Study of genetic diversity in *Puccinellia nuttalliana* based on agronomic/morphological traits and AFLP molecular markers

A Thesis

Submitted to the College of Graduate Studies and Research

in Partial Fulfillment of the Requirements

for the Degree of Master of Science

in the Department of Plant Sciences

University of Saskatchewan

Saskatoon

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ABSTRACT

Native prairie grasses of western Canada have the potential for development as turf and forage grass cultivars for semiarid environments. Nuttall's salt-meadow, or alkali grass (*Puccinellia nuttalliana* (Shultes) Hitchc.), is a native grass species in North America well known for its salt tolerance. Little information is available about the genetic diversity of natural populations of this species. Understanding the genetic diversity of this species is a prerequisite for developing populations for forage or turf use in western Canada. The objectives of this study were to assess the variation in agronomic/morphological characters and AFLP markers of collections of *Puccinellia* and identify promising populations and genotypes for turf and forage utilization.

A four replicate randomized complete block field nursery of twenty-four collections from western Canada was established in 2010. Plant height, tiller number, crown diameter, dry matter yield, seed yield, and leaf related characters were measured for each collection in the summers of 2011 and 2012. Considerable phenotypic variation was detected among and within the twenty-four populations. Promising populations and genotypes were identified with respect to their superior turf and forage related characteristics.

The amplified fragment length polymorphism (AFLP) technique was used to assess the comparative genetic diversity of the collections. Five AFLP primer pairs were employed to screen 15 genotypes from each population, and 185 polymorphic AFLP bands were scored for each sample. Their frequencies of occurrence ranged from 0.02 to 0.99 with a mean of 0.61. The analysis of molecular variance revealed more than 96% of the total AFLP variation resided within populations. Populations were not highly differentiated with only 4% of the total AFLP variation residing among populations. A Mantel test revealed a significant but low correlation between genetic and geographic distances ($r=0.293$; $P=0.024$) and non-significant correlation between genetic and phenotypic distances ($r=0.070$; $P=0.282$). Implications for *P. nuttalliana* conservation, germplasm sampling, and cultivar development are discussed.

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to my advisor Dr. Bruce E. Coulman for providing me such a wonderful opportunity to study in Canada. His gentle guidance and valuable direction over the past three years have made this experience educational as well as truly enjoyable. I would also like to thank my advisory committee members, Dr. Yong-Bi Fu, Dr. Yuguang Bai and Dr. Pierre Hucl, for their perspectives and suggestions throughout my research program. My thanks are also extended to Dr. Kevin Falk for acting as the external examiner.

Thanks to Cheryl Duncan and Tim Nelson in the forage breeding lab of the AAFC Saskatoon Research Centre for their technical assistance and direction. Thanks to Gregory Peterson, Carolee Horbach, and Vicky Roslinsky for their technical support in molecular screening.

I would like to thank the Department of Plant Sciences and Agriculture and Agri-Food Canada for funding support and for providing me such a stimulating environment during my research and study. Financial support from Ducks Unlimited Canada is gratefully acknowledged.

I also want to express my thanks to my fellow graduate students, the excellent faculty and the wonderful main office staff members of the Department of Plant Sciences.

I thank my parents whose lifetime of support and encouragement has brought me to where I am today. Thanks to my friends and roommates whom I am very fortunate to have known and spent time with.

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LIST OF ABBREVIATIONS

Abbreviation	Meaning
AFLP	Amplified Fragment Length Polymorphism
PCR	Polymerase Chain Reaction
AMOVA	Analysis of Molecular Variance
RFLP	Restriction Fragment Length Polymorphisms
RAPD	Random Amplified Polymorphic DNA
SSR	Simple sequence repeats
PCoA	Principal Coordinate Analysis
UPGMA	Unweighted Pair-group Method with Arithmetic averages
LSD	Least Significant Differences
NPB	Number of polymorphic bands
MBF	Mean band frequency
WPV	Within-population variation

1 Introduction

Native grass species of the Canadian prairie are of great importance providing wildlife habitat, forage resources for livestock, and ground cover. Currently, little effort has been made to assess the genetic diversity of native grass species and high seed cost and inadequate seed availability have restricted their use in vegetation or restoration programs in western North America (Walker and Shaw 2005). There is a challenge in improving seed production while simultaneously maintaining genetic diversity for adaption across a range of environments (Smith and Whalley 2002).

Nuttall's salt meadow or alkali grass (*Puccinellia nuttalliana* (Shultes) Hitchc.) is a native, salt tolerant cool season grass species. It is an octoploid ($2n=8x=56$), outcrossing, perennial species grown for vegetation along roadsides and for forage. This species is widely distributed in temperate and boreal western North America, ranging from Wisconsin to British Colombia, and south to Kansas and California. It is very tolerant of high salinity in soils across a wide geographic range due to its low maintenance requirements, low growth habit, and natural competitiveness (Mintenko et al. 2002). *P. nuttalliana* belongs to the family *Poaceae*, subfamily the *Pooideae* and tribe *Poeae* (Davis 1991). The classification of species of *Puccinellia* is controversial because the genus has reduced floral parts which make for fewer distinguishing morphological characters (Hitchcock 1969).

Knowledge of the genetic diversity of available germplasm is useful for the identification of superior populations for the selection of desirable plants. Morphological and agronomic evaluations are of value in studies on germplasm evolution and in identifying differences among populations. Together with molecular markers, morphological traits are used to study genetic diversity, to develop conservation strategies, and to develop new germplasm (Sharma et al. 2011).

Variable environmental conditions will lead to selection of populations with favorable alleles for each site, creating considerable genetic variation within and among populations (Vanhala et al. 2004). Knowledge of relationships between populations is very useful for the selection of germplasm with maximum genetic distance which may result in heterosis when interpollinated. Geographic information on collections is helpful especially when other information on genotypes

is unavailable. The Euclidean distance between the standardized points representing the genotypes or populations can be used as a measure of relatedness.

Maintenance of genetic diversity is a principal concept in rangeland conservation and restoration, with the belief that genetic variation will increase the probability of population persistence (Gustafson et al. 2004). Molecular markers have played an important role in aiding the assessment of genetic diversity in a number of grass species (Baloch et al. 2010, Uzun et al. 2011). The Amplified Fragment Length Polymorphism (AFLP) technique is an effective method for this purpose, given its wide genome coverage with no requirement for prior genome information. AFLPs combine restriction digestion and PCR (Polymerase Chain Reaction) and provide an effective means of detecting several polymorphisms in a single assay. It is equally applicable to all species (cross-pollinated and self-pollinated species) and is highly reproducible and heritable in the study of genetic variation among and between closely related taxa, including blue bunch wheatgrass (*Pseudoroegneria spicata* [Push] A. Love) (Larson et al. 2001), crested wheatgrass (*Agropyron* spp. Gaerth.) (Mellish et al. 2002), little bluestem (*Schizachyrium scoparium* [Michx.] Nash)(Fu et al. 2004a) and fringed brome (*Bromus ciliates* L.) (Fu et al. 2005).

The phi-statistic (Φ_{st}), calculated using the Analysis of Molecular Variance (AMOVA), has been successfully used to determine genetic distances between populations of many out-crossing species. As an out-crossing species, each population of *P. nuttalliana* represents a collection of related genotypes rather than a single genotype; therefore there is more variation within a population of this species than in annual self-pollinating cereal crops. Greater within-population variation than between-population variation is often observed in out-crossing species. For example, 85.1% within-population AFLP variation was observed among 14 cultivars of smooth bromegrass (Ferdinandez and Coulman 2004) and 88% within-population variation among 12 populations of crested wheatgrass (Mellish et al. 2002). AFLP markers can provide useful measures of genetic variability in native grasses (Koopman 2005).

Differences between genotypes with regard to morphological characters or agronomic characters would either indirectly, or directly, represent the differences at the DNA level and then provide

information about genetic relationships. The evaluation of different populations collected from various Canadian prairie regions would provide an opportunity to select promising genotypes.

The objectives of the present study are 1) to evaluate agronomic/morphological variation and differences among and within *P. nuttalliana* collections from twenty-four locations in the Canadian great plains region grown in a common nursery; 2) to assess the genetic diversity and relationship of these populations using AFLP markers; 3) and to select genotypes or populations to produce populations which would be useful for forage or turf purposes. This will provide information for the appropriate development and conservation of western Canadian *P. nuttallliana* germplasm.

2 Literature review

2.1 *Puccinellia nuttalliana*

2.1.1 Distribution and adaptation

Puccinellia species colonize harsh alkaline arctic environments and are widely distributed in arctic and temperate areas worldwide. *P. nuttalliana* is a perennial grass species distributed principally in temperate and boreal western North America, ranging from Wisconsin to British Colombia, and south to Kansas and California. It is widespread on high pH soils in arid and semi-arid environments due to its salinity tolerance and natural competitiveness. It is often associated with areas of low site productivity and is considered as a facultative halophyte (Beyschlag et al. 1996, Macke and Ungar 1971) since it can complete its life cycle in non-saline environments. This plant is among the most salt tolerant C_3 grasses in North America (Macke and Ungar 1971, Harivandi et al. 1983, Ashraf et al. 1986, Salo et al. 1996, Mintenko et al. 2002, Tarasoff et al. 2010).

2.1.2 Taxonomy

Genus *Puccinellia*, also known as alkali grass, belongs to family *Poaceae* (Davis 1983), subfamily *Pooideae* and tribe *Poeae*. *Puccinellia* comprises 25-30 northern temperate and arctic species (Gould 1968, Hitchcock 1969). The grass *Puccinellia* (subfam. *Pooideae*) is one in which species boundaries are controversial (Hitchcock 1969). These species are often difficult or impossible to distinguish from one another because there are few morphological characters to use in this grass genus with reduced floral parts. Natural subdivisions of the genus are not clearly delineated, but a morphologically intergrading complex of species related to *P. nuttalliana* (Schult.) A. Hitchc. may be recognized (Davis 1983). This species can be identified by a combination of characters that include bract shapes, ratios of bract lengths, and trichome characters (Davis 1983).

2.1.3 Morphological characteristics of *P. nuttalliana*

P. nuttalliana is a caespitose slender perennial growing from 25-80 cm tall. It has hollow, erect, narrow glabrous or slightly scabrous culms. Blades are usually involute at maturity, strongly ribbed and scabrous on the upper surface. The leaf length ranges from 1-15 cm and the width

from 0.8-2.6 mm. Numerous, flat, short basal leaves grow from the crown. Lemmas are narrow and usually pointed at the apex, 2-2.5 mm long.

The inflorescence is an open panicle 7-26 cm long. The inflorescence is made up of a few thin, spreading branches and becomes reflexed as the fruit matures. There are 2-7 florets per spikelet, often with hairs on the florets. Anthers are variable in size. The seeds are 0.9-1.5 mm long.

In cool temperate climates, the active growth period of *P. nuttalliana* begins in early May, with a maximum biomass achieved by late July. Regrowth after harvest is relatively slow. *P. nuttalliana* is quite palatable to ruminant animals in its early growth stages; however, as maturity advances, palatability declines (Campbell et al. 1966).

Following wind pollination, seed development of *P. nuttalliana* begins in late June (Olejniczak and Lembicz 2007). The panicle produces a considerable seed crop with from four to ten seeds per spikelet. The seeds are small and overlap each other in the spikelet and are ripe and start to shatter by mid-July.

Like most of the caespitose species of the genus, *P. nuttalliana* is considered as an almost strict sexually reproducing plant, being self-incompatible and predominantly cross-fertilizing (Davis & Manos 1991). Plants that have a low rate of selfing would be likely to experience high levels of introgression or hybridization. Thus populations would be heterogeneous and genotypes would have a high level of heterozygosity due to hybridization. The observation that some plants in one population have similar genotypes to plants in other populations suggests that there is introgression of genes between populations through pollen or seed spread (Consaul et al. 2008).

More than half of the *Puccinellia* species have been found to be polyploid (Davis and Consaul 2007). Similar to other genera of the subfamily Pooideae, *P. nuttalliana* has a base chromosome number of seven. Various chromosome numbers have been reported for *P. nuttalliana* and related species; however, most of the evidence suggests that *P. nuttalliana* is an octoploid (Church 1949, Davis 1991).

2.1.4 Use as turf

Puccinellia was first considered for use as a turfgrass in Illinois (Sanks 1971) and Colorado (Butler 1972). Cultivar "Fults" from weeping alkaligrass (*Puccinellia distans* [Jacq.] Parl.) has been used as a turf grass since the 1980s. Butler et al. (1974) listed three *Puccinellia* spp. as being the most valuable for turf: weeping alkaligrass, Nuttall's alkaligrass and Lemmon's alkaligrass (*P. lemmoni* [Vasey] Scribn). *Puccinellia* species are mainly suited for low-maintenance turf, and they have been used successfully along roadsides, boulevards, and rights-of-way where winter snows bring about frequent salt applications (Casler and Duncan 2003).

2.2 Forage and turf related phenotypic characteristics and their associations

Plant breeders are often interested in improving a plant species for several traits, or to identify simply inherited traits that are related to complex traits of interest. Thus the correlation between various traits is of interest. The estimation of the degree of association between two traits can help to determine the change brought about in a given character when selection is practiced on another trait and to examine the relationship between characters in a natural population (Falconer and Mackay 1996, Lynch and Walsh 1998).

2.2.1 Dry matter yield

The ultimate value of perennial grasses used as forage for agriculture is the quantity and quality of livestock production. Most forage grass breeding programs have focused on selection for higher yield. As a complex character, forage yield is influenced by genetic effects as well as the interaction of genotype with the environment. Selection for improvement of the components of yield or other morphological characters may be an effective way of improving yield. Forage dry matter yield is often closely related to one or more morphological traits. Tiller number was found to be of great importance in determining dry matter yield of many perennial grasses, like tall fescue (*Festuca arundinacea* Schreb.) (Nelson et al. 1977) and perennial ryegrass (*Lohium perenne* L.) (Thomson et al. 1973). Forage yield of perennial ryegrass was positively correlated with leaf length (Rhodes, 1969, 1972). Selection of plants with larger tillers and leaf blades in reed canary grass and tall fescue has resulted in increased forage yields (Carlson et al. 1983, Nelson and Sleper 1983).

Characteristics that have been identified to make grasses more competitive include vigour and greater plant height, and rapid and large leaf area development and duration (Christensen 1995, Huel and Hucl 1986, Lemerle et al. 2001). A number of studies (Whan et al. 1991, Turner and Nicolas 1998, Botwright et al. 2007) have reported genetic increases in early vigour to be associated with greater biomass and grain yield for wheat grown in Mediterranean environments.

2.2.2 Tillers

Tillers are shoots produced from the base of the stem of herbaceous species and are the primary structural units of a mature plant. A grass plant is a collection of tillers that arise from a single primary tiller or crown (Moore and Moser 1995). Tiller number has been found to be an important character related to forage yield in smooth bromograss (Tan et al. 1977), perennial ryegrass (Lazenby and Rogers 1964) and tall fescue (Nelson et al. 1977). Environment is an important factor in tiller development of perennial grasses (Moore and Moser 1995). In most temperate grass species, tillers must undergo a period of vernalization before floral induction occurs (Langer 1979). Therefore, it is primarily the tillers initiated the previous fall that develop to maturity in the first growth of the subsequent growing season. Stress in the previous fall can result in a significant reduction in the number of tillers that become reproductive.

2.2.3 Leaves

When characterizing abiotic stress tolerance of grass species, it is important to evaluate the traits related to growth of plants. Leaf blades are a major component of yield and nutritive value in forage grasses. Because leaves determine radiation interception and are the main photosynthetic organs, reduction in leaf expansion and function are directly related to yield reduction under stress conditions such as salinity. Productivity is directly related to leaf area expansion and duration (Taleisnik et al. 2009). Salinity creates a water deficit, thus morphometric variables that influence transpirational water loss, such as leaf size, shape, and number, may be associated with salt tolerance within a species.

Generally, there are four stages in grass leaf development: (1) establishment of the cell division zone, (2) establishment of the cell elongation zone, (3) linear growth, and (4) cessation of cell division and gradual decrease in the leaf elongation rate until elongation ceases (Skinner and Nelson 1994). Leaf characters may describe a large portion of the variation in forage yield. Leaf

appearance during seedling development is critical for spring growth and is strongly related to root development (Moser et al. 1993). The appearance and development of leaves of perennial grasses is necessary to achieve a positive carbon balance to support further regrowth development after defoliation (Davies and Morgan 1988). Leaf elongation is essential for maintaining the productivity of grasslands over seasonal changes (Durand et al. 1999).

2.2.4 Seed yield

Seed yield is an important factor in the commercial success of forage grass cultivars (Marshall and Wilkins 2003). Seed yield is a complex trait affected by several yield components and the environment (Boelt and Studer 2010). Langer (1980) reported that the number of spikes per unit area affect seed yield in ryegrass and cereals. Veronesi and Falcinelli (1988) found that seed yield was positively related to dry matter yield and plant height in tall fescue. However seed yield was found not to be related to plant height in switchgrass (Newell and Eberhart 1961).

2.2.5 Turf quality

Turf breeders select plants to synthesize populations that will maintain a dense, high-quality turf that can be mowed close to the ground. Finer (narrower) and shorter leaf blades, finer (thinner) stems, and shorter internodes contribute to a dense and high-quality turf. Other morphological traits contributing to high quality turf include later heading, higher tiller density, lower growth habit, lower crown placement and greater persistence. Dark leaf colour is a desirable aesthetic quality characteristic of turf grasses in the North America, but not in Europe, where a lighter green colour is preferred. Colour is traditionally evaluated by visually rating turf plots on a scale of 1 to 9, with 1 representing yellow or brown turf and 9 representing dark green turf (Richardson 2004).

Spreading ability is an important criterion since it estimates the ability of the plant to cover the soil surface. Lebsock and Kalton (1954) concluded that spread is the most important component in yield improvement in smooth bromegrass, a rhizomatous forage grass. In caespitose species, crown diameter gives an indication of the spreading ability and the capability of the plant to produce tillers. Large diameter crowns were found to enhance growth and water uptake and therefore enhance drought tolerance in tall fescue (Torbert et al. 1990, Huang and Gao 2000).

For turf utilization, plants with high biomass and large tillers appear to be more resistant to wear damage (Shildrick and Peel 1984, Lush 1990, Parr 1981). Data on turf biomass and density can provide information on turf response to management factors and are useful for comparing genotypes (Lush and Rogers 1992). Tiller density is a critical trait since increased density would lead to decreased invasion of weed species.

2.2.6 History of forage and turf breeding

Humans have been indirectly selecting cultivated crops based on their desirable traits for thousands of years. However, forage grasses have a relatively short history as intensively cultivated crops. Therefore, in all the common grass species, there exists a wide diversity of natural adaptations to climatic and geographic conditions. Rapid improvement began from the mid-20th century (Vogel and Sleper 1994), with the discovery and exploitation of naturally occurring ecotypes (Breese and Hayward 1972). The ultimate goal of forage grass breeding is to produce adapted cultivars that have high economic value. The improvement of forage species is aimed at major goals such as dry matter yield, seed yield, persistence and tolerance to environmental stress (Wilkins and Humphreys 2003).

The science of turf breeding began in 1962, with the development of a small number of perennial ryegrass, tall fescue, fine fescue (fine-leaved *Festuca* spp.) and Kentucky bluegrass (*Poa pratensis* L.) populations with narrower leaves, smaller tillers, higher tiller density, darker colour and greater tolerance to low mowing height than the typical forage phenotypes of the seed sources (Meyer and Funk 1989). These plants originated from unimproved forage type populations which were not adapted to frequent mowing. Turf breeding led to the development of perennial grasses with traits which proved useful for human enterprise and recreation.

Due to high fertilizer prices and water shortages, there has been increased interest in low-maintenance turf cultivars, which have reasonable turf quality and require less fertilizer and water. Native grasses of the Western Canadian Plains may be good candidates for low maintenance turf (Holzworth 1990). *P. nuttalliana* has evolved in an arid continental climate in saline soils, so it has potential for low maintenance turf in more stressful environments.

2.2.7 Breeding methods for perennial grass

Most important perennial forage and turf grasses used are cross-pollinated perennial grasses. These grasses are wind pollinated and have small floral parts that are difficult to emasculate. The inheritance of traits is complicated since most forage grasses are polyploid. Most traits are controlled by numerous genes, few of which have been identified or mapped due to complex inheritance. In addition, individual plants in populations are highly heterozygous. As a result, the most effective breeding systems for cross-pollinated forage grasses are heterogeneous population improvement systems (Vogel and Pedersen 1993). Perennial grass cultivars are generally synthetic cultivars produced by intercrossing a number of selected genotypes to maintain an acceptable level of heterozygosity (Vogel and Pederson 1993, Breese and Hayward 1972). The aim of population improvement is to increase the frequency of genes with favourable expression for characters of interest while avoiding the effects of inbreeding depression (Breese and Hayward 1972). Therefore, the optimal number of plants chosen to form a synthetic must be determined. A larger number of selected plants would result in lower genetic improvement and a more variable population while a smaller number may increase the possibility of inbreeding depression in further generations.

In a specific region where no prior breeding work has been done with a species, it is necessary to collect, assemble and evaluate germplasm for its ability to meet agricultural, turf, or conservation need, which is defined as ecotype selection (Vogel and Pederson 1993). This process can lead to the rapid discovery of excellent genotypes and the synthesis and release of new cultivars.

Forage and turfgrass population improvement usually involves recurrent selection and the most commonly used recurrent selection methods are mass and phenotypic selection (Casler et al. 1996). Mass selection effectively maintains population size and results in gradual advance over generations. Phenotypic recurrent selection involves evaluating a large number of individuals, followed by isolation and intercrossing of the best individuals. In mass selection, the best individuals are identified and seed is harvested from them; however, a percentage of the pollination of selected individuals would be from pollen from unselected plants. Other breeding methods include progeny tests, between and within family selection, and recurrent multistep family selection (Vogel and Pederson 1993).

The success of phenotypic selection is determined by whether it is possible to distinguish the breeding value of a plant from its phenotype. Environmental effects and gene interactions reduce the effectiveness of phenotypic selection. For quantitative characters controlled by many genes, selection effectiveness will be improved by a large initial population size, a high intensity of selection, some degree of pollination control, and several cycles of selection (Breese and Hayward 1972).

2.3 Genetic variation

For selection of improved populations, there has to be adequate genetic variation in breeding populations (Nguyen and Sleper 1983). Analyzing genetic diversity is necessary to identify core accessions that are useful for specific breeding purposes and to determine genetic relationships among germplasm sources.

2.3.1 Traditional methods for studying genetic diversity

Measurements of morphological variation have been widely used in assessing genetic diversity of plant populations (Karp et al. 1996). Morphological characters are the external expression of the organism, and in the natural environment, morphological characters are not only influenced by genetic factors, but also by environmental factors. Plant populations can adapt to transformed environments by altering their morphology. Intra-specific variation in plant populations is generally considered to be the key to this adaptation (Schut et al. 1997). Studies on the morphological variation of plant species from different habitats could indicate plant adaptations to these environments and help us understand the variation within and among populations. Therefore, morphological variation analysis can determine genotypic variation and local differentiation (Schaal et al. 1991).

Davis (1983) identified a set of 27 characters in the polyploid *P. nuttalliana* complex that expressed low phenotypic plasticity and significant genetic variation under various levels of salt concentration and water availability.

2.3.2 Molecular methods for studying genetic diversity

Traditionally, a combination of morphological and agronomic traits has been used to measure genetic diversity. However, environment may greatly influence these characters and not reflect

true genetic similarity or differences. While the value of morphological or other traits as evaluation measures may vary according to the intended use of the material, molecular characterization of genetic diversity provides base information which can be used to select a range of accessions for different breeding programs (Roldan-Ruiz et al. 2001). Molecular markers can provide information needed to select genetically diverse parents for developing breeding and mapping populations. Molecular markers have played an important role in aiding the assessment of genetic diversity in a number of grass species (Baloch et al. 2010, Uzun et al. 2011). This is the first study to probe molecular genetic diversity in *P. nuttalliana* populations collected in Western Canada.

Genetic diversity in plant populations has been detected using allozyme electrophoresis (Gonzalez-Astorga et al. 2004) and DNA markers, such as Restriction Fragment Length Polymorphisms (RFLP) (Gauthier et al. 2002), Random Amplified Polymorphic DNA (RAPD) (Gonzalez-Rodriguez et al. 2005), Amplified Fragment Length Polymorphism (AFLP) markers (Vos et al. 1995) and microsatellite markers (Edwards et al. 1991, Fu et al. 2003). Allozyme markers were first used in 1960s. Each protein band in an electrophoretic gel usually represents a direct gene product. Allozyme markers can detect heterozygotes and are suitable for low complexity samples. However, they have limited resolution, genome coverage and numbers.

DNA-based markers have been used to characterize genotypes in crop development programs since the 1980s. RFLP involves electrophoretic comparison of the size of defined restriction fragments derived from genomic DNA. RFLP markers are co-dominant and very informative, but this technique is species-specific and labour intensive. RAPD analysis involves PCR-based markers which employ single 10-nucleotide primers of arbitrary nucleotide sequence to amplify anonymous PCR fragments from genomic template DNA. RAPD markers have been used in many forage grasses for investigating clonal genetic diversity and population structure (Chai and Sticklen 1998). The RAPD technique is highly efficient in generating large numbers of polymorphisms because it screens a large amount of the genome. Besides, RAPDs do not require prior sequence information. However, RAPDs cannot distinguish between homozygotes and heterozygotes and are very sensitive even to slight changes in the amplification conditions. Simple sequence repeats (SSRs) are short repeats of DNA sequences. SSRs are highly variable,

reproducible, stable co-dominant markers, making them ideal for diversity studies (Harper et al. 2003). However, the development of SSRs requires knowledge of the genome of the species.

The AFLP technique combines restriction digestion and PCR and provides an effective means of detecting several polymorphisms in a single assay. It relies on selective PCR amplification of restriction fragments from a total digest of genomic DNA without knowledge of genome information. Adapter and restriction site sequence are used as target sites for primer annealing in PCR amplification of restriction fragments. The selective amplification is of only those fragments in which the primer extensions match the nucleotides flanking the restriction sites. Sets of restriction fragments may be visualized by PCR without knowledge of nucleotide sequence (Vos et al. 1995).

The AFLP technique is a very powerful DNA fingerprinting technique for DNA of any origin or complexity, given its wide genome coverage and no requirement for prior genome information. It is equally applicable to all species (cross- and self-pollinated species) and is highly reproducible and heritable with an overall error rate of <2% (Tohme et al. 1996, Vos et al. 1995). AFLP markers have been successfully used to determine genetic diversity in many forage and turf grass species, including crested wheatgrass (Mellish et al. 2002) and little bluestem (Fu et al. 2004). AFLP markers have also been used to detect genetic diversity and genetic structure between and within populations (Besse et al. 1998, Schmidt and Jensen 2000, Arroyo Garcia et al. 2002, Juan et al. 2004, Kim et al. 2005, Wilson et al. 2005, Abbott et al. 2007).

In comparison with RAPD, AFLP markers are more efficient in detecting polymorphisms (Fuentes et al. 1999, Powell et al. 1996). They are as reproducible as RAPDs but they require more DNA (0.3-1.0 μg per reaction) and are more technically demanding than RAPDs. Ferdinandez et al. (2002) found that the estimates of genetic variation among bromegrass species from AFLPs and RAPDs were similar but the AFLP method appeared to be more informative due to the higher number of polymorphic loci.

To summarize, although AFLPs behave as dominant markers, they are useful for diversity analyses as informative, robust and highly effective markers.

2.3.3 Analysis of genetic variation within and among populations

Population genetic variation is the total genetic variation among individuals within a population (Young et al. 1996). Genetic variation within and between populations of crop species is of interest for plant breeders and population geneticists. It is necessary to estimate the extent of variation within and between populations of a species to analyze the genetic structure of germplasm (Breese and Hayward 1993), to predict potential genetic gain in a breeding program (Moreno-Gonzalez and Cubero 1993), and to plan conservation and exploitation of plant genetic resources (Dawson et al. 1994).

Population genetic structure within a species has traditionally been studied using allele frequency variations over a population (Vos et al. 1995). F-statistics have been used to describe the hierarchical level of subdivided populations, indicating genetic drift and also the estimation of the magnitude of gene flow (Wright 1951, 1965, Hooftman et al. 2004, Gonzalez-Astorga et al. 2004).

Excoffier et al. (1992) developed a statistical method called analysis of molecular variance (AMOVA), which produces estimates of variance components and F-statistic analogs as Φ-statistics. It reflects the correlation of genetic variance within and among populations by converting individual distance matrices into an analysis of variance (Excoffier et al. 1992, Huff et al. 1993). AMOVA was first developed specifically for RFLP data and Huff et al. (1993) applied it to RAPD data. For an AMOVA, the existence of each polymorphic band is represented as 0 or 1. Then, the degree of difference between any two individuals is shown by a distance metric based on Euclidean Distance. Last, an analysis of variance is performed by partitioning the total sum of squares of the genetic distance into variation among different sources of variation.

Φ_{ST} is the correlation of random haplotypes within populations relative to that of random pairs of haplotypes drawn from the whole species (Excoffier et al. 1992). In order to test the relationship between isolated geographic distance and genetic diversity, pairwise genetic distances or Φ_{ST} among populations are obtained to produce matrices of genetic distances. A lower Φ_{ST} indicates relatively more similarity between any two groups (populations, genotypes, etc.). The Mantel test

(Mantel 1967) is then used to test whether genetic distances between populations are positively correlated to geographical distances.

Multivariate analytical techniques are widely used in analysis of genetic diversity of morphological and molecular markers in evaluating morphological and genetic differentiation within and among populations. Among these algorithms, cluster analysis and principal coordinate analysis (PCoA) are most commonly employed and appear particularly useful (Brown-Guedira et al. 2000). The Unweighted Paired Group Method Using Arithmetic averages (UPGMA), based on inter-population genetic distance matrices, is the most commonly adopted clustering algorithm in grass species. The primary purpose of cluster analysis is to group individuals or populations based on the characteristics they possess, so that individuals with similar descriptions are mathematically gathered into the same cluster (Hair et al. 1995).

PCoA is a scaling method that starts with a matrix of similarities or dissimilarities between a set of individuals and produces a low-dimensional graphical plot of the data in such a way that distances between points in the plot are close to original dissimilarities (Mohammadi and Prasanna 2003). It is usually performed based on Euclidean distance matrices to evaluate the genetic association of individuals or populations.

2.3.4 Sampling size in genetic diversity assessment

Most traits of polyploid species are controlled by numerous genes and thus few genes have been determined or mapped due to their complex inheritance. Determination of genetic diversity in *P. nuttalliana* and other outcrossing species is further complicated by the fact that genetic variation exists among populations as well as among individuals within populations. For this reason, genetic diversity studies on outcrossing species have been traditionally conducted by separately profiling DNA from a number of individuals within each population. Allele frequencies estimated within a bulk of individuals representing a given population were very similar to those estimated from individuals (Kilian et al. 2012).

Since both among and within variation are important in grass species, sampling a higher number of individuals from fewer sites may be more effective in capturing genetic diversity than sampling a larger number of sites with just a few individuals from each site (Boller and

Vetelainen 2010). A preliminary survey is an effective way to determine the appropriate sample sizes because the real distributions of variation in natural populations can vary among species. Huff et al. (1993) found that a sample size of 12 individuals per populations was large enough to establish the pattern of variation among populations in outcrossing buffalograss (*Buchloe dactyloides* (Nutt.) Engelm). Only ten individuals per population were used when studying perennial ryegrass (Huff et al. 1998). However, it was also recommended that larger sample sizes up to 25 individuals within each population would be more effective in characterizing population to population variation within regional ecotypes.

2.3.5 Geographical patterns of genetic variation

The existence of populations in different environmentally variable geographic locations and evolutionary processes can shape the genetic variation of a species through gene flow, genetic drift and natural selection. The Mantel test has been used to test the local environmental effect on genetic differentiation by measuring the association of genetic distance and geographical distance (Mantel et al. 2003). Fu et al. (2005) found a significant association between AFLP variability and geographic location of fringed brome populations. Still et al. (2005) observed genetic divergence along a north-south climatic gradient in narrow-leaved purple coneflower (*Echinacea angustifolia*).

3 Materials and Methods

3.1 Plant material

Collections of *P. nuttalliana* were made by staff of Ducks Unlimited Canada. Seeds or plants were collected from 24 locations in western Canada (Table 3.1, Fig 3.1). In September of 2008, collections were made at 16 sites with ten plants being collected at each site (2008 Collections). These plants were transplanted (10 cm pots) in the greenhouse, grown throughout the winter and contaminating species removed. The plants were established in a field nursery in May, 2009 and seeds harvested from individual plants in August of 2009. In September of 2009, collections of seeds were made at eight additional locations (2009 Collections). Seeds were harvested from 1 – 10 plants at each site for a total of 73 plants.

Seeds from five plants from each of the 24 collection locations (120 lines) were germinated in root trainers in the greenhouse in spring of 2010 and seedlings were transplanted to the field in June, 2010 in a spaced-planted design, with plants on 1 m centres. Each plot consisted of three plants from the 120 collected lines and there were four replications in a randomized complete block design. Total plant number was 120 lines ×3 plants/plot ×4 replicates for a total of 1440. There were 60 plants from each of the 24 collection sites.

The nursery was established at the research farm of the Agriculture and Agri-Food Canada (AAFC) Saskatoon Research Centre on a Sutherland clay loam soil (Typic Haploboroll). Morphological and agronomic data were collected in the 2011 and 2012 field seasons. Plant material for molecular analysis was sampled in September of 2010 from all individuals in block one of the nursery, followed by freeze-drying, and storage at -80℃. Thus, there were 15 plants from each collection site; however, due to mortality, there were only seven plants from Schuler North, the only collection from Alberta. Therefore, Schuler North was eliminated from the molecular analysis.

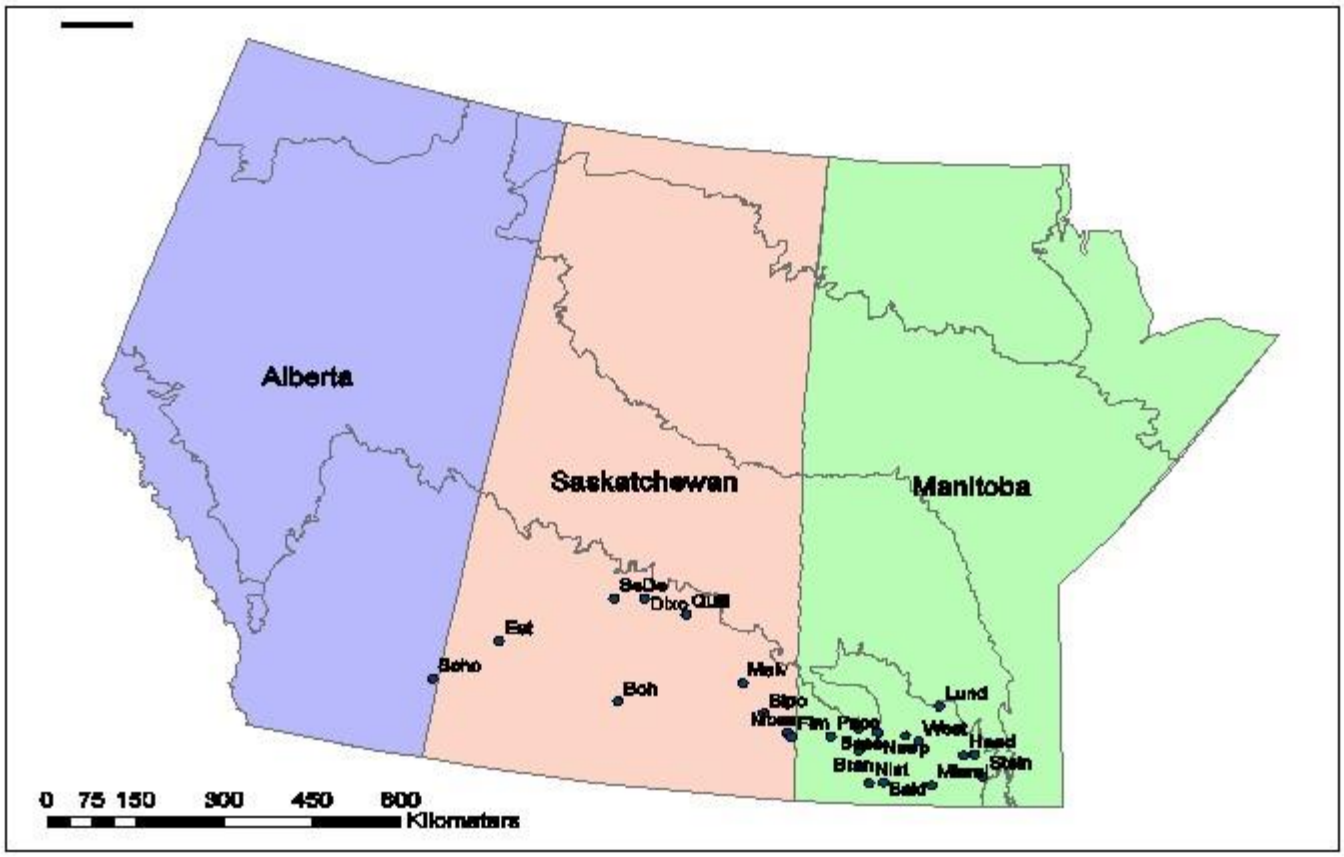


Figure 3.1 Locations of 24 *Puccinellia nuttalliana* populations

Table 3.1 Location information for 24 *Puccinellia nuttalliana* populations

Location (province)	Collection year	Latitude (N)	Longitude (W)
Baldur (MB)	2008	49.38°	99.31°
Basswood (MB)	2008	50.28°	99.96°
Brandon (MB)	2008	49.91°	99.93°
Lundar/Coldwell (MB)	2009	50.71°	98.06°
Westbourne09 (MB)	2009	50.19°	98.84°
Headingly (MB)	2008	49.88°	97.45°
Miami (MB)	2008	49.36°	98.18°
Miniota (MB)	2008	50.31°	101.03°
Neepawa (MB)	2008	50.22°	99.50°
Ninette (MB)	2008	49.36°	99.65°
Westbourne (MB)	2008	50.10°	98.53°
Winnipeg @ Empress & Wel (MB)	2009	49.90°	97.19°
Steinbach (MB)	2009	49.53°	96.98°
Parks Corner (MB)	2008	50.13°	100.61°
Moosomin (SK)	2008	50.14°	101.65°
Quill Lake (SK)	2008	52.01°	104.38°
Eston (SK)	2009	51.15°	108.77°
Fleming (SK)	2009	50.08°	101.53°
Schuler north (AB)	2009	50.34°	110.15°
Boharm (SK)	2009	50.40°	105.72°
Melville (SK)	2008	50.93°	102.82°
Dixon (SK)	2008	52.20°	105.45°
St. Denis (SK)	2008	52.14°	106.21°
Bird's Point (SK)	2008	50.44°	102.25°

3.2 Data collection

3.2.1 Field data collection

The characters and methods of measurement for the analyses of variation among the 24 *P. nuttalliana* populations are shown in Table 3.2. Data for the traits spring growth, regrowth, tiller number, tiller height, crown diameter, leaf angle and leaf colour were collected in years 2011 and 2012. Data for the traits leaf length and leaf width were collected in year 2011. Data for dry matter yield and seed yield were collected in year 2012. Entire plants in each plot were cut to determine the fresh biomass and seed yield. After weighing to determine the fresh biomass, samples of each plot were dried at 34℃ and seeds were threshed by hand. To adjust the weights

for dry matter biomass, additional whole plant samples were taken from guard rows that were dried at 60℃ and dry matter yields were determined.

Table 3.2 Descriptors used to assess the observed characters in the analyses of variation among 24 *Puccinellia nuttalliana* populations

Characters	Method of Measurement	Time of Field Measurement
1. Tiller height (cm)	Measured on one random plant per plot after anthesis, from the ground level to the top of the tallest tiller	Early July
2. Spring growth	1-5, visually evaluated, 1-poor growth, 5-vigorous growth	Late May
3. Leaf angle	1-5, visually evaluated, 1-horizontal, 5-vertical	Late May
4. Tiller number (N)	Total number of tillers per plant	Mid July
5. Dry weight (g $plot^{-1}$)	Total aboveground dry matter yield, measured on each plot	Mid July
6. Seed yield (g $plot^{-1}$)	Total seed yield, measured on each plot	Mid July
7. Regrowth	1-5, 1-poor growth, 5-vigorous growth, visually evaluated after defoliation	Early September
8. Leaf colour	1-9, visually evaluated, 1-yellow, 5-light green, 7-blue green, 9-dark green	Early September
9. Leaf length (cm)	Measured on six fully expanded leaves of one plant in each plot after plants fully headed	Early July
10. Leaf width (cm)	Measured on six fully expanded leaves at the widest part of each leaf in each plot after plants fully headed	Early July
11. Crown diameter (cm)	The diameter of each plant's crown measured two weeks after defoliation	Late August

3.2.2 DNA extraction and AFLP procedure

Two to three leaves from each collected sample were placed in a 1.5mL micro-centrifuge tube with three 2 mm glass beads. The tubes were placed on a horizontal shaker until the leaf tissue was ground to a fine powder. The DNA was extracted the samples by using a DNeasy^{TM} Plant Mini Kit (QIAGEN Inc. Mississauga, Ontario) according to the manufacturer's directions. Then extracted DNA was quantified by fluorimetry using Hoechst 33258 stain (Sigma Chemical Co., St. Louis, Missouri), followed by dilution to 25 ng μL^{-1} for AFLP analysis.

The AFLP analysis was performed using the AFLP Analysis System 1 (Life Technologies, Burlington, ON) according to Vos et al. 1995. There were four main steps: (i) restriction digestion of the 250ng genomic DNA sample with *Eco*RI and *Mse*I restriction enzymes and ligation of adapters with T4 DNA ligase to the restriction fragments to create primary templates; (ii) pre-amplification reaction included one *Eco*RI primer and one *Mse*I primer with an additional single nucleotide at the 3' end; (iii) selective amplification of the pre-amplified fragments with *Mse*I and *Eco*RI primer labeled with [γ^{35}P], both including three selective nucleotides at the 3' end; Ten *Eco*RI:*Mse*I primer pairs were initially screened on twelve leaf samples and the five most informative ones were selected for this AFLP analysis; (iv) fragments were electrophoresed in the automated ABI 3100 Genetic Analyzer Visualization and sizing of fragments were performed by ABI FeneScan 3.1 software. To minimize technique-related and scoring errors, two duplicate samples of one individual were arranged on one gel as control.

3.3 Statistical analysis

3.3.1 Morphological and agronomic characters

Data were analysed using appropriate procedures of SAS software (SAS Institute Inc. 2011). An analysis of variance (ANOVA) was performed among 24 populations and among 5 genotypes within each population for each trait using the Mixed Model procedure. Population, year, and their interaction were assumed to be fixed effects and replications were assumed to be a random effect and significance declared at α=0.05. Values of measured variables were averaged across both years if no significant year × population interaction (P>0.05) was detected.

Data for each year were analyzed separately due to significant year × population interactions for most characteristics. For analysis of individual years, genotypes and populations were considered as fixed effects and block was considered as a random effect. In each analysis, if the ANOVA indicated significant differences at the $p \leq 0.05$ level, the means were compared by calculating least significant differences (LSD) at $p \leq 0.05$. Normalities of distribution ($p > 0.05$, 5% significance level) were checked by using the Shapiro-Wilk test (Shapiro and Wilk 1965). Homoscedasticity was also checked before analysis. The data for the traits spring growth, regrowth, leaf angle and leaf colour were transformed by the square root transformation to

achieve normality. Pearson correlation coefficients were calculated using the CORR procedure of SAS version 9.3.

To determine whether there were differences between collection years of the 24 *P. nuttalliana* populations based on the 11 field observed characters, an analysis of variance (ANOVA) was performed on two years' collections for each trait using the Mixed Model procedure. Seed collection year was considered as a fixed effect and block was considered as a random effect. Normality of distribution ($p>0.05$, 5% significance level) was checked by using the Shapiro-Wilk test (Shapiro and Wilk 1965). The data for the traits spring growth, regrowth, leaf angle and leaf colour were transformed by the square root transformation to achieve normality.

A data matrix of 24 populations using the means of 11 morphological/agronomic characteristics in both years was constructed (only the characters that showed significant variability among populations were investigated in the clustering analysis). The data matrix was standardized and analysed using the Euclidean coefficient. The data matrix was subjected to cluster analysis using an unweighted pair-group method with arithmetic mean (UPGMA) analysis. Because of the presence of population × year interactions, the cluster analysis was applied separately to data from each year. The results and conclusions from the separate cluster analyses were similar for the two years (Appendix C, Appendix D); therefore, a single set of analyses, on the basis of population means over two years, was used.

The 24 population means for all eleven variables were subjected to principal coordinate analysis (PCoA) to examine the relationship among the 24 populations. All the analyses were performed using NTSYS-pc 2.10 software (Rohlf 1997).

3.3.2 Molecular analysis

For each gel generated from each primer pair, the numbers of observable and polymorphic AFLP bands were counted. The goal was to select primer combinations that would yield polymorphic banding patterns that could be scored without ambiguity. Polymorphic AFLP bands with sufficient intensity for all the samples were selected and manually scored as present (1) or absent (0) and missing data points were assigned as 9. The analysis of AFLP polymorphisms on the selected scored bands was carried out by counting the number of polymorphic bands (NPB) and

generating the summary statistics on the mean band frequencies (MBF) with respect to primer pair and population. The within-population variation (WPV) (the average number of differences between all pairs of haplotype within a population) was estimated using Arlequin 3.0 (Excoffier et al. 2005).

To assess AFLP variation across various populations and regions, an AMOVA was performed using Arlequin version 3.1 (Excoffier et al. 2005). This analysis not only allows the partition of the total AFLP variation into within-and among- population variation components but also provides a measure of intergroup genetic distances as the proportion of the total AFLP variation residing between any two populations (called Phi statistic (Φ_{st}); Excoffier et al. 1992, Huff et al. 1998). Models involving various types of structuring (population and region) were applied. Significance of resulting variance components and intergroup genetic distances was tested with 9999 random permutations. The two collection years were analyzed separately as to different collection methodology (i.e. plants vs seeds).

To assess the genetic associations of the *P. nuttalliana* plants representing the 23 populations, the inter-population distance matrices were analyzed using NTSYS-pc 2.10e and clustered with the UPGMA based on the Euclidean distance matrices. Where ties occurred, a find option was used in the UPGMA analysis. The 2008 and 2009 collections were clustered separately in the UPGMA analysis. A principal coordinate analysis was conducted with NYSYS 2.10e using the binary matrix of all the individuals as the input matrix. The scatter plot was determined by the first two principal components.

Matrix correlations between genetic distances and geographic and phenotypic distances were estimated with the Mantel test (Mantel 1967) using the GenAlEx 6.5 software with 9999 permutations. For the genetic distance matrix, the Φ_{st} pairwise differences between populations were used from the AMOVA procedure. The geographic distance matrix was generated from the latitude/longitude decimal form. Phenotypic data were normalized first to generate the matrix.

4 Results and Discussion

4.1 Phenotypic and agronomic variation of 24 *P. nuttalliana* populations

4.1.1 Variation among 24 *P. nuttalliana* populations

The year × population interaction was significant ($p<0.05$) for spring growth, regrowth, leaf angle, tiller height and leaf colour, thus, the individual years were analysed separately for these five characters (Table 4.1). Where year × population interactions were non-significant, data were averaged across years for each collected population. Population × year interactions have been found to be significant ($p<0.05$) on field observed characters in many perennial grasses including *P. nuttalliana* and side-oats grama (*Bouteloua curtipendula* (Michx.) Torr.) (Tarasoff et al. 2007, Schellenberg et al. 2012).

The ANOVA revealed that there were highly significant ($p<0.001$) differences among the 24 *P. nuttaliana* populations in all measured field traits in 2011 and 2012, except the trait of leaf colour in 2012 for which differences were significantly ($p<0.05$) different (Table 4.2).

Table 4.1 *F* value from the analysis of variance of seven observed characters for 24 *P. nuttalliana* populations measured in 2011 and 2012 using PROC MIXED.

	d.f.	Spring growth	Leaf angle	Tiller height	Tiller number	Crown diameter	Leaf colour	Regrowth
Population	23	18.40 ***	20.69 ***	18.40 ***	5.15 ***	10.83 ***	3.92 ***	6.47 ***
Year	1	20.02 ***	43.91 ***	20.02 ***	164.34 ***	357.50 ***	143.37 ***	2186.17 **
Population× year	23	1.79 *	3.27 ***	1.79 *	1.01	1.28	2.08 **	2.05 **

Variance ratio *** P≤0.001; **P≤0.01; *P≤0.05;

Table 4.2 *F* values from the analyses of variance among 24 *P. nuttalliana* populations for 11 field characters measured in 2011 and 2012

Field observations	**2011**	**2012**
Tiller height	10.18***	10.53***
Tiller number	4.33***	7.06***
Crown diameter	5.33***	7.37***
Dry matter yield	-	8.92***
Seed yield	-	9.57***
Leaf length	18.77***	-
Leaf width	10.40***	-
Spring growth	11.25***	9.00***
Regrowth	5.49***	2.36***
Leaf angle	8.80***	14.54***
Leaf colour	4.19***	1.77*

Variance ratio *** P≤0.001; **P≤0.01; *P≤0.05;

4.1.1.1 Height, tiller number and crown diameter

The populations Eston (91.7cm) and Steinbach (84.0cm) were significantly ($p<0.05$) taller than all of the other 21 populations with the exception of Westbourne09 (Table 4.3). The population St. Denis was significantly ($p<0.05$) shorter than the others in 2011. In 2012, the population Steinbach (82.6cm) was significantly ($p<0.05$) taller than most of the other populations. The populations St. Denis (53.0 cm) and Schuler North (53.4cm) were significantly ($p<0.05$) shorter than the others. The mean height over all populations in 2011 was significantly greater than in 2012. Similar results were found in other research on *P. nuttalliana* with significantly taller plants and greater biomass in year 1 following establishment than year 2 (Tarasoff et al. 2007).

Tiller numbers per plant were averaged over the two years due to non-significant year × population interaction. The population Westbourne09 and Eston had significantly ($p<0.05$) more tillers than other populations except for St. Denis, which had numerically the third highest number of tillers (Table 4.3). The population Schuler North had a significantly ($p<0.05$) lower number of tillers than all the other populations.

Crown diameters were averaged over the two years (Table 4.3) due to non-significant year × population interaction. Crown diameter ranged from 15.7cm to 27.6cm, with a mean of 22.5cm. The populations Eston (27.6cm) and Westbourne09 (27.2cm) had significantly ($p<0.05$) wider

crowns than other populations with the exception of St. Denis, Neepawa and Quill Lake. Crowns of the populations Fleming and Schuler North were narrower than other populations in both years.

Table 4. 3 Height, tiller number and crown diameter of 24 *P. nuttalliana* populations

Population	Height (cm)		Tiller number (N)	Crown diameter (cm)
	2011	2012	2-yr mean[&]	2-yr mean
Eston	91.6	78.7	293	27.6
Westbourne09	79.5	72.5	317	27.2
Steinbach	84.0	82.6	215	19.9
Lundar	79.1	72.6	230	21.3
Quill Lake	75.9	72.2	257	23.9
Bird Point	75.2	71.4	259	22.0
Boharm	75.1	76.2	223	22.0
Parks Corner	74.7	70.6	201	20.3
Melville	74.5	71.0	231	23.3
Baldur	74.4	73.7	224	23.1
Miniota	73.7	75.7	211	22.0
Brandon	72.9	73.7	218	22.2
Empress	72.3	70.5	220	22.3
Westbourne	72.1	67.3	230	22.6
Headingly	71.8	71.9	249	22.4
Ninette	70.8	70.2	238	22.3
Fleming	70.7	66.3	186	18.7
Moosomin	70.7	68.5	194	21.5
Miami	69.6	73.1	247	22.8
Basswood	68.7	67.1	253	23.8
Neepawa	68.0	70.0	237	24.0
Dixon	67.0	65.1	210	22.4
Schuler North	65.6	53.3	74	15.7
St. Denis	53.2	53.0	277	26.4
Mean	73.2 A[+]	70.5 B	228	22.5
LSD (0.05)	9.19	7.25	44.56	3.30
SE	0.54	0.48	0.02	0.02
CV%	10.0	8.2	17.12	11.75

N number of tillers per plant

[+] Overall population means in individual years followed by the same letter do not differ significantly (p=0.05)

[&] Two-year data for tiller number and crown diameter were averaged due to non-significant year × population effect (p>0.05)

4.1.1.2 Dry matter yield and seed yield

The population Schuler North was excluded from the analysis of dry matter yield and seed yield because of plant mortality which reduced yield which was expressed on a plot basis. Dry matter yield of the 23 populations ranged from 172 g plot^{-1} to 369 g plot^{-1}, with a mean of 262 g plot^{-1} (Table 4.4). The population Westbourne09 had significantly ($p<0.05$) higher dry matter yield (369 g plot^{-1}) than the other 19 populations with the exception of Eston, Bird's Point, Basswood and Quill lake.

Seed yield of the 23 *P. nuttalliana* populations ranged from 38.6 g plot^{-1} to 100.8 g plot^{-1}, with a mean of 55.2 g plot^{-1}. The population Eston had significantly ($p<0.05$) higher seed yield than all the other populations (Table 4.4). The population Westbourne09 (82.5 g plot^{-1}) had the second highest seed yield but was not significantly different from St. Denis and Basswood. Seed yield was only harvested in year 2 of the present study. A decrease in the average seed yield of *P. nuttalliana* from year 2 compared to year 1 was found in other studies (Tarasoff et al. 2007), so it is possible that higher seed yields would have been obtained in year 1 of the present study.

Table 4.4 Dry matter yield and seed yield of 23 *Puccinellia nuttalliana* populations in 2012

Population	Dry matter yield (g plot^{-1})	Seed yield (g plot^{-1})
Westbourne 09	369	82.5
Eston	334	100.8
Bird's Point	302	62.6
Basswood	296	65.0
Quill Lake	291	57.6
Melville	289	59.5
Empress	289	54.3
St. Denis	280	74.7
Lundar	270	57.7
Steinbach	270	49.3
Miami	268	56.1
Brandon	255	54.8
Fleming	250	42.3
Boharm	237	36.6
Ninette	243	45.4
Baldur	232	47.6
Westbourne	241	46.7
Headingly	240	51.4
Neepawa	238	50.2
Miniota	219	48.9
Dixon	216	42.9
Parks Corner	214	43.4
Moosomin	172	38.6
Mean	262	55.2
LSD (0.05)	68.62	16.22
SE	5.65	1.33
CV%	21.7	24.2

4.1.1.3 Spring growth and regrowth

Most populations grew vigorously in spring and the mean scores of spring growth in 2011 and 2012 were 3.68 and 3.83, respectively (Table 4.5). The population Eston (4.76) had significantly ($p<0.05$) higher spring growth scores than all the other populations in 2011, followed by St. Denis (4.22), Quill Lake (4.17) and Westbourne09 (4.13). The populations Eston (4.53) and St. Denis (4.55) had significantly ($p<0.05$) higher spring growth scores than the most of the other populations in 2012.

The mean scores of regrowth vigour following cutting in 2011 were much higher than those in 2012 for all 24 populations (Table 4.5). The population Westbourne09 (4.05, 1.92) had significantly ($p<0.05$) higher regrowth scores than most of the other populations in both years.

Almost all of the 24 populations had considerable spring growth in both years, but they all had significantly ($p<0.05$) lower regrowth scores in 2012 than in 2011. Mintenko et al. (2002) found that regrowth after mowing was decreased when precipitation was low. However, in the present study, regrowth vigour in 2012 was lower even though there was higher rainfall in 2012 than in 2011 (Appendix G). Fulkerson and Donaghy (2001) suggested that plant density or tiller number was not affected by defoliation during the first year following the establishment year but persistence would be expected to decrease with defoliation over time. Mintenko et al. (2002) found that *P. nuttalliana* showed poor tolerance to mowing stress across years with a 46% decrease of plant cover after three years. The low regrowth vigour of *P. nuttalliana* at the end of the third year of the stand in the present study, even under adequate precipitation, may indicate that this species may be a short-lived perennial.

Table 4.5 Spring growth and late summer regrowth of 24 *P. nuttalliana* populations

Population	Spring growth		Regrowth	
	2011	2012	2011	2012
	----------1-5 Scale[§]----------			
Eston	4.76	4.53	3.90	1.68
St. Denis	4.22	4.55	3.67	1.50
Quill Lake	4.17	3.95	3.63	1.51
Westbourne09	4.13	4.33	4.05	1.92
Ninette	3.78	3.75	3.35	1.58
Miniota	3.75	3.51	3.14	1.61
Westbourne	3.73	3.70	3.21	1.47
Headingly	3.71	3.77	3.43	1.51
Melville	3.68	3.87	3.53	1.85
Neepawa	3.68	4.01	3.39	1.56
Bird's Point	3.67	3.78	3.48	1.74
Basswood	3.63	4.03	3.53	1.53
Baldur	3.58	3.82	3.33	1.61
Parks Corner	3.58	3.68	3.12	1.50
Miami	3.55	3.87	3.43	1.58
Brandon	3.54	3.72	3.44	1.67
Moosomin	3.53	3.23	3.13	1.49
Empress	3.52	3.85	3.40	1.65
Steinbach	3.49	3.58	2.86	1.46
Lundar	3.48	4.00	3.33	1.71
Dixon	3.46	3.85	3.14	1.49
Boharm	3.26	4.21	3.25	1.77
Fleming	3.24	3.27	3.02	1.25
Schuler North	2.37	1.76	1.74	1.04
Mean	3.68 B[+]	3.83 A	3.37 A	1.59 B
LSD (0.05)	0.45	0.53	0.53	0.35
SE	0.02	0.03	0.03	0.02
CV%	9.8	11.2	12.8	17.6

[§] Spring growth and regrowth rating scale: 1-5 (1=poor growth, 5=vigorous growth).

[+] Overall population means in individual years followed by the same letter do not differ significantly (p=0.05)

4.1.1.4 Leaf characters

Leaf length ranged from 6.55mm to 11.95mm, with mean of 9.96mm in 2011. The populations Eston (11.95mm) and Steinbach (11.86mm) had the longest leaves, yet the difference was not significant from most of the other populations (Table 4.6).

Leaf width ranged from 0.21mm to 0.34mm, with mean of 0.27mm (Table 4.6). The population Eston (0.34mm) also had significantly ($p<0.05$) wider leaves than most other populations. The populations Schuler North and Boharm had relatively short and narrow leaves while the populations St. Denis and Neepawa had short, but wide leaves.

Most populations had a light green or blue-green leaf colour, with mean scores of 6.22 and 5.46 in 2011 and 2012, respectively. The populations Eston (6.97) and Westbourne09 (6.87) had a deeper green colour than all the other populations in 2011 (Table 4.6), while Schuler North had a lighter green colour. In 2012, Schuler North and Miniota had lighter green leaves than the majority of the populations. The average score of leaf colour in 2011 was significantly higher than the score in 2012. This may be explained by more vigorous regrowth following cutting in 2011, as leaf colour measurements were taken in early September.

The mean leaf orientation scores were 3.13 and 3.41 over the 24 populations in 2011 and 2012 (Table 4.6). This indicates that the populations generally had a more vertical habit of growth and this was more marked in 2012. The St. Denis population showed the most horizontal growth habit in both years (2.07 and 1.70), having a significantly lower score than all other populations. The populations Fleming and Ninette showed the most vertical growth habit in both years.

Table 4.6 Leaf characters of 24 *Puccinellia nuttalliana* populations

Population	Leaf length	Leaf width	Leaf colour		Leaf angle	
	2011	2011	2011	2012	2011	2012
	-------- cm -------	------- cm --------	-------1-9 Scale[§]--------		-------1-5 Scale[#]--------	
Eston	11.95	0.34	6.97	5.54	2.69	3.56
Steinbach	11.86	0.30	5.70	5.48	3.52	3.63
Westbourne 09	11.36	0.26	6.87	5.51	3.08	3.60
Quill Lake	10.99	0.28	6.45	5.57	3.40	3.76
Ninette	10.98	0.27	6.10	5.78	3.52	3.82
Brandon	10.97	0.27	6.32	5.61	3.52	3.72
Parks Corner	10.64	0.27	5.97	5.38	3.42	3.73
Miami	10.57	0.27	6.01	5.01	3.27	3.49
Lundar	10.29	0.27	6.21	5.46	3.32	3.61
Headingly	10.27	0.26	6.41	5.46	3.07	3.39
Baldur	10.15	0.26	5.98	5.83	3.27	3.63
Birds' Point	10.14	0.27	6.32	5.46	3.23	3.68
Empress	10.07	0.27	6.37	5.86	3.18	3.38
Melville	10.03	0.27	6.55	5.44	3.13	3.40
Basswood	9.93	0.26	6.47	5.42	2.77	3.24
Fleming	9.86	0.25	5.85	5.26	3.49	4.17
Minolta	9.82	0.27	5.98	4.81	3.08	3.50
Moosomin	9.72	0.26	5.58	5.37	3.30	3.66
Westbourne	9.29	0.28	6.07	5.40	2.80	3.22
Dixon	9.01	0.28	6.04	5.57	2.86	2.86
Neepawa	8.97	0.31	6.08	5.78	3.05	3.50
Schuler North	7.77	0.21	4.68	4.37	3.37	2.73
Boharm	7.00	0.24	6.34	5.73	3.05	2.45
St. Denis	6.55	0.30	6.72	5.46	2.07	1.70
Mean	9.96	0.27	6.22 A[+]	5.46 B	3.13 B	3.41 A
LSD (0.05)	1.86	0.04	0.89	0.76	0.54	0.74
SE	0.07	0.001	0.04	0.04	0.02	0.03
CV%	14.9	12.2	11.6	11.2	13.8	17.3

[§] Colour rating scale: 1-9 (1=brown, 5=light green, 7=blue green, 9=dark green).
Leaf angle rating scale: 1-5 (1=horizontal, 5=vertical).
[+] Overall population means in individual years followed by the same letter do not differ significantly (p=0.05)

4.1.1.5 Association between characters

There were significant ($p<0.001$) positive correlations between many of the characters (Table 4.7). A detailed study on association of characters can provide a better of understanding of which traits contribute directly or indirectly to improved forage yield or turf quality.

A high significant correlation coefficient ($r=0.87$) was found between dry matter yield and seed yield. Other studies have shown conflicting results in the relationship of these two characters in perennial grasses. Dewey and Lu (1959) found a highly significant positive correlation between plant weight and seed yield in crested wheatgrass, while Schaaf et al. (1962) found no correlation of these two traits in the same species. In the present study, the populations Eston and Westbourne09 had both higher dry matter and seed yields than the other populations. On the other hand, the populations Fleming and Boharm had low seed yields but intermediate dry matter yields.

Dry matter yield and tiller number were significantly and positively related ($r=0.53$). Both high tiller number and dry matter yield were found in the populations Eston and Westbourne09 (Table 4.3). Tiller number has been found to be of importance in determining dry matter yield of many perennial grasses, like tall fescue (Nelson et al. 1977), perennial ryegrass (Thomson et al. 1973; Yamada et al. 2004) and napier grass (*Pennisetum purpureum* Schum) (Zhang et al. 2009). The positive relationship between tiller number, height and dry matter yield indicates that large, vigorous plants are high yielding, as one would expect.

On the other hand, increased tiller number per plant is usually associated with reduced weight of individual tillers (Lemaire and Chapman 1996); however, in the present study, it appears that tiller number was a more important factor in dry matter yield determination than tiller weight. Zarrough and Nelson (1980) suggested that tiller weight may become more important than tiller number once the number of tillers in a stand stabilizes. The populations Fleming and Steinbach had low tiller number but intermediate dry matter yield (Table 3.6 and Table 3.8). Knight (1961) reported that low tiller number was associated with both high- and low-yield in orchard grass in different lines.

Tiller height was significantly related to dry matter yield (r=0.50). The populations Eston and Westbourne09 had taller plants, and higher dry matter yield than other populations, and also greater tiller numbers. Tiller number, however, was not significantly correlated to tiller height. Not all taller populations had higher dry matter yields, such as the Steinbach population. Lush and Rogers (1992) found that dry matter yield was positively related to height in perennial ryegrass used as turf. There were significant and positive associations between tiller height and leaf angle (r=0.56) and leaf length (r=0.75). The populations Eston and Steinbach were taller and had longer leaves than other populations, while the shorter populations, St.Denis and Neepawa had shorter leaves. Similar result was found in napiergrass with positive correlations between leaf length and tiller height (John 2008).

Leaf colour was significantly and positively correlated to almost all of the measured characters. Thorogood (2003) stated that turf visual quality, shoot density, percentage ground cover and leaf texture were all correlated and contributed to the turf quality of perennial ryegrass. Plants with high dry matter yield tend to be taller, have greater numbers of tillers, larger crown diameter and greater regrowth vigor. The positive association among different traits indicates that the improvement of one trait may simultaneously improve other desired traits. Correlated traits that are easy to visualize and score can facilitate the rapid selection of *P. nuttalliana* populations with improved forage and turf characteristics.

Table 4.7 Coefficients of correlation between 11 observed traits for *Puccinellia nuttalliana* populations in 2011 and 2012

	Spring growth	Leaf angle	Leaf colour	Leaf length	Leaf width	Crown diameter	Regrowth	Tiller height	Tiller number	Seed yield
Leaf angle	ns									
Leaf colour	0.87***	ns								
Leaf length	ns	0.74***	ns							
Leaf width	0.74***	ns	0.54**	ns						
Crown diameter	0.95***	ns	0.84***	ns	0.65**					
Regrowth	0.89***	ns	0.86***	ns	0.50*	0.88***				
Tiller height	ns	0.56**	ns	0.75***	ns	ns	0.47*			
Tiller number	0.57**	ns	0.55**	ns	0.49*	0.56**	0.51*	ns		
Seed yield	0.88***	ns	0.78***	0.42*	0.69**	0.87***	0.78***	ns	0.64**	
Dry matter yield	0.85***	ns	0.85***	0.49*	0.55**	0.79***	0.87***	0.50*	0.53**	0.87***

[+]*p<0.05, **p<0.01, ***p<0.0001, ns-non significant

4.1.2 Variation within populations

There was significant ($p<0.05$) variation among genotypes for certain traits within some populations in 2011 and 2012 (Table 4.8, Table 4.9). Means of individual genotypes within populations that showed significant differences are presented in Appendix E and F. A number of these genotypes may be useful for the synthesis of new populations with improved turf or forage performance.

4.1.3 Selection of populations with superior forage and turf characteristics

The present study has characterized populations of *P. nutalliana* collected across the prairie region of western Canada. Individual populations have been identified which have superior forage or turf characteristics and could be used as germplasm for these purposes. In addition, superior genotypes have been identified in some populations which could be selected to synthesize new cultivars for forage or turf purposes.

The *P. nuttalliana* populations with potential as turfgrasses were those that showed vigorous spring growth and regrowth after mowing, dark leaf colour, wide crown diameter (i.e. spreading ability), relatively short plant height and horizontal leaf angle. In addition, lines should have reasonable seed yields, so that seed costs would be reasonable. Four of five genotypes from the population St. Denis had consistently short tiller height, wide crown diameter, horizontal leaf orientation, darker leaf colour, and this population had one of the highest seed yields. The one negative point is that the leaves of this population are relatively wide and fine leaves are preferred for turf. Genotype 72 from the population St. Denis should not be considered as promising genotype for turf as it had relatively tall and vertical plants. Other individual genotypes that show potential for synthesizing new cultivars for turf purposes include genotypes 7 and 9 from the Basswood population, genotype 17 from the Dixon population, genotypes 51 and 52 from the Neepawa population, genotype 57 from the Ninette population and genotypes 76 and 78 from the Westbourne population (Appendix E and F). These genotypes had relatively narrow leaves, vigorous spring growth, and wide crown diameter. Other turf-related traits that were not measured in the present study include low crown placement, disease resistance, and persistence under frequent mowing. In the present study, disease resistance was indirectly evaluated through spring and regrowth vigor and leaf color ratings. Disease free plants would be

expected to have greater vigor and deeper green leaves. Further evaluations of *P. nuttalliana* for turf characters should include these other characters, and evaluations done over more years would determine the longevity of stands of this species.

The populations Eston and Westbourne09 had consistently the tallest plants, highest forage dry matter yield and high seed yields; thus they show potential for forage use. It would be useful to determine the forage quality of these lines. Individual genotypes that show potential for synthesizing new cultivars for forage purposes include genotype 21 from the Bird Point population, genotype 31 from the Melville population, genotype 39 from the Miami population, genotype 60 from the Ninette population, genotypes 68 and 69 from the Quill Lake population, genotype 94 from the Empress population and genotype 98 from the Steinbach population. These genotypes possess comparable dry matter yield and plant height to the populations Westbourne09 and Eston.

In many studies of genetic variation of perennial grasses, estimates come from a single location and a two or three year time period (Schellenberg et al. 2012), as was the case for the present study. Longer-term trials on *P. nuttalliana* at additional locations would better characterize genotype × environment interactions and also genotype persistence.

Future studies including saline soil sites would be useful. *P. nuttalliana* was found to be less competitive (i.e. shorter plants, lower number of tillers, lower biomass and yield) when plants were grown in normal soil than when grown in saline soil (Grime 1979, Tarasoff et al. 2007). Multiple locations, especially including normal and saline soil conditions, would explain better the contribution of genotype × environment to phenotypic diversity.

Table 4.8 *F* value from the analyses of variance within each of the 24 *P. nuttalliana* populations for 9 observed traits in 2011

	Spring growth (1-5)	Leaf orient (1-5)	Tiller number (N)	Plant height (cm)	Regrowth (1-5)	Leaf colour (1-9)	Crown diameter (cm)	Leaf length (cm)	Leaf width (cm)
Baldur	1.12	2.15	0.88	1.29	0.95	2.66*	1.47	3.11*	2.64*
Basswood	2.12	4.01**	0.70	1.45	1.15	0.53	1.16	4.75**	4.74**
Brandon	0.56	0.79	0.49	2.15	0.70	0.31	0.41	9.95***	7.67***
Dixon	1.87	15.94***	0.91	8.32***	2.19	2.40	1.10	9.24***	10.60***
Bird's Point	5.30**	0.39	7.23**	6.05**	2.60*	2.75*	5.55***	4.68**	0.81
Headingly	1.11	1.49	1.13	1.73	0.05	1.03	0.92	4.98***	0.73
Melville	0.90	1.91	1.39	3.13	0.67	0.19	0.89	1.25/	1.75
Miami	3.32*	4.88**	4.15*	0.26	1.06	1.24	2.52*	4.07**	1.24
Miniota	1.16	0.71	0.55	1.42	4.48**	3.94**	1.22	2.30	0.95
Moosomin	1.66	0.16	1.03	1.56	0.36	1.01	1.63	1.52	2.92*
Neepawa	3.40**	2.21	1.61	1.56	3.94**	2.26	8.76***	16.06***	12.75***
Ninette	4.52**	3.77**	2.51	6.88**	9.29***	6.31***	1.85	5.84**	4.90**
Parks Corner	3.84**	1.52	0.95	4.60*	3.39**	2.26	3.94**	1.97	3.96**
Quill Lake	1.69	2.08	1.54	0.89	5.63***	1.90	4.73**	10.56/***	5.96***
St. Denis	2.30	5.16**	0.84	19.84***	3.12*	2.08	0.31	16.68***	4.03/***
Westbourne	1.36	9.53***	0.38	4.47*	2.28	0.63	1.30	8.08***	2.67*
Lundar	1.36	1.36	2.96	1.17	3.01*	1.86	1.09	1.13	5.82***
Westbouren09	1.00	1.06	2.26	0.14	1.56	2.02	0.56	3.91**	2.28
Empress	3.39*	2.29	0.41	4.29*	1.17	1.43	1.44	10.47***	3.52**
Steinbach	2.44	4.17***	0.36	9.85***	3.64*	5.47***	4.02**	5.41**	32.67***
Eston	0.72	2.34	0.31	0.68	1.33	0.33	0.23	1.50	3.28*
Fleming	4.95**	2.99*	1.58	0.17	3.54*	3.63*	0.65	5.71**	1.46
Schuler North	1.70	1.25	2.35	1.44	1.67	3.52	8.02*	2.29	3.83**
Boharm	0.82	3.91**	1.01	0.76	0.59	0.54	2.58*	3.03*	2.99*

Variance ratio ***P≤0.001; **p≤0.01; *P≤0.05;

Table 4.9 *F* values from the analyses of variance within each of the 24 *P. nuttalliana* populations for 9 observed traits in 2012

	Spring growth (1-5)	Leaf orient (1-5)	Tiller number (N)	Plant height (cm)	Dry weight (g plot^{-1})	Seed yield (g plot^{-1})	Regrowth (1-5)	Leaf colour (1-9)	Crown diameter (cm)
Baldur	0.79	0.76	0.80	2.16	1.45	1.51	0.57	1.46	1.00
Basswood	0.82	1.58	1.48	0.47	1.80	3.13*	0.56	3.17*	0.16
Brandon	1.01	1.34	0.78	0.39	0.83	1.68	1.18	1.44	2.93*
Dixon	1.44	15.59***	4.29**	6.31**	0.91	1.44	2.30	4.06**	1.45
Bird's Point	2.04	1.43	3.94**	2.82	0.68	0.37	0.94	0.57	3.26*
Headingly	0.21	0.79	0.18	0.07	0.09	0.61	1.89	2.21	0.61
Melville	0.96	1.86	0.40	3.50*	3.87*	3.36*	1.20	2.48	1.51
Miami	0.43	3.15*	1.37	4.45*	0.79	0.59	1.02	2.68*	1.51
Miniota	1.40	1.67	2.07	2.72	2.85	3.59*	1.02	3.14*	2.66*
Moosomin	0.71	1.39	2.56*	2.14	0.78	0.82	0.73	1.50	1.02
Neepawa	1.14	1.56	2.21	2.65	0.09	0.55	3.54*	0.46	7.81***
Ninette	6.31***	4.09**	3.64**	5.18**	14.16***	8.35**	1.56	3.05*	4.33**
Parks Corner	0.63	0.67	0.88	0.25	0.16	0.22	2.21	3.49*	0.46
Quill Lake	2.67*	6.09***	2.88*	1.39	1.56	1.85*	0.95	1.14	8.68***
St. Denis	5.17**	31.19***	0.49	12.12***	2.89	5.09**	2.11	8.89***	0.70
Westbourne	0.80	29.72***	2.09	3.04*	1.37	1.82*	1.32	6.05***	2.67*
Lundar	0.63	3.68*	1.82	2.99	1.11	0.10	0.58	0.44	0.48
Westbouren09	1.07	1.32	0.45	1.18	0.19	0.76	0.02	1.20	0.85
Empress	4.56**	1.83**	3.11*	0.68	6.15**	2.63*	2.34	4.38**	4.31**
Steinbach	4.98**	4.12**	4.30**	0.93	1.65	2.48*	1.24	0.83	2.62*
Eston	1.15	2.69*	0.39	0.53	0.09	0.29	2.38	0.97	0.82
Fleming	0.77	3.10*	1.65	0.25	1.06	0.34	0.34	1.77	1.89
Schuler North	4.04*	0.90	4.17**	2.30	0.10	0.26	1.34	1.98	1.46
Boharm	0.46	2.18	1.46	0.79	0.29	0.99	3.24*	2.10	1.65

Variance ratio ***P≤0.001; **p≤0.01; *P≤0.05;

4.1.4 Comparison of collection methods

The collections made in the fall of 2008 were plants. Plants of the 16 populations collected in 2008 were planted together in a field nursery in 2009 and seeds harvested from the plants to be used in the present study. Thus there was interpollination in the nursery among plants of all populations. In the 2009 collections, seeds used in the present study were harvested directly from each location, thus there was no interpollination between populations from different locations. The analysis of variance made on morphological and agronomic traits after grouping the 24 populations based on two collection years revealed significant ($p<0.05$) differences only for the trait plant height in both years (Table 4.10). The 2008 Collections had significantly shorter plants and leaves than 2009 Collections. There were also differences in spring growth and leaf length between the two collections in 2011 only. Whether these differences were a result of genetic differences among the lines collected from the different locations in each year, or the seed production method, cannot be determined.

Table 4.10 Comparison of the mean values for the field observed traits between the two collection years in 2011 and 2012

Field observed characters	2008 Collections	2009 Collections	2008 Collections	2009 Collections
	2011		2012	
Total height (cm)	70.9 b[+]	78.0 a	69.6 b	72.2 a
Spring growth	3.70 b	3.64 a	3.82 a	3.85 a
Leaf angle	3.11 a	3.20 a	3.39 a	3.46 a
Tiller number (N)	169 a	167 a	255 a	246 a
Dry matter yield (g plot^{-1})	-	-	248.8 a	264.9 a
Seed yield (g plot^{-1})	-	-	52.8 a	55.9 a
Regrowth	3.37 a	3.36 a	1.58 a	1.60 a
Leaf colour	6.19 a	6.27 a	5.42 a	5.53 a
Leaf length (cm)	9.88 b	10.17 a	-	-
Leaf width (cm)	0.27 a	0.27a	-	-
Crown diameter (cm)	25.4 a	25.6 a	20.2 a	19.5 a

[+] Means in a row within a year with the same letter do not differ significantly ($P>0.05$)

4.1.5 Relatedness of P. nuttalliana populations based on morphological and agronomic characters

The relationships among the 24 *P. nuttalliana* populations revealed by UPGMA cluster analyses based on 11 morphological and agronomic characters are presented in Fig. 4.2. Six sub-clusters were identified, being separated from each other by normalized Euclidean distances of 0.2 or greater.

The first group includes seven populations from Manitoba and two populations from Saskatchewan whose plants show relatively low dry matter yield and seed yield. The second group includes the population Moosomin whose plants show medium height and low seed yield. The third group includes five populations from Manitoba and three populations from Saskatchewan whose plants show vigorous spring growth, high dry matter yield and seed yield. The fourth group includes two populations from Manitoba and two populations from Saskatchewan whose plants have an erect habit and high tiller density. The last two cluster groups represented the most phenotypically distinct populations. The population Schuler North from Alberta was the most unique one with poor spring growth and summer regrowth, while the plants of the population St. Denis show a horizontal growth habit, wide crown diameter and low tiller height.

The third group included most of the populations that had relatively high scores of the following traits: plant height, dry matter yield, seed yield, tiller number, spring growth and crown diameter. These traits are more characteristic of plants that would be useful for forage purposes. Collectively, these populations represent potentially valuable germplasm for population improvement and breeding of *P. nuttalliana* as a forage crop.

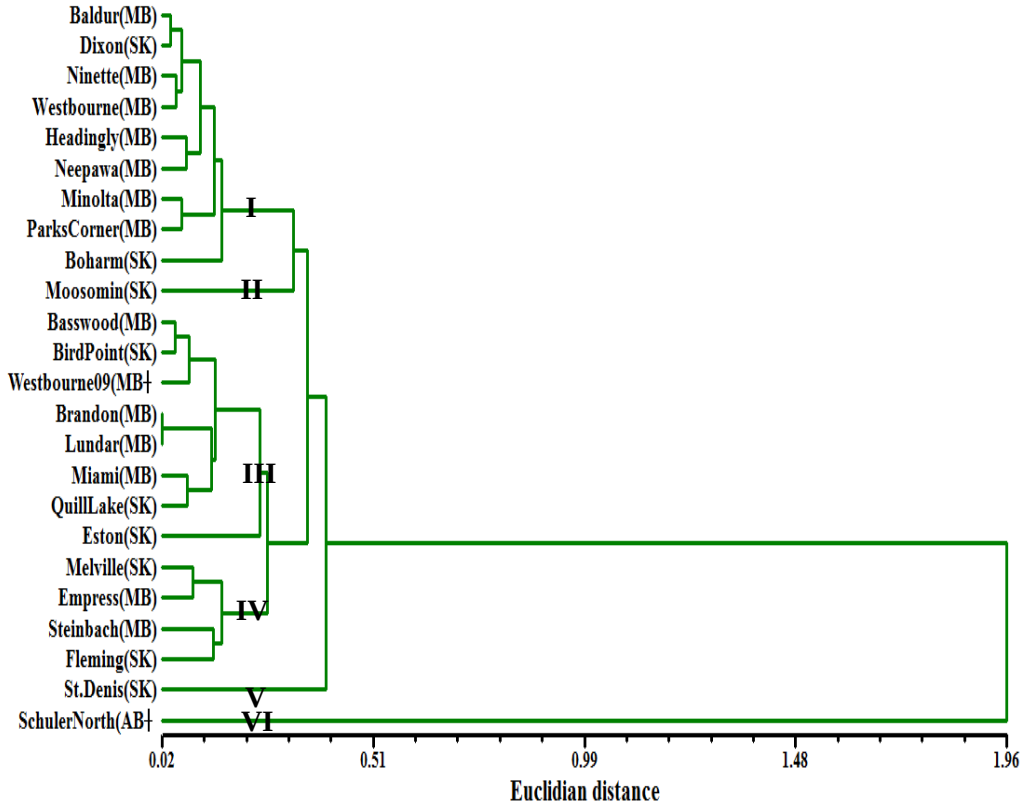
Valuable characters for turf purposes include short plant height, high tiller density and medium yield. Sub-cluster Group six (St. Denis) and some populations in Group one meet these criteria as promising populations for turf.

The collection from Schuler North had the lowest scores of spring growth and regrowth. This population has relatively little value in a breeding program designed to improve the forage or turf production of *P. nuttalliana*. However, this population does illustrate the low end of the range of phenotypic variability of this species on the Canadian Prairie.

The UPGMA analysis failed to group populations based on their geographic regions, which is not surprising due to the large variation within *P. nuttalliana* populations for observed characteristics.

Figure 4. 1 Dendrogram of the 24 *P. nuttalliana* populations revealed by UPGMA cluster analysis of generic similarity based on 11 phenotypic/agronomic characters

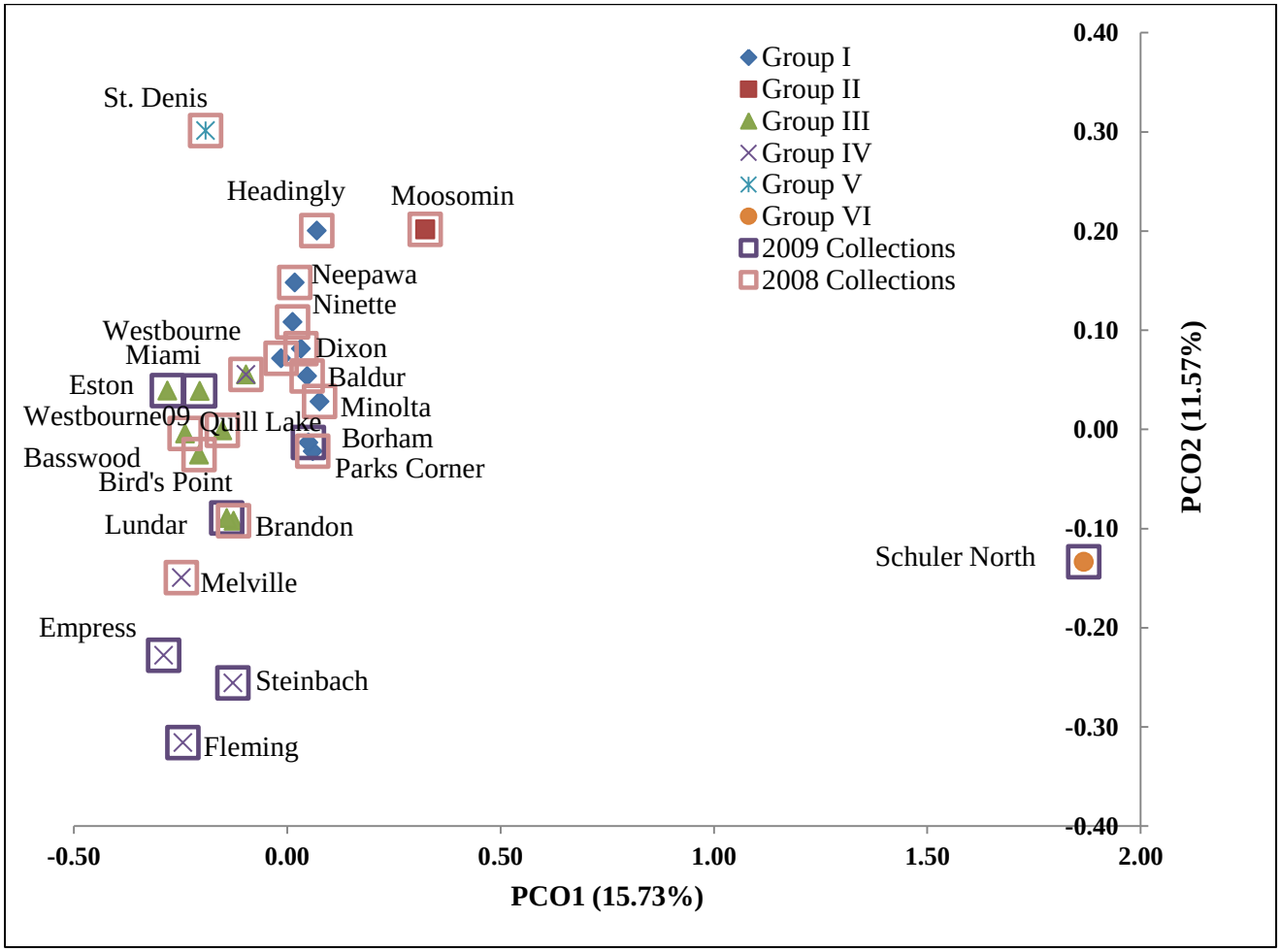
MB- Manitoba; SK-Saskatchewan; AB-Alberta



The results of PCoA (Fig. 4.2) supported the results of the UPGMA. The 24 populations were clustered into six groups based on the UPGMA dendrogram and were similarly defined by the first two principal coordinates which accounted for 27.3% of the total variation. Visually, the PCoA showed reasonably distinct groups which coincided with the groupings of the UPGMA dendrogram, with Schuler North being the most unique population.

The populations were also grouped based on different collection years. There were no distinguishable groupings of collections made in 2008 or in 2009. This is not surprising since there were very few differences between the means of the populations collected in each year for the measured characters.

Figure 4.2 Distribution of 24 *Puccinellia nuttalliana* populations on the first two principal coordinates PC1 and PC2 of the PCoA performed for field observed traits



4.2 AFLP variation

4.2.1 AFLP Polymorphisms

Each of the *Eco*RI:*Mse*I primer combinations amplified 150 to 234 bands ranging from 80 to 590 bp with the majority of the bands within the 100 to 300 bp range. A majority (84%) of the amplified bands were polymorphic while only 185 polymorphic bands could be scored without ambiguity. The observed band frequencies ranged from 0.017 to 0.966 with an average of 0.601 (Table 4.11). For each primer pair, statistics (mean, minimum, and maximum) of the band frequencies are given in Table 4.11. The lowest mean frequency was found with the primer pair E+AGG/M+CGC (39%), while the highest mean frequency was observed with the primer pair E+AAC/M+CAG (77%). Thus, a wide range of AFLP variation was detected in this grass species.

The large number of polymorphic bands detected by the five AFLP primer pairs indicates the utility of AFLP markers in the assessment of genetic variability in *P. nuttalliana*. These scored bands were randomly sampled from the whole *P. nuttalliana* genome, but their exact genome coverage is unknown. No individuals were found with identical marker profiles.

The frequency of scored bands was also determined for the two collection years separately (Table 4.12). The overall trend of the frequencies for individual collections was similar to the overall band frequency while the mean value of each primer pair's frequency for 2008 was slightly higher than for 2009.

Table 4.11 AFLP variation patterns in *P. nuttalliana* populations for each primer pair

Primer pair	No. of AFLP bands		Frequency of scored bands		
	Total	Scored[+]	Mean	Minimum	Maximum
E+AAC/M+CAG	180	43	0.767	0.344	0.957
E+ACC/M+CAG	192	37	0.637	0.032	0.966
E+ACG/M+CTA	234	33	0.603	0.032	0.963
E+AGG/M+CGC	187	33	0.390	0.023	0.919
E+AGG/M+CTC	223	39	0.607	0.017	0.966
All	1016	185	0.601	0.090	0.954

Note: [+] polymorphic AFLP bands scored without ambiguity

Table 4.12 Amplified fragment length polymorphism (AFLP) variation patterns in *P. nuttalliana* populations from collections in 2008 and 2009 for each primer pair

Primer pair	Frequency of scored bands					
	2008 Collections			2009 Collections		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
E+AAC/M+CAG	0.801	0.352	0.983	0.725	0.339	0.920
E+ACC/M+CAG	0.649	0.026	0.974	0.623	0.045	0.929
E+ACG/M+CTA	0.628	0.030	0.987	0.567	0.018	0.937
E+AGG/M+CGC	0.408	0.013	0.915	0.352	0.018	0.893
E+AGG/M+CTC	0.628	0.017	0.996	0.574	0.009	0.920
All	0.623	0.088	0.971	0.568	0.086	0.920

4.2.2 Patterns of variation

Genetic variation for 23 individual *P. nuttalliana* populations was quantified in this study by determining the percentage of polymorphic bands, the mean band frequency, and the within-population variation as calculated from the AMOVA sum of squares (Table 4.13). Within individual populations, the number of scored polymorphic bands ranged from 134 to 170 with an average of 156, the mean band frequency ranged from 0.546 to 0.685 with an average of 0.625 and the within-population variation ranged from 42.17% to 63.54% with an average of 53.46%. Both the number of bands and their frequencies in each population were taken into account in the estimation of the overall within-population variation.

The AMOVA over all of the 23 *P. nuttalliana* populations indicated that the within-population variation accounted for a greater proportion (96.93%) of the total variance than among-populations within regions variation (2.47%) and among regions variation (0.60%); however, there were significant differences among populations ($P<0.001$) based on the permutation test (Table 4.14). The genetic distance between pairs of populations ranged from 0.0001 to 0.1920, with an average of 0.04253 (data not shown). The high within-population variation estimate shows a high level of heterozygosity and genetic variation among the individual *P. nuttalliana* plants.

The AMOVA of populations which were collected in the two different years also showed a greater proportion of within-population variation than the among-population variation, respectively. The within-population variation of the 2008 collections (97.86%) was similar to the 2009 collections (95.96%).

Table 4.13 Amplified fragment length polymorphism (AFLP) variation in 23 *Puccinellia nuttalliana* populations collected at different locations in western Canada

Location (province)	Collection Year	Latitude (N)	Longitude (W)	NP	NPB	MBF	WPV
Baldur (MB)	2008	49.4°	99.3°	15	160	0.628	51.66
Basswood (MB)	2008	53.2°	100.0°	15	151	0.593	53.16
Brandon (MB)	2008	49.9°	99.9°	15	159	0.685	46.13
Lundar/Coldwell (MB)	2009	50.7°	98.1°	15	156	0.663	50.99
Westbourne 09(MB)	2009	50.2°	98.8°	15	144	0.600	53.31
Headingly (MB)	2008	49.9°	97.5°	12	139	0.637	45.73
Miami (MB)	2008	49.4°	98.2°	14	154	0.637	49.91
Miniota (MB)	2008	50.3°	101.0°	15	168	0.610	57.46
Neepawa (MB)	2008	50.2°	99.5°	15	148	0.645	52.51
Ninette (MB)	2008	49.4°	99.7°	15	134	0.661	42.17
Westbourne (MB)	2008	50.1°	98.5°	14	159	0.573	56.58
Winnipeg/Empress (MB)	2009	49.9°	97.2°	15	154	0.640	54.53
Steinbach (MB)	2009	49.5°	97.0°	15	148	0.655	48.82
Parks Corner (MB)	2008	50.1°	100.6°	15	158	0.625	54.38
Moosomin (SK)	2008	50.1°	101.7°	15	154	0.640	47.03
Quill Lake (SK)	2008	52.0°	104.4°	15	157	0.606	54.74
Eston (SK)	2009	51.2°	108.8°	15	170	0.607	63.05
Fleming (SK)	2009	50.1°	101.5°	15	161	0.655	55.85
Boharm (SK)	2009	50.4°	105.7°	15	160	0.546	61.52
Melville (SK)	2008	50.9°	102.8°	15	162	0.634	54.91
Dixon (SK)	2008	52.2°	105.5°	15	164	0.579	58.93
St. Denis (SK)	2008	52.1°	106.2°	15	157	0.582	63.54
Bird’s Point (SK)	2008	50.4°	102.3°	15	165	0.669	52.74

Notes: NP, the number of plants assayed; NPB, the number of polymorphic AFLP bands scored; MBF, mean band frequency; WPV, within-population variation calculated from the sum of squares of AMOVA.

Table 4.14 Analysis of molecular variance (AMOVA) based on AFLP data among 23 *P. nuttalliana* populations from different provinces and different collection years

	Source	d. f.	SS	Variance components	% variation	F_{ST}
Region	Among regions	1	64.002	0.166	0.60	
	Among populations within regions	21	773.978	0.683	2.47	0.0304*
	Within populations	317	8485.914	26.769	96.93	
	Total	339	9323.894	27.618		
2008 Collections	Among populations	15	522.062	0.576	2.14	0.0214*
	Within populations	219	5769.381	26.344	97.86	
	Total	234	6291.443	26.920		
2009 Collections	Among populations	6	279.743	1.260	4.35	0.0435*
	Within populations	98	2716.533	27.720	95.96	
	total	104	2996.276	28.980		

d.f. – degrees of freedom; % variation – the fraction of total variation contributed by each nested component; * – P=0.0001 (n = 10,000 permutations);

Since *P. nuttalliana* is a highly self-incompatible, cross-pollinating, polyploid species, individual plants maintain a higher-than-expected frequency of heterozygosity. Losses of genetic variability due to genetic drift can be counterbalanced by genetic recombination to release new genetic variability (Casler et al. 2007). Comparable high levels of AFLP variation have been reported for other highly outcrossing grass species such as crested wheatgrass (Mellish et al. 2002), and smooth and meadow brome (Ferdinandez and Coulman 2002).

The *P. nuttalliana* populations were weakly differentiated with only 3.07% of the total AFLP variation being between populations (i.e. 0.60% among regions and 2.47% among populations within regions). Most of the total AFLP variation (96.93%) resided within the *P. nuttalliana* populations. The among populations variation, although a minor part of the total variation, was statistically significantly. A high percentage of the total variation being within-population, ranging from 65%-95%, has been previously observed for outcrossing perennial grass species (Casler et al. 2007). Comparable results for Canadian native grass populations were found in plains rough fescue (*Festuca hallii* [Vasey] *Piper*) with only 6.5% of variation

residing among thirty populations (Qiu et al. 2009) and in little bluestem with 7.2% of total variation residing among six natural populations (Fu et al. 2004).

Self-incompatibility systems generally maintain 3 to 10 times more genetic variability within populations than among populations (Gustafson et al. 2004, Huff et al. 1993, Xu et al. 1994). The observation of the low among-population variation may indicate gene flow via pollen and seed among the populations at various sites. Further information on gene flow among *P. nuttalliana* populations in Canadian prairie region and its effect on genetic diversity are needed (Hutchison and Templeton 1999).

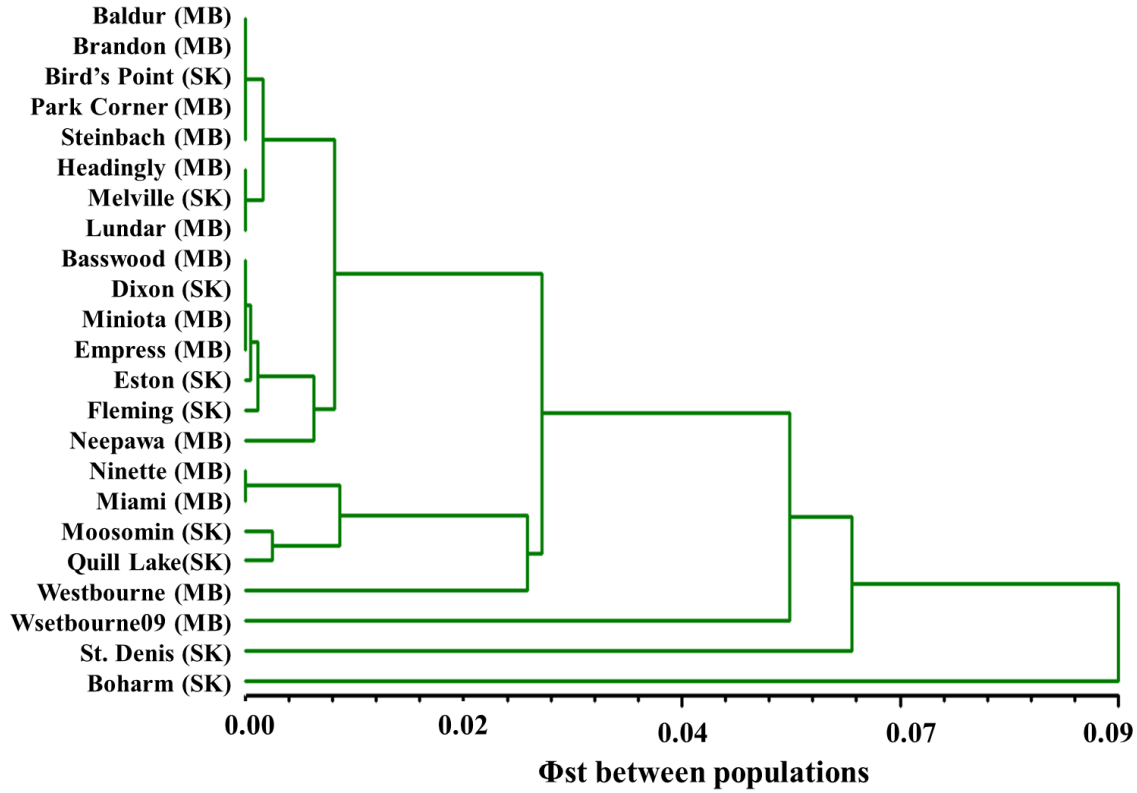
Only AFLP variation was analyzed in this octoploid species. Since AFLP are dominant markers there is no distinction between homozygous dominants and heterozygotes. Co-dominant markers like microsatellites may provide more detailed information on the genetic variation of this species.

4.2.3 Genetic relatedness of *P. nuttalliana* collections

A UPGMA dendrogram was constructed to determine the relationships among the 23 *P. nuttalliana* populations based on inter-population distance (Fig 4.3). Populations originating from Saskatchewan were spread throughout the cluster dendrogram, not showing any specific tendency to be clustered together. For example, the two populations Eston and St. Denis were grouped together with populations from Manitoba. The cluster dendrogram illustrated the lack of a relationship between geographic proximity and genetic relatedness. For example, there was a very close relationship between some geographically distant populations. There were also several collections which showed no AFLP variation in pairwise comparisons (i.e. phi statistic was 0).

Figure 4.3 Dendrogram constructed from 185 AFLP markers observed on 23 *Puccinellia nuttalliana* populations using the UPGMA clustering algorithm

MB- Manitoba; SK-Saskatchewan;

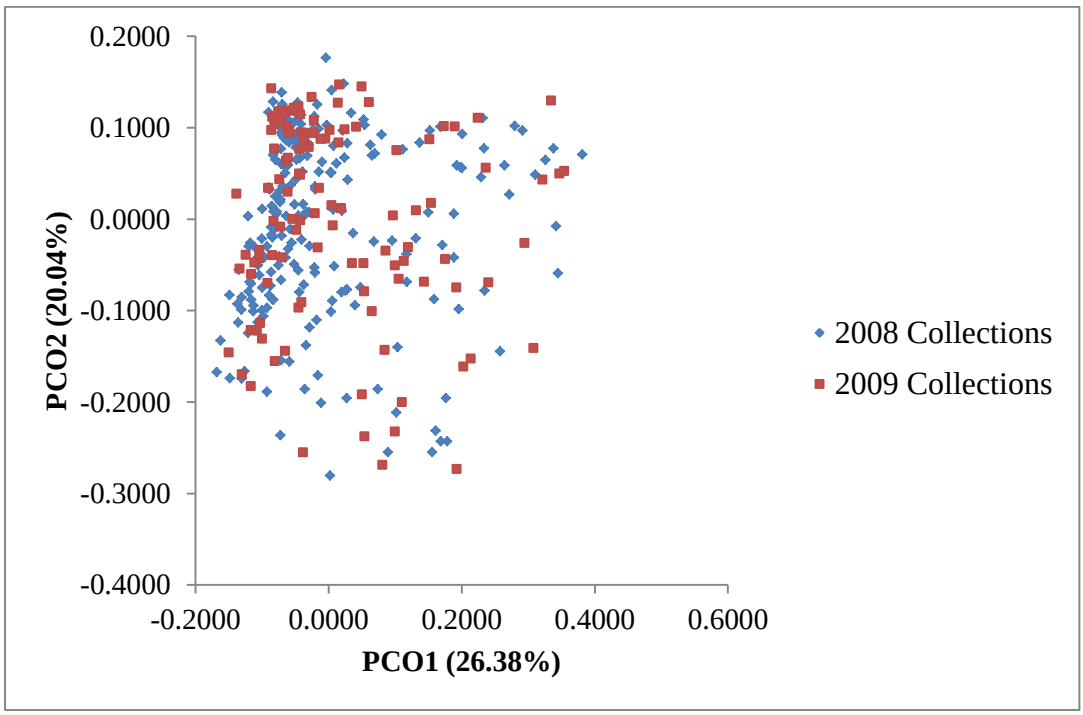


For the PCoA, collections were divided into two groups based on the two collection years. The PCoA (Figure 5.4) illustrated the overlapping distributions of the two collection groups. The first (Dimension =26.38%) and second (Dimension =20.04%) axes explained 46.42% of the variance in the PCoA correlation matrix of relationships among 340 genotypes collected in 23 locations. Similar to the analysis of morphological/agronomic characters, both the PCoA and UPGMA indicated little or no relationship between geographic and genetic distances for AFLP markers.

Wind-facilitated pollen flow or animal facilitated seed dispersal may result in gene migration over a short or long distance. The weak association between the AFLP variation and geographical distance may indicate that migration is an important factor in the low genetic differentiation of *P. nuttalliana* populations across the Canadian Great Plains. Selection for adaptation to specific environment factors, such as soil type and habitat, may also result in population differentiation (Bradshaw 1972, Linhart and Grant 1996). Since all the populations assessed in this study were located in the Canadian Great Plains, analysis of more

populations collected across the full range of the species may reveal greater population differentiation.

Figure 4.4 Principal coordinate analysis plot of 340 individuals representing the 23 populations of 2008 and 2009 collections of *P. nuttalliana*.



4.2.4 Differences between collection methods

Figure 4.5 revealed the relationship among 16 *P. nuttalliana* populations collected in 2008. The analysis split the 16 populations into three sub-clusters. The lack of geographic relationships among the populations, with a very close relationship between some geographically distant populations was detected. Figure 4.6 revealed the relationship among 7 *P. nuttalliana* populations whose seeds were collected in 2009. The Boharm population was clustered separately, while the Eston and Fleming populations from Saskatchewan were clustered with collections from Manitoba. This is the same result found in the analysis done over the two collection years (Figure 4.3).

Half of the 2008 collections showed no AFLP variation in pairwise comparisons (i.e. phi statistic was 0) and the greatest phi statistic distance was 0.05. On the other hand, the greatest phi statistic distance among the 2009 collections was 0.09. The smaller distance in 2008 collections may be due to the interpollination of all collections to produce seed, which did not occur for the 2009 collections. Or, it is possible that the populations collected in the 2009 collections had a higher degree of differentiation than those collected in 2008. It is not possible to determine which of these is correct, since the collections in the two years were from different sites. The only site that was common between the two years was Westbourne; however, the actual sites of collection in 2008 and 2009 at this location were around 24 kilometers apart.

Figure 4.5 Dendrogram constructed from 185 AFLP markers observed on 16 *Puccinellia nuttalliana* populations collected in 2008 using the UPGMA clustering algorithm
MB- Manitoba; SK-Saskatchewan

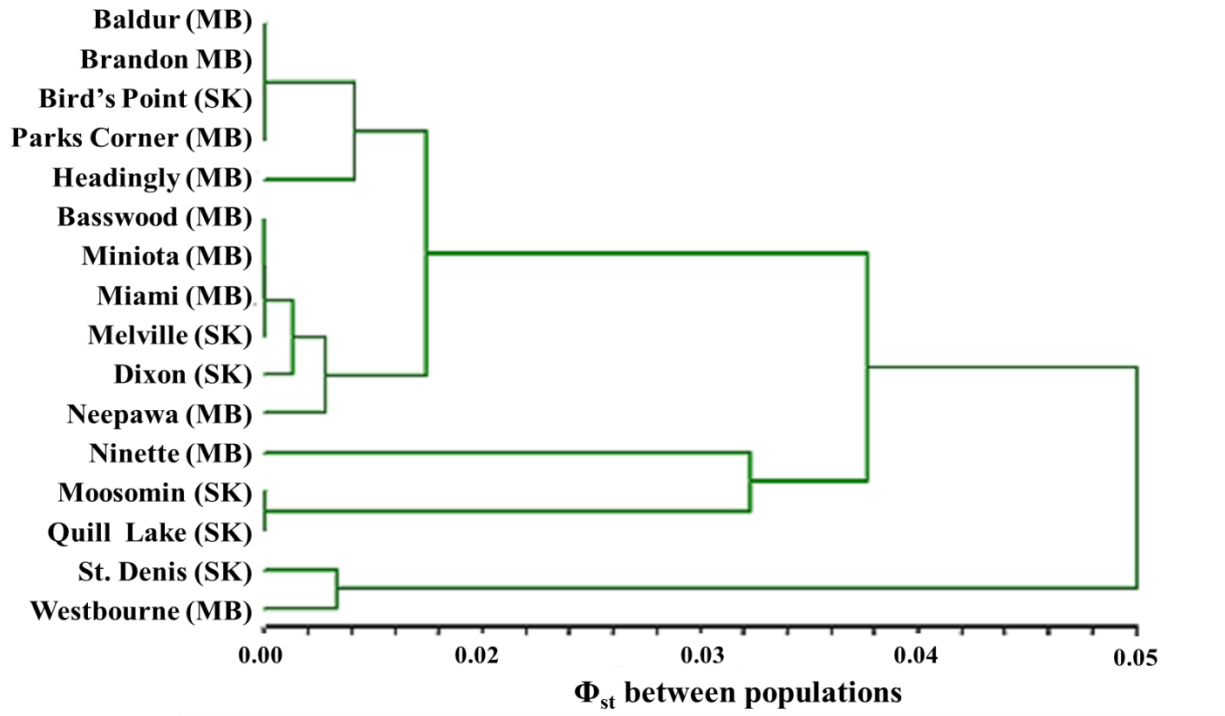
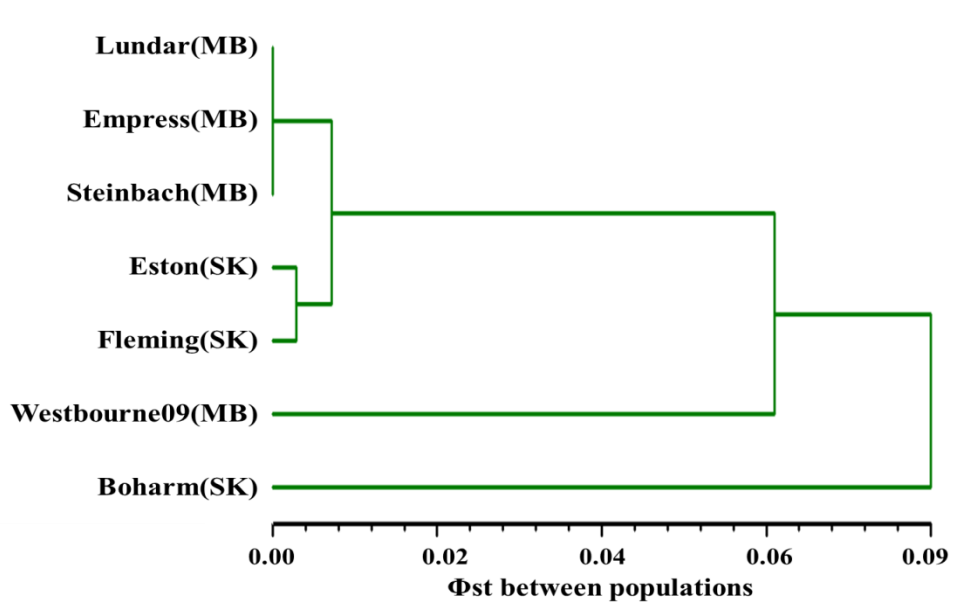


Figure 4.6 Dendrogram constructed from 185 AFLP markers observed on 7 *Puccinellia nuttalliana* populations collected in 2009 using the UPGMA clustering algorithm
MB- Manitoba; SK-Saskatchewan;



The overall trend of allele frequencies was similar between the 2008 and 2009 collections, thus it appears that the interpollination among 2008 collections has not influenced the allele frequency and genetic diversity in this study. Bradley and Johnson (1997) found that genetic diversity, such as heterozygosity and allelic richness, did not change with respect to different seed collection methods in annual ryegrass (*Lolium multiflorum* Lam.). Johnson (1998) also found similar genetic structures of original ryegrass seed populations by different regeneration methods and there was no loss of alleles that are at a frequency of at least 0.05. López and Oliveira (2009) found that significant changes in allele frequencies and agronomic/morphological characters were observed in Italian ryegrass under two different seed regeneration methods; however, genetic diversity was maintained and there was no loss of common alleles.

4.2.5 Correlation between genetic and geographic distances

The correlation between the population pair-wise genetic differences based on phi-statistics and their geographical distances (km) was performed for the 23 populations (Fig. 4.7). Genetic distances were significantly but weakly associated with geographic distances ($r=0.293$, $P=0.024$). The Mantel test supports the results of UPGMA and PCoA (Fig. 4.3 and 4.4).

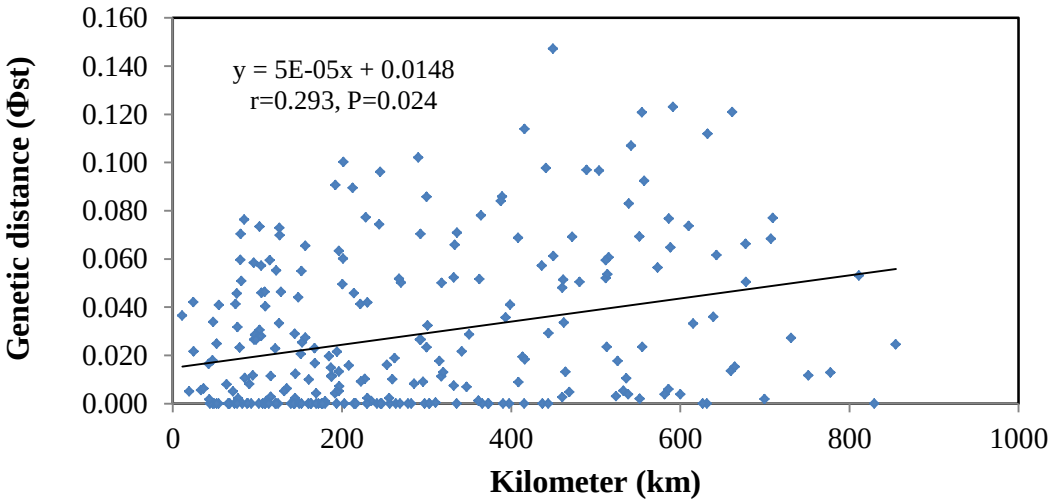
A weak but significant association between genetic and geographic distance has also been found in plains rough fescue ($r=0.39$, $P<0.004$) (Qiu et al. 2009). Non-significant association between genetic diversity estimates and geographic distance was observed in other studies, for example for big bluestem (*Andropogon geradii*) and Indian grass (*Sorghastrum nutans*) (Gustafson et al. 2004).

The Mantel test has also been successfully used to test the significant effects of local environment on genetic differentiation by measuring the association between genetic distance and geographic distance, for fringed brome ($r=0.68$, $P<0.0001$) (Fu et al. 2005). For fringed brome there was low within-population (47.38%) variation and limited gene flow via pollen and seed among the populations, likely explaining the differentiations among the populations.

Genetic differentiation of salt tolerant *P. nuttalliana* populations may not be significantly associated with precipitation or temperature differences, but may rather be associated with salinity level in soils within the geographical range. Therefore, future studies on the association

between genetic distance and salinity level of original collection sites may provide more information on geographical variation of *P. nuttalliana*.

Figure 4.7 Associations between genetic distances measured by the Phi statistic and geographical distances in kilometers of 23 *P. nuttalliana* populations as determined by a Mantel test



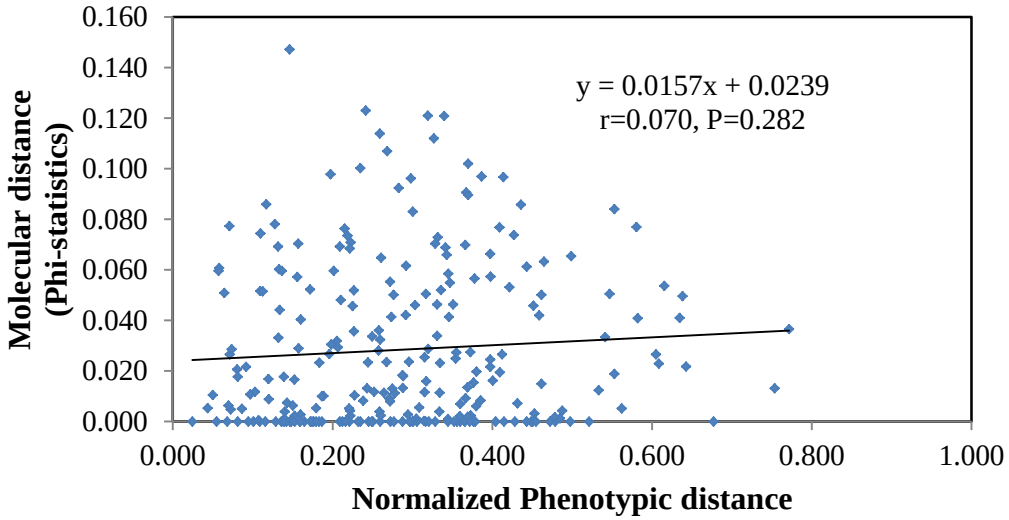
4.2.6 Correlation between genetic and phenotypic distance

Normalized phenotypic distances, taking into account all eleven phenotypic variables, were not significantly associated with molecular genetic distance generated from Phi-statistics (r=0.070, P=0.282) for the 23 populations (Fig. 4.8).

Many morphological and agronomic traits are influenced by environmental factors that may affect their expression. AFLP markers are random in the genome and have not been associated with any agronomic or morphological traits in *P. nuttalliana* Thus, it is not surprising that there is no relationship between phenotypic and molecular variation. A significant relationship between phenotypic traits and molecular markers can be observed if the markers are selected based on their linkage to particular known loci (Persson and Gustavsson 2001). A lack of correlation between morphological and genetic distances has been established in other perennial grass species as well, such as cocksfoot (*Dactylis glomerata* L.) (Shanjani et al. 2012). The lack of significant association between phenotypic and molecular diversity in this study indicated that

P. nuttalliana germplasm utilization should be based on both diversity measurements to obtain comprehensive diversity information.

Figure 4. 8 Scatterplot of normalized phenotypic distance vs. molecular distance (Phi-statistics) of 23 *P. nuttalliana* populations



4.2.7 Sampling for breeding programs and conservation

Genetic and geographic information on *P. nuttalliana* populations is important for their potential utilization in conservation programs and for turf or forage cultivar development. The assayed *P. nuttalliana* populations appear to be maintaining a high diversity, both in AFLP markers and morphological/agronomic traits. Xu et al. (1994) stated that the optimal number to adequately sample the genetic diversity within a bulk is 15 plants, which is the number that was used in the present study. The lack of a major differentiation of populations over the geographic region, and the high within population variation, suggests that collecting over the entire region would not be required for diversity studies, conservation, or establishing breeding populations. Sampling a small number of sites would capture a large amount of the total genetic variation. Future variation studies on *P. nuttalliana* would be more effective by sampling a small number of individuals from each population over a wider area of the distribution of this species. Further studies on habitat type and evolutionary forces such as pollen flow and seed dispersal would be useful to better understand the variation patterns of *P. nuttalliana*.

5 Summary and Conclusions

In the present study, the genetic diversity of 24 *P. nuttalliana* populations was analyzed using both agronomic/morphological traits and AFLP markers. The agronomic and morphological study was based on plant height, tiller number, dry matter yield, seed yield, crown diameter, growth habit (spring and late summer regrowth), leaf length and width, leaf colour and leaf angle characteristics. Both analyses revealed high diversity among and especially within populations. This information is useful in identifying promising populations and genotypes for synthesizing improved populations which would be useful for forage or turf purposes. The study also contributes to our knowledge of population differentiation in native grass species.

5.1 Morphological and agronomic variation

There was significant variation among and within *P. nuttalliana* populations for all the measured morphological and agronomic characters. Most of the variation was found within populations; although variation was significant between populations, they were not highly differentiated. Almost all the populations possessed vigorous spring growth but poor growth recovery following defoliation. No clear geographic pattern of population differentiation was observed among 24 *P. nuttalliana* populations collected from the Canadian Great Plains based on morphological and phenotypic characters.

Several promising populations were identified. The plants in the populations Eston and Westbourne09 were taller, had greater basal diameter, highest tiller numbers and high biomass and seed yields. These two populations show potential for forage uses. The St. Denis population had shorter plants with a large crown diameter, high tiller numbers, darker leaf colour, and a more horizontal growth habit. This population has many of the desirable characteristics of turf grasses.

A number of promising genotypes which could be used to synthesize new cultivars with improved forage or turf performance were identified. These genotypes came from a number of different populations. Gaining a broader knowledge of *P. nuttalliana* genetic resources, especially from other regions of Canada and North America should be helpful in enriching genetic resources.

5.2 AFLP marker analysis

Using the AFLP technique, significant within- and among population variation was found in the *Puccinellia* collections. Within population variation accounted for approximately 96% of the total variation. There was a significant correlation between genetic distance and geographic distance; however, its magnitude was small, so geographically distant populations were not always the most genetically different.

These results suggest that sampling a relatively small number of populations and individuals in each population across the Canadian prairie region would capture a large portion of the genetic diversity of this species in this region. Thus assembling populations for breeding programs or for germplasm conservation will not require sampling of numerous sites and plants.

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7 Appendix

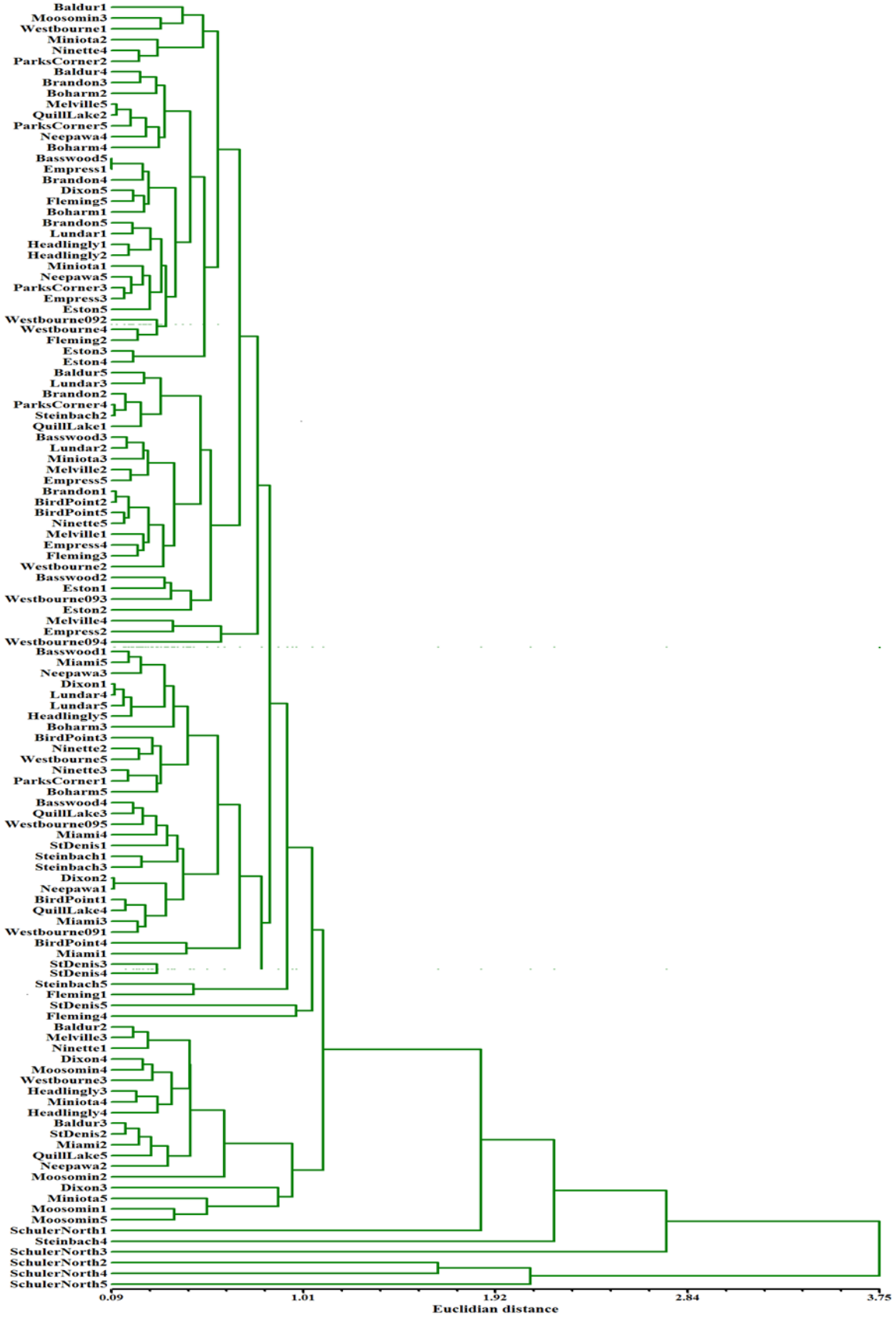
Appendix A. Means (s.d.) and ranges for 11 morphological and agronomic characters of 24 Puccinellia nuttalliana populations

Population	Spring growth (1-5)	Leaf angle (1-5)	Tiller number (N)	Plant height (cm)	Crown diameter (cm)	Seed yield (g plot^{-1})	Dry weight (g plot^{-1})	Leaf length (cm)	Leaf width (cm)	Regrowth (1-5)	Leaf colour (1-9)
Baldur	3.70 (1.01) (1-5)	3.45 (0.83) (1-5)	223 (77) (29-323)	75.4 (7.15) (59-94.5)	23.2 (7.67) (6.4-45.7)	47.6 (18.65) (11.6-78.2)	231.9 (69.85) (106.5-352.4)	10.15 (2.98) (3.2-17.2)	0.26 (0.06) (0.10-0.40)	2.52 (1.24) (1-5)	2.92 (1.35) (2-8)
Basswood	3.88 (0.96) (1-5)	3.07 (1.08) (1-5)	254 (157) (51-510)	67.3 (13.52) (7-92)	23.8 (9.29) (2.5-48.3)	65.3 (26.96) (18.0-119.0)	296.2 (122.25) (87.3-511.0)	9.93 (3.07) (3.1-16.7)	0.26 (0.09) (0.10-0.55)	2.59 (1.31) (1-5)	5.98 (1.36) (1-9)
Brandon	3.61 (1.03) (1-5)	3.53 (0.92) (1-5)	232 (96) (131-487)	75.1 (7.27) (56.5-91.4)	22.1 (8.54) (2.5-48.3)	54.8 (16.99) (23.2-84.6)	255.0 (80.28) (119.8-440.8)	10.97 (3.62) (2.9-18.5)	0.27 (0.07) (0.10-0.50)	2.63 (1.27) (1-5)	6.00 (1.39) (1-9)
Dixon	3.61 (1.04) (1-5)	2.92 (1.11) (1-5)	247 (144) (8-538)	67.2 (12.31) (40.0-96.0)	22.4 (8.38) (5.1-49.5)	42.9 (22.95) (15.9-90.9)	215.7 (103.95) (77.0-424.7)	9.01 (3.09) (3.0-18.2)	0.28 (0.08) (0.10-0.50)	2.39 (1.26) (1-5)	5.81 (1.46) (2-9)
Bird's Point	3.73 (1.09) (1-5)	3.46 (0.78) (1-5)	340 (203) (153-782)	73.7 (9.78) (53.5-95.55)	22.2 (8.59) (5.1-48.3)	62.6(25.93) (20.3-131.1)	302.3 (137.21) (95.6-627.2)	10.14 (3.27) (3.2-18.5)	0.27 (0.07) (0.10-0.50)	2.66 (1.29) (1-5)	5.94 (1.55) (2-9)
Headingly	3.74 (1.05) (1-5)	3.23 (0.92) (1-5)	249 (128) (56-478)	73.4 (10.64) (41.0-94.0)	22.4 (7.48) (2.5-38.1)	51.4 (33.07) (1.7-132.2)	240.3 (120.73) (61.1-532.9)	10.27 (2.66) (3.8-17.0)	0.26 (0.07) (0.10-0.45)	2.60 (1.30) (1-5)	5.96 (1.35) (1-9)
Melville	3.78 (1.05) (1-5)	3.26 (0.90) (1-5)	227 (130) (20-405)	73.8 (8.90) (46.1-86.1)	23.4 (7.40) (2.5-39.4)	59.5 (19.93) (30.8-100.4)	288.5 (86.10 (132.0-403.4))	10.03 (2.97) (3.2-16.4)	0.27 (0.08) (0.10-0.50)	2.73 (1.30) (1-5)	6.02 (1.40) (1-9)
Miami	3.71 (1.03) (1-5)	3.36 (0.86) (1-5)	175 (100) (38-380)	73.2 (8.21) (55.0-91.4)	22.9 (8.44) (2.5-48.3)	56.1 (30.86) (15.2-116.6)	268.2 (129.88) (82.5-518.2)	10.57 (3.28) (3.0-18.0)	0.27 (0.07) (0.10-0.45)	2.58 (1.29) (1-5)	5.53 (1.58) (1-9)
Miniota	3.62 (0.91) (1-5)	3.29 (0.80) (1-5)	243 (149) (80-620)	76.5 (7.49) (64.0-94.7)	22.1 (8.31) (7.6-48.3)	48.9 (27.06) (13.8-135.3)	219.4 (115.63) (45.5-532.4)	9.82 (2.58) (3.1-16.3)	0.27 (0.07) (0.10-0.40)	2.51 (1.15) (1-5)	5.48 (1.62) (1-9)
Moosomin	3.38 (1.18) (1-5)	3.48 (0.83) (1-5)	154 (68) (38-286)	70.7 (7.45) (57-85.05)	21.7 (9.26) (2.5-49.5)	38.6 (19.83) (7.0-69.7)	172.1 (88.80) (35.0-314.2)	9.72 (3.12) (2.4-19.9)	0.26 (0.06) (0.10-0.40)	2.45 (1.27) (0-5)	5.47 (1.55) (1-9)
Neepawa	3.84 (0.98) (1-5)	3.27 (1.21) (1-5)	267 (162) (64-540)	70.2 (10.86) (49-89.25)	24.0 (8.52) (7.6-35.6)	50.2 (18.68) (6.7-79.5)	237.9 (109.61) (26.7-476.0)	8.99 (3.52) (1.9-16.5)	0.31 (0.10) (0.10-0.60)	2.53 (1.32) (1-5)	5.93 (1.52) (1-9)
Ninette	3.77 (1.03) (1-5)	3.67 (0.91) (2-5)	228 (132) (35-512)	72.3 (9.96) (45-100.8)	22.3 (7.74) (2.5-40.6)	45.4 (21.93) (14.5-106.3)	242.9 (111.62) (66.5-483.0)	10.98 (3.07) (4.0-17.7)	0.27 (0.06) (0.10-0.40)	2.56 (1.26) (1-5)	5.63 (1.80) (1-9)
Parks Corner	3.62 (1.10) (2-5)	3.61 (0.95) (1-5)	219 (158) (61-668)	74.2 (6.57) (60.2-86.1)	20.4 (6.70) (7.6-45.7)	43.4 (23.15) (9.4-80.9)	213.9 (111.14) (44.5-447.8)	10.64 (3.24) (4.0-18.2)	0.27 (0.08) (0.10-0.50)	2.39 (1.23) (1-5)	5.66 (1.52) (1-9)

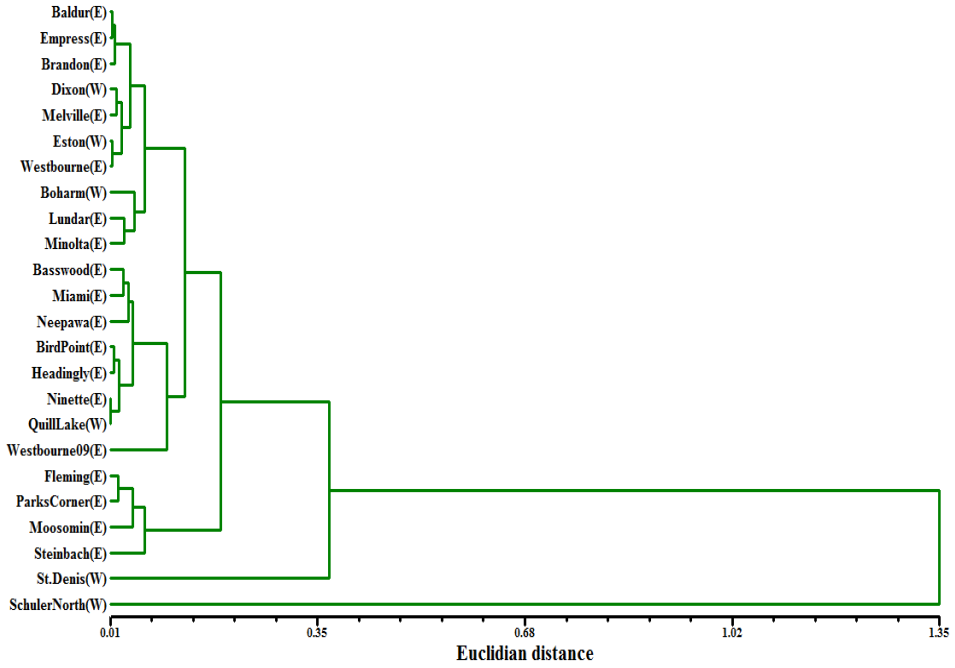
Population	Spring growth (1-5)	Leaf angle (1-5)	Tiller number (N)	Plant height (cm)	Crown diameter (cm)	Seed yield (g plot^{-1})	Dry weight (g plot^{-1})	Leaf length (cm)	Leaf width (cm)	Regrowth (1-5)	Leaf colour (1-9)
Quill Lake	4.06 (0.97) (1-5)	3.58 (1.04) (1-5)	168 (112) (69-495)	75.9 (11.01) (40-106)	24.0 (7.66) (9.7-48.3)	57.6 (31.50) (9.3-130.2)	291.1 (139.13) (46.5-599.8)	10.99 (3.53) (2.5-19.1)	0.28 (0.06) (0.10-0.45)	2.70 (1.46) (1-5)	6.05 (1.64) (1-9)
St. Denis	4.38 (0.72) (1-5)	1.88 (1.26) (1-5)	275 (99) (43-400)	54.1 (14.34) (35-87)	26.5 (7.04) (6.9-55.9)	74.7 (29.91) (22.7-143.8)	279.6 (109.66) (91.6-576.4)	6.55 (3.69) (1.8-22.0)	0.30 (0.08) (0.15-0.50)	2.62 (1.32) (1-5)	6.11 (1.59) (1-9)
Westbourne	3.71 (1.12) (1-5)	3.04 (1.35) (1-5)	175 (57) (67-251)	70.9 (13.17) (39-92)	22.7 (8.09) (6.4-55.9)	46.7 (29.91) (7.0-93.6)	240.7 (111.95) (25.0-480.4)	9.29 (3.49) (1.6-18)	0.28 (0.08) (0.10-0.50)	2.42 (1.37) (0-5)	5.77 (1.63) (2-9)
Lundar/ Coldwell	3.74 (1.05) (1-5)	3.46 (0.94) (1-5)	271 (129) (78-520)	77.4 (10.17) (54-95)	21.3 (8.41) (2.5-47.0)	57.7 (17.37) (18.2-78.6)	270.3 (71.01) (105.7-357.9)	10.29 (3.25) (3.5-19.5)	0.27 (0.07) (0.10-0.45)	2.54 (1.21) (1-5)	5.84 (1.48) (1-9)
Westbourne09	4.23 (0.86) (1-5)	3.34 (0.86) (1-5)	298 (138) (100-561)	76.9 (7.35) (62-96)	27.2 (7.95) (10.26-55.9)	82.5 (28.09) (25.8-128.7)	368.6 (154.70) (114.4-796.5)	11.36 (4.42) (2.4-20.4)	0.26 (0.08) (0.10-0.50)	3.05 (1.35) (1-5)	6.22 (1.66) (0-9)
Empress	3.68 (1.05) (1-5)	3.28 (1.02) (1-5)	206 (64) (106-321)	72.6 (7.69) (53-87)	22.4 (9.92) (5.1-53.3)	54.3 (20.43) (27.1-102.6)	288.6 (113.78) (108.2-479.9)	10.07 (3.56) (3.0-18.3)	0.27 (0.06) (0.15-0.40)	2.55 (1.26) (1-5)	6.12 (1.44) (1-9)
Steinbach	3.54 (1.00) (1-5)	3.58 (0.85) (1-5)	243 (167) (62-619)	84.6 (9.99) (61-101)	20.0 (7.55) (6.4-43.2)	49.3 (25.87) (4.4-111.6)	269.5 (130.67) (78.1-595.1)	11.86 (3.59) (3.4-21.0)	0.30 (0.10) (0.10-0.60)	2.21 (1.21) (1-5)	5.59 (1.51) (1-9)
Eston	4.64 (0.71) (1-5)	3.12 (0.87) (2-5)	302 (180) (11-526)	85.9 (11.92) (49.5-111)	27.6 (7.23) (10.2-47.0)	100.8 (35.83) (28.2-163.9)	333.9 (130.52) (94.9-514.3)	11.95 (4.11) (3.3-21.1)	0.34 (0.08) (0.10-0.50)	2.82 (1.34) (1-5)	6.32 (1.34) (2-9)
Fleming	3.25 (0.94) (1-5)	3.83 (0.88) (2-5)	126 (72) (30-267)	69.4 (7.73) (48.3-84.84)	18.8 (6.77) (5.1-44.5)	42.3 (19.79) (14.3-75.1)	250.1 (121.10) (99.8-480.7)	9.86 (3.43) (2.4-18.8)	0.25 (0.07) (0.10-0.40)	2.20 (1.19) (1-5)	5.59 (1.50) (1-9)
Schuler north	1.98 (1.26) (1-5)	2.97 (1.15) (1-5)	157 (148) (9-404)	56.39 (14.11) (34-82)	14.7 (8.74) (3.8-33.0)	11.4 (21.70) (0.0-79.8)	41.4 (69.63) (4.6-274.3)	7.50 (2.92) (2.4-14.1)	0.21 (0.08) (0.10-0.40)	1.29 (0.65) (1-5)	4.77 (1.83) (1-9)
Boharm	3.73 (0.86) (2-5)	2.75 (1.00) (1-5)	204 (73) (100-331)	77.0 (9.78) (48-96.6)	22.1 (6.38) (5.1-38.1)	36.6 (14.75) (20.4-±67.0)	236.6 (72.73) (115.1-323.3)	7.00 (2.32) (2.5-12.7)	0.24 (0.08) (0.10-0.50)	2.56 (1.26) (1-5)	6.04 (1.60) (1-9)
Overall mean	3.76	3.27	231	72.9	22.8	53.9	254.3	9.91	0.27	2.50	5.82

s.d. – standard deviation;

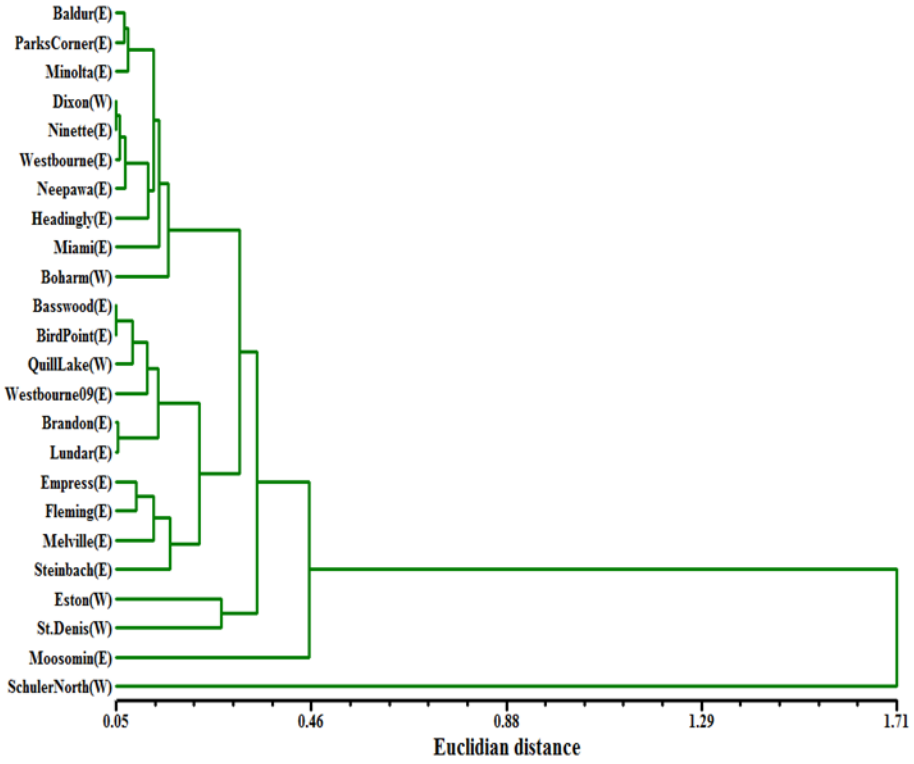
Appendix B. Dendrogram of the 120 genotypes representing the 24 *P. nuttalliana* populations revealed by UPGMA cluster analysis of generic similarity based on mean values of nine phenotypic characters observed in 2011 and 2012



Appendix C. Dendrogram of the 24 *P. nuttalliana* populations revealed by UPGMA cluster analysis of generic similarity based on nine phenotypic characters observed in 2011



Appendix D. Dendrogram of the 24 *P. nuttalliana* populations revealed by UPGMA cluster analysis of generic similarity based on nine phenotypic characters observed in 2012



Appendix E. Means of individual genotypes in populations for which differences were significant ($p<0.05$) in certain traits in 2011

	Spring growth (1-5)	Leaf angle (1-5)	Plant height (cm)	Tiller number (N)	Regrowth (1-5)	Crown diameter (cm)	Leaf colour (1-9)	Leaf length (cm)	Leaf width (cm)
Population Ninette									
Genotype 60	4.46 a[+]	4.13 a	82.5 a	215 a	2.97 a	27.3 a	6.58 a	13.31a	0.32 a
Genotype 57	3.96 ab	3.38 b	69.0 b	165 ab	2.78 a	26.9 a	6.11ab	10.28 b	0.26 b
Genotype 58	3.75 b	3.25 b	69.5 b	137 b	1.99 b	25.4 ab	5.26 b	10.80 b	0.25 b
Genotype 59	3.63 b	4.17 a	59.3 c	138 b	2.33 b	20.4 ab	5.68 b	9.59 b	0.25 b
Genotype 56	3.04 c	3.42 b	73.6 ab	202 ab	2.05 b	23.5 b	4.33 c	10.94 b	0.28 b
Population St. Denis									
Genotype 71	4.75 a	1.42 c	46.0 b		2.86 a		4.77 a	5.62 c	0.30 a
Genotype 75	4.58 ab	1.29 c	43.7 b		2.79 ab		6.96 a	4.03 c	0.31 a
Genotype 73	4.33 b	1.29 c	44.3 b		2.32 c		5.49 b	5.33 c	0.30 a
Genotype 74	4.33 b	2.04 b	46.0 b		2.51 abc		5.63 b	7.34 b	0.33 a
Genotype 72	3.92 c	3.38 a	81.2 a		2.45 bc		5.61 b	10.42 a	0.25 b
Population Bird's Point									
Genotype 21	4.29 a			199a	3.04 a	31.8 a		9.78 bc	
Genotype 23	4.08 ab			195 a	2.73 ab	24.1 b		10.80 ab	
Genotype 25	3.67 bc			171 a	2.54 ab	25.6 b		12.05 a	
Genotype 22	3.33 c			151 a	2.21 b	21.2 c		9.04 c	
Genotype 24	3.25 c			86 b	2.42 b	22.4 bc		9.03 c	
Population Dixon									
Genotype 20		3.38 a			2.13 bc		5.76 a	7.63 c	0.22 c
Genotype 19		3.38 a			2.79 a		6.15 a	9.21 b	0.30 b
Genotype 16		3.31 ab			2.53 ab		5.97 a	9.98 ab	0.25 c
Genotype 18		2.92 b			1.84 c		4.77 b	11.19 a	0.30 ab
Genotype 17		1.38 c			2.41 ab		6.37 a	7.03 c	0.34 a

	Spring growth (1-5)	Leaf angle (1-5)	Plant height (cm)	Tiller number (N)	Regrowth (1-5)	Crown diameter (cm)	Leaf colour (1-9)	Leaf length (cm)	Leaf width (cm)
Population Melville									
Genotype 33		3.58 a	74.8 ab						0.27 ab
Genotype 34		3.38 a	72.8ab						0.24 b
Genotype 35		3.33 a	80.5 a						0.28 ab
Genotype 31		3.28 a	77.8 a						0.30 a
Genotype 32		2.74 b	66.8 b						0.27 ab
Population Quill Lake									
Genotype 69	4.42 a	3.96 a		198 ab		28.8 ab		12.91 a	
Genotype 68	4.33 ab	2.75 b		181 ab		31.5 a		9.44 b	
Genotype 67	4.00 abc	3.63 a		146 bc		25.1 bc		10.05 b	
Genotype 70	3.88 bc	3.71 a		229 a		27.5 ab		9.25 b	
Genotype 66	3.67 c	3.86 a		128 b		21.6 c		13.33 a	
Population Empress									
Genotype 94	4.25 a	3.54 a	75.4 a		3.04 a	25.3 ab	7.00 a	8.02 c	0.24 c
Genotype 93	3.88 ab	3.33 ab	80.5 a		2.50 b	20.9 bc	5.91 b	12.87 a	0.29 a
Genotype 91	3.75 ab	2.83 b	66.3 bc		2.33 b	26.0 a	6.13 b	8.20 c	0.28 ab
Genotype 95	3.46 bc	3.00 b	65.6 c		2.28 b	20.0 c	5.50 b	10.86 b	0.29 ab
Genotype 92	3.08 c	3.71 a	73.9 abc		2.44 b	19.6 c	6.04 b	10.41 b	0.26 bc
Population Miniota									
Genotype 42		1.90a			2.24 b	26.7 ab	4.77 b	9.19 b	
Genotype 43		1.84 ab			2.25 b	24.8 ab	5.25ab	9.60 b	
Genotype 45		1.80 ab			1.90 b	22.0 b	4.95 b	9.52 b	
Genotype 41		1.75 b			2.97 a	28.6 a	5.93 a	11.13 a	
Genotype 44		1.72 b			2.32 b	24.7 ab	5.97 a	9.77 ab	

[+] Means within columns and populations followed by the same letter are not significantly different ($p \geq 0.05$)

Appendix F. Means of individual genotypes in population for which differences were significant (p<0.05) in certain traits in 2012

	Spring growth	Leaf angle	Dry biomass	Plant height	Tiller number	Seed yield	Regrowth	Crown diameter	Leaf colour
Population Ninette									
Genotype 60	4.46 a[+]	4.13 a	396 a	79.7 a	312 a	76.8 a	2.97 a	24.7 a	6.58 a
Genotype 57	3.96 ab	3.38 b	246 b	66.6 b	316 a	37.8 b	2.78 a	22.7 ab	6.11ab
Genotype 58	3.75 b	3.25 b	219 bc	67.4 b	262 a	44.8 b	1.99 b	16.7 c	5.26 b
Genotype 59	3.63 b	4.17 a	165 c	63.1 b	171 b	34.9 b	2.33 b	16.8 c	5.68 b
Genotype 56	3.04 c	3.42 b	189 bc	75.5 a	236 ab	32.7 b	2.05 b	17.5 bc	4.33 c
Population St. Denis									
Genotype 71	4.75 a	1.42 c		51.5 b		81.9 b	2.86 a		4.77 a
Genotype 75	4.58 ab	1.29 c		43.7 b		115.0 a	2.79 ab		6.96 a
Genotype 73	4.33 b	1.29 c		47.7 b		62.2 b	2.32 c		5.49 b
Genotype 74	4.33 b	2.04 b		50.3 b		58.7 b	2.51 abc		5.63 b
Genotype 72	3.92 c	3.38 a		76.4 a		55.8 b	2.45 bc		5.61 b
Population Bird's Point									
Genotype 21	4.29 a			84.3 a	386 a		3.04 a	23.4 a	
Genotype 23	4.08 ab			73.0 ab	385 a		2.73 ab	17.8 b	
Genotype 25	3.67 bc			68.2 ab	249 b		2.54 ab	19.2 ab	
Genotype 22	3.33 c			69.8 b	219 b		2.21 b	15.2 b	
Genotype 24	3.25 c			66.6 b	218 b		2.42 b	19.1 ab	
Population Dixon									
Genotype 20		3.38 a		68.3 b	223 a		2.13 bc		5.76 a
Genotype 19		3.38 a		66.8 b	249 a		2.79 a		6.15 a
Genotype 16		3.31 ab		78.1 a	284 a		2.53 ab		5.97 a
Genotype 18		2.92 b		67.9 b	129 b		1.84 c		4.77 b
Genotype 17		1.38 c		49.3 c	267 a		2.41 ab		6.37 a

	Spring growth	Leaf angle	Dry biomass	Plant height	Tiller number	Seed yield	Regrowth	Crown diameter	Leaf colour
Population Melville									
Genotype 33		3.58 a	200 b	69.4 bc		37.5 b			
Genotype 34		3.38 a	322 a	73.6 ab		63.9 a			
Genotype 35		3.33 a	250 ab	78.0 a		53.8 ab			
Genotype 31		3.28 a	350 a	78.0 a		77.7 a			
Genotype 32		2.74 b	320 a	64.9 c		64.5 a			
Population Quill Lake									
Genotype 69	4.42 a	3.96 a			381a	67.2 ab		25.0 a	
Genotype 68	4.33 ab	2.75 b			352 a	86.3 a		24.7 a	
Genotype 67	4.00 abc	3.63 a			248 ab	46.4 ab		19.8 b	
Genotype 70	3.88 bc	3.71 a			259 ab	49.7 ab		17.9 bc	
Genotype 66	3.67 c	3.86 a			181 b	38.6 b		15.1 c	
Population Empress									
Genotype 94	4.25 a	3.54 a			276 a	68.0 a	3.04 a	20.6 ab	7.00 a
Genotype 93	3.88 ab	3.33 ab			249 a	52.8 ab	2.50 b	14.6 c	5.91 b
Genotype 91	3.75 ab	2.83 b			222 ab	51.6 b	2.33 b	21.2 a	6.13 b
Genotype 95	3.46 bc	3.00 b			271 a	52.4 b	2.28 b	14.4 c	5.50 b
Genotype 92	3.08 c	3.71 a			160 b	46.7 b	2.44 b	16.3 bc	6.04 b
Population Miniota									
Genotype 42		1.90a			186 ab	31.3 b	2.24 b	16.1 b	4.77 b
Genotype 43		1.84 ab			276 a	81.5 a	2.25 b	19.5 ab	5.25ab
Genotype 45		1.80 ab			163 b	33.0 b	1.90 b	15.9 b	4.95 b
Genotype 41		1.75 b			254ab	55.4 ab	2.97 a	22.4 a	5.93 a
Genotype 44		1.72 b			237 ab	43.4 b	2.32 b	17.7 b	5.97 a

[+] Means within columns and populations followed by the same letter are not significantly different ($p \geq 0.05$)

Appendix G. Monthly average temperature (℃) and total precipitation (mm) in Saskatoon in 2011 and 2012