasses*. American Society of Agronomy, Madison, WI.
- Asay, K. H. and Johnson, D. A. 1980.** Screening for improved stand establishment in Russian wild ryegrass. *Can. J. Plant Sci.* **60**: 1171-1177.
- Asay, K. H. and Mayland, H. F. 1991.** Genetic variances for dry matter yield, nitrogen content, and nitrogen yield in crested wheatgrass-alfalfa mixtures. *J. Range Manage.* **44**: 418-42.
- Asay, K. H., Chatterton, N. J., Jensen, K. B., Wang, R. R-C., Johnson, D. A., Horton, W. H. and Young S. A. 1997.** Registration of 'CD-II' crested wheatgrass. *Crop Sci* **37**: 1023.
- Asay, K. H., Dewey, D. R., Gomm, F. B., Horton W. H. and Jensen, K. B. 1986.** Genetic progress through induced and natural tetraploids in crested wheatgrass. *J. Range Manage.* **39**: 261-263.
- Asay, K. H., Dewey, D. R., Gomm, F. B., Johnson D.A. and Carlson J. R. 1985.** Registration of 'Hycrest' crested wheatgrass. *Crop Sci.* **25**: 368-369.
- Asay, K. H., Jensen K. B., Hsiao, C. and Dewey, D. R. 1992.** Probable origin of standard crested wheatgrass, *Agropyron desertorum* Fisch ex Link, Schultes. *Can.*

J. of Plant Sci. 72: 763-772.

**Asay, K. H., Johnson, D. A., Jensen, K. B., Chatterton, W. H., Hansen, N. J., Horton, W. T. and Young, S. A. 1995a.** Registration of 'Vavilov' Siberian crested wheatgrass. *Crop Sci.* 35: 1510.

**Asay, K. H., Johnson, D. A., Jensen, K. B., Chatterton N. J., Horton, W. H., Hansen, W. T. and Young, S. A. 1995b.** Registration of Douglas crested wheatgrass. *Crop Sci.* 35: 1510-1511.

**Barkworth, M. E. and Dewey, D. R. 1985.** Genomically based genera in the perennial *Triticeae* of North America: Identification and membership. *Amer. J. Bot.* 72: 767-776.

**Barrett, B. A. and Kidwell, K. K. 1998.** AFLP-Based genetic diversity assessment among wheat cultivars from the Pacific Northwest. *Crop Sci.* 38: 1261-1271.

**Berdahl, J. D. and Barker, R. E. 1984.** Selection for improved seedling vigor in Russian wild ryegrass. *Can. J. Plant Sci.* 64: 131-138.

**Berdahl, J. D. and Ries, R. E. 1996.** Development and vigor of diploid and tetraploid Russian wildrye seedlings. *J. Range Manage.* 50: 80-84.

**Berdahl, J. D., Wilton, A. C. and Frank, A. B. 1989** Survival and agronomic performance of 25 alfalfa cultivars and strains interseeded into rangeland. *J. Range Manage.* 42: 312-315.

**Bretagnolle, F. and Lumaret, R. 1995.** Bilateral polyploidization in *Dactylis glomeratus* L. subsp. *lusitanica*: occurrence, morphological and genetic characteristics of first polyploids. *Euphytica* 84: 197-207.

**Canode, C. L. and Law, A. G. 1978.** Influence of fertilizer and residue management on grass seed production. *Agron. J.* 70: 543-546.

**Casal, J. J., Deregibus, V. A. and Sanchez R. A. 1985.** Variations in tiller dynamics and morphology in *Lolium multiflorum* Lam. vegetative and reproductive plants as affected by differences in red/farred irradiation. *Ann. Bot.* 56:553-559.

**Casler, M. D. and Hovin, A. W. 1985.** Predicting forage yield from morphological traits in reed canarygrass. *Crop Sci.* 25: 783-787.

**Casler, M. D. and Vogel, K. P. 1999.** Accomplishments and impact from breeding for increased forage nutritional value. *Crop Sci.* 39: 12-20.

**Casler, M. D., Berg, C. C., Carlson, I. T. and Sleper, D. A. 1997.** Convergent-

Divergent selection for seed production and forage traits in orchardgrass: III. Correlated responses for forage traits. *Crop Sci.* 37: 1059-1065.

Casler, M. D., Pedersen, J. F., Eizenga, G. C. and Stratton, S. D., 1996. Germplasm and cultivar development. Pages 413-469 in Moser L. E., Buxton, D. R. and M. D. Casler, eds. *Cool Season Forage Grasses*. American Society of Agronomy, Madison, WI.

Coulman, B. E. and Knowles, R. P. 1974. Variability for in vitro digestibility of crested wheatgrass. *Can. J. Plant Sci.* 54: 651-657.

Dewey, D. R. 1961. Polyhaploids of crested wheatgrass. *Crop Sci.* 1: 249-254.

Dewey, D. R. 1969. Hybrid between tetraploid and hexaploid crested wheatgrasses. *Crop Sci.* 9: 787-791.

Dewey, D. R. 1981. Forage resources and research in northern China. Page 60 in *Agronomy abstracts*. American Society of Agronomy, Madison WI.

Dewey, D. R. 1984 The genomic system of classification as a guide to intergeneric hybridization with the perennial Triticeae. Pages 209-271 in J. P. Gustafson, ed. *Gene Manipulation in Plant Improvement*. Plenum Press, New York, NY.

Dewey, D. R. and Asay, K. H. 1982. Cytogenetic and taxonomic relationships among three diploid crested wheatgrasses. *Crop Sci.* 22: 645-650.

Dewey, D. R. and Lu, K. H. 1959. A correlation and path-coefficient analysis of components of crested wheatgrass production. *Agron. J.* 5: 515-517.

Dewey, D. R., and Pendse, P. C. 1967. Cytogenetics of crested wheatgrass triploids. *Crop Sci.* 7: 345-349.

Dewey, D. R., and Pendse, P. C. 1969. Hybrids between *Agropyron desertorum* and induced tetraploid *Agropyron cristatum*. *Crop Sci.* 8: 607-611.

Diz, D. A., Wofford, D. S. and Schank, S. C. 1994. Correlation and path-coefficient analyses of seed-yield components in pearl millet x elephantgrass hybrids. *Theor. Appl. Genet.* 89:112-115.

Elliot C. R. and Bolton J. L. 1970. *Licensed varieties of cultivated grasses and legumes*. Canada Department of Agriculture Publ. 1405. Ottawa, ON.

Excoffier, L., Smouse, P. E. and Quattro, J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: applications to human mitochondrial DNA restriction data. *Genetics* 131: 479-491.

- Fehr, W. R. 1987.** Heritability. Pages 95-105 *in* Principles of Cultivar Development vol 1: Theory and Technique. Macmillan Inc. New York, NY.
- Frank, A. B. and Hofmann, L. 1994.** Light Quality and stem numbers in cool-season grasses. *Crop Sci.* **34**: 468-473.
- Hoshikawa, K. 1969.** Underground organs of the seedlings and the systematics of Gramineae. *Bot. Gaz.* **130**: 192-203.
- Hsiao, C., Asay, K. H. and Dewey, D. R. 1989.** Cytogenetic analysis of interspecific hybrids and amphiploids between two diploid crested wheatgrasses, *A. mongolicum* and *A. cristatum*. *Genome* **32**: 1079-1084.
- Hsiao, C., Wang R. R.-C. and Dewey D. R. 1986.** Karyotype analysis and genome relationships of 22 diploid species in the tribe *Triticeae*. *Can. J. Genet. Cytol.* **28**: 109-120.
- Huff, D. R. 1997.** RAPD characterization of heterogeneous perennial ryegrass cultivars. *Crop Sci.* **37**: 557-564.
- Huff, D. R., Peakall, R. and Smouse, P. E. 1993.** RAPD variation within and among natural populations of outcrossing buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.]. *Theor. Appl. Genet.* **86**: 927-934.
- Hunt, O. J. and Miller, D. G. 1965.** Coleoptile length, seed size, emergence in intermediate wheatgrass (*Agropyron intermedium* (Host) Beauv.). *Agron. J.* **57**: 192-195.
- Hyder, D. N., Everson, A. C. and Bement, R. E. 1971.** Seedling morphology and seedling failures with Blue Grama. *J. Range Manage.* **24**: 287-292.
- Jefferson, P. G. 1993.** Seedling growth analysis of Russian wildrye. *Can. J. Plant Sci.* **73**: 1009-1015.
- Jessen, D. L. and Carlson, I. T. 1985.** Response to selection for seed and forage traits in smooth bromegrass. *Crop Sci.* **25**: 502-505.
- Jewiss, O. R. 1972.** Tillering in grasses - Its significance and control. *J. Br. Grass. Soc.* **27**: 65-83.
- Knowles, R. P. 1950.** Studies of combining ability in bromegrass and crested wheatgrass. *Sci. Ag.* **30**: 275-300.
- Knowles, R. P. 1955.** A study of variability in crested wheatgrass. *Can. J. Bot.* **33**: 534-546.

- Knowles, R. P.** 1959. Performance of crested wheatgrass synthetics in advanced generation. *Agron. J.* **51**: 521-524.
- Knowles, R. P.** 1990. Registration of Kirk crested wheatgrass. *Crop Sci.* **30**: 749.
- Lafond, G. P. and Baker, R. J.** 1986. Effect of genotype and seed size on speed of emergence and seedling vigour in nine spring wheat cultivars. *Crop Sci.* **26**: 341-345.
- Lamb, J. F. S., Vogel, K. P. and Reece, P. E.** 1984. Genotype and genotype x environment interaction effects on forage yield and forage quality of crested wheatgrasses. *Crop Sci.* **24**: 559-564.
- Lawrence, T.** 1963. A comparison of methods of evaluating Russian wild ryegrass for seedling vigour. *Can. J. Plant Sci.* **43**: 307-312.
- Lawrence, T., Slinkard A. E., Ratzlaff C. D., Holt N. W. and Jefferson P. G.** 1990. Tetracan, Russian wild ryegrass. *Can. J. Plant Sci.* **70**:311-313.
- Looman J. and Best K. F.** 1987. *Budd's Flora of the Canadian Prairie Provinces.* Agriculture Canada Publ. 1662. Ottawa, ON.
- Marriot, C. A., Fisher, M. J., Hood, K. J. and Smith M. A.** 1997. Persistence and colonization of gaps in sown swards of grass and clover under different sward managements. *Grass and Forage Sci.* **52**: 156-166.
- Mayland, H. F., Asay, K. H. and Clark, D. H.** 1992. Seasonal trends in forage quality of crested wheatgrass. *J. Range Manage.* **45**: 369-374.
- McIntyre, G. I.** 1987. The role of water in the regulation of plant development. *Can. J. Bot.* **65**: 1287-1298.
- McIntyre, G. I.** 1997. The role of nitrate in the osmotic and nutritional control of plant development. *Aust. J. Plant Physiol.* **24**: 103-118.
- Mian, M. A. R. and Nafziger, E. D.** 1994. Seed size and water potential effects on germination and seedling growth of winter wheat. *Crop Sci.* **34**:169-171.
- Moore, K. J. and Moser, L. E.** 1995. Quantifying developmental morphology of perennial grasses. *Crop Sci.* **35**: 37-43.
- Mueller, R. J. and Richards, J. H.** 1986. Morphological analysis of tillering in *Agropyron spicatum* and *Agropyron desertorum*. *Ann. Bot.* **58**: 911-921.
- Murphy, J. S. and Briske, D. D.** 1992. Regulation of tillering by apical dominance:

- Chronology, interpretive value and current perspectives. *J. Range Manage.* **45**: 419-429.
- Nelson, C. J.** 1996. Physiology and developmental morphology. Pages 87-125 in Moser, L. E., Buxton, D. R. and M. D. Casler, eds. *Cool Season Forage Grasses*. American Society of Agronomy, Madison, WI.
- Newman, P. R. and Moser, L. E.** 1988. Grass seedling emergence, morphology and establishment as affected by planting depth. *Agron. J.* **80**: 383-387.
- Nevskii S. A.** 1934. Tribe XIV. *Hordeae* Benth. Pages 590-722 in Komarov V. L. ed. *Flora USSR*. The Botanical Institute of the Academy of Sciences of the USSR, Leningrad USSR.
- Nguyen, H. T. and Sleper, D. A.** 1983a. Genetic variability of seed yield and reproductive characters in tall fescue. *Crop Sci.* **23**: 621-626
- Nguyen, H. T. and Sleper, D. A.** 1983b. Theory and application of half-sib matings in forage grass breeding. *Theor. Appl. Genet.* **64**: 187-196.
- Nyquist, W. E.** 1991. Estimation of heritability and prediction of selection response in plant populations. *Critical Rev. in Plant Sci.* **10**: 235-322.
- Olson, B. E. and Richards, J. H.** 1988a. Annual replacement of the tillers of *Agropyron desertorum* following grazing. *Oecologia* **76**: 1-6.
- Olson, B. E. and Richards, J. H.** 1988b. Spacial arrangement of tiller replacement in *Agropyron desertorum* following grazing. *Oecologia* **76**: 7-10.
- Olson, B. E. and Richards, J. H.** 1989. Crested wheat grass growth and replacement following fertilization, thinning and neighbor plant removal. *J. Range Manage.* **42**: 93-97.
- Olson, K. C. and Malechuk, J. C.** 1988. Heifer nutrition and growth on short duration grazed crested wheatgrass. *J. Range Manage.* **41**: 259-263.
- Paul, S., Wachira, F. N., Powell, W. and Waugh, R.** 1997. Diversity and genetic differentiation among populations of Indian and Kenyan tea (*Camellia sinensis* (L. O. Kuntze) revealed by AFLP markers. *Theor. Appl. Genet.* **94**:255-236.
- Pesek, J. and Baker, R. J.** 1971. Comparison of predicted and observed responses to selection for yield in wheat. *Can. J. Plant Sci.* **51**: 187-192.
- Peterson, C. M., Klepper, B. and Rickman, R. W.** 1989. Seed reserves and seedling development in winter wheat. *Agron. J.* **81**: 245-251.

- Poehlman, J. M. and Sleper, D. A. 1995.** Breeding cross-pollinated forage crops. Pages 387-415 in *Breeding Field Crops*. Iowa State University Press, Ames, IO.
- Ray, I. M. and Harms, J. P. 1994.** Heritabilities of morphological and agronomic traits in western wheatgrass. *J. Range Manage.* **47**: 60-63.
- Ray, I. M., Frank A. B. and Berdahl, J. D. 1994.** Genetic variances of agronomic traits in tetraploid crested wheatgrass under competitive conditions. *Crop Sci.* **34**: 1436-1439.
- Ray, I. M., Frank A. B. and Berdahl, J. D. 1997.** Genetic variance of agronomic and morphological traits of diploid crested wheat grass. *Crop Sci.* **37**: 1503-1507.
- Richards, J. H., Mueller, R. J. and Mott, J. E. 1988.** Tillering in tussock grasses in relation to defoliation and apical bud removal. *Ann. Bot.* **62**: 173-179.
- Rogler G., A. 1954.** Seed size and seedling vigor in crested wheatgrass. *Agron. J.* **46**: 216-220.
- Rogler, G. A. and Lorenz, R. J. 1983.** Crested wheatgrass: Early history in the United States. *J. Range Manage.* **36**: 91-93.
- Roland-Riuz, I., Dendauw, J., Van Bockstaele, E., Depicker, A., and De Loose M. 2000.** AFLP markers reveal high polymorphic rates in ryegrass (*Lolium* spp.). *Mol. Breed.* **6**: 125-134.
- Rolhf, F. J. 1998.** NTSYS-pc. Numerical taxonomy and multivariate analysis system, Version 2.01. Applied Biostatistics, New York.
- Ronald, P. S., Brown, P. D., Penner, G. A., Brule-Babel, A. and Kibite, S. 1999.** Heritability of hull percentage in oats. *Crop Sci.* **39**: 52-57.
- Rumbaugh, M. D., Johnson, D.A. and Van Epps, G. A. 1982.** Forage yield and quality in a Great Basin shrub, grass, and legume pasture experiment. *J. Range Manage.* **35**: 604-609.
- SAS Institute, 1985.** SAS user's guide: Statistics. 5<sup>th</sup> ed. SAS Institute, Inc. Cary, NC.
- Schaaf, H. M. and Rogler, G. A. 1963** Breeding Crested Wheatgrasses for seed size and yield. *Crop Sci.* **3**: 347-350.
- Schaaf, H. M., Rogler, G. A. and Lorenz, R. J. 1962.** Importance of variation in forage yield, seed yield, and seed weight to the improvement of crested wheatgrass. *Crop Sci.* **2**: 67-71.

- Schneider, S., Roessli, D. and Excoffier, L. 2000.** Arlequin ver 2.000: A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
- Schulz-Schaeffer, J., Allerdice, P. W. and Creel, G. C. 1963.** Segmental allopolyploidy in tetraploid and hexaploid *Agropyron* species of the crested wheatgrass complex (section *Agropyron*). *Crop Sci.* **3**: 525-530.
- Schut, J. W., Qi, X. and Stam, P. 1997.** Association between relationship measures based on AFLP markers, pedigree data and morphological traits in barley. *Theor. Appl. Genet.* **95**: 1161-1168.
- Sharma, S. K., Knox, M. R. and Ellis, T. H. N. 1996.** AFLP analysis of the diversity and phylogeny of *Lens* and its comparison with RAPD analysis. *Theor. Appl. Genet.* **93**: 751-758.
- Sicher, R. C., Kremer, D. F., Harris, W. G. and Baenziger, P. S. 1984.** Photosynthate partitioning in diploid and autotetraploid barley (*Hordeum vulgare*). *Physiol. Plant.* **60**: 239-246.
- Smoliak S. and Dormar, J. F. 1985.** Productivity of Russian wild rye and crested wheatgrass and their effect on prairie soil. *J. Range Manage.* **38**: 403-405.
- Steel, R. G. and Torrie, J. H. 1960.** Principle and Procedures of Statistics. McGraw-Hill Publishing New York, NY.
- Stammers, M., Harris, J., Evans, G. M., Hayward, M. D. and Forester, J. W. 1995.** Use of random PCR (RAPD) technology to analyse phylogenetic relationships in the *Lolium/Festuca* complex. *Heredity* **74**: 19-27.
- Sugiyama, S. 1995.** The relationship between growth and development of vegetative shoots in genotypes of tall fescue (*Festuca arundinacea* Schreb.). *Ann. Bot.* **76**: 553-558.
- Svitashev S., Salomon, B., Bryngelsson, T. and, von Bothmer, R. 1996.** A study of 28 *Elymus* species using repetitive DNA sequences. *Genome.* **39**: 1093-1101.
- Tai, W. and Dewey, D. R. 1966.** Morphology, cytology and fertility of diploid and colchicine- induced tetraploid crested wheatgrass. *Crop Sci.* **6**: 223-226.
- Taylor R. J. and McCoy, G. A. 1973.** Proposed origin of tetraploid crested wheatgrass based on chromatographic and karyoptic analyses. *Am. J. Bot.* **60**: 576-583.
- Trupp, C. R. and Carlson, I.T. 1971.** Improvement of seedling vigour of smooth brome grass (*Bromus inermis* Leyss.) by recurrent selection for high seed weight.

- Crop Sci. 11: 225-229.
- Tzvelev, N. N. 1976.** Tribe 3. Triticeae Dum. Pages 105-206, in Poaceae URSS. Nauka Publishing House, Leningrad, USSR.
- VanToai, T. T., Peng, J. and St Martin, S. K. 1997.** Using AFLP markers to determine the genomic contribution of parents to populations. Crop Sci. 37: 1370-1373.
- Vogel, K. P. and Pedersen, J. F. 1993.** Breeding systems in cross-pollinated perennial grasses. Plant Breed. Rev. 11: 251-274.
- Vogel, K. P. and Sleper, D. A. 1994.** Alteration of plants via genetics and plant breeding Pages 891-921 in G. C. Fahey ed. Forage Quality, Evaluation and Utilization.
- Vogel, K. P., Arumaganathan, K. and Jensen, K. B. 1999.** Nuclear DNA content of perennial grasses of the *Triticeae*. Crop Sci. 39: 661-667.
- Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, J. P., Peleman, J., Kuiper, M. and Zabeau, M. 1995.** AFLP: A new fingerprinting technique for DNA fingerprinting. Nucleic Acids Res. 23: 4407-4414.
- Waldron, B. L., Ehlke, N. J., Wyse, D. L. and Vellekson, D. J. 1998.** Genetic variation and predicted gain from selection for winter hardiness and turf quality in perennial ryegrass topcross population. Crop Sci. 38: 812-822.
- Welker, J. M. and Briske, D. D. 1991.** Clonal biology of the temperate caespitose, graminoid *Schizachyrium scoparium*: a synthesis with reference to climate change. Oikos 63: 357-365
- Yee, E., Kidwell, K. K., Sills, G. R. and Lumpkin, T. A. 1999.** Diversity among selected *Vigna angularis* (Azuki) accessions on the basis of RAPD and AFLP markers. Crop Sci. 39: 268-275.
- Zarroug, K. M. and Nelson, C. J. 1980.** Regrowth of genotypes of tall fescue differing in yield per tiller. Crop Sci. 20: 540-544.
- Zarroug, K. M., Nelson, C. J. and Coutts, J. H. 1983a.** Relationship between tillering and forage yield of tall fescue. I. Yield. Agron. J. 23: 333-337.
- Zarroug, K. M., Nelson, C. J. and Coutts, J. H. 1983b.** Relationship between tillering and forage yield of tall fescue. II. Pattern of tillering. Agron. J. 23: 338-342.