Genetic Diversity of Blue Grama (*Bouteloua gracilis*) and Little Bluestem (*Schizachyrium scoparium*) as Affected by Selection

by

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A Thesis

Submitted to the Faculty of Graduate Studies

in partial Fulfilment of the Requirements for

the Degree of

DOCTOR OF PHILOSOPHY

Department of Plant Science
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FACULTY OF GRADUATE STUDIES

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GENERAL ABSTRACT

There is a renewed interest in the use of native grasses in North America for agronomic, environmental, and ecological purposes. The preservation and maintenance of genetic diversity in native plants has also become an important issue in North America and the world, particularly with respect to restoration of native habitat. The scarcity of remnant seed sources of native plants adds to the challenge of restoration efforts. The need for commercial sources of genetically diverse native grass seed prompted the present research. The objectives of this study were threefold: 1) To determine variation in seed yield traits of 11 blue grama and 14 little bluestem plant collections from sites across southern Manitoba, Canada; 2) To develop a simple and effective selection method to improve seed yield potential and maintain genetic diversity in blue grama and little bluestem; 3) To use RAPD molecular markers to assess genetic diversity among and within the 11 blue grama plant collections from southern Manitoba, and to determine shifts in genetic diversity caused by selection.

Genetic diversity of blue grama and little bluestem was evaluated using individual plant measurements on the original collections. The random amplified polymorphic DNA (RAPD) assay was used to analyze genetic shifts using low-selection-intensity ecovar, and high-selection-intensity cultivar blue grama populations.

Analysis of individual plant measurements revealed variation in plant size, culm number, seedhead number, seed yield, caryopsis weight, and anthesis date among all original blue grama and little bluestem plant collections. Genetic variation was found for

all measured seed yield component traits, and variation among collections was consistent between years, which suggested that collections were genetically distinct.

Four selection criteria – seed yield, harvest index, kernel index, and a 'combined index' (for blue grama = [kernel index] x [seed yield]; for little bluestem = [kernel index] x [harvest index]), and three methods of pooling (stratified selection with equal pooling, unstratified with equal pooling, and mass selection) for improving seed production potential on a per plant basis were compared. Simple mass selection using low selection intensity produced noticeable potential shifts in unselected traits based on phenotypic correlations. Selection criteria showed greater effect on potential shifts of unselected traits compared to pooling method. Selection for caryopsis yield in the form of Kernel Index was found to produce the least potential genetic shift. Selection with a combined index that included seed yield and kernel index for blue grama and one combining kernel index with harvest index for little bluestem, and equal pooling across all plant collections, was shown to improve overall seed production potential while maintaining genetic diversity.

A genetic study of the blue grama populations utilized RAPDs. The populations compared included the Original, which was comprised of a random sample of individuals from all original plant collections obtained from the 11 sites across southern Manitoba; the Ecovar, which was derived from a polycross containing equal an number of selections from all plant collections selected for seed yield and kernel index; and the Cultivar, which was derived from a polycross of individuals selected by a simple mass selection without regard to equal representation from all original plant collections. The RAPD analysis revealed high levels of genetic variation in the Original plant collections. Ecovar

and Cultivar populations showed similar levels of genetic variation despite differences in selection intensity and genetic contribution from the original collections. However, the Ecovar population was much closer in genetic distance to the Original collections and had fewer shifts in RAPD band frequencies compared to the Original than the Cultivar population.

Results from this research showed high levels of genetic variation in naturally occurring populations of blue grama and little bluestem, and that this variation can be used in selection programs. RAPD marker analysis revealed that it is possible to select for agronomically limiting traits and simultaneously maintain high levels of naturally occurring genetic diversity.

ACKNOWLEDGMENTS

This graduate research project and thesis was completed through the blessings of the Lord God. I am grateful to my dad for his silent encouragement and support. I was very blessed to have S.Ray Smith Jr. as my major advisor, who provided the opportunity and support for this project, and for the example he sets professionally, personally, and spiritually. I thank Drs. Rachael Scarth, Brian Fristensky, and Bruce Ford for serving on my advisory committee, and Dr. Tom Jones for serving as my external examiner. I extend thanks to the many friends in the Plant Science department throughout my tenure. Special thanks to Mr. Matt Fruehm, Dr. Doug Cattani, Dr. Collins Kimbeng, and the 'Forage and Turfgrass' crews (particularly Yasas Ferdinandez, Alex Avecilla, Dinen Subramaniam, and Leonard Kehler) for their help with labor and their friendship.

DEDICATION

I dedicate this thesis and degree to my mom and dad. To my dad for his driving encouragement to learn, and to my mother for everything she sacrificed.

FOREWORD

This thesis is written in manuscript style. All tables and figures were formatted according to requirements for the journal Crop Science. It consists of three manuscripts and concludes with a general discussion. The first manuscript (Manuscript 1: Seed yield variation in blue grama and little bluestem plant collections in southern Manitoba, Canada) has been accepted for publication in Crop Science. The subsequent manuscripts will also be submitted to appropriate peer-reviewed journals.

INTRODUCTION

Before agricultural settlement, the Great Plains was a vast expanse of grassland covering most of central North America. This region was subdivided into the short-grass, mixed-grass, and tall-grass prairies based on the pre-exisiting vegetative types. These distinct vegetation types evolved according to the availability of moisture (Brown, 1979). By the 1930's, there had been a tremendous decline in natural grassland communities due to drought, overgrazing, and tillage, accompanied by massive wind and water erosion. The value of native plant species for recovering land and controlling erosion was quickly recognized. Continued initiatives to reclaim and revegetate eroded and disturbed areas since the 1930's have created a need for better understanding of native plant species. Presently, there are only approximately 4% of the original tallgrass prairie remaining, with a decline in Manitoba of 99.9% (Steinauer and Collins, 1996).

There are two major limitations that currently hinder commercial utilization of native plant materials in the northern Great Plains. The first limitation is availability of seed sources. Although numerous improved cultivars of native grass species have been developed in the USA, cultivar releases in Canada are limited (Crowle, 1970; Smoliak, 1980; Smoliak, 1983). Consequently, imported seed from the USA has often been used in Canada, but these materials are less adapted to northern latitudes, resulting in reduced longevity of established stands. The alternative to using imported seed is utilization of locally adapted seed sources harvested from remnant native stands. Unfortunately, seed yields are often low and too expensive for large-scale use.

Ecological concerns over the genetic integrity of planted materials are the second major limitation to native plant utilization. Ideally, target planting sites should make use of locally adapted seed sources (Millar and Libby, 1989) in order to avoid introduction of non-local gene pools. The argument for using local seed sources is based on the ecotype concept introduced by Turesson (1922), who proposed that isolated populations of a species may become genetically differentiated from other isolated populations due to selection caused by differences in habitat pressures. The distinct ecotypes are therefore considered to be genetically compatible with their environment. Because of this, the accepted geographic range for movement of native plant materials is limited (Atkins and Smith, 1967). When local seed sources are not available, genetic diversity in the seed source to be planted becomes an important consideration.

The combined limitations of poor seed availability and the need for maintaining genetic integrity have created a need for native plant germplasm improved for commercial production while maintaining high levels of genetic diversity. The term 'Ecovar' has been trademarked by Ducks Unlimited Canada to describe such ecological varieties, a concept originally conceived by Erling Jacobsen of the USDA Natural Resources Conservation Service (NRCS). Theoretically, ecovars will allow establishment of native plants over a much wider geographic range than possible with local seed harvests.

In the present research, blue grama (*Bouteloua gracilis* [H.B.K.] Lag. ex Steud.) and little bluestem (*Schizachyrium scoparium* [Michx.] Nash) were the subject species for ecovar development. They are both warm-season, outcrossing grasses and are two of the dominant native grass species in the Great Plains (Brown, 1979; Anderson and

Aldous, 1938; Riegel 1941; Miller, 1967). Previous research studies have provided fundamental knowledge of genetic variation in blue grama and little bluestem populations (McMillan, 1956; McMillan, 1959 a,b; Miller, 1967; Cornelius, 1947; Anderson and Aldous, 1938; Riegel 1940; Riegel, 1941), but these studies were conducted 30 to 60 years ago, and limited to populations obtained from the USA.

OBJECTIVES

The overall objective of the present research is to provide a basic framework for future study of native grass species in the northern Great Plains of North America, through a better understanding of genetic diversity and genetic shifts in natural and selected native plant populations. The specific objectives of this research were:

1. To determine variation in seed yield traits of blue grama and little bluestem plant collections obtained from sites across southern Manitoba, Canada.

2. To develop a simple and effective selection method to improve seed yield potential and maintain genetic diversity in blue grama and little bluestem using individual plant measurements.

3. To assess the level of genetic diversity among and within blue grama plant collections from southern Manitoba, Canada, and determine shifts in genetic diversity caused by selection using RAPD molecular markers.

LITERATURE REVIEW

The following review of pertinent literature is organized into sections designed to familiarize the reader with the species Blue grama (*Bouteloua gracilis* (H.B.K.) Lag. ex Steud.) and little bluestem (*Schizachyrium scoparium* [Michx.] Nash). A general description of these species is first provided, followed by a description of each species' growth and developmental characteristics. Agronomic characteristics and production parameters associated with each species are then provided, followed by a review of their documented genetic diversity. This review of literature concludes with an overview of population variation and genetic shifts, and the use of molecular techniques to characterize genetic variation and their shifts.

Blue Grama and Little Bluestem: Species Description and Distribution

Blue grama (*Bouteloua gracilis* (H.B.K.) Lag. ex Steud.) and little bluestem (*Schizachyrium scoparium* [Michx.] Nash) are important warm-season native grass species of the Great Plains region (Hitchcock, 1950; Steinauer and Collins, 1996). In the earlier literature, little bluestem had been referred to as *Andropogon scoparius* (Hubbard, 1917). Gould (1975) provided a general distribution of blue grama as ranging from Wisconsin to Alberta, Canada, south to Missouri, Texas, southern California and Mexico, with some reports of occurrence in the eastern states. For little bluestem, Gould (1956) described a general distribution encompassing much of North America, from Canada to Mexico, and absent only throughout Nevada and the Pacific coastal states.

Blue grama is a low-growing bunchgrass which is recognized by its fine leaves and one-sided purplish spikes (Figure. 1.a). Blue grama is predominantly outcrossing (Miller, 1967; Riegel, 1941) but apomixis has also been speculated to occur. Besides propagation from seed, blue grama spreads from established plants via short root stalks and expansion of the basal area by tillering. Stoloniferous types of blue grama have also been found (Stubbendieck et al., 1973) in which vegetative spread may be even more pronounced.

Like blue grama, little bluestem is a bunchgrass which spreads predominantly by tillering and short root stalks, as well as by seed (Figure 1.b). Identifiable features of this species include flat, bluish basal shoots and glabrous leaf blades which tend to fold. Morphological characteristics of little bluestem are highly variable (Hubbard, 1917). This species is also outcrossing (Anderson and Aldous, 1938; Miller, 1967) with raceme

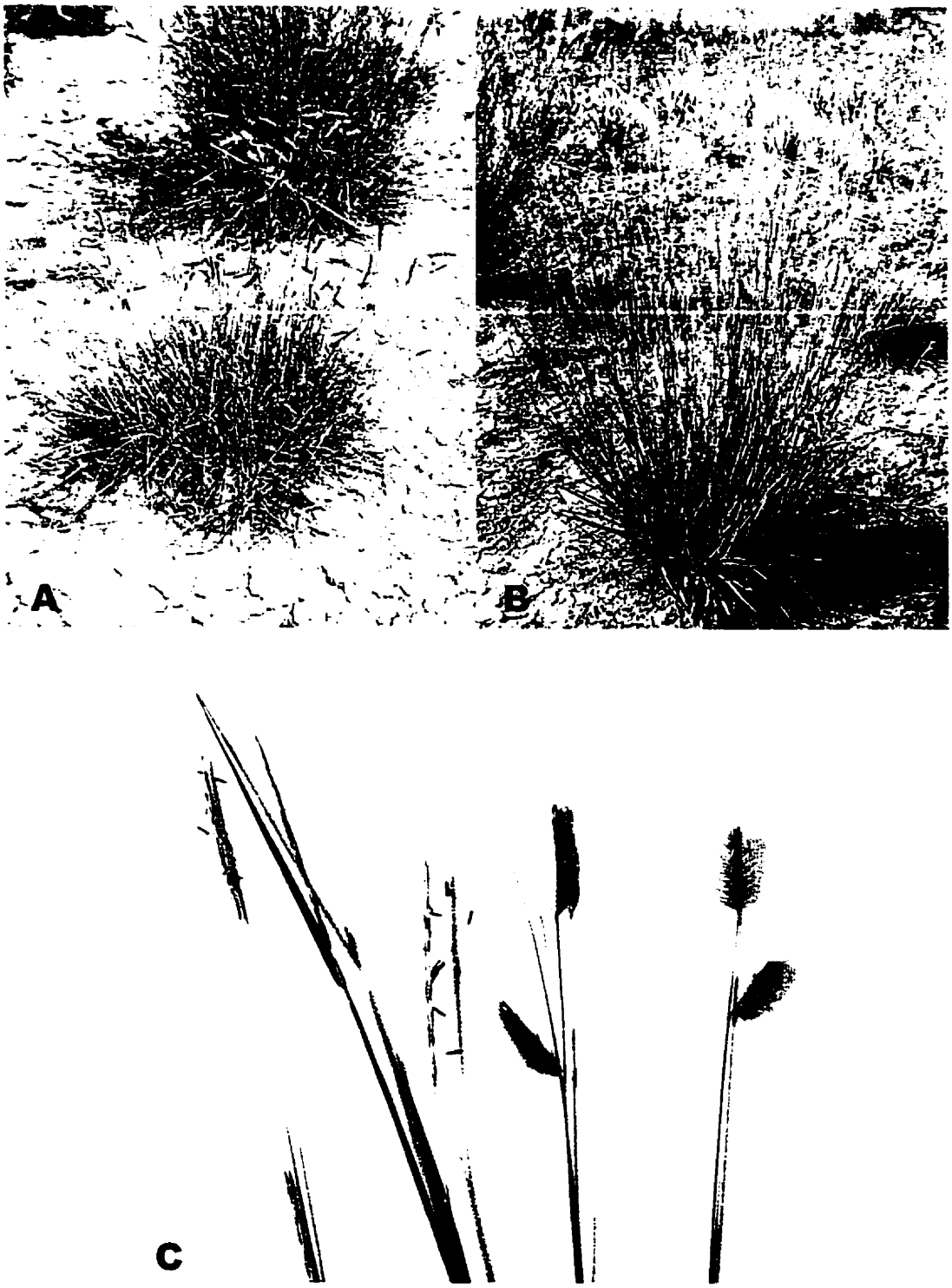


Figure 1. **A)** Blue grama plant. **B)** Little bluestem plant. **C)** Inflorescences of little bluestem and blue grama.

inflorescences in which spikelets occur in pairs of one sessile and one perfect. Upon seed ripening, the sessile spikelet detaches from the infloresence with the pedicel and a section of the rachis remaining attached (Gould, 1975).

Species Growth and Development

Blue grama

Seedling Emergence and Morphology

Riegel (1941) provides a thorough description of blue grama development and growth, and notes that seeds germinate about 3 days to 2 weeks after planting, with seminal roots that can reach 14 cm in depth and remains functional for 4 to 6 weeks. The emergence type exhibited by this species is the elongating first internode-coleoptile type of emergence similar to oat (*Avena sativa*) (Sims et al., 1973). This type of seedling morphology in blue grama positions the crown just 2 mm below the soil surface, and thus water uptake into the leaves is limited to the conducting capacity of the very thin subcoleoptilar internode (Hyder et al., 1971). Deeper plantings have been found to cause lengthening of the subcoleoptilar internode and reduce its ability to conduct water for the seedling (Carren et al., 1987a). Bypassing this limitation requires adventitious roots which are initiated during a moist period of 2 to 4 days following a 2 to 8-week germination period (Wilson and Briske, 1979) and production of 3 to 4 leaves (Riegel, 1941). Carren et al. (1987a) observed that deep planting (> 2 cm) of blue grama resulted in delayed seedling emergence and less vigorous seedlings. However, the effects of deep planting were less pronounced with larger caryopses which were found to produce vigorous seedlings. Further study of blue grama

caryopsis weight and planting depth under marginal soil moisture conditions by Carren and colleagues (1987b) demonstrated that larger caryopses planted at 2-cm depth resulted in optimum seedling emergence and that shallower (1 cm) plantings were less successful because of lack of moisture near the soil surface.

Plant Establishment

Successful establishment of blue grama depends on the growth of adventitious roots. Following seedling emergence of blue grama, adequate adventitious root growth needed for seedling establishment is most probable only at temperatures higher than 15°C and relative humidity of not less than 96% humidity (Briske and Wilson, 1977; Briske and Wilson, 1978). Wilson (1981) observed increases in adventitious root growth with an increase in sub-soil temperature from 20 to 30°C, and recommended planting blue grama when the soil can warm up to at least 15°C for adequate adventitious root growth. For adventitious root initiation, buds below the crown must be exposed to light while seedlings are at least at the 3-leaf stage (Roohi et al., 1991). The longevity of seedlings is therefore restricted to only the seminal root that has been observed to remain functional from 9 to 22 weeks under greenhouse conditions (Weaver and Zink, 1945; Van Der Sluijs and Hyder, 1974). Dessication, however, is frequent under field conditions. Dehulling of blue grama seeds followed by treatment with indoleacetic acid have been found to expedite the development of adventitious roots and provides a potential means of enhancing stand establishment (Roohi and Jameson, 1991).

Nason et al. (1987) conducted selection for high shoot weight and water uptake in blue grama using a base population of the cultivars 'Lovington' and 'Hachita', and two

accessions of composites from Nebraska and Kansas, and Kansas and Texas. Seedling shoot weights were found significantly correlated with caryopsis weight ($r = 0.40$) and with water uptake ($r = 0.81$). Their recurrent selection of the top 10% of the population (15 selections from a base population of 150) increased shoot weight by 21% over three cycles, which proved to be a more efficient means of improving establishment potential of blue grama seedlings compared to measurement of water uptake.

Blue grama is a warm-season species whose growth is favoured by higher temperatures. Seedling growth rates were enhanced by warmer day-night temperatures of 25-20°C (Fulbright et al., 1985). This warm-season species requires the accumulation of approximately 1300 GDD to establish 5 leaves (Frank and Hoffman, 1989). Tiller development from axillary buds is also enhanced by higher cumulative temperatures and longer daylengths (Stubbendieck and Burzlaff, 1971).

Despite adequate levels of seed production and quality, blue grama does not appear to propagate extensively by seed in the natural environment (Savage, 1939 after Hyder et al. 1971). The specific conditions needed for seedling emergence and establishment, and competition from surrounding plants are probably reasons for this observation. Riegel (1941) attributed the apparent lack of blue grama colonization by seed to the limited distance in which the seeds are dispersed. Riegel (1943) also demonstrated the impact of weed competition and pest infestation, and believed that natural establishment of blue grama in the wild may be fairly limited. Miller (1967) suspected that the blue grama plants he studied were vegetative descendants of the original colonized parents. The natural occurrence and propagation of blue grama as vegetative stands suggest that sampling of plants rather than seed would allow characterisation of genetic diversity that reflects genetic

adaptation of the population to the surrounding habitat conditions. Sampling of plants would also be advantageous to seed sampling because it would be unbiased towards differences in flowering and maturity between plants.

Adaptative Characteristics

The growth and maturation of blue grama is highly affected by seasonal conditions. Following seed ripening, blue grama proceeds towards dormancy with the drying and browning of its foliage. Observations of space-planted blue grama in Winnipeg indicated that the plants begin to go dormant around early September. While dormant, blue grama is capable of surviving severe winters to -35°C and is tolerant of temperatures as high as 43°C (Schwarz and Reaney, 1989). Following establishment, blue grama increases in its ability to withstand drought with age (Briske and Wilson, 1980) and becomes one of the most drought tolerant of native grass species (Majerus, 1975). This adaptive feature is probably attributed to its extensive fibrous root system. The roots of a blue grama plant proliferate primarily within 5 cm of the plant and within a depth of 10 cm according to ^{14}C-labelled observations of Coffin and Lauenroth (1992), and the total root biomass may extend beyond a depth of 90 cm. They also noted that despite marked overlap of root systems between neighboring plants, blue grama plants did not show alterations in their root distribution and morphology, suggesting relative insensitivity to plant competition. Drought tolerance in blue grama is attributed to its utilisation of non-structural carbohydrates (Khan and Wilson, 1984), and variation in drought tolerance of seedlings suggests the potential for selection (Wilson and Sarles, 1978).

Photoperiod Response and Anthesis

Photoperiodic response studies of *Bouteloua* by Olmsted (1943) indicated that blue grama is a long-day species that showed optimal flowering under a 16-hr photoperiod. The source of blue grama under observation by Olmsted (1943) was suggested to have an influence on the plant's photoperiodic response, as it was found that optimal responses occurred under day-lengths similar to their source of origin. Benedict (1940) observed from a greenhouse study of blue grama found that flowering could occur both at 18- and 20-hr days as well as 8- or 10-hr days providing that the temperature was kept at 24°C. Under these conditions, they observed the number of days to flowering from the date of planting ranged from 70 to 113. Peak pollen shed in blue grama was observed to occur in the early morning between 4:50 and 5:50 am, evaluated in Lincoln, Nebraska (Jones and Newell, 1946). The investigators also indicated that flowering was inhibited at temperatures below 15°C. At the other extreme, flowering of blue grama under high temperatures (40°C) results in frequent pollen abortion (Kneebone, 1957).

Little bluestem

Seedling Emergence and Morphology

Although little bluestem does not exhibit the morphological characteristics and germination requirements of blue grama, this species displays seed dormancy that has not been reported in blue grama. Little bluestem obtained from Missouri and Kansas was found to display prolonged seed dormancy under various storage conditions (Coukos, 1944). Germination was observed only after 2 months of storage in cloth bags and jars under room

conditions, but appreciable percent germination was observed after 18 months of storage following harvest. They also observed that seed dormancy was prolonged and viability was preserved for up to 38 months under cold-room storage (~ 3°C). Following germination, little bluestem displays the elongating-coleoptile type of seedling emergence.

Plant Establishment

Prior studies with little bluestem make no mention of difficulties in establishing this grass from seed. The larger seed size of little bluestem compared to blue grama, and its elongating-coleoptile type of emergence, give rise to seedlings that do not have major morphologically imposed limitations to water uptake and establishment. Following seedling establishment, little bluestem plant development goes through 5 major steps: (1) tiller formation, (2) spring vegetative growth, (3) culm elongation, (4) flowering and maturation, and (5) fall vegetative (Smith and Leinweber, 1971). The successful establishment of little bluestem, as with blue grama, may be assessed by its ability to survive through winter.

Adaptative Characteristics

Although little bluestem does not display the type of seedling morphology associated with blue grama, its ability to colonize by seed may be due to low seed set. Poor seed production was observed in a space-planted nursery of 16 little bluestem ecotypes in Kansas during 1940 to 1942 where an average fertility of 21.6% (expressed as number of caryopses per number of spikelets in a seedhead) was observed over the three years (Cornelius, 1947).

In comparison, little bluestem is less drought tolerant than blue grama (Majerus, 1975), but it has adapted to a wide range of soil and temperature conditions (Hitchcock, 1950). With a rooting system capable of reaching 2.4 m in depth, it can tolerate dry conditions to some extent and, like blue grama, it is also valuable for soil erosion control (Cornelius, 1946). In contrast to blue grama, however, little bluestem appears to be more productive on finer-textured soils (Waller et al. 1975). Despite its general association with more mesic environments, little bluestem in North Dakota was observed to be consistently associated with slopes, where conditions were often dryer than in the lower depressions (White, 1961).

Photoperiod Response and Anthesis

Little bluestem was recognized as a long-day species with a minimum photoperiod requirement of 13 hrs for southern populations (36° latitude and southward) and 15 hrs for more northern populations (37° latitude and northward) for adequate plant growth, culm elongation, and flower initiation (Larsen, 1947).

Blue Grama Production Parameters

The importance of native grasses such as blue grama and little bluestem to revegetation was emphasized during the drought of the 1930's. Branson (1941) studied seed production of several native grass species including blue grama and little bluestem during the drought of 1939 at Fort Hays, Kansas. Examination of seed harvests of these grasses throughout the drought-affected region revealed the limited capacity of remnant stands to supply adequate seed quantities because of limited availability of soil moisture. Seed collections from the 1939 drought showed very low caryopsis yields compared to

collections made in 1937. It was concluded that availability of soil moisture was the most crucial factor affecting seed production as it was required during flowering and seed maturation.

Under conditions of limited soil moisture, competition between plants for available resources can be expected. The importance of inter-plant competition in blue grama was examined by Aguilera (1992), who observed that adult plants competed extensively for available resources, affecting the establishment of nearby seedlings. Chemical thinning of stands with glyphosate has been evaluated as a means to reduce plant competition within a stand and was found to double seed yields from 5.6 kg/ha to 13.0 kg/ha compared to untreated stands (McGinnies, 1984). The increase in yields was attributed to the improved water and nutrient availability following the reduction in plant competition. Conversely, Kneebone (1957) demonstrated that N-fertilization could improve blue grama seed yields even at the minimal rate of 50 lbs N/acre, compared to no fertilization. However, seed yields in his study were probably also enhanced by irrigation, as yields were not found to increase appreciably with higher fertilization rates.

Little Bluestem Production Parameters

The production parameters of little bluestem reflect its natural occurrence in more mesic environments compared to blue grama. For instance, little bluestem has been found to be less tolerant of low soil moisture conditions than blue grama (Majerus, 1975), which suggests that this species may be absent in drier areas such as knolls where blue grama may occur. The influence of available soil moisture on the distribution of little bluestem was observed on clayey soils in South Dakota (White, 1961).

Edaphic influences on little bluestem productivity also include aspects of soil depth and fertility. Higher plant productivity of little bluestem was observed in areas with soil depths of 30-45-cm compared to areas of 15 cm or less, in an Ohio relict prairie (Dalgarin and Wilson, 1975). Greater soil moisture availability was probably associated with the greater soil depths that also probably fostered better root development than in shallower soils. With respect to soil fertility, Waller et al. (1975) observed that little bluestem productivity, measured as plant dry matter, was 10 times greater on more fertile clay soil compared to that for less fertile sandy soil. Little bluestem was found to be proficient in P-uptake and performs well on P-deficient soils (Wuenscher and Gerloff, 1971). Under conditions of plant competition, little bluestem may not fare as well as other species that exhibit greater ability to exploit unpenetrated soil regions (Van Auken et al., 1994), and the subsequent competition for light also becomes important (LaGory et al., 1982).

Genetic Variation in Blue Grama and Little Bluestem

Ploidy Variation in Blue grama

Cytological studies of blue grama have shown variation in ploidy. Snyder and Harlan (1953), from their examination of single blue grama plants from 108 sites in western Texas and eastern New Mexico and one in western Oklahoma, found diploid types of $2n$ = 20, tetraploids $2n$ = 40, hexaploids $2n$ = 60, and a previously unrecognized aneuploid $2n$ = 42. The single collection from Oklahoma was found to consist of $2n$ = 84 types showing regular bivalent formation. Snyder and Harlan's (1953) findings suggested that members of blue grama have a basic chromosome number of x = 10, with varying occurrence of auto-

and allo-polyploids. In contrast, prior work by Fults (1942) indicated a basic chromosome number of $x = 7$, with $2n = 77$ being the highest chromosome complement for blue grama previously recorded. However, the basic chromosome number of $x = 7$ is generally rejected because $2n = 14$ and $2n = 28$ types of blue grama have never been found. More recent investigation of chromosome number in blue grama revealed the presence of pentaploids ($2n = 50$) that had never before been reported (Tsuchiya et al., 1992).

McGinnies et al. (1988) revealed that the occurrence of different ploidy types can be found on a smaller geographic scale. These researchers examined variation in blue grama from a single pasture and found tetraploids, pentaploids and hexaploid types, with tetraploids occurring in the highest frequency. Snyder and Harlan (1953) had previously suggested that polyploid types of blue grama probably exhibit a wider range in distribution than diploids. Phenotypic variation in blue grama may therefore be partly attributed to ploidy differences as plant phenotype has been associated with variation in ploidy levels, in which larger plants are generally associated with higher ploidy (Halloran and Pennell, 1982).

Ploidy Variation in Little bluestem

In little bluestem, cytological work revealed a basic chromosome number of $x = 10$ (Gould, 1968). Although Church (1929) previously characterized little bluestem as an octoploid, it is generally accepted as a diploid with a chromosome complement of $2n = 40$ (Hunter,1934; Dewald and Jalal, 1974). Furthermore, based on examination of little bluestem from northeastern North Dakota and northwestern Minnesota, Dewald and Jalal (1974) concluded that this species is an alloploid, as it showed incomplete homolcgy among

its progenitor genomes, but where bivalents were prevalent. They also found that pollen sterility was negatively correlated with chromosome abberrations, but that these were not significant. In contrast, irregular chromosome pairing was previously found to produce partial sterility with 20-30% of pollen being inviable (Church, 1929).

Population Variation in Blue Grama

The wide geographic distribution of blue grama across diverse habitat types is expected to produce genetically diverse populations of this species according to their habitat adaptation. Riegel (1940) observed differences in forage and seed production of 9 blue grama seed populations from different states in the USA ranging from North Dakota to New Mexico when grown in one environment at Kansas. Most notable differences were in height and forage production of northern compared to southern collections, where northern populations were generally smaller and produced less forage. Moreover, the southern collections produced wider and longer leaves, and a higher degree of tiller production than the northern group. The blue grama collections from the central region displayed superior seed production compared to the northern and southern groups. He also noted that, in general, the best seed producers were lower in forage yield and vice versa.

Variation among blue grama plants also occurs at a much smaller geographic scale. McGinnies et al. (1988) observed that blue grama occurrence in a single native pasture consisted of patches of tall and short plants, which was probably due to differences in genotype as the relative height differences were maintained in a common greenhouse environment. These blue grama plants ranged from 202 to 719 in mean culm

number, 45 to 76 cm in height, and 39 to 93 g in plant dry weight, and from 11 June to 20 July in anthesis dates. This indicates the large amount of genetic variation present in blue grama between and within spatially separated populations.

Soil factors have shown an influence on variability of blue grama performance. For example, the trend in flowering typically exhibited by populations from different latitudes with earlier flowering in northern populations, as seen in little bluestem and other native grasses, was not always apparent in blue grama in which variability in soil moisture was suspected as a confounding factor (McMillan, 1959b). Coffin and Lauenroth (1992) examined seed production of blue grama at 10 locations in a northern Colorado grassland to represent different soil textures and grazing intensities. They found that soil texture alone, and in combination with grazing, exerted a large influence on blue grama distribution and seed production. More seed was produced on coarser-textured soils because of the enhanced water infiltration and its subsequent plant availability in the soil profile. Observations of growth and seed yield of blue grama and little bluestem, among other native grasses in natural prairie community types at Fort Hays, Kansas from 1939 to 1941, showed variation between years in seed production and plant growth (Brown, 1943). Differences in seedhead fertility were observed in both blue grama and little bluestem occurring in the different habitat types, and could possibly be attributed to moisture characteristics of the soil environment as Branson (1941) previously indicated.

Population Variation in Little Bluestem

Previous investigations with little bluestem have also demonstrated population differentiation caused by environmental parameters. Population differentiation according to Turesson's (1922) description can occur from differences in habitat parameters including photoperiod. Larsen's (1947) study of little bluestem plants representing an extensive geographical range from North Dakota to Texas and Connecticut to Wyoming, in both greenhouse and outdoor garden environments, showed that plants from different sources were strikingly different in their photoperiodic responses to the day-length at the assembled location of Chicago. McMillan's (1956) study of little bluestem collections representing 5 community sites in Nebraska showed that northern collections flowered earlier, and western most collections flowered 2 weeks earlier than the eastern most collections. Further studies by McMillan (1959.a,b) using transplanted collections of several native grass species from wide geographical sources in the southern USA demonstrated the general trend that early flowering was exhibited by more northern and western collections, and later flowering seen in southern and eastern collections. His observations of plants in their natural habitat generally agreed with transplant garden observations. In conclusion, he postulated that the distribution of these plant species was a function of both genetics and habitat differences in which the selective influence of climate was also paramount. Population differentiation between habitat types was found to generate smaller, earlier flowering, less leafy plants in northern collections of little bluestem evaluated by Anderson and Aldous (1938). These researchers also found high levels of variation in leaf area, basal diameter, plant height, and maturity between and

within populations, which afforded them the opportunity to improve forage productivity by selection over 3 generations.

The effect of habitat selection across a geographic gradient has often been related to latitudinal differences in producing population differentiation. McMillan (1964) found morphological differentiation in little bluestem and two other warm-season grasses to be associated with their geographic origin. Southern populations were taller and exhibited early spring activity and later flowering and fall dormancy compared to northern populations. His previous study of native grass collections in a transplant garden in Nebraska indicated that each prairie community may differ from each other because of variations in their habitat (McMillan, 1959). McMillan (1964) again confirmed with little bluestem the latitudinal cline in maturity of plant collections grown in a transplant garden. He observed that southern populations had early spring activity and later flowering while northern populations flowered earlier. Cornelius (1947) examined little bluestem from North Dakota, Wyoming, Nebraska, Iowa, Kansas, Oklahoma, North Carolina, Arizona, and Texas in a common plant nursery at Manhattan, Kansas and similarly observed earlier and later flowering in the northern and southern populations, respectively. Flowering date and plant dry weight were also found to be positively correlated. McMillan (1964) concluded that the observed ecotypic differentiation in flowering behavior was the result of recurrent selection exerted by habitat pressures.

Selection pressures on little bluestem populations are sometimes inconspicuous. For example, Roos and Quinn's findings (1977) suggested that population differentiation in little bluestem could be influenced by the age of the grass stand. They examined 6 little bluestem populations from fields ranging from 2 to 40 yrs of age and found that

older fields exhibited later anthesis and lower reproductive effort compared to younger ones. However, these investigators did not fully consider the effects of plant competition as older stands are expected to experience higher inter-plant competition for available resources, which may consequently affect plant growth and seed production. In addition, Carman and Briske (1984) examined the impact of long-term grazing on morphological and genetic variation of naturally occurring little bluestem using isozyme data. They found that both grazed and non-grazed populations exhibited high levels of genetic variation, and that shorter plants with many smaller tillers were a result of selection towards grazing tolerance.

Population Differentiation and the Ecotype Concept

Definition of Ecotype

Turesson (1922) recognized the genotypic response of plants to environmental parameters and postulated that genetic differentiation between isolated groups of individuals occupying contrasting habitats could produce distinct populations. Hence, rather than referring to a species in the holistic sense, Turesson proposed the term, '*ecotype*', to refer to an ecological unit of the species occupying a particular habitat. The general textbook definition given by Raven et al. (1992) defined an ecotype as " a locally adapted variant of an organism, differing genetically from other ecotypes ". In addition, Janick (1986) mentions that an ecotype's "integrity is maintained by ecological barriers,... separated by differing habitat preferences, thus producing different geographic distributions". These

basic definitions emphasize the role of population isolation and selection from habitat pressures on a population.

Factors Affecting Population Differentiation

Spatially separated plant populations are often subjected to different environmental parameters of biotic and abiotic conditions in which natural selection may occur. In relative isolation, plant populations of a species occupying different habitats adapt to prevailing conditions through the gradual selection of favoured genotypes (Bennington and McGraw, 1995). However, natural selection can occur at microgeographic scales, and are often associated with soil characteristics (Nevo et al., 1988a; Nevo et al., 1988b; Nevo et al., 1986). It was shown that ecotypic differentiation in this species resulted in the formation of two distinct gene pools.

There are numerous biotic and abiotic factors affecting plant population genetic structure (Loveless and Hamrick, 1984). In grass species such as blue grama and little bluestem, differentiation between naturally occurring populations is particularly determined by factors including their outcrossing breeding system, the degree of their isolation, which affects rates of inter-population gene flow, and their effective population size. Effective population size is defined as the number of individuals in a population that effectively contributes to the intra-population gene flow (Loveless and Hamrick, 1984), and will often be less than the total population size due to factors such as dormancy and asynchrony in flowering.

Plant species mating systems affect the degree of gene flow and levels of genetic variation within populations (Loveless and Hamrick, 1984; Barbier, 1990). Outcrossing

produces greater genetic variation within populations than with selfing (Laytin and Ganders, 1984). In outcrossing buffalograss and little bluestem, higher levels of genetic variation within populations were found compared to variation between populations (Huff et al., 1993; Huff et al., 1999). With outcrossing, population differentiation is likely to be hindered as pollen exchange tends to facilitate gene flow between populations and increases effective population size (Loveless and Hamrick, 1984). However, the buffalograss and little bluestem populations analysed by Huff et al. (1993; 1999) were found to be significantly different, and suggests that mechanisms associated with their perennial habit and isolation can negate the effects of gene flow. Outcrossing also facilitates the colonization of new areas by enhancing genetic diversity of founders following genetic bottlenecks (Rice and Jain, 1985). By contrast, selfing species tend to show higher degrees of differentiation that can occur at the level of local subdivision into distinct patches as effective population sizes are much reduced (Loveless and Hamrick, 1984; Knapp and Rice, 1996).

Utilization of Native Grasses

According to Steinauer and Collins (1996), only about 4% of the original pre-settlement tallgrass prairie remains. They indicate that tallgrass prairie in Manitoba has declined from a historic area of 600 000 ha to approximately 300 ha; a 99.9% decline. Wildlife habitat restoration objectives of Ducks Unlimited Canada, a non-profit conservation organization (http://www.ducks.ca), have addressed the need for re-vegetation of target grassland areas.

The conservation of genetic diversity has become an important issue in the area of restoration ecology in the prairies. Reclamation of land for wildlife habitat restoration (Duebbert et al., 1981; Knapp and Rice, 1996), revegetation and soil erosion control (Gaffney and Dickerson, 1987; Fults, 1936), and highway beautification in the US (Greener Roadsides, 1997) have created greater awareness of the ecological and aesthetic advantages of native plant species over introduced plant materials. Native plant species are uniquely adapted to the environmental extremes of a particular region, and naturally occupy local ecological niches. For this reason, locally adapted plant populations are generally recommended for restoration purposes (Millar and Libby, 1989; Knapp and Rice, 1994). Brown (1943) indicated that native grass species were the best choices for revegetation because of their adaptation. Similarly, Cornelius (1946) demonstrated the value of native grass species in their potential to provide both livestock forage and soil erosion control. Cooper (1957) highlighted some basic deemed important for successful conservation planting of native species. His experience led him to recommend that plant materials should only be moved within 250-300 miles north and 100-150 miles south of their point of origin to remain within their region of adaptation. However, they also indicate that the range of adaptation for some species and ecotypes may be quite narrow.

The utilization of native grass species is limited by the availability of remnant grass stands from which locally adapted seed can be harvested, and hence there is a need for commercial seed production of these species. Furthermore, improvement of seed yield, conventionally measured simply as the content of spikelets or florets, can be misleading because of infertility. Therefore, caryopsis yield is a better indication of the value of seed production in native grass species (Kneebone, 1957; Branson, 1941).

Variation in caryopsis weight allowed Cuany et al. (personal communication) to increase blue grama caryopsis weight from 47.2 mg/100 to 55.1 and 65.3 mg/100 over two selection cycles. The absence of breeding efforts with blue grama and little bluestem in Canada has resulted in the current import of cultivars of these species from the USA.

Value of Genetic Diversity in Plant Communities

Genetic diversity is desirable for the associated stability of population performance over environments by means of genetic buffering and reducing genetic vulnerability (Poehlman and Sleper, 1995; Fehr, 1991). In a more local context, genetic diversity among individuals within a population enhances their ability to occupy different micro-environments as seen in wheat (Nevo et al., 1988.a), *Phragmites* (Koppitz et al., 1997), and oat (Hamrick and Holden, 1979). Similarly, genetic differentiation on the population level accommodates larger scale geographic and environmental differences (Ferguson et al., 1998). In heterogeneous environments with inherent soil moisture, fertility, and topographical limitations, genetic diversity is important for stand establishment and long-term persistence.

Selection and Genetic Shifts

The combined objective of selection for maintenance of genetic diversity and improvement of key limiting traits has inherent difficulties. Firstly, selection causes a reduction in genetic variation with each selection cycle (Falconer and Mackay, 1996; Hallauer, 1970). For example, selection for forage production in little bluestem produced

a decline in variation in flowering time (Anderson and Aldous, 1938). Secondly, shifts in the phenotypic values of traits other than the one(s) of interest may also occur as a result of correlated responses (Stojsin and Kannenberg, 1994; Conner and Sterling, 1996; Coors and Mardones, 1989). The degree of correlated responses in unselected traits is dependent on the genetic correlation between the selected and unselected traits and the selection intensity applied (Fehr, 1991). In native grasses intended for use in land reclamation/revegetation, shifts in traits caused by correlated responses may result in the eventual loss of morphological and physiological features suited for adaption to heterogeneous and marginal environments.

The goal of achieving genetic gain combined with genetic diversity is a challenge because of the negative relationship between selection and genetic diversity. Few breeding methodologies directed at improving desirable plant traits in conjunction with maintenance of high levels of genetic diversity have been reported in the literature. In wheat, blending high-performance lines having subtle differences in genetic loci of interest into multi-lines has been used as a means to improve stability of performance over years and environments (Marshall and Brown, 1973). Disease resistance to multiple pathogens/pathotypes can similarly be achieved by pyramiding the respective resistance genes into a common recipient through back-cross series (Beaver and Macchiavelli, 1998). Another approach, used in tree breeding, involved adjusting selection to consider co-ancestry of selected genotypes to maximize contribution of genetic diversity from parental populations (Zheng et al., 1997). Vogel and Pedersen (1993) described a breeding strategy referred to as 'ecotype selection', whereby genetic diversity of naturally occurring ecotypes could be exploited by selection within and between accessions for rapid cultivar development. However, the

specific objective of minimizing genetic shifts and/or maximizing genetic diversity was not a consideration in 'ecotype selection'.

Characterization of Genetic Diversity with Molecular Markers

Commonly Used Molecular Markers

The advent of DNA-based methods of genotypic assay have enabled the characterization of variation in individuals and populations without confounding environmental effects. Differences in genetic loci can be determined at the nucleotide level and often visualized as the presence and absence of a particular DNA sequence. A method involving restriction endonuclease digestion and hybridization of DNA produces informative restriction fragment length polymorphisms (RFLPs; Walton, 1990), while methods based on the polymerase chain reaction (PCR) (Saiki et al., 1988) produce distinguishable random amplified polymorphic DNA fragments (RAPD; Williams et al. 1990; Welsh and McClelland, 1990) and polymorphisms in motifs of simple sequence repeats (SSRs; Westman and Kresovich, 1999). The latter PCR-based methods have become highly automated for increased throughput. A patented technology that combines the advantages of RFLPs with the efficiency of PCR is the AFLP (Amplified Fragment Length Polymorphism) method of genetic assay (Vos et al., 1995). Comparisons between the efficiencies of RFLPs, AFLPs, SSRs and RAPDs can be found in the literature (Russell et al., 1997; Nagaoka and Ogihara, 1997).

The utility of Random Amplified Polymorphic DNAs (RAPDs)

The RAPD technique is technically simple, efficient, and accommodates high throughput (Deragon and Landry, 1992), which led to its wide adoption by researchers. The PCR amplification of genomic regions, using short oligonucleotide primers of arbitrary sequence, provides an almost unlimited source of informative genetic polymorphisms (Williams et al. 1990; Welsh and McClelland, 1990). Also, the sensitivity of PCR in DNA amplification minimizes the need for large quantities of tissue and target genomic DNA (Saiki et al., 1988). The molecular genetic profiles of individuals are free of confounding environmental effects, which present a certain advantage of RAPD compared to morphological measurements and isozyme analysis. Applications of RAPD have included cultivar distinction (Gidoni et al., 1994), marker-assisted selection (Kelly and Miklas, 1998), linkage mapping (Lacou et al., 1998; Thompson et al., 1997; Rajapakse et al., 1995), and conservation biology (Rossetto et al., 1999). The application of RAPD for the characterization of genetic diversity has been well documented in grasses (Huff et al. 1993; Peakall et al. 1995; Huff, 1997; Huff et al., 1999; Gunter et al., 1996; Ferdinandez, 1999) as well as in other species (Vierling and Nguyen, 1992; Chalmers et al., 1992; Nesbitt et al., 1995; Crochemore et al., 1996).

The Advantages of RAPDs

The use of RAPDs presents certain advantages over other DNA-based marker systems such as RFLPs and SSRs for surveying genetic diversity. The technology is technically simple and the use of anonymous oligonucleotide primers to generate RAPD genetic profiles does not require prior knowledge of target DNA sequences as is the case

with RFLPs and SSRs. Hybridization of RAPD primers can therefore occur randomly, interspersed throughout the genome, and is not limited to only expressed genes as is the case with isozyme analysis. Therefore, RAPDs provide a random sample of total genetic variation of the target organism, making it particularly suitable for surveying genetic diversity of populations.

The Limitations of RAPDs

The simplicity of the RAPD method is accompanied by inherent limitations. Polymorphisms detected with the RAPD method are qualitative and scored as the presence or absence of an amplified fragment. Therefore, RAPDs are considered to be dominant markers, which is a disadvantage in genetic studies because of the inability to detect heterozygosity as can be done with analysis of co-dominant marker systems of isozymes (Wendel and Weeden, 1989) and RFLPs (dos Santos et al., 1994).

Sources of error have been encountered with the use of RAPDs. Because the generation of RAPDs is accomplished using oligonucleotide primers of anonymous sequence, the amplified fragments are also of unknown nucleotide sequence. These RAPD bands are separated on a gel on the basis of molecular weight (i.e. fragment size) and electrical charge. Therefore, a visible band may potentially contain amplified fragments of similar molecular weight that differ in nucleotide sequence. Furthermore, band intensities reflect amplified fragment dosage; and less intense bands may be irreproducible (Heun and Helentjaris, 1993). Differences in RAPD band intensity have been observed from assay of leaf versus root tissue in soybean (Chen et al., 1997). Two associated sources of error commonly attributed to RAPDs are band reproducibility and

scoring error (Skroch and Nienhuis, 1997). Staub et al. (1996) assessed additional sources of error in using RAPDs including age of tissue assayed, pathogen infection, intra-population contamination, and variation between stocks of PCR reagents.

MANUSCRIPT 1

Seed Yield Variation in Blue Grama and Little Bluestem Plant Collections in Manitoba, Canada

ABSTRACT

Inadequate seed supplies of adapted blue grama (*Bouteloua gracilis* [H.B.K.] Lag. ex. Steud.) and little bluestem (*Schizachyrium scoparium* [Michx.] Nash.) cultivars limits the use of these two species in the northern Great Plains of Western Canada. This study examined variation in seed yield traits of 11 blue grama and 14 little bluestem plant collections obtained from southern Manitoba, Canada to facilitate development of improved germplasm of these species. Measurements of seed yield traits including harvested air-dried biomass, culm number, seedhead number, seed yield, caryopsis weight, and kernel index were taken from randomized complete block spaced-plant nurseries. Tests were conducted for 2 yr at Winnipeg and 1 yr at Carman, Manitoba, Canada. Collections within both species differed significantly for all traits. Significant collection x year interaction (Winnipeg) and collection x location interactions were present for biomass and seed yield, and many of the seed yield-component traits. While significant, the interactions resulted in few changes in rank among collections. Generally, the most northern collection in both species showed earlier anthesis, produced less biomass, and had lower seed yield compared to more southern collections. These findings indicate that indigenous plant collections of blue grama and little bluestem show high levels of genetic diversity for biomass and seed yields and seed yield components, as

indicated by at least 80% of the observed variation was due to differences between plant collections. These findings show opportunity for the development of adapted cultivars with enhanced forage and seed production capability for the region.

INTRODUCTION

Native grasses are important for soil erosion control (Cornelius, 1946) and revegetation of wildlife habitat. Blue grama and little bluestem are dominant warm-season grass species in the Great Plains (Hitchcock, 1950) and are recognized for their adaptedness and drought tolerance (Majerus, 1975). Utilization of blue grama and little bluestem is currently limited in western Canada by availability of adapted seed sources. Presently, commercial quantities of blue grama and little bluestem seed are often obtained from U.S. sources where they are sold as cultivars or simply by species name. Moreover, locally adapted seed sources preferred by many ecologically-minded restorationists can only be collected from remnant patches of naturally occurring blue grama and little bluestem plants, but this approach yields small seed quantities and is expensive. An economically viable source of adapted seed of blue grama and little bluestem is needed in western Canada.

Literature on agronomic studies of blue grama and little bluestem is limited and often out-dated. Prior studies of blue grama and little bluestem in North America originated in the U.S., which led to cultivar development of these species. Blue grama and little bluestem are outcrossing species (Anderson and Aldous, 1938; Riegel, 1941; Miller, 1967) in which populations with high levels of genetic diversity are found across a broad

geographical range. McGinnies et al. (1988) found that blue grama plants from a single pasture in Colorado showed high levels of variability in number and height of reproductive culms, plant biomass, basal diameter, and anthesis date. Coffin and Lauenroth (1992) found that differences in seed yield of blue grama between range sites in north-central Colorado was greatly influenced by soil texture and animal grazing. McMillan (1956) evaluated 16 little bluestem ecotypes in Kansas and found differences in morphology and seed yield between populations obtained from sites ranging from North Dakota and Colorado to Kansas. He found that the local Kansas ecotypes produced the highest seed yield, while northern ecotypes from near Waterton, North Dakota showed earlier anthesis and low seedset, whereas southern ecotypes with late maturation succumbed to autumn frosts. Selection in blue grama has improved different seed yield traits such as culms plant^{-1} (Lovington and Hachita), spikes culm^{-1} (Lovington), spikelets spike^{-1} (Hachita), and fertility (PM-K1482) (Wilson et al., 1981). Similar studies characterizing morphology and seed yield in blue grama and little bluestem populations in Canada could not be found in the literature. Ecotypic variation in blue grama and little bluestem populations caused by habitat differences, as described by Miller (1967), demonstrates the need to characterize populations of these species in western Canada.

The objective of this study was to quantify variation in seed yield traits between blue grama and little bluestem plant collections obtained from sites across southern Manitoba, Canada. This information should facilitate the development of adapted cultivars with improved seed yield potential for the region.

MATERIALS AND METHODS

Plant Collections

Plant material for this study was obtained in late August of 1992 by the field staff of Ducks Unlimited Canada. Blue grama and little bluestem plants were randomly collected wherever they were found during a collection expedition in southern Manitoba, Canada. Following the only guideline of stopping at interspersed distances of at least 30 km, a total of 11 and 14 locations were sampled for blue grama and little bluestem, respectively. Approximate geographic locations of plant collection sites are shown in Figure 1. Two blue grama collections were obtained near Goodlands, MB (designated GDL1 and GDL2) because of a marked difference in elevation (152 m) between the sampled areas. Similarly for little bluestem, two collections were made from an area near Hartney because of marked differences in soil salinity (designated HTY and HTY-Saline). Characteristics of the sites are given in Table 1. Intact plants were dug from collection sites, transported to the University of Manitoba field research station in Winnipeg (49° 49′ N, 97° 7′ W), and directly transplanted to a field nursery.

Experimental Design

Winnipeg

The plant collections were arranged in a randomized complete block design with nine replications for blue grama and seven for little bluestem. The plant collections were divided into experimental units of plots comprised of five individual plants spaced 1 m.

The rows were also 1 m apart. The total plant number was 495 (5 plants x 11 collections x 9 replicates) for blue grama and 490 (5 plants x 14 collections x 7 replicates) for little bluestem. The experimental area of each species was flanked by a single row of border plants. The soil was a Cumulic Regosol with a high clay content. Winterkill in 1993 reduced plant numbers to 470 and 483 for blue grama and little bluestem, respectively.

Carman

In late June of 1994, clones of all plants of both species were used to duplicate the Winnipeg experiments at the University of Manitoba Carman Field Research Station (49° 30′ N, 98° 00′ W) in Manitoba on an Orthic Black Chernozem soil, sandy in texture, with re-randomization of the treatments. The plants were allowed to establish during the 1994 growing season.

Data Collection

Growing-degree day (GDD) calculations and individual plant measurements were made at the Winnipeg site in 1994 and 1995, and at the Carman site only in 1995. Growing-degree days were calculated from air temperature data as GDD = ([maximum daily temperature + minimum daily temperature]/2) − (15°C) and were accumulated over the period of 1 May to 31 August. A base temperature of 15°C was used to represent the approximate minimum temperature needed for initiation of anthesis in blue grama (Benedict, 1940). We used this same base temperature for little bluestem. Number of calendar days to first anthesis was determined based on the starting date of 1 May, which

was prior to visible emergence of new shoots. Plant height was measured as the height of the tallest reproductive culm near the center of the plant after anthesis. Crown diameter was determined as the average of two measurements of crown width taken at right angles after anthesis. Plants were individually harvested by cutting at 18-cm stubble height, which included all mature culms. Harvesting of both blue grama and little bluestem commenced when approximately 70 to 80% of the culms showed signs of seedhead ripening.

Harvested plants from all location-years were kept in paper bags and allowed to air dry at approximately 27°C under continuously blowing fans in a closed metal shed, and dried plants were subsequently processed. Total harvested biomass of each plant was determined. By visual estimation, approximately 50% of each plant was used to estimate the following on a per plant basis: number of culms = (number of culms counted in 50% sample) x 2; seedhead number = (number of seedheads counted in 50% sample) x 2; seed yield = (seed threshed from 50% sample) x 2. With blue grama, an additional sample of 20 culms with ripened, unshattered seedheads were randomly selected to determine number of seedheads 20 culms^{-1} and caryopsis extraction. Because seed shattering was a greater concern with little bluestem, 30 mature culms were sampled. From the 20 and 30 reproductive culms of blue grama and little bluestem, respectively, seedheads were removed and weighed, and spikelets were subsequently removed by hand. The spikelets were further processed using a belt thresher, which had a moving rubber belt against a rolling drum in which the spikelets were fed 3 to 4 times to separate the caryopses from the chaff. Some caryopses were broken, but were collected for weight determination as the belt was brushed clean after each run. Caryopses were separated from the chaff by running them 2 to 3 times through a column air blower. The end-cap of the column was constantly agitated to

maintain consistent air flow through the column. The 'kernel index' (referred to by Raeber and Kalton (1956) as a 'fertility index') was calculated as follows:

$$\text{Kernel Index (KI)} = \frac{Caryopsis\ yield(mg)}{Unthreshed\ Seedhead\ Weight(mg)} \times 100\% \qquad [1]$$

The weight of 100 caryopses was also determined to the nearest 0.1 mg for the 1994 Winnipeg harvest.

Data Analysis

Data were first tested for normality using the SAS UNIVARIATE procedure (SAS Institute Inc. 1988). Values for all measured characters taken from blue grama and little bluestem plants in 1994 and 1995 in Winnipeg and Carman were square-root transformed to improve normality (W:normal >0.9) for valid execution of the analysis of variance (ANOVA). Statistical analyses were performed on a plot-mean basis. Plant collection means were compared with LSDs using replicate means of plant collections. The plant collection x year interaction was determined from the ANOVA using means of plant collections for each of the measured traits, and Spearman's rank correlation of plant collection means was used to determine the change in relative performance between collections over 1994 and 1995 in Winnipeg. Interactions of plant collections with transplant locations (i.e, environments) and location main effects were analyzed with ANOVA using 1995 measurements from Winnipeg and Carman.

RESULTS AND DISCUSSION

Winnipeg Measurements

Seed Production Over Years

An analysis of 1994 and 1995 Winnipeg data showed differences between plant collections for all measured traits in both years (Tables 2 and 3). For plant traits showing significant collection x year interaction, means for plant collections were compared separately for each year (Table 2). In blue grama, significant collection x year interactions were found for harvested biomass, days to anthesis, and kernel index. For little bluestem, significant interactions were found for seedhead number culm^{-1}, seed yield plant^{-1}, and kernel index plant^{-1}. Where collection x year interactions were non-significant, the data were averaged across years for each plant collection (Table 3). Averaging the data between the 2 yr provided a general description of variation between the plant collections and was supported by observations that seed yields of native grass stands in western Canada can be highly sporadic (Kilfoyle, 1995, personal communication).

Growth and development of warm-season grasses such as blue grama and little bluestem are affected by seasonal growing temperatures (Frank and Hofmann, 1989; Gillen and Ewing, 1992). There were contrasting conditions in temperature and precipitation between 1994 (GDD = 473, precipitation = 416 mm) and 1995 (GDD = 619, precipitation = 229 mm) in Winnipeg, however the plant collections showed significant rank correlation between years (Table 3). Although cooler conditions in 1994 produced larger plants of blue grama, seed yield plant^{-1} was lower than in 1995 (data not shown). Little bluestem

produced higher average plant biomass and seed yield in 1995 compared to 1994. Precipitation differences between years could affect seed yield as greater availability of soil moisture promotes anthesis in blue grama and little bluestem (McMillan, 1959a; Kneebone, 1957). Seed production would therefore be maximized with enhanced pollination as both are outcrossing species (Anderson and Aldous, 1938; Riegel, 1941; Miller, 1967). Although Majerus (1975) found that little bluestem did not perform as well as blue grama under low soil moisture conditions, we observed that drier conditions coinciding with higher temperatures favored little bluestem seed yields, as seen in 1995 (Table 2). Conversely, blue grama seed and culm production under a relatively cooler but wetter season in 1994 was greater than under a dryer and warmer season in 1995. Whether blue grama seed yields between 1994 and 1995 involved differences in caryopsis size was not determined. Most of the difference in biomass and seed yields in little bluestem and blue grama between years was due to changes in culm production in all plant collections, as culms are an important component of seed yield and had the greatest contribution to plant biomass.

Seasonal differences between 1994 and 1995 did not significantly affect rankings among the blue grama and little bluestem plant collections for any seed yield trait except kernel index (Table 3). Kernel index for both species showed a low and non-significant rank correlation between years, suggesting that this trait is phenotypically plastic between plant collections. The few changes in rank among plant collections between 1994 and 1995 for the majority of measured traits may be a reflection of each population's adaptation to its original habitat. In general, these findings show that annual fluctuations in temperature and moisture conditions affect seed production potential in blue grama and

little bluestem, but extreme changes in plant performance between years in the form of significant plant collection x year interaction were not prevalent.

Days to Anthesis and Biomass Yields

In this study, the most northerly blue grama and little bluestem collections from RSL showed the earliest anthesis dates (Tables 2 & 3). The range in anthesis dates among plants within collections was generally similar, and is likely a genetic attribute of their adaptation to the photoperiods of their original habitats (Turesson, 1922). McMillan (1956) indicated that flowering in little bluestem is highly determined by genetic factors and that variation in flowering between planting sites may arise from genotype x environment interactions. Consequently, when native plant materials are moved too far latitudinally from their point of origin, they may show poor establishment, seed production, and even survival (Atkins and Smith, 1967).

As seen for anthesis date, considerable variation occurred in biomass among collections of blue grama and little bluestem (Table 2 & 3). Plant-harvested biomass was comprised mostly of reproductive culms, with a greater contribution from foliage to the biomass in little bluestem than in blue grama. In both 1994 and 1995, the blue grama LDR collection ranked highest for plant biomass while RSL ranked the lowest (Table 2). For little bluestem, SWS produced the greatest plant biomass whereas INT-1 and RSL produced the lowest. Variation between plant collections in this study agreed with a general trend seen in previous studies in the U.S. that more northern collections comprised of smaller plants with earlier anthesis dates (McMillan, 1956; McMillan, 1959a; McMillan, 1959b; Larsen, 1947; Cornelius, 1947).

Yield Component Traits

Differences in number of days to anthesis among plant collections did not, in general, always reflect differences in plant culm production. In both blue grama and little bluestem, the number of culms plant^{-1} was lowest in the northern RSL collection. In blue grama, GDL-2 and RSL produced relatively fewer culms plant^{-1} and showed early dates of anthesis, but DGL produced the most culms plant^{-1} despite also having an early anthesis date. Similarly, HOL and RSL little bluestem collections showed low culm production and early anthesis date, but SRB and INT-2 also showed high culm production with early anthesis. Clearer trends may be evident with additional plant collections covering a wider geographical range than in this study. Selection for culms plant^{-1} in blue grama has previously produced the cultivars Lovington and Hachita (Wilson et al., 1981).

Blue grama and little bluestem showed variation in seedheads culm^{-1} among plant collections, with greater variation seen in little bluestem (Table 3). We observed that blue grama and little bluestem plants produced varying numbers of seedheads culm^{-1} within and among plants. The number of seedheads culm^{-1} within and among plants ranged from 1 to 4 for blue grama, and from 3 to 16 for little bluestem (data not shown). However, plants generally showed a predominance of culms with a fixed number of seedheads which may reflect the genetic influence of this trait. Improvement of seedhead number culm^{-1} in blue grama has already been accomplished in the cultivar, Lovington (Wilson et al., 1981), and the large variation for this trait seen in little bluestem indicates the same potential for improvement.

Variation in seed yield components translated to variation in seed yield plant^{-1} among blue grama and little bluestem collections (Tables 2 and 3). In little bluestem, plant

collection x year interaction for seed yield per plant was significant, so the means were reported separately for 1994 and 1995 (Table 2). In both years, INT-1 produced the lowest average seed yield plant^{-1} and SWS yielded the highest among little bluestem collections. Noting the absence of plant collection x year interactions for blue grama seed yield, the highest seed yield plant^{-1} in blue grama was observed in the LDR collection and the lowest was seen in the RSL collection, averaged across years (Table 3). The tendency for northern collections to show low seed yields plant^{-1} was also evident in the RSL and INT-1 little bluestem collections.

Seed yield in native grasses is only as important as the proportion that is germinable. Kernel index measures the proportion of biomass allocated to caryopses in the inflorescence, and is distinct from a measure of fertility of inflorescence determined as the percentage of florets that produce caryopses (Trupp and Slinkard, 1965). Trupp and Slinkard (1965) used the term 'fertility index' rather than 'kernel index', and found it to be highly correlated with fertility of inflorescence in intermediate wheatgrass. The term 'fertility index' was not used here because size and number of caryopses produced per inflorescence was not taken into account in this study. In blue grama, the LDR collection produced the highest kernel index while S10 produced the lowest (Table 3). In little bluestem, RSL had the highest average kernel index while HTY and its nearby neighbor with more saline soil, HTY-S, had the lowest kernel indexes. The potential for improvement of caryopsis yield is particularly important because these species are usually sold and planted on a pure live-seed basis.

Also of significance to native grass species utilization is caryopsis size, for which variation in 100-caryopses weight was seen among plant collections of both blue grama

and little bluestem (Table 3). The mean 100-caryopses weights among all blue grama plants ranged from 17.4 to 64.1 mg in 1994 (data not shown). Wilson et al. (1981) reported a similar range from 25 to 70 mg 100 caryopses^{-1} among individual plants from four blue grama collections. For little bluestem, 100-caryopses weights among all individual plants ranged from 46.8 to 163.1 mg in 1994 (data not shown). In comparison, Roos and Quinn (1977) obtained 100 caryopsis weights ranging from 74.5 to 98.0 mg among plants from two young (2-3 yr) and two old (35-40 yr) little bluestem populations in New Jersey. The large amount of variation in caryopsis weight seen in this study indicates that selection for this trait can be effective with selection within and/or among plant collections as indicated earlier by Wilson et al. (1981). Native grasses can benefit from larger caryopses as this enhances seedling emergence and stand establishment (Carren et al., 1987a; Carren et al., 1987b).

Plant Collection x Location Interaction

Blue grama and little bluestem collections were evaluated in Winnipeg and Carman, Manitoba, to determine plant collection x location interactions of seed yield component traits (Table 4). Plant collection x location interactions were evident in both species. The low interaction mean square for seedhead weight in blue grama was significant whereas the interaction mean square for collection effects was not. The phenotypic plasticity of plant collections, as shown by significant collection x location interactions, should therefore be considered when breeding native grasses for desired regions.

Mean squares from the ANOVA showed differences in magnitude of plant collection effects relative to the interaction effects, and alludes to the genetic differences between collections (Table 4). For blue grama, mean squares for plant collection was usually about twice as large, and up to almost nine times as large as the interaction mean squares. For little bluestem, there were fewer significant collection x location interactions, and ratios between plant collection- and interaction-mean squares were even larger than in blue grama. The larger plant collection effects relative to small interaction effects suggests a strong genetic basis for the variation between collections at an ecotype level. Occurrence of distinct blue grama and little bluestem populations in Manitoba would facilitate development of adapted cultivars of these species for western Canada.

CONCLUSION

Differences in seed production and component traits were found among plant collections of blue grama and little bluestem obtained from southern Manitoba, Canada. Generally, the most northerly collections showed earlier anthesis dates, produced less biomass, and had lower seed yield compared to more southerly collections. There was no significant change in rank between collections evaluated at Winnipeg for two years for most traits, but significant collection x year interactions were found for plant harvested biomass, culm height, kernel index, and days to anthesis in blue grama, and seedhead number 30 culms^{-1}, seed yield 30 culms^{-1}, and kernel index in little bluestem. Significant plant collection x location interactions were probably due to ecotypic differences between plant collections. These findings indicate that indigenous plant collections of blue grama

and little bluestem show high levels of genetic diversity for biomass, seed yields and seed yield components, which provide opportunity for development of adapted cultivars with enhanced forage and seed production capability for the region.

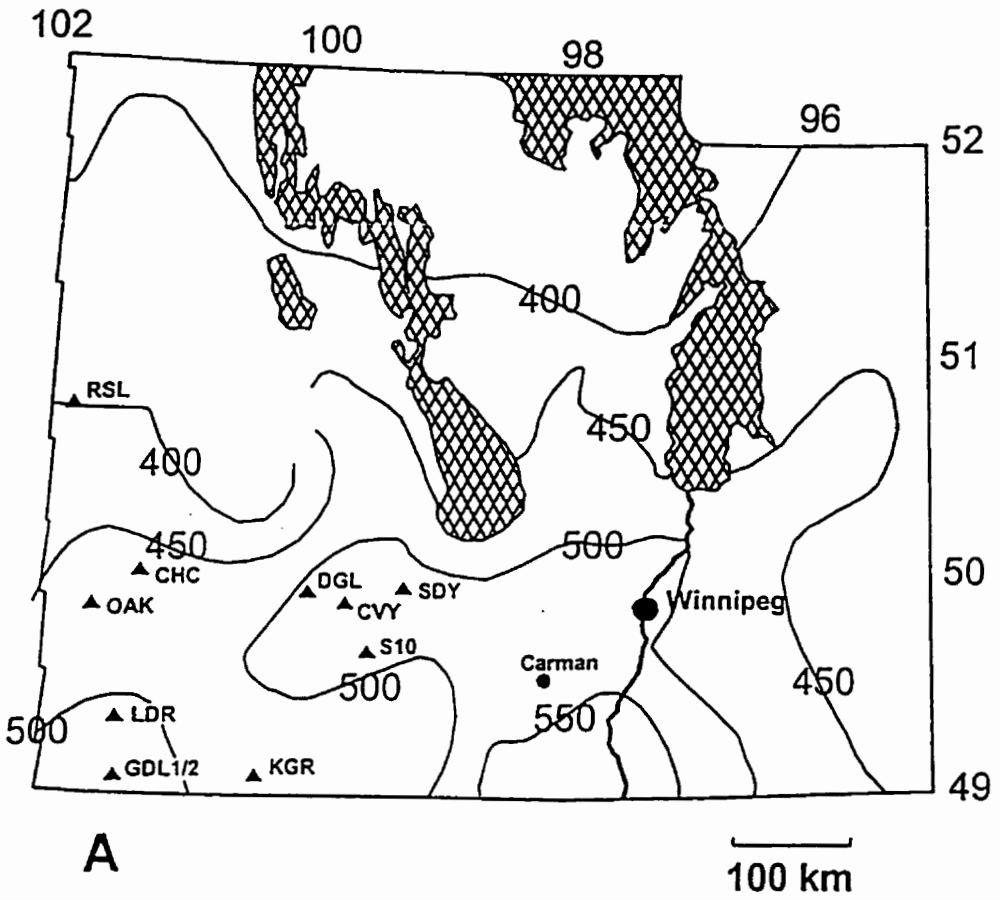


Figure 1.a. Blue grama plant collection sites with respect to zones of growing degree days (GDD) in southern Manitoba, Canada. Calculation of GDD used a base temperature of 15°C and accumulated between 1 May and 31 August. Degrees longitude and latitude are indicated on the figure margins.

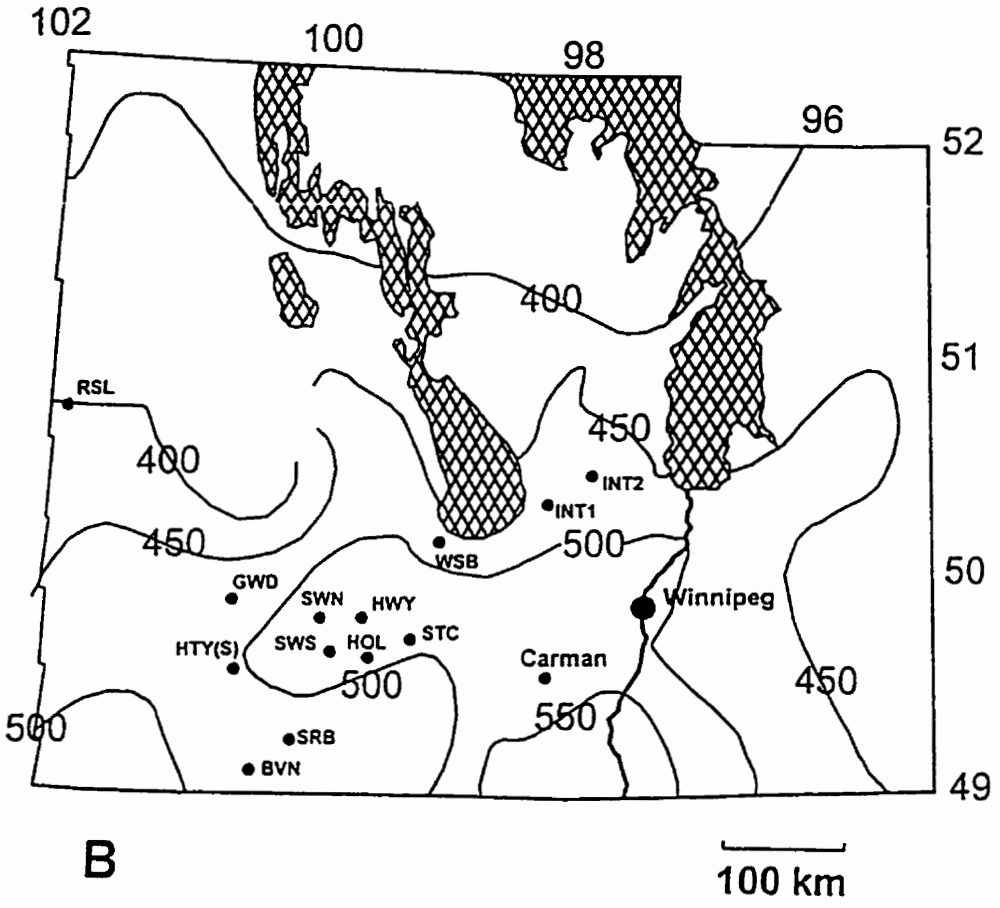


Figure 1.b. Little bluestem plant collection sites with respect to zones of growing degree days (GDD) in southern Manitoba, Canada. Calculation of GDD used a base temperature of 15°C and accumulated between 1 May and 31 August. Degrees longitude and latitude are indicated on the figure margins.

Table 1. Characteristics of 11 blue grama and 14 little bluestem collection sites in southern Manitoba, Canada†.

Collection site	Soil texture‡	Moisture rating	Exposure rating	Slope
Blue grama				—%—
CHC: Cactus Hills	S-L	poor	north	0
CVY: Carberry	S	poor	south	2
DGL: Douglas	S-L	poor	south	0-5
GDL1: Goodlands	S-L	poor	south	5
GDL2: Goodlands§	L	fair	south	0-2
KGR: Kruger	fine S-L	poor	west/north	2
LDR: Lauder	S	poor	west	0-2
OAK: Oak Lake	S/S-L	fair	south/west	5-10
RSL: Russel	thin S-L/G	poor	south	2-5
S10: Glenboro	S	poor	west/south	0-5
SDY: Sydney	S	poor	north	5-10
Little bluestem				
BVN: Boissevain	C/C-L	good	south	0
GWD: Griswold	S/S-L	fair	south	0
HOL: Holland	C-L	good	south	0-3
HTY: Hartney	S-L/S	poor	west	5
HTY-S: Hartney (saline)	S-L/C	fair	south	0
HWY: Highway	S	poor	south	10
INT1: St. Laurent	L/C-G	fair	west	2-5
INT2: Inwood	C/G	good	west	2-5
RSL: Russel	S-L	poor	south	0-2
SRB: Souris River	C/G	good	south	2-5
STC: St. Claude	S	poor	north	0-2
SWN: Swan River	S	poor	south	0
SWS: Glenboro	S	poor	south/east	5-10
WSB: Westbourne	C-L/G	fair	south	10

† No salinity at any site except HTY-S with an electrical conductivity rating of 10+.

‡ Soil texture visually assessed using a combination of designations: S = sandy; L = loamy; C = clay; G = gravel; combinations of designations indicate a range of variable soil texture within a collection site.

§ Higher elevation than GDL1 by approximately 152 m.

Table 2. Means for plant measurements showing significant collection X year interactions among 11 blue grama and 14 little bluestem plant collections in Winnipeg, Manitoba in 1994 and 1995.

	Harvested biomass plant^{-1}†		Days to anthesis from 1 May		Kernel index plant^{-1}‡	
	1994	1995	1994	1995	1994	1995
Blue Grama	g		d		%	
LDR	77.9	64.8	79.5	90.8	30.3	42.5
OAK	76.1	57.6	80.7	91.3	27.4	15.1
CHC	67.7	60.5	79.6	89.7	26.9	17.4
CVY	67.6	64.3	77.7	87.8	29.4	16.5
DGL	62.9	58.4	76.3	85.5	27.6	19.1
S10	53.8	41.3	78.9	92.9	28.4	13.7
SDY	49.6	47.5	76.1	86.4	31.4	20.8
GDL-2	46.1	41.6	75.5	83.2	31.2	20.9
GDL-1	43.2	45.5	75.3	83.6	33.0	21.0
KGR	40.4	31.3	74.7	84.3	32.4	21.6
RSL	21.7	28.4	73.9	78.5	29.0	25.1
LSD (0.05)	12.8	10.6	2.1	2.4	3.0	3.0

	Seedheads culm^{-1}		Seed yield plant^{-1}		Kernel index plant^{-1}‡	
	1994	1995	1994	1995	1994	1995
little bluestem	no.		g		%	
SWS	8.9	11.3	32.3	46.1	17.6	16.8
BVN	6.0	5.6	25.5	28.8	13.9	20.0
HWY	8.3	11.2	23.2	35.9	22.2	20.6
STC	7.7	9.3	23.1	24.0	22.7	24.2
SWN	8.0	9.5	22.1	28.8	17.8	20.1
HTY	6.0	6.8	22.2	30.0	14.4	18.1
SRB	7.0	7.9	21.5	28.8	18.3	20.3
GWD	7.5	7.4	20.1	19.8	17.5	20.0
HTY-S	6.2	8.1	17.0	19.6	15.0	17.6
INT-2	5.9	5.9	14.5	15.6	15.6	23.6
WSB	6.9	6.9	15.1	22.8	16.7	18.4
HOL	6.8	6.8	13.9	18.7	17.8	21.8
INT-1	5.3	5.3	9.5	14.9	16.1	23.0
RSL	5.7	5.7	12.5	17.1	22.3	31.0
LSD (0.05)	0.8	0.8	4.2	6.5	2.9	2.9

† Harvested biomass plant^{-1} = total plant air-dry weight from harvesting at an 18-cm stubble height.

‡ Kernel Index plant^{-1} = caryopsis yield (g)/unthreshed seedhead weight (g) x 100%.

Table 3. Means and rank correlations (1994 vs. 1995) for blue grama and little bluestem plant collections from Manitoba evaluated in 1994 and 1995 at Winnipeg for traits not showing significant collection × year interactions.

Plant Collection	Harvested biomass plant^{-1}	Days to anthesis from 1 May	Culm height	Culms plant^{-1}	Seedheads culm^{-1}	Weight seedhead^{-1}	Seed yield plant^{-1}	Kernel index plant^{-1}	100 caryopsis weight†
Blue Grama	— g —	— d —	— cm —	——— no. ———		— mg —	— g —	— % —	— mg —
LDR	72.1	83.3	55.5	237	2.3	52	20.9	36.1	46.2
OAK	66.5	82.1	53.2	205	2.2	55	19.1	21.3	50.6
CHC	64.1	84.4	53.5	212	2.4	50	19.4	21.8	46.4
CVY	65.9	81.7	57.8	216	2.2	53	19.9	23.0	44.6
DGL	60.2	79.6	56.1	248	2.2	49	19.5	23.3	43.9
S10	48.2	85.0	49.0	178	2.2	51	15.2	20.7	45.5
SDY	48.5	83.7	53.4	199	2.1	54	17.5	26.1	46.7
GDL-2	43.4	76.9	51.2	187	2.1	48	15.4	26.1	48.0
GDL-1	44.2	82.3	49.4	225	2.1	44	16.9	26.7	44.6
KGR	35.8	81.4	44.8	205	2.0	42	13.9	27.0	45.8
RSL	24.9	78.5	46.3	143	2.1	41	10.3	27.2	45.0
LSD (0.05)	9.9	1.7	2.5	35	0.1	6	3.1	2.5	2.5
Spearman ρ	0.87**	0.88**	0.66*	0.74*	0.95**	0.71*	0.60**	0.58	——

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

† Weight of 100 caryopses was from the 1994 harvest.

Continued next page

Table 3. Continued.

	Harvested biomass plant^{-1}	Days to anthesis from 1 May	Culm height	Culms plant^{-1}	Seedheads culm^{-1}	Weight seedhead^{-1}	Seed yield plant^{-1}	Kernel index plant^{-1}	100 caryopsis weight†
Little Bluestem									
SWS	138.0	78.1	86.2	293	10.1	——	39.2	17.3	103.9
HWY	102.8	76.9	80.8	224	9.7	——	29.9	21.7	116.7
BVN	95.9	75.0	75.5	302	6.4	——	27.5	17.2	85.7
HTY	94.6	73.2	75.7	342	6.4	——	26.0	16.1	86.0
SWN	88.1	74.5	79.7	223	8.7	——	26.0	18.6	104.0
STC	84.5	75.5	83.0	222	8.4	——	23.1	23.4	109.0
SRB	83.4	71.2	73.8	282	7.5	——	24.8	19.3	90.5
GWD	71.4	74.4	73.8	212	7.5	——	20.3	18.8	84.6
WSB	68.5	73.2	72.5	221	7.4	——	19.1	17.7	98.8
INT-2	61.4	70.5	69.8	255	5.9	——	16.1	19.8	101.3
HTY-S	60.0	73.3	66.6	220	7.1	——	18.4	16.2	84.5
HOL	57.1	74.2	76.5	176	7.2	——	16.4	19.8	101.4
RSL	50.8	68.6	68.8	190	6.0	——	14.8	26.5	106.7
INT-1	49.8	70.6	72.9	200	5.5	——	12.3	19.4	90.6
LSD (0.05)	16.5	1.6	4.9	44	0.9	——	4.7	2.8	19.0
Spearman ρ	0.91**	0.92**	0.89**	0.83**	0.93**	——	0.61*	0.40	——

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

† Weight of 100 caryopses was from the 1994 harvest.

Table 4. Mean squares from analysis of variance of 1995 seed yield traits for blue grama and little bluestem collections from southern Manitoba and planted in Winnipeg and Carman, Manitoba.

		Blue grama							
Source	df	Harvested biomass plant^{-1}	Culms plant^{-1}†	Weight of 20 culms	Seedheads 20 culms^{-1}	Seed yield 20 culms^{-1}	Seed yield plant^{-1}	Seedhead weight	Kernel index plant^{-1}‡
		g	no.	g	no.	g			%
Collection (C)	10	45.92***	47.19***	2.48***	0.23***	0.32***	45.92**	0.004***	7.33***
Environment (E)	1	570.35***	1148.66***	0.27	0.22**	0.12*	570.35***	0.002	320.02***
C X E	10	9.90***	26.90**	0.28***	0.05	0.12***	9.90**	0.002**	2.92***

		Little bluestem							
Source	df	Harvested biomass plant^{-1}	Culms plant^{-1}†	Weight of 30 culms	Seedheads 30 culms^{-1}	Seed yield 30 culms^{-1}	Seed yield plant^{-1}	Seedhead weight	Kernel index plant^{-1}‡
		g	no.	g	no.	g			%
Collection (C)	10	65.13***	75.40***	7.53***	124.66***	2.35***	21.37***	—	5.69***
Environment (E)	1	2181.13***	4649.84***	0.25	11.44	1.08*	330.68***	—	122.75***
C X E	13	17.86***	30.81**	0.19	5.22	0.11	4.13***	—	0.86

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively.

† Culms per plant were all reproductive.

‡ Kernel Index = cayopsis yield (g)/unthreshed seedhead weight (g) x 100.

MANUSCRIPT 2

Selection Methods to Improve Seed Production and Genetic Diversity in Native Grasses

ABSTRACT

Limited availability of adapted seed sources of native grass species has hindered their use in soil stabilization, habitat revegetation, and native rangeland seeding. Genetic diversity is desired in native grass seed sources to enhance establishment and adaptation to the planting sites. The objective of this study was to determine the most efficient selection protocol to improve seed yield and maintain genetic diversity in native grass Ecovars. Collections of 495 blue grama and 490 little bluestem plants were made from 11 and 14 locations across southern Manitoba, Canada, respectively. Measurements of seed yield component traits were made over 2 yr in Winnipeg and 1 yr in Carman, Manitoba. Potential genetic gains and shifts from selection were examined using four selection criteria aimed at improving seed production potential on a per plant basis (seed yield, harvest index, kernel index, and a 'combined index' of kernel index with harvest index) and three methods of pooling selections to minimize genetic shifts and maximize genetic diversity. The results indicate that the selection methods were independent of environmental effects due to low genotype x environment interactions. Phenotypic correlations provided a means to predict shifts in associated unselected traits. Selection criteria had greater influence on shifts in associated traits than pooling methods. Development of native grass Ecovars should focus on traits of interest, to an equal or

greater degree than pooling method, to obtain both maximum genetic gain and maintain genetic diversity.

INTRODUCTION

The preservation and maintenance of genetic diversity has become an important issue in the discipline of restoration ecology in the Great Plains region of the USA and Canada. Reclamation of land for wildlife habitat restoration (Jacobson et al., 1994; Duebbert et al., 1981; Knapp and Rice, 1996), revegetation and soil erosion control (Gaffney and Dickerson, 1987; Fults, 1936), and highway beautification (Greener Roadsides, 1997) has created greater awareness of the ecological and aesthetic advantages of native plant species over the use of introduced plant materials. Native plant species are uniquely adapted to the environmental extremes of a particular region through time, and naturally occupy local ecological niches. For this reason, locally adapted plant populations are generally recommended for restoration purposes (Millar and Libby, 1989; Knapp and Rice, 1996). However, the limited source of seed obtainable from remnant prairie stands is insufficient and often too expensive to meet the demands of large scale seeding projects. This limitation has resulted in the use of commercially available native grass cultivars that some have criticized as being genetically uniform based on the assumption that they were developed through traditional mass selection and derivation from a limited germplasm base.

Genetic diversity is desirable for the associated stability of population performance over environments by means of genetic buffering and reducing genetic

vulnerability (Poehlman and Sleper, 1995; Fehr, 1991). In a more local context, genetic diversity among individuals within a population enhances the population's ability to occupy different micro-environments as seen in wheat (Nevo et al., 1988 a,b), phragmites (Koppitz et al., 1997), and oat (Hamrick and Holden, 1979). Similarly, genetic differentiation on the population level accommodates larger scale geographic and environmental differences (Ferguson et al., 1998). In heterogeneous environments, with inherent soil moisture, fertility, and topographical limitations, genetic diversity is important for stand establishment and long-term persistence. In the 1980's, Erling Jacobson of the USDA Natural Resource Conservation Service, conceived the term *Ecovar* for 'ecological varieties' to address the need for native plant germplasm where maintenance of genetic diversity was as important as improvement for specific traits. Although this term was not accepted in the USA, Ducks Unlimited Canada has since trademarked 'Ecovar™' (refer to Ducks Unlimited Canada's *Native Plant Solutions* for trademark and legal details) to describe genetically diverse native plant germplasm. A number of Ecovars have now been developed through collaborative projects with university, provincial, and federal plant breeders. Through this organization, approximately 4000 ha yr^{-1} is planted to native grasses, forbs, and shrubs for wildlife habitat. They also recognize a much larger commercial potential for this type of product.

Achieving genetic gain combined with genetic diversity is a common goal of plant breeders, but few breeding methodologies have focused on high levels of genetic diversity. Multilines in wheat, blends of high-performance lines having subtle differences in genetic loci of interest, have improved stability of performance over years and environments (Marshall and Brown, 1973). Disease resistance to multiple

pathogens/pathotypes can similarly be achieved by pyramiding the respective resistance genes into a common recipient through a series of backcrosses (Beaver and Macchiavelli, 1998). Another approach used in tree breeding considered relatedness of selections as opposed to the traditional practice of restricting parental contribution to the selected population simply based on plant performance (Zheng et al. 1997).

Vogel and Pedersen (1993) described a breeding strategy similar to the Ecovar concept as 'ecotype selection', whereby genetic diversity of naturally occurring ecotypes could be exploited by phenotypic selection within and between accessions for rapid cultivar development. However, the specific objective of minimizing genetic shifts and/or maximizing genetic diversity was not a consideration in 'ecotype selection'.

The combined objective of selection for maintenance of genetic diversity and improvement of key limiting traits has inherent difficulties. Firstly, selection reduces genetic variation for selected traits with each selection cycle (Falconer and Mackay, 1996; Hallauer, 1970). Secondly, shifts in the phenotypic values of traits other than the one(s) of interest may also occur as a result of correlated responses (Stojsin and Kannenberg, 1994; Conner and Sterling, 1996; Coors and Mardones, 1989). In little bluestem, selection for forage characteristics reduced variation in flowering time (Anderson and Aldous, 1938), a shift which would affect a diverse population's ability to outcross. In native grasses intended for use in land reclamation/revegetation, such shifts may result in the loss of adaptive features that make them suited for heterogeneous and marginal environments.

The purpose of this study was to examine the practicality of Ecovar development of native grass species. The specific objective was to develop a simple and effective

selection method to improve seed yield potential and maintain genetic diversity in blue grama (*Bouteloua gracilis* [H.B.K.] Lag. ex. Steud.) and little bluestem (*Schizachyrium scoparium* [Michx.] Nash.) using hypothetical synthetic populations constructed from individual plant measurements.

MATERIALS AND METHODS

Plant Collections

Blue grama and little bluestem plants were randomly collected from multiple locations in southern Manitoba, Canada in late August of 1992 by the field staff of Ducks Unlimited Canada (Figures 1A and 1B. pp.46 & 47). Collection locations were separated by a minimum of 30 km, and a total of 11 and 14 locations were sampled for blue grama and little bluestem, respectively. Intact plants were excavated from each collection site using a minimum 1-m separation distance between adjacent plants, and transplanted at the University of Manitoba field research station at Winnipeg (49° 49′ N, 97° 7′ W).

Plant Nurseries

Winnipeg

The plant collections were arranged in a randomized complete block design with nine replications for blue grama and seven for little bluestem. Plots were comprised of five individual plants spaced on 1-m centers. The total plant number was 495 (5 plants x 11 collections x 9 replicates) for blue grama and 490 (5 plants x 14 collections x 7 replicates)

for little bluestem. The experimental area of each species was flanked by a single row of border plants. The soil was a Cumulic Regosol with a high clay content. Plant mortality over the winter of 1992-93 reduced plant numbers to 470 and 483 for blue grama and little bluestem, respectively. Detailed plant measurements were taken in 1994 and 1995.

Carman

In late June of 1994, clones of all plants of both species were used to duplicate the Winnipeg plant nurseries at the University of Manitoba Carman Field Research Station (49° 30′ N, 98° 00′ W) on an Orthic Black Chernozem soil, sandy in texture, with re-randomization of the treatments. The plants were allowed to establish during the 1994 growing season and measurements were taken in 1995.

Measurements

Number of calendar days to first anthesis was determined based on the starting date of 1 May, which was prior to visible emergence of new shoots. Plant height was measured as the height of the tallest reproductive culm near the center of the plant after anthesis. Crown diameter was determined as the average of two measurements of crown width taken at right angles after anthesis. Plants were individually harvested by cutting at an 18-cm stubble height which included all mature culms. Harvesting of both blue grama and little bluestem commenced when approximately 70-80% of the culms showed signs of seedhead ripening by visual estimation of caryopsis medium to hard-dough stage, browning and drynesss of inflorescences. In blue grama, ripened spikes were sandy colored and arched to

accommodate seed dislodge, while in little bluestem, racemes were open and the spikelet appendages gave them a "fluffy" appearance.

Harvested plants from all location years were kept in paper bags and allowed to air-dry at approximately 27°C under continuously blowing fans in a closed metal shed. Total harvested biomass of each plant was determined. Fifty percent of each plant was used to estimate the following on an individual plant basis: number of culms = (number of culms counted in 50% sample) x 2; seedhead number = (number of seedheads counted in 50% sample) x 2; seed yield = (seed threshed from 50% sample) x 2. With blue grama, an additional sample of 20 culms with ripened, unshattered seedheads was randomly selected for determination of number of seedheads 20 culms^{-1} and caryopsis extraction. Because seed shattering was a greater concern with little bluestem, 30 mature culms were sampled. From the 20 and 30 reproductive culms of blue grama and little bluestem, seedheads were removed and weighed, and spikelets were subsequently removed by hand. The spikelets were further processed using a belt thresher having a moving rubber belt against a rolling drum in which the spikelets were fed 3-4 times to separate the caryopses from the chaff. Some caryopses were broken, but these were collected for weight determination as the belt was brushed clean after each run. Caryopses were separated from the chaff by running them 2-3 times through a column air blower. The end-cap of the column was constantly agitated to maintain consistent airflow through the column. A 'kernel index' was calculated as follows:

$$\text{Kernel Index (KI)} = \frac{\textit{Caryopsis yield(mg)}}{\textit{Unthreshed Seedhead Weight(mg)}} \times 100\% \qquad [1]$$

The weight of 100 caryopses was also determined to the nearest 0.1 mg for the 1994 Winnipeg harvest.

'Ecovar' and 'Cultivar' Selection

Selection Criteria

Various selection criteria for improving seed production in crop species have been examined and successfully applied. In this research, four selection criteria were used. The most direct selection criterion for improving seed production potential is seed yield plant^{-1}. However, seed yield plant^{-1} does not take variation in plant size or dry matter production into consideration because larger plants typically produce more seed. Therefore, harvest index was used so that smaller plants were not neglected in selection. In their objective to improve seed production in smooth bromegrass (*Bromus inermis*), Raeber and Kalton (1956) used a 'fertility index', referred to here as 'kernel index', as their selection criterion to improve yield of viable caryopses in smooth bromegrass rather than just overall seed weight. In the present research, a selection index was developed which considered the above criteria with the added specific objective of improving overall seed yield. Using information from field observations and the literature, a 'Combined Index' was devised to meet the objective of improving seed production potential with minimum genetic shifts.

(1) Seed yield plant^{-1}

(2) Harvest Index (HI) = $\frac{Seed\ Yield\ Plant^{-1}\ (g)}{Harvested\ Biomass\ Plant^{-1}(g)}$ x 100%

(3) Kernel Index (KI) = $\frac{Caryopsis\ yield(mg)}{Unthreshed\ Seedhead\ Weight(mg)}$ x 100%

(4) Combined Index (CI): for blue grama: KI x Seed yield plant^{-1}

for little bluestem: KI x HI

These 'Combined Indices' were developed based on intuitive expectations of response to selection. The CI for blue grama combined KI with seed yield because the 1 to 4 seedheads per culm in this species are borne near the culm terminus, and the seed yield of this species did not appear to be affected by other culm characteristics such as height or number of nodes based on field observations. For little bluestem, the CI was adjusted to include HI instead of seed yield per plant because seedheads in these species are borne at the culm nodes, and it was surmised that seed yield could therefore be directly influenced by culm height. Using HI in the Combined Index for little bluestem was intended to minimize shifts in culm characteristics.

Ecovar Pooling Methods

Using the above criteria for selection, 'Ecovar' and 'cultivar' groups were partitioned from the original population of plant collections. A cultivar group was included for the purpose of comparing the Ecovar approaches with a simple mass selection method commonly used for rapid cultivar development. For the cultivar group, the highest-ranking 25 individuals were selected among the entire original population, regardless of plant collection, to represent a 5% selection intensity.

In contrast to the cultivar, the goals of the Ecovar method were to minimize loss of genetic diversity due to selection, and maximize genetic diversity. Two parameters were considered for the Ecovar method. First, a lower selection intensity (20%) was used for the Ecovar compared to the cultivar method to help minimize loss of diversity from selection. Second, equal numbers of plants were pooled from all plant collections to offset potential reductions in variation due to selection and to maximize genetic diversity. Two Ecovar pooling methods utilized equal pooling (Ecovar [1] and [2] below) and one did not.

Ecovar [1]: The top ranking individual from each plant collection in each experimental block.

For blue grama: 11 collections x 9 blocks = 99 selections.

For little bluestem: 14 collections x 7 blocks = 98 selections.

Ecovar [2]: The top ranking 9 and 7 individuals per plant collection for blue grama and little bluestem, respectively, without experimental blocking.

Ecovar [3]: The top ranking 99 and 98 individuals among blue grama and little bluestem, respectively, regardless of blocking or plant collection.

The different methods of pooling for the Ecovar were designed to use both stratified and unstratified selection procedures. In method one (Ecovar-1), restricted selection was conducted within experimental replications and plant collections. This pooling method was an extension of restricted phenotypic selection as described by Burton (1974), who used subdivisions of the plant nursery as the selection unit, equivalent to blocking, to minimize effects of environmental variation. However, the Ecovar-1 method further required contribution of superior individuals from all plant collections, and therefore redefined the selection unit as a combination of blocking and plant collection. In method two (Ecovar-2), restricted selection occurred only among plant collections and disregarded experimental blocking. Ecovar selection methods 1 and 2 both maintained equal representation of every original plant collection. In contrast, the third pooling method (Ecovar-3) was simply a mass selection method having a lower selection pressure than for the cultivar method. This last method was included to evaluate the necessity of equal representation of plant collections as in methods 1 and 2, and whether simply using lower selection pressure would minimize genetic shifts as well as methods 1 and 2.

Data analysis

The 1994 and 1995 data from Winnipeg and the 1995 data from Carman comprised the 3 environment-years. All individual plant data combined over 2 yr in Winnipeg and 1 yr in Carman, Manitoba, were subjected to Analysis of Variance (ANOVA) (SAS Institute, 1989) to determine the presence of plant collection x environment-yr interaction. To generate populations of equal size for comparison purposes, bootstrap re-sampling of pseudo-groups executed 100 times was used to generate the Ecovar and cultivar populations. The bootstrapped Ecovar and cultivar population means and variances were found to be almost identical to the actual group means and variances, so population comparisons were subsequently done using actual values. To assess the impact of plant collection x environment-yr interaction, Spearman's rank correlations of group ranks among Ecovars and cultivars between environment-yrs were computed. The generated Ecovar and cultivar populations were then compared with the overall unselected original population, which comprised all plant collections, using one-tailed t-tests. Associations between seed yield component traits were examined with phenotypic correlations calculated from covariances of expected mean squares for plant collections using 1994 and 1995 measurements in Winnipeg where plant collection and years were assumed to have random effects. Data from Carman was excluded from the calculation of phenotypic correlations because of plant mortality. Potential genetic shifts, observed as changes in trait correlations caused by Ecovar and cultivar selections, were determined by the Mantel test of matrix correspondence using the NT-SYSpc v.1.05 software with the assumption that correlation matrixes of the Ecovars and cultivars were independent.

RESULTS AND DISCUSSION

Features of an Ecovar

This research was conducted to address the present need for commercial supplies of genetically diverse native grass seed for western Canada and the north central United States. The objective of this study was to evaluate the combination of pooling strategies and selection criteria for improving viable seed yield of blue grama and little bluestem, while maintaining genetic diversity and minimizing genetic shifts following selection. The Ecovar pooling methods 1 and 2 maintained equal representation of all 11 blue grama and 14 little bluestem plant collections. In comparison, mass selection used with the cultivar method and Ecovar pooling method 3 produced populations with unequal representation of the plant collections (Table 1). Greater representation was obtained with Ecovar pooling methods than the cultivar method because of the former's lower selection intensity. Although complete representation of all plant collections was not obtained from the cultivar method and Ecovar-3 pooling, most plant collections were still represented even if by only one individual in these groups. Little bluestem cultivar selection resulted in representation of fewer plant collections than for blue grama. This indicated that greater selection was conducted between plant collections than within for little bluestem, perhaps due to accentuated differences between plant collections for this species as shown previously (Phan and Smith, 2000). It is postulated that equal genetic representation from all plant collections is the best strategy to maximize genetic diversity.

Consistency of Ecovar Selection Based on ANOVA and Rank Correlations

In native grass breeding, as with any other breeding program, the development of cultivars or Ecovars should not be affected by year or environment from which data were used to identify superior genotypes. Ideally, a germplasm base could be evaluated and selection of the best individuals conducted in one year. However, genotype-by-environment-yr interactions confound the true genotype of an individual. The analysis of data in this study using 3 environment-yrs showed no plant collection x environment-yr (C x E) interaction for most measured traits (Table 2). Little bluestem showed fewer interactions than blue grama. In both species, there was a C x E interaction for harvested plant biomass and culm spread, but the interaction mean squares were smaller than the separate plant collection and environment mean squares. This is in contrast to an earlier report of C x E interaction for most traits in blue grama and, to a lesser degree, in little bluestem across two of these environments-yrs (Phan and Smith, 2000). The change may be due to the added degree of freedom in the present analysis after including an additional environment-yr.

The effectiveness of phenotypic selection can often be hindered by phenotypic plasticity of the trait(s) of interest. In the Ecovar development context, the presence of C x E interaction would affect efficiency of selection for desired traits because individual plants, or entire collections, that rank high in one year or environment may rank low in different years and/or environments. The method of ecotype selection as described by Vogel and Pedersen (1993) considered the confounding effects of environment and recommended the evaluation of germplasm over multiple environment-yrs. Despite the absence of C x E interactions for most traits in this study, the significant interaction found

for plant-harvested biomass indicated that phenotypic selection involving this trait may require additional environment-yrs of field data for effective selection.

Interpretation of C x E provides a basis for predicting whether selection will be effective, but it does not provide a good indication about the consistency or reliability of Ecovar selection between years and environments. Therefore, actual comparisons of Ecovar and cultivar-selected groups were conducted using Spearman's rank correlation with data from different environments (Table 3). It was found that for almost all traits, there was significant correlation of group ranks between all environments. Only plant kernel index and days to anthesis in blue grama showed a discrepancy between C x E interaction and rank correlation results. Kernel index in blue grama showed low rank correlation between 1994 and 1995 in Winnipeg, and no C x E interaction between Winnipeg and Carman, which suggested a low environmental influence on this trait.

Despite significant plant collection x environment interaction for harvested biomass for both species, any plasticity in this trait did not affect the rank among the Ecovar and cultivar-selected groups. The rank correlation results showed that selection of plants between and within the original plant collections to form Ecovar and cultivar groups using data from different environment-yrs would essentially produce similar groups of selections. Therefore, this analysis suggested that one random environment-yr of data was sufficient for Ecovar development, providing that plant mortality among collections was low and random, in the absence of unusual weather phenomena. However, it should be stressed that measurements be made on fully established plants at least one yr following transplanting for plants to express their full genetic potential.

Phenotypic Correlations Between Selected and Unselected Traits

The four selection criteria evaluated for Ecovar development were aimed at improving seed production potential with minimal shifts in unselected traits, and thereby maintaining genetic diversity for these unselected traits. The degree of genetic shift in an unselected trait is determined by the genetic correlation between selected and unselected traits, its heritability, and the selection intensity utilized (Falconer and Mackay, 1996). Shifts in unselected traits caused by correlated responses have been shown in previous studies (Stojsin and Kannenberg, 1994; Conner and Sterling, 1996; Coors and Mardones, 1989). There were positive phenotypic associations between many of the measured seed yield traits in blue grama and little bluestem (Table 4). In both species, plant-harvested biomass was highly correlated ($r > 0.9$, $P = 0.05$) with seed yield, which could be attributed to the positive association of biomass with number of culms plant^{-1}, seedhead number, and culm height. This suggested that including harvested biomass as a component in Ecovar selection criteria could cause shifts in several of the traits associated with it. Similarly, seed yield plant^{-1} in both species was correlated with seed yield component traits, which also suggests that genetic shifts in several of its correlated component traits may be expected.

Plant selections must have sufficient overlap in flowering time for effective inter-pollination to enhance and maintain within-population genetic diversity. The number of days to anthesis was found to be highly associated with many plant traits. This trait was not considered in any of the selection criteria evaluated because it was thought that greater genetic shifts may occur if selecting for uniform anthesis. For instance, shifts towards earlier flowering may result in subsequent onset of plant dormancy and reduce

the period of plant productivity while later flowering may jeopardize proper plant maturation and seed production prior to first frost.

Only kernel index and caryopsis weight showed low and non-significant correlation with other measured traits. The rationale of using kernel index as a criterion in Ecovar selection was to improve the proportion of viable seed in harvested seed yield (Raeber and Kalton, 1956) and simultaneously take advantage of the lack of association between this trait and other seed yield-component traits. In little bluestem, caryopsis weight was correlated only with kernel index, and in blue grama, caryopsis weight was not correlated with any other measured trait. Wilson et al. (1981) found similar results in blue grama and suggested that it would be possible to simultaneously select for high caryopsis weight and high seed yield. The high correlation between caryopsis weight and kernel index may be attributed to the observed association between seedhead size and spikelet number, the latter being an indirect measure of the former. In contrast, caryopses seedhead^{-1} in blue grama measured on a weight basis can vary due to both caryopsis size and caryopsis number. Therefore, kernel index does not discriminate between plants having seedheads with few large caryopses and those with many small caryopses. The lack of association of kernel index and caryopsis weight with other seed yield component traits suggested that these two traits may be ideal for Ecovar selection, because they can be selected for with minimum shifts in other component traits.

Although single-trait selection would produce a higher selection response than multi-trait selection, as demonstrated in smooth bromegrass (Carpenter and Casler, 1990), additional requirements were included in the 'Combined Index'. The present deficiency in adapted seed sources makes the use of native grass species too expensive

for large-scale planting projects, the main objective of selection was to improve overall caryopsis yield. Because caryopsis yield is a function of seed yield and seedhead fertility, the 'Combined Index' for blue grama was comprised of kernel index and seed yield. For little bluestem, kernel index was combined with harvest index rather than seed yield because field observations indicated that smaller, lower seed-yielding plants would otherwise be excluded from selection. This would occur because taller little bluestem plants were found to produce more seedheads on their reproductive culms. This was not a concern with blue grama because field observations of this species indicated that regardless of culm height, seedhead number per culm only ranged from 1 to 4.

Potential Genetic Shifts

Changes in Trait Correlations Following Selection

Selection may produce genetic shifts in the form of changes in trait correlations. Genetic shifts due to correlated responses may produce improved populations in which correlations between traits differ from those prior to selection. Because correlated response is a function of genetic correlation between selected and unselected traits (Falconer, 1993), a trait having higher correlation with the selected trait will experience a greater shift due to selection than one with a lower correlation. Therefore, it can be expected that plant traits having a higher association with the selected trait will shift towards a higher correlation with the trait of selection, while traits initially not highly correlated with the trait of selection would experience a neutral shift in their correlation. It is therefore important to make observation of plant growth and before considerations on selection criteria are made.

To determine shifts of this nature, the Mantel test of matrix correspondence was used to compare simple correlation matrixes for traits of the original population and those of the Ecovar and cultivar selections (Table 5). A high Mantel-correlation between matrices indicates no significant difference between matrices. In general, trait correlations in the cultivar groups shifted the most. The average correlation of matrices across the 3 environment-yrs were all low and non-significant in blue grama, and in little bluestem, with the exception of harvest index for little bluestem. In contrast, almost all Ecovar pooling methods showed few shifts in trait correlations in both species. Only Ecovar pooling methods 2 and 3 in blue grama, when seed yield was the selection criterion, showed non-significant correlations with the original population in two out of the three comparisons. These comparisons indicate that the cultivar groups have a greater likelihood of experiencing genetic shifts in the form of changes in trait correlations than the Ecovar groups.

Effect of Selection Criteria

A comparison of the different Ecovar pooling methods within each selection criterion indicated differences in potential shifts in trait means (Table 6). Calculated values averaged over the 3 environment-yrs may be over-estimates of true population means because they depend on trait heritabilities. However, over-estimations would allow easier detection of any potential shifts in unselected traits that may occur during cycles of phenotypic selection. Effects of the different selection criteria were observed from differences among the cultivar groups in both blue grama and little bluestem. As expected from the analysis of phenotypic correlations, selection for seed yield and harvest index produced shifts in many of the unselected traits in both species. It was

shown that selection for forage yield in bahiagrass increased morphological trait measurements by as much as 423% after 16 cycles of recurrent restricted phenotypic selection (Werner and Burton, 1991) and shifted populations towards taller plants with fewer rhizomes and increased susceptibility to winter injury (Pedreira and Brown, 1996). Among the unselected traits in this study, selection for harvest index resulted in negative shifts in comparison to the original unselected population, and selection for seed yield produced positive shifts in both species.

The Combined Indices also produced shifts in unselected traits in the Ecovar groups of both blue grama and little bluestem. These shifts reflected the combination of a desirable trait having few correlations (kernel index) with a trait that had many correlations (seed yield plant^{-1} and harvest index) that was used in making selections. However, it was observed that Ecovar selection using the Combined Indices produced different amounts of undesired shifts depending on the pooling method. Ecovar pooling method 1 was seen as most effective in shifting seed yield component traits but not overall plant attributes including plant size (i.e. harvested biomass), number of culms, culm height (only in little bluestem), and culm spread. Stojsin and Kannenberg (1994) found in their study with corn that many of the indirectly selected traits were predominantly controlled by additive genes. If the same can be assumed for blue grama and little bluestem, it is expected that unselected traits would change in the same cumulative manner as directly selected trait(s) with each cycle of phenotypic selection.

Kernel index was used as a selection criterion because past research has long indicated that low seed yield in many grass species is due to undeveloped embryos (Blake, 1939; Stoddart and Wilkinson, 1938). When used as the sole selection criterion,

kernel index showed the fewest associated shifts in unselected traits, as predicted from the correlation analysis. There were observed shifts only in seedhead number 30 culms^{-1} in little bluestem and 100 caryopsis weight and days to anthesis in blue grama. Conner and Sterling (1996) indicated with outcrossing species that vegetative and floral plant traits could be independently selected for based on greater correlations within respective floral and vegetative trait groups than between groups. Therefore, the relative independence of kernel index from other traits, which should minimize shifts in unselected traits, make it the best selection criterion for Ecovar development of blue grama and little bluestem among those evaluated in this study.

Effect of Ecovar Pooling Method

Some of the selection criteria caused large shifts in unselected traits despite attempts to reduce shifts by using different methods for pooling genetic diversity (Table 6). The rationale behind Ecovar pooling method 1, where the best plants from each plant collection were selected in each experimental block, was to use a restricted phenotypic selection method which takes into consideration blocking effect on plant collection. Ecovar pooling method 2 did not consider blocking, and the best plants within each plant collection were selected to form the Ecovar group. Both Ecovar pooling methods 1 and 2 maintained equal representation from all plant collections. In contrast, Ecovar pooling method 3 was simply mass selection for the purpose of including only the best representatives. The outcrossing nature of blue grama and little bluestem (Anderson and Aldous, 1938; Riegel, 1941; Miller, 1967) lend themselves to genetic recombination between selected genotypes. Therefore, it is conceivable that the generated Ecovar

groups may contain more genetic diversity than any single plant collection following intercrossing.

The Ecovar pooling methods showed differences in potential shifts of unselected traits, depending on the selection criterion (Table 6). In both blue grama and little bluestem, culms plant^{-1} and days to anthesis showed the least shift among Ecovar pooling methods. Ecovar pooling method 1 produced no shift in seed yield per plant in blue grama compared to pooling methods 2 and 3. In little bluestem, selection for seed yield as the criterion did not produce much difference in shifts between the three Ecovar pooling methods. It is possible that uniformity of the plant nursery with respect to gradient and soil fertility (data not shown) reduced the effectiveness of blocking. Therefore, maximizing selection between blocks may have occurred at the expense of not selecting superior individuals occurring within a block.

Using the 'combined index' as a selection criterion, blue grama showed fewest shifts from using Ecovar pooling method 1 compared to methods 2 and 3, and in little bluestem, Ecovar pooling methods 1 and 2 showed fewer shifts compared to method 3. In blue grama, only the 'combined index' showed an advantage to using pooling method 1 over methods 2 and 3 by producing fewer shifts. The 'combined index' used for little bluestem produced fewer overall shifts than in blue grama. This could probably be attributed to the substitution of harvest index into the 'combined index' for little bluestem in comparison to seed yield used for blue grama. The observed difference in potential shifts in unselected traits between the 'combined indexes' of the two species was therefore probably due to differences in correlations associated with seed yield compared to harvest index.

In contrast to the other selection criteria, kernel index did not show much difference in genetic shifts between the three Ecovar pooling methods in either blue grama or little blustem. Observed shifts associated with kernel index only included traits which were directly associated with kernel index determination, such as 100 caryopsis weight. Only culm height with pooling method 2 was seen to shift in blue grama, using kernel index as the selection criterion. These results suggest that the method of Ecovar pooling is only secondary in importance to selection criteria. However, Ecovar pooling methods may show a greater influence at the molecular genetic level at which genetic recombination between selections occur.

Ecovar pooling using solely seed yield and harvest index as selection criteria produced the most shifts in unselected traits compared to the other selection criteria. But more importantly, the shifts associated with selection using harvest index were with traits that did not contribute to improving seed yield. In contrast, Ecovar pooling from selection with seed yield shifted most of the seed yield component traits in both blue grama and little bluestem. In little bluestem, Ecovar pooling method 3 produced the most shifts in unselected traits including culm height, culm spread, and days to anthesis, whereas methods 2 and 3 produced more undesirable shifts than method 1 in blue grama. The Ecovar pooling methods involving seed yield and harvest index as selection criteria generally produced similar populations with many undesirable shifts in unselected traits.

The present comparison of Ecovar pooling methods with each selection criterion showed that the combination of pooling methods and selection criteria differed in their ability to meet the needs of an Ecovar. Overall, kernel index was the selection criterion that produced the fewest shifts in unselected traits, but it was limited in its ability to

improve seed yield. However, the Combined Index, used in conjunction with Ecovar pooling method 1, was regarded as the best combination of approaches to develop Ecovars.

Phenotypic data used in our analysis of potential genetic shifts due to correlated traits is only one aspect of this study. Data from a RAPD (Random Amplified Polymorphic DNA) study conducted on blue grama Ecovar and cultivar populations is currently being analysed to determine shifts in genetic diversity due to selection at the DNA level.

Additional Considerations to Ecovar Breeding

The concept of Ecovar breeding is a novel one and therefore the selection methods outlined in this research provide a framework from which to construct a breeding model. As such, there remain additional considerations that have not been specifically addressed in this study. These considerations include the need for overlapping periods of flowering between selected plants to ensure crossing, while maintaining overall variation in flowering time. Another consideration is potential incompatibility of ploidy levels among selected individuals from the different plant collections that may affect seed development and viability. There are reported variations in ploidy levels in little bluestem (Church, 1929; Hunter, 1934; Nielson, 1939; Dewald and Jalal, 1974) and stronger evidence for different ploidy levels in blue grama (McGinnies et al., 1988; Fults, 1942; Snyder and Harlan, 1953). Variations in ploidy level need to be investigated. Overall, the results of this study indicate that Ecovar breeding is feasible

using equal pooling of representatives from plant collections, in conjunction with appropriate selection criteria.

CONCLUSION

This study was aimed to provide a knowledge base and an initial frame-work with which to develop improved and genetically diverse native grass Ecovars. Analysis of data from 3 environment-yrs provided insight into potential genetic gains and shifts from selection using four selection criteria aimed at improving seed production potential, and three methods of pooling designed to minimize genetic shifts and maximize genetic diversity. The results indicated that Ecovar selection using data from different years and environments produced consistently similar populations. Therefore, effective selection can be made based on only one or two environment-yrs of data. Phenotypic correlations provided a means to predict shifts in associated unselected traits. The four selection criteria had greater influence on shifts in associated traits than the Ecovar pooling methods. The results of this study indicated that Kernel Index was the best selection criterion to maintain genetic diversity, but that a combined index with seed yield offer a valid compromise to meet the breeding objectives. Development of native grass Ecovars should therefore focus on traits of interest and their genetic correlations to an equal or greater degree than pooling methods in order to obtain both maximum gain and genetic diversity.

Table 1. Number of plants selected from 11 blue grama and 14 little bluestem collections from southern Manitoba for Cultivar and Ecovar-3 synthetic groups.

	Blue grama							
	Cultivar based on†:				Ecovar-3 based on†:			
Plant collection	CI	KI	HI	SY	CI	KI	HI	SY
CHC	0	0	1	1	13	5	4	12
CVY	3	2	1	2	10	3	4	12
DGL	4	1	2	5	5	6	5	8
GDL1	4	6	3	1	9	18	15	9
GDL2	2	4	1	1	10	14	14	6
KGR	0	3	3	1	6	12	15	5
LDR	5	3	2	4	13	9	5	14
OAK	3	3	0	5	13	7	1	0
RSL	0	1	5	0	1	9	12	15
S10	2	2	2	1	7	3	10	8
SDY	3	0	5	4	12	13	14	10
total	25	25	25	25	99	99	99	99

	Little bluestem							
	Cultivar based on†:				Ecovar-3 based on†:			
Plant collection	CI	KI	HI	SY	CI	KI	HI	SY
BVN	0	0	1	4	3	1	7	13
GWD	0	1	4	1	4	3	11	7
HOL	4	5	2	0	7	10	8	3
HTY	0	0	1	0	2	2	4	8
HTYS	4	2	1	0	5	3	13	7
HWY	2	3	2	2	12	13	7	10
INT1	0	0	0	0	1	4	0	0
INT2	2	3	1	0	6	5	4	2
RSL	6	3	5	0	16	13	12	0
SRB	3	1	2	1	9	7	10	9
STC	2	6	1	3	13	14	2	10
SWN	1	1	1	3	7	11	2	9
SWS	1	0	2	11	7	5	11	20
WSB	0	0	2	0	6	7	7	0
total	25	25	25	25	98	98	98	98

† Abbreviations: CI = Combined Index; KI = Kernel Index; HI = Harvest Index; SY = Seed Yield on a per plant basis.

Table 2. ANOVA mean squares of plant measurements of blue grama and little bluestem plant collections from southern Manitoba, Canada, and evaluated in Winnipeg (1994 & 1995) and Carman (1995), Manitoba.

Source	df	Harvested biomass plant^{-1}†	Culms plant^{-1}	Seedheads 20 culms^{-1}	Seed yield 20 culms^{-1}	Kernel index plant^{-1}‡	Culm height	Culm spread	Days to anthesis§
		Blue grama							
Plant Collection	10	15743.1**	57396.8**	519.6**	8.4**	625.6**	1888.5**	5734.5**	565.4**
Environment	2	92886.1**	530334.1**	705.4**	28.2**	17775.0**	7059.3**	21512.9**	20316.3**
Collection x Env't	20	2128.7*	15534.8ns	28.8ns	1.7ns	144.4ns	260.8**	1355.9*	421.9**
		Little bluestem							
Source		Harvested biomass plant^{-1}†	Culms plant^{-1}	Seedheads 30 culms^{-1}	Seed yield 30 culms^{-1}	Kernel index plant^{-1}‡	Culm height	Culm spread	Days to anthesis§
Plant Collection	13	30835.6**	113992.3**	150688.8**	39.3**	599.1**	1489.8**	6834.3**	414.2**
Environment	2	341339.0**	1851239.3**	189328.3**	10.9**	4199.1**	38128.1**	306185.7**	83.1ns
Collection X Env't	26	5499.3**	18908.6ns	7545.6ns	1.4ns	93.6ns	322.8ns	1426.5*	24.0ns

† Harvested biomass plant^{-1} comprised of plant air-dried weight from harvesting at an 18-cm stubble height.
‡ Kernel index plant^{-1} = caryopsis yield (g)/unthreshed seedhead weight (g) x 100.
§ Days to first anthesis was counted from 1 May.
*, **, *** significant at P = 0.05.

Table 3. Spearman rank correlation of blue grama and little bluestem Ecovar and Cultivar means selected using separate data-sets from three environment-years in Manitoba, Canada (n = 16).

Environment-year comparison	Blue grama							
	Harvested biomass plant^{-1}	Culms plant^{-1}	Culm height	Seedheads 20 culms^{-1}	Seed yield 20 culms^{-1}	Spikelet Index†	Kernel index§	Days to anthesis from 1 May
'94Wpg vs. '95Wpg	0.995***	0.990***	0.936***	0.731***	0.871***	0.877***	0.430	0.458
'94Wpg vs. '95Car	0.956***	0.982***	0.899***	0.782***	0.850***	0.828***	0.230	——
'95Wpg vs. '95 Car	0.966***	0.972***	0.985***	0.407	0.778***	0.736***	0.903***	——
	Little bluestem							
	Harvested biomass plant^{-1}	Culms plant^{-1}	Culm height	Seedheads 30 culms^{-1}	Seed yield 30 culms^{-1}	30 Culm weight	Kernel index†	Days to anthesis from 1 May
'94Wpg vs. '95Wpg	0.880***	0.953***	0.959***	0.564*	0.562*	——	0.807***	0.556*
'94Wpg vs. '95Car	0.836***	0.830***	0.911***	0.672**	0.896***	——	0.921***	——
'95Wpg vs. '95 Car	0.694**	0.872***	0.897***	0.706**	0.654**	0.598*	0.746***	——

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

† Spikelet Index = seed yield (g)/unthreshed seedhead weight (g) x 100%.

§ Kernel index = caryopsis yield (g)/unthreshed seedhead weight (g) x 100%.

Table 4. Phenotypic correlation coefficients for seed yield traits in blue grama and little bluestem plants collected in southern Manitoba, Canada.

Blue Grama	Culms plant^{-1}	Seedheads 20 culms^{-1}	Seedheads plant^{-1}	Seed yield plant^{-1}	Kernel index plant^{-1}	100 Caryopsis weight	Culm height	Crown diameter	Days to anthesis
Harvested biomass plant^{-1}†	0.78*	0.82*	0.88*	0.97*	-0.49	0.31	0.91*	0.82*	0.89*
Culms plant^{-1}‡		0.42	0.98*	0.86*	-0.10	0.01	0.62*	0.92*	0.53
Seedheads 20 culms^{-1}			0.60*	0.71*	-0.66*	0.20	0.82*	0.50	0.86*
Seedheads plant^{-1}				0.92*	-0.24	0.06	0.74*	0.91*	0.66*
Seed yield plant^{-1}					-0.35	0.30	0.86*	0.82*	0.85*
Kernel index plant^{-1}§						-0.15	-0.45	-0.34	-0.62*
100 Caryopsis wt							0.15	0.06	0.48
Culm height								0.63*	0.77*

Little Bluestem	Culms plant^{-1}	Seedheads 30 culms^{-1}	Seedheads plant^{-1}	Seed yield plant^{-1}	Kernel index plant^{-1}	100 Caryopsis weight	Culm height	Crown diameter	Days to anthesis
Harvested biomass plant^{-1}†	0.69*	0.72*	0.96*	0.99*	0.02	0.12	0.85*	0.35	0.82*
Culms plant^{-1}‡		0.07	0.78*	0.70*	-0.49	-0.41	0.31	0.84*	0.27
Seedheads 30 culms^{-1}			0.67*	0.71*	0.42	0.51	0.82*	-0.22	0.82*
Seedheads plant^{-1}				0.96*	-0.09	0.02	0.73*	0.50	0.69*
Seed yield plant^{-1}					0.03	0.10	0.81*	0.37	0.80*
Kernel index plant^{-1}§						0.76*	0.33	-0.48	0.15
100 Caryopsis wt							0.44	-0.38	0.31
Culm height								-0.04	0.83*

* Correlation significant at the 0.05 probability level.

† Harvested biomass was total plant dry wt. obtained from harvesting at an 18-cm stubble height.

‡ Culms plant^{-1} were all reproductive culms obtained from the harvested plant.

§ Kernel Index = caryopsis yield (g)/unthreshed seedhead weight (g) x 100.

Table 5. Mantel test of matrix correspondence between simple correlation matrixes of blue grama and little bluestem Ecovar and Cultivar selections and the original unselected population for 3 environment-yrs†.

Population‡	Blue grama				Little bluestem			
	Wpg 1994	Wpg 1995	Car 1995	$\bar{x}$	Wpg 1994	Wpg 1995	Car 1995	$\bar{x}$
Cultivar-CI	0.55	0.39	0.82*	0.59	0.69	0.86*	0.81*	0.79
Cultivar-KI	0.67	0.72	0.97**	0.79	0.68	0.82*	0.76	0.75
Cultivar-SY	0.37	0.28	0.60	0.42	0.46	0.66	0.58	0.57
Cultivar-HI	0.57	0.58	0.79	0.65	0.78	0.84*	0.89*	0.84*
Ecovar-1-CI	0.82*	0.74	0.90**	0.82*	0.94**	0.97**	0.98**	0.97**
Ecovar-2-CI	0.80*	0.71	0.89*	0.80*	0.89*	0.99**	0.96**	0.95**
Ecovar-3-CI	0.80*	0.70	0.91**	0.80*	0.90**	0.94**	0.94**	0.92**
Ecovar-1-KI	0.91**	0.95**	0.87*	0.91**	0.92**	0.99**	0.99**	0.97**
Ecovar-2-KI	0.83*	0.89*	0.84*	0.85*	0.88*	0.97**	0.93**	0.93**
Ecovar-3-KI	0.85*	0.90**	0.86*	0.87*	0.86*	0.96**	0.94**	0.92**
Ecovar-1-SY	0.86*	0.80*	0.93**	0.86*	0.80*	0.94**	0.97**	0.91**
Ecovar-2-SY	0.84*	0.69	0.84*	0.79	0.69	0.89*	0.90**	0.83*
Ecovar-3-SY	0.77	0.82*	0.76	0.79	0.62	0.86*	0.92**	0.80*
Ecovar-1-HI	0.91*	0.93**	0.87*	0.91**	0.85*	0.96**	0.93**	0.91**
Ecovar-2-HI	0.86*	0.91**	0.87*	0.88*	0.91**	0.96**	0.96**	0.95**
Ecovar-3-HI	0.83*	0.88*	0.88*	0.86*	0.91**	0.95**	0.97**	0.95**

† Degree of matrix similarity is * = 'good' for $0.8 \; r < 0.9$ and ** = 'very good' for $r \geq 0.9$ (Rohlf and Fisher, 1968).

‡ CI = Favored Index; KI = Kernel Index; SY = Seed Yield; HI = Harvest Index; Ecovar pooling methods 1 = best plant per ecotype per experimental block; 2 = top ranking 9 and 7 plants per ecotype for blue grama and little bluestem, respectively; 3 = top ranking 99 and 98 plants for blue grama and little bluestem, respectively, regardless of plant collection.

Table 6. Blue grama and little bluestem Ecovar and Cultivar group means compared to the Original unselected population, averaged over 3 environment-years.

Population	Blue grama mean†									
	Harvested biomass	Culms plant^{-1}	Culm height	Culm spread	Seedheads 20 culms^{-1}	Seed yield 20 culms^{-1}	Spikelet index	Kernel index	100 Caryopsis weight	Days to anthesis from 1 May
	g	no.	cm	cm	no.	g	%		mg	no.
Original	44.32	188	54.0	66.8	43	1.82	78.8	25.5	46.2	77.1
Cultivar-SI	53.84*	184	57.5**	72.8	46	2.72***	80.5	32.2***	51.9***	76.6
Cultivar-KI	39.38	190	54.8	66.8	43	1.77	83.2***	38.2***	49.6**	74.0**
Cultivar-SY	56.62**	173	58.4**	72.7	46*	2.95***	77.9	25.4	51.0***	78.1
Cultivar-HI	20.14***	127***	48.8***	55.9***	45	2.12**	79.0	28.0*	48.7	75.1
Eco-1-SI	44.23	181	55.6*	70.1	45*	2.11***	80.3**	30.0***	49.1***	76.9
Eco-2-SI	51.64**	194	57.1***	70.8*	45*	2.23***	80.5***	31.6***	50.3***	76.9
Eco-3-SI	56.63***	200	58.2***	73.4***	45*	2.31***	80.3**	31.1***	50.6***	77.4
Eco-1-KI	43.48	189	54.9	69.2	44	1.89	81.4***	31.5***	48.7***	76.0
Eco-2-KI	47.97	197	55.6*	69.1	43	1.91	81.4***	33.6***	48.9***	76.2
Eco-3-KI	43.61	193	54.2	66.8	43	1.81	81.6***	34.3***	48.9***	75.3**
Eco-1-SY	48.07	181	55.2	71.3**	45**	2.24	78.5	25.5	48.8***	78.3
Eco-2-SY	53.03***	190	56.3**	70.5*	46***	2.37***	78.6	26.8*	49.7***	77.9
Eco-3-SY	57.34***	190	57.6***	73.5***	46***	2.47***	78.8	25.8	50.1***	78.4*
Eco-1-HI	34.40***	168*	52.7	63.2*	44	2.03***	80.0*	27.1**	46.8	76.6***
Eco-2-HI	29.32***	154***	51.6***	60.3***	44	1.98**	79.8*	26.8*	46.3	76.1
Eco-3-HI	23.22***	141***	49.1***	57.0***	43	1.94	80.3**	27.6***	46.2	75.1***

Table 6. Continued.

Population	Little bluestem mean†									
	Harvested biomass	Culms plant^{-1}	Culm height	Culm spread	Seedheads 30 culms^{-1}	Seed yield 30 culms^{-1}	30 Culm weight	Kernel index	100 Caryopsis weight	Days to anthesis from 1 May
	— g —	— no. —	— cm —	— cm —	— no. —	— g —	— g —	— % —	— mg —	— no. —
Original	68.81	211	70.0	79.8	228	2.83	10.6	18.1	97.9	73.5
Cultivar-SI	49.81**	176*	63.4**	70.7	215	3.04	8.4**	27.3***	109.9	73.0
Cultivar-KI	63.11	217	70.0	85.6	200*	2.54	9.4	30.5***	109.3	72.1
Cultivar-SY	111.58***	195	75.6*	94.7**	386***	5.34***	19.6***	17.3	109.7	77.8***
Cultivar-HI	43.64***	148***	63.2**	65.0**	214	3.11	7.6***	20.6*	97.6	73.2
Eco-1-SI	75.68	205	71.8	83.0	252***	3.41***	12.1***	22.0***	102.3	73.8
Eco-2-SI	77.61	207	71.1	84.1	259***	3.55***	12.7**	22.8***	102.4	73.5
Eco-3-SI	83.93***	197	74.4***	83.8	287***	4.01***	12.3**	22.6***	111.3***	75.5***
Eco-1-KI	72.88	225	70.9	83.4	222	2.85	10.6	23.4***	110.3***	73.5
Eco-2-KI	72.81	224	71.1	82.9	220	2.87	10.8	25.0***	102.5	73.5
Eco-3-KI	63.63	201	69.9	79.8	214	2.77	10.2	26.3***	107.3*	72.4
Eco-1-SY	79.31**	198	71.9	84.4	275***	3.69***	13.3***	18.5	101.9	73.8
Eco-2-SY	82.81***	201	71.6	83.0	287***	3.91***	14.1***	18.3	98.8	73.7
Eco-3-SY	94.28***	200	75.7***	86.0*	314***	4.34***	15.7***	18.5	106.8*	76.1***
Eco-1-HI	59.58*	201	66.1**	75.4	221	2.95	9.0	18.8	96.1	72.7
Eco-2-HI	59.98*	195	64.6***	74.2	228	3.09	9.0***	18.6	98.0	73.4
Eco-3-HI	55.40***	182**	64.3***	72.3**	225	3.07	8.9**	19.0	96.0	73.2

*, **, *** Significantly different from the Original population at the 0.05, 0.01, and 0.001 probability levels, respectively.

† Means were calculated on per plant basis (population sizes were: Cultivar = 25, Ecovar = 99 and the Original = 470 for blue grama and 488 for little bluestem).

MANUSCRIPT 3

Genetic variation and Effect of Selection in Blue Grama measured with RAPDs

ABSTRACT

Little is known about the genetic diversity of natural blue grama (*Bouteloua gracilis* [H.B.K.] Lag. ex. Steud.) populations at the DNA level, nor the effects caused by selection in this important native grass species. The objective of this research was to characterize genetic diversity among natural blue grama populations and to determine genetic shifts caused by selection using the RAPD markers. The plant material used for this research was collected from 11 sites in southern Manitoba, Canada and comprised of 495 plants, which represented the Original population. The Ecovar population was selected from the Original with lower selection intensity (20%) and equal representation from each collection, and the Cultivar population was selected from the Original with simple mass selection and higher selection intensity (5%). Twelve 10-mer primers were identified which yielded 60 polymorphic bands. Analysis of Molecular Variance determined genetic variation between- and within- populations, and relationships between populations were assessed by UPGMA cluster analysis. Calculated genetic distances and Canonical Variates Analysis determined genetic shifts caused by selection. High and uniform levels of genetic diversity were found in each plant collection, with 97.8% of the variation found within the collections, but the collections could be distinguished. Comparison of the Ecovar and Cultivar with the Original indicated similar levels of genetic variation. However, the Cultivar population was three times more distant from the

Original than the Ecovar, and showed more shifts in frequency of RAPD bands. These results indicate that high levels of genetic diversity are present in natural blue grama populations that can be utilized for breeding. Additionally, genetic diversity can be maintained following selection, but genetic shifts may occur in certain alleles.

INTRODUCTION

Native plant species have gained renewed interest in research and plant breeding programs in recent years. There is particular interest in native grass species for use in a number of areas including soil stabilization (Cooper, 1957), mine site reclamation (Gaffney and Dickerson, 1987), wildlife habitat restoration (Duebbert et al., 1981) and improvement for use as high-quality forage (Vogel and Pedersen, 1993). One of the greatest limitations to expanded native grass utilization is lack of commercial seed quantities. In the USA and Canada, plant breeders have addressed this limitation with the development of cultivars for improved seed production (Crowle, 1970; Smoliak and Johnson, 1980; Smoliak and Johnson, 1983). However, maintaining genetic diversity and ecological integrity also needs to be considered (Knapp and Rice, 1996). Blue grama (*Bouteloua gracilis* H.B.K. Lag. ex Steud.) is a species that has particular value for reclamation and ground cover because it naturally occurs over a wide range in North America (Hitchcock, 1950). Blue grama is highly outcrossing (Riegel, 1941; Miller, 1967) and is ecologically suited to marginal environments with inherent soil moisture and fertility limitations.

Maintaining genetic diversity in populations improved by selection has been addressed with the development of 'ecovars' (i.e. ecological varieties). Ecovar selection protocols have been evaluated for blue grama and little bluestem (*Schizachyrium scoparium* [Michx.] Nash) to identify the most efficient method to improve seed production, pool genetic diversity, and to minimize genetic shifts due to correlated responses during selection (Smith and Phan, 1999). Although genetic advance in selected traits has been predicted, ecovar development is a novel approach that requires further evaluation.

The use of RAPDs (Random Amplified Polymorphic DNA) and application of the Analysis of Molecular Variance (AMOVA) developed by Excoffier et al. (1992) have been well documented for their utility in characterization of genetic diversity in grass populations (Huff et al., 1993; Peakall et al., 1995; Huff, 1997; Huff et al., 1999; Gunter et al., 1996; Ferdinandez, 1999) and other species (Nesbitt et al., 1995; Crochemore et al., 1996). With RAPDs, the polymerase chain reaction amplifies short genomic regions using primers of arbitrary sequence (Williams et al., 1990; Welsh and McClelland, 1990). This provides a random sample of total genetic variation at the DNA level not limited to only expressed genes, and therefore it is especially practical for surveying genetic diversity. The ability to generate molecular genetic profiles of individuals that are free of confounding environmental effects is an important advantage of RAPDs compared to using only morphological measurements or even isozyme analysis (Parker et al., 1998) for characterizing genetic diversity.

Prior studies on genetic shifts attributed to selection have been documented for crops such as oat (Rodgers et al., 1983), maize (Coors and Mardones, 1989; Helms et al.,

1989; Stojsin and Kannenberg, 1994), and bahiagrass (Pedreira and Brown, 1996; Werner and Burton, 1991), but these involved analysis of morphometric and/or isozyme data. For our purposes, utilization of RAPDs to characterize genetic diversity and associated genetic shifts from selection in blue grama will be a first for this species and will provide valuable information which can serve to develop a model system for future development, breeding and utilization of under-exploited native grass species.

The objective of this research was to assess the level of genetic diversity among blue grama populations obtained from natural collections in southern Manitoba, Canada and to determine shifts in genetic diversity caused by two methods of selection using RAPD markers.

MATERIALS AND METHODS

Blue Grama Populations Assayed

Three blue grama populations were used in the present study. The first population, 'Original', was comprised of 495 plants obtained from 11 collection sites in southern Manitoba (Figure 1A p.46) and the second and third were improved populations, 'Ecovar' and 'Cultivar' which were derived from the Original following one cycle of selection (Smith and Phan, 1999). The Original population was sampled by collecting vegetative tillers from the original plants grown in a spaced-plant nursery. These tillers were propagated in the greenhouse in 1:2:1 soil, sand, and peat mixture and the pots grouped according to collection site (i.e. location from which they were obtained). The Ecovar was selected for seed yield per plant in combination with a measure of caryopsis

yield, and was comprised of 99 plants equally representing all 11 plant collections (See Manuscript 2). The Cultivar was selected for the same traits but was comprised of 25 plants and did not have equal representation from the 11 plant collections. Seed was harvested from the 99 Ecovar and 25 Cultivar parental plant selections following open-pollination in isolated polycross nurseries. A random sample of seed was germinated from the maternal Ecovar and Cultivar plants on filter paper in petri dishes moistened with 0.5% KCl solution and put under a 16 hr/8 hr day-night photoperiod at 25°C. For the RAPD assay, 96 seedlings each were randomly selected among the 99 Ecovar and 25 Cultivar petri dishes. The seedlings were transplanted into individual pots and kept in the greenhouse. Young leaf tissue was clipped from the greenhouse-propagated plants to fill the volume of a 2 ml centrifuge tube, kept on ice and immediately lyophilized. The best tissue samples were obtained from fresh re-growth 3 days following clipping.

DNA Extraction and Quantification

The following protocol was modified from one used for *Brassica napus* at the Agriculture and Agri-Food Canada Saskatoon Research Center. DNA was extracted from approximately 50 mg of lyophilized leaf tissue from each individual plant. The dry leaves were clipped as finely as possible into 2 ml centrifuge tubes, 4-5 glass beads were added, and the tubes were mechanically shaken for 30 minutes to produce a fine powder. Six hundred µl of hot (95°C) extraction buffer [2 M Tris-HCl (pH 8.0), 3 M KCl, 0.5 M EDTA (pH 8.0), 14 % w/v SDS] was added to each tube of powdered tissue. The tubes were heated at 95°C for 15 minutes with vortexing every 2 minutes, followed by placement in ice for 2 minutes. After cooling, the tubes were centrifuged at 13 000 rpm

at 4°C for 12 minutes, 400 µl of clean supernatant was extracted from each and pipetted into fresh tubes. Four µl of RNAse [at 10 mg/ml] was added to each tube, and incubated at 37°C for 20 minutes. The DNA was precipitated with 800 µl cold isopropanol and mixed by inverting the tubes. The DNA precipitate was pelleted by centrifuging at 13000 rpm for 5 minutes and the supernatant was removed and the tubes allowed to air dry. The DNA was re-suspended with 400 µl of 70% EtOH and pelleted by centrifuging again for 5 minutes and the supernatant removed. Subsequent DNA washing was performed by re-dissolving the pellet in 150 µl water at 50°C, centrifuging for 10 minutes and transfering the supernatant into fresh tubes for re-precipitation with 30µl NaOAc + 600µl 95% EtOH. Finally, the clean DNA was pelleted by centrifuging for 5 minutes and dissolved in 100 µl water at 50°C for 30 minutes or until DNA was completely dissolved. The DNA was quantified by fluorimetry using Labsystems Flouroskan II with Hoechst 33258 staining, then diluted to equal concentrations of 2.5 ng/µl and stored at 4°C, and the excess DNA was kept frozen at –20°C.

The quality of the genomic DNA was assessed by gel electrophoresis using a 0.8% agarose gel [80 ml water, 20 ml TBE (5x), 800 mg agarose]. A 10 µl sample was taken from 10 DNA extracts and electrophoresced at 20 V for 12 hr. Although minor smearing occurred along the path of electrophoretic movement caused by the presence of low molecular weight fragments, the majority of the DNA extracted was of high molecular weight and therefore usable for PCR.

PCR Reactions

Each PCR reaction utilized 10-ng DNA, 1-U of Taq DNA polymerase (BRL, Mississauga, Canada), 2.5-mM $MgCl_2$, 200-uM of each dNTP and 0.2-μM primer. The DNA amplification protocol was 95 ^{0} C – 1.5 min (1 cycle); 95 ^{0} C-0:20 sec; 36^0 C –60 sec; ramp 1^0 C/sec to 72^0 C –60 sec (35 cycles); 72^0 C- 7 min (1 cycle). All PCR products were separated by electrophoresis in 2% (w/v) agarose gels in 1X TAE ran at 100 V for 3 hr. Gels were then stained with 70 μl ethidium bromide diluted in 700 ml water and photographed on a digital gel documentation system. A DNA mass ladder (GIBCO BRL) was used as the standard, and molecular weights of electrophoresced fragments was determined using Stratagene's EagleSight–EagleEye gel analysis software.

Primer screening

A set of 8 genotypes, randomly chosen from 8 of the Original collections, were used to screen for informative primers. Twelve primers that produced robust electrophoretic profiles with scorable bands were selected from the UBC series of 10-mer primers of random sequence (UBC series, University of British Columbia). Scorable bands were robust bands that exhibited polymorphism as present in one individual but absent in another.

RAPD profile reproducibility

The RAPD profiles were tested for reproducibility using 12 individuals from the Original population. A separate DNA extraction was performed on each individual along

with PCR reactions using 6 of the informative primers. Agarose gel electrophoresis was conducted as previously described and the individual profiles were compared with those from the previous DNA extraction and electrophoresis. The newly extracted DNA was also subjected to PCR simultaneously with the corresponding samples from the previous extraction and electrophoresced side by side. The profiles from the two separate extractions were identical for all individuals and primers used in the repeatability test.

Data analysis

Polymorphic RAPD bands were scored as 1 (band present) or 0 (band absent) for each individual and stored as a binary matrix. Genetic similarity was calculated as a simple matching coefficient (SMC) (Sokal and Michener, 1958), as used by Thompson et al. (1998), using NT-SYS-pc v.2.02 (Rohlf, 1997) where similarity between individual pairs *i* and *j* was:

$$S_{ij} = (a + d)/(a + b + c + d) \qquad [1]$$

Where a = number of 1-1 matches, b = number of 1-0 matches, c = number of 0-1 matches, and d = number of 0-0 matches. The similarity matrixes were then reformatted into proper text files using a computer program written by Mr. Dinen Subramaniam (available through the author), and imported into MS-Excel™ spreadsheet for conversion to dissimilarities ($1 - S_{ij}$) and then into Euclidean distances $(1 - S_{ij})^{0.5}$. The distance matrixes were used as input files for the Analysis of Molecular Variance (AMOVA) (Excoffier et al., 1992). A balanced data-set of 56 scored bands was produced by

removing missing scores which resulted in population sizes of 91, 92, and 94 for the Original, Ecovar and Cultivar, respectively. The AMOVA was performed on the 11 blue grama collections comprising the 'Original' using a balanced data set of 8 individuals and 56 scored bands per collection.

Euclidean distance matrixes were used as input files for pair-wise comparisons using the AMOVA from which total RAPD variation was partitioned into between- and within- population components, and statistical significance was obtained by permutational methods (Excoffier et al., 1992). The number of permutations to test for significance was set at 500, which was found to be no different from setting it at 1000. The proportion of variation partitioned between populations (the phi-statistic, Φ_{st}) from AMOVA was used as a measure of inter-population distance following Huff et al. (1993) and Huff (1997), and these were used to perform cluster analysis using the Unweighted Pair-Group Method of arithmetic Averages (UPGMA) on all 11 plant collections and on the Original, Ecovar and Cultivar populations. All dendrograms were constructed using NTSYS-pc.

RAPD variation in an individual assayed was calculated according to Nienhuis et al. (1994) as:

$$\text{Total marker variance} = \Sigma(n \times p \times q)/(n-1) \qquad [2]$$

Where n = number of individuals in a population, and p and q refer to the frequency of the presence and absence of a band, respectively. The mean RAPD variation of individuals in each population was then compared using the LSD procedure in SAS (SAS Institute, 1988). The Ecovar and Cultivar population sizes in the balanced data set were further reduced to 80 and 79, respectively (to accommodate the software limit of 250

individuals), for canonical variates analysis (CVA) performed with the SYN-TAX v.5 computer software, and a scattergram of individuals in the three populations plotted. The CVA generated univariate F-tests for each RAPD band which enabled identification of scored bands that were important in discriminating between the populations. Because of the large number of degrees of freedom for each RAPD band, many F-ratios were statistically significant, therefore only bands with F-ratios ≥ 10 were isolated.

RESULTS

RAPD Polymorphisms

A total of 89 primers were initially screened, and most of them generated robust RAPD profiles with many polymorphic bands. Many primers generated a large number of bands of varying thickness, molecular weight, and intensity, and it was impractical to effectively score all amplified products reliably. Therefore, only robust polymorphic bands from a subset of 12 primers were used for the population assays. An example of a gel is shown in Figure 1. A total of 60 polymorphic bands were scored from the 12 primers, with the number of bands scored per primer ranging from 3 to 10 (data not shown). The scored bands were comprised of fragments of molecular weights ranging from 330-1500 base-pairs, and differences in band thickness and electrophoretic intensity were not considered in the data analysis.

Genetic diversity of plant collections

The RAPD profiles generally showed uniform levels of genetic variation within each plant collection (Table 1). Genetic variation within plant collections ranged from 15.54 to 16.84. Mean RAPD variation per individual was similar among all 11 blue grama collections and ranged from 0.229 to 0.249. Furthermore, the mean genetic distance between individuals was similar across all plant collections with a range from 0.577 to 0.615. The maximum genetic distances between individuals were also similar among collections. However, a larger range from 0.130 to 0.500 was observed for minimum genetic distance between individuals within collections. All plant collections were found to have high within-collection genetic variation which accounted for 97.8% of the total, and the proportion among populations accounted for only 2.2%. Despite the low inter-collection variation, there were differences among the different blue grama collections at the 0.01 probability level as determined by the Analysis of Molecular Variance (AMOVA). The AMOVA sums of squares were found to be affected by differences in group sizes of the collections being analysed and therefore a balanced data set was used. However, AMOVA results were similar from analysis both with and without missing data.

A dendrogram constructed using inter-collection distances (phi-statistic, Φ_{st}) from AMOVA is shown in Figure 2. Four main clusters could be distinguished from the dendrogram; one cluster comprised of CHC and CVY, the next cluster comprised of GDL1 and GDL2 to which the addition of DGL formed a main cluster, followed by clustering of KGR, LDR, RSL, and SDY, and then a final cluster with OAK and S10.

Genetic relationship between Original, Ecovar, and Cultivar

Pair-wise AMOVA comparisons of the Original population with the Ecovar and Cultivar indicated population differences as shown in Table 2. The Original population contained the highest genetic variation as expected, and the Ecovar and Cultivar had lower and almost identical levels of variation. However, a comparison of the mean genetic variation per individual showed that the Ecovar mean was intermediate between the higher value of the Original and the lower value of the Cultivar and did not differ from them, while the Cultivar and the Original were different.

The univariate F-tests on individual RAPD bands from canonical variates analysis (CVA) indicated that a handful of RAPD bands had very high discriminating power between the Original, Ecovar, and Cultivar populations (Table 3). A comparison of the Original and the Ecovar revealed only 2 highly discriminating bands from 2 primers whereas the Original versus the Cultivar revealed 8 highly discriminating bands from 7 primers. The main comparison of all three populations could be discriminated with a core set of 4 bands which were essentially a subset derived from the individual pair-wise comparisons of the Original with the Ecovar and with the Cultivar. These informative bands were found to occur in different frequencies among the three populations. The only two bands delineating the Original and the Ecovar were found to be in much lower frequencies in the Cultivar, with decreases of 84% and 57% in the frequency of these bands. In the comparison between the Original and the Cultivar, frequencies of 5 out of

the 8 bands were found to be lower in the Cultivar by as much as 62% (primer 346, band e), and the remaining 3 were higher by as much as 71% (primer 564, band b).

The genetic relationships between the Original and the Ecovar and Cultivar populations were effectively depicted as a scattergram of all individuals (Figure 3) and as a dendrogram of the three populations (Figure 4). Despite the high levels of variation present in the data, over 99% of the variation between populations could be accounted for by the first two canonical variates. The scattergram was therefore highly effective in showing the delineation between the three populations. There was very minor overlap among the populations as the 95% confidence circles for each population were well separated from one another. The dendrogram better illustrates the genetic relationships between the three populations, and summarizes them as a close clustering of the Ecovar with the Original while the Cultivar remained separate. Interpopulation distances (Φ_{st}) obtained from the AMOVA indicated that although the Ecovar and Cultivar both differed from the Original, the Cultivar was found to have shifted in distance three further than the Ecovar (Φ_{st} Original – Ecovar = 0.006; Φ_{st} Original – Cultivar = 0.019, Figure 4).

DISCUSSION

RAPD variation of plant collections

Polymorphic RAPD bands were easily obtained from an initial primer screening, which showed that the RAPD technique is a highly effective tool in characterizing genetic diversity in blue grama. The easily detectable variation in RAPD profiles indicates the high level of genetic diversity in this species as expected by its cross-

pollinating breeding system (Miller, 1967; Riegel, 1941) and high ploidy levels (McGinnies et al., 1988). As with other RAPD marker results from cross-pollinating species, no two individuals were found to be identical. The only reported exception occurred with smooth bromegrass (*Bromus inermis*) (Ferdinandez, 1999). Because there were no unique identifiable bands associated with any specific population, genetic analysis of the populations utilized differences in band frequencies.

The plant collections assayed for RAPD genetic diversity represented naturally occurring stands of blue grama in southern Manitoba that were at least 30 km apart from each other (Phan and Smith, 2000). RAPD analysis revealed high levels of genetic diversity between and within the plant collections (Table 1). The observed clustering of the blue grama plant collections (Figure 2) appeared to be independent of geographic origin, which suggests the importance of habitat differences in shaping the genetic characteristics of a population. A RAPD analysis of genetic variation in little bluestem led Huff et al. (1999) to suggest that site differences in fertility and ecological history can promote genetic differentiation between populations.

In the present study, the pattern of dendrogram clustering did not follow the expectation that plant collections closest to one another geographically were expected to form a cluster. For instance, KGR and LDR (two of the most southern collections) were conspicuously closely linked to each other, but also linked to the northern collection, RSL. Phan and Smith (2000) reported that for seed yield traits, LDR and RSL represented the high and low extremes among the plant collections for most measurements. The RAPD data did not show the observed disparity between LDR and RSL that was observed for morphological measurements. This was similar to Nesbitt et

al.'s (1995) finding with eucalyptus, in which marked morphological differences between populations did not necessarily translate to marked differences in RAPD phenotype. The two collections that were closest geographically, GDL1 and GDL2 (<1000 m distance, 152 m elevation), clustered closely as expected.

Most of the RAPD variation in the plant collections (96.6%) was made up of within-collection genetic diversity, and all 11 plant collections exhibited very similar levels of genetic variation. Our findings agree with the general trend for other RAPD studies of outcrossing grass species, including smooth and meadow bromegrass (Ferdinandez, 1999), buffalograss (Peakall et al., 1995; Huff et al., 1993), perennial ryegrass (Huff, 1997), and little bluestem (Huff, 1999), in which the majority of genetic variation was attributed to within-collection variation. In contrast, prior evaluation based on morphology data of the blue grama plant collections used in this study showed that collections were clearly different, with genetic variation between collections accounting for over 86% of the total observed phenotypic variation when the plant collections were grown in a common nursery (Phan and Smith, 1997). The fact that RAPD profiles are not limited to expressed genes may also be a limitation to characterizing genetic diversity, as it would tend to be biased towards higher genetic variation within individuals and populations, whereas morphological information pertains to 'real' genetic differences resulting from environmental selection on expressed genes. In spite of the low between-collection variation observed with the RAPD data, the differences between collections were confirmed with the Analysis of Molecular Variance (AMOVA; $P < 0.002$). Although sample size from each collection used in the analysis was small ($n = 8$), the ability to detect differences shows the distinctiveness between collections as reported

based on morphology data (Phan and Smith, 2000) (Figure 2). High levels of genetic variation exhibited between the plant collections demonstrate that naturally occurring blue grama populations from relatively small separation distances can provide a source of diverse germplasm for breeding work.

Mean values for genetic variation and genetic distance were similar between individuals within the collections (Table 1). Similar levels of genetic diversity among the blue grama collections suggests low levels of differential selection pressure due to habitat differences among the sampled sites. Another possible reason for the relatively uniform level of genetic variation among the collections may be the potential for gene flow between populations via seed and/or pollen. The maximum genetic distance between individuals within a collection ranged from 0.68 to 0.78, and the minimum distances ranged from 0.13 to 0.48 among all plant collections. Values for maximum genetic distance between individuals within a collection indicates the potential for wide crosses occurring within a population, and therefore the potential for enhanced genetic diversity in progeny populations (Kisha et al., 1997). The small range in maximum genetic distance values suggests that all the blue grama collections possessed the same potential for wide crosses, and hence another reason for observed similarity in levels of genetic variation. The indication that genetic diversity in each collection was similar while collections were also deemed different suggests that the types of selection pressures between collection sites may be different based on habitat differences, while outcrossing and perenniality of the species maintained the observed levels of genetic diversity. These habitat differences included soil texture and moisture availability, as noted by Phan and Smith (2000), which may have contributed to genetic differentiation between collections.

Blue grama is a warm-season grass species whose distribution reaches its northern fringe in southern Manitoba (Hitchcock, 1950). Therefore, the levels of genetic diversity observed in these plant collections may represent only a small sample of a potentially larger source of genetic diversity in more southern latitudes. The similar levels of genetic diversity among plant collections suggest the lack of population bottlenecks, which are often associated with founder effects during geographic expansion of a species (Knapp and Connors, 1999). However, the assayed collections remain limited in geographic scope, and more effective comparisons could be made by including collections from both the northern and southern latitudes. Population differences in morphology and flowering between widely separated collections of blue grama and little bluestem in the U.S.A. have been well documented (McMillan, 1956; McMillan, 1959a,b; Miller, 1967; Cornelius, 1947).

The differences observed in minimum genetic distances between individuals suggests that either the genetic structure of the collections were different or that efficiency in collecting plants from each site differed, perhaps due to habitat heterogeneity. Although plant collections may show similar mean levels of genetic diversity, the plant collections having higher minimum distances between individuals may reflect more efficient sampling and a likelihood of capturing greater genetic diversity since plants would be less closely related. Because blue grama colonizes exposed areas primarily by seed (Coffin and Lauenroth, 1992), a minimum distance between plants should be implemented when sampling plants from natural populations so that closely related individuals are not over-represented. This is especially important in

Ecovar breeding where within-collection diversity may contribute to the maintenance of genetic diversity.

Genetic shifts caused by selection

Ecovar development is based on the premise that improvements to a germplasm can be made by selection while minimizing shifts in genetic diversity. In the context of prairie revegetation and wildlife habitat restoration, a seed source with a high degree of genetic diversity may be important for sustainability of newly established stands (Knapp and Rice, 1996). The blue grama Ecovar used in this study was developed based on the premise that high levels of genetic diversity are important. The purpose of the Cultivar population used in this research was to assess the effectiveness of the Ecovar selection method and to determine potential shifts in genetic diversity between these two selection intensities.

Ecovar and Cultivar selection produced populations that were different from the Original (Table 2). As expected, the Original maintained a higher level of genetic diversity than the Ecovar and Cultivar populations. However, there did not appear to be any difference in the genetic variation between the Ecovar and Cultivar. The Ecovar and Cultivar populations assayed in this study were derived from only one selection cycle using selection intensities of 20% and 5% for the Ecovar and Cultivar, respectively. Also, the proportions and amounts of genetic contribution to the Ecovar and Cultivar gene pools were different, with the Cultivar containing lower and unequal representation from all the plant collections. It is possible that the difference in selection intensities was negligible for producing an observable difference between the two populations. In

addition, the highly outcrossing nature of blue grama, and the enhanced genetic diversity promoted by it, may have effectively negated the difference in selection intensity between the two selection methods.

In contrast, the minimum genetic distance between individuals within a population was higher in the Ecovar than the Cultivar. This was possibly the effect of including selected individuals from a wider genetic background, which increases the potential for wider crosses to occur. Johnson (1998) demonstrated with annual ryegrass that a more balanced representation of genetic backgrounds from the parental generation was most effective in maintaining the genetic intregrity of a regenerated population. This suggests that the balanced representation of all 11 plant collections in the Ecovar would maintain more genetic diversity from the Original population than the Cultivar. Another observed difference between the Ecovar and Cultivar was the three-fold magnitude shift in genetic distance of the Cultivar from the Original compared to the Ecovar (Figure 4), which suggested that the Ecovar pooling strategy was effective in retaining genetic similarity to the Original.

Despite the negligible difference in genetic variation within the Ecovar and Cultivar, genetic shifts from the Original were indeed observed as changes in RAPD band frequencies (Table 3). It was found that 4 RAPD bands from 4 different primers could distinguish between the Original, Ecovar, and Cultivar populations, based on differences in the frequencies of these bands among the assayed populations. It is known that mass selection shifts population genetic structure towards higher frequencies of desirable genes associated with the desired trait(s), with the consequence of a reduction in genetic variation. In this study, only two major RAPD bands observed in the Ecovar showed a

large change in frequency from the Original, both being downward shifts. In comparison, eight RAPD bands in the Cultivar were found to have shifted in frequency; five decreased in frequency, and three increased. The increase in frequency of the three bands seen in the Cultivar may reflect the selection of favorable alleles associated with seed production. The two bands showing downward shifts in the Ecovar were also included with those in the Cultivar, which suggested that the decline in frequency of these particular alleles was caused by excluding certain individuals from the Original. The fewer bands showing major shifts in frequency in the Ecovar compared to the many bands showing shifts in the Cultivar suggests that equal pooling of representatives from all plant collections in the Ecovar effectively retained more genetic diversity than the Cultivar.

CONCLUSIONS

These RAPD results showed high levels of genetic variation among and within 11 blue grama plant collections obtained in southern Manitoba, Canada. Genetic diversity of these populations did not appear to be related to geographic distance between sites of origin. The Ecovar and Cultivar populations derived from the cumulative 11 plant collections (i.e. the Original) showed similar levels of genetic variation. However, greater genetic shift was observed in the Cultivar for genetic distance from the Original and in number of RAPD bands showing large shifts in frequency. These results demonstrate that the Ecovar breeding method was effective in maintaining genetic diversity and genetic similarity to the Original.

Figure 1. Blue grama plant collection sites with respect to zones of growing degree days (GDD) in southern Manitoba, Canada. Calculation of GDD used a base temperature of 15°C and accumulated between 1 May and 31 August. Degrees longitude and latitude are indicated on the figure margins.

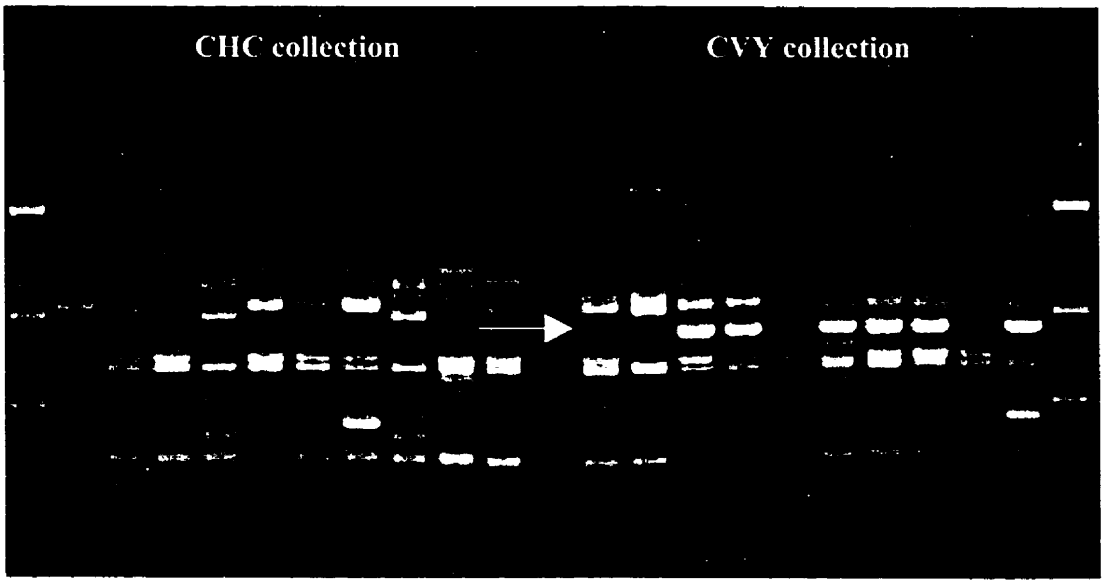


Figure 2. Agarose gel depicting RAPD differences between blue grama plant collections. The two outer-most lanes represent standard DNA mass ladders. The arrow indicates a band present in the CVY collection but absent in the CHC collection.

Table 1. RAPD genetic diversity of 'Original' blue grama plant collections obtained from southern Manitoba, Canada.

Blue grama plant collection†	Genetic variation within a population‡	Mean RAPD variation per individual¶	Genetic distance between individuals		
			Maximum	Minimum	Mean
CHC	16.84	0.249	0.719	0.316	0.609
CVY	16.69	0.239	0.742	0.447	0.602
DGL	16.33	0.237	0.719	0.192	0.615
GDL1	16.11	0.238	0.683	0.290	0.594
GDL2	16.56	0.236	0.696	0.290	0.602
KGR	15.54	0.229	0.696	0.130	0.577
LDR	15.54	0.232	0.707	0.500	0.584
OAK	15.67	0.243	0.742	0.259	0.578
RSL	15.84	0.243	0.701	0.447	0.602
SDY	16.84	0.240	0.753	0.484	0.623
S10	16.84	0.236	0.777	0.300	0.594

† Sample size of collections used in the analysis was balanced to 8 individuals per collection with 56 RAPD bands.

‡ Population RAPD variation was obtained from AMOVA sums of squares.

¶ RAPD variation per individual was calculated according to Nienhuis et al. (1994).

§ Genetic distances were Euclidean converted from simple matching coefficients.

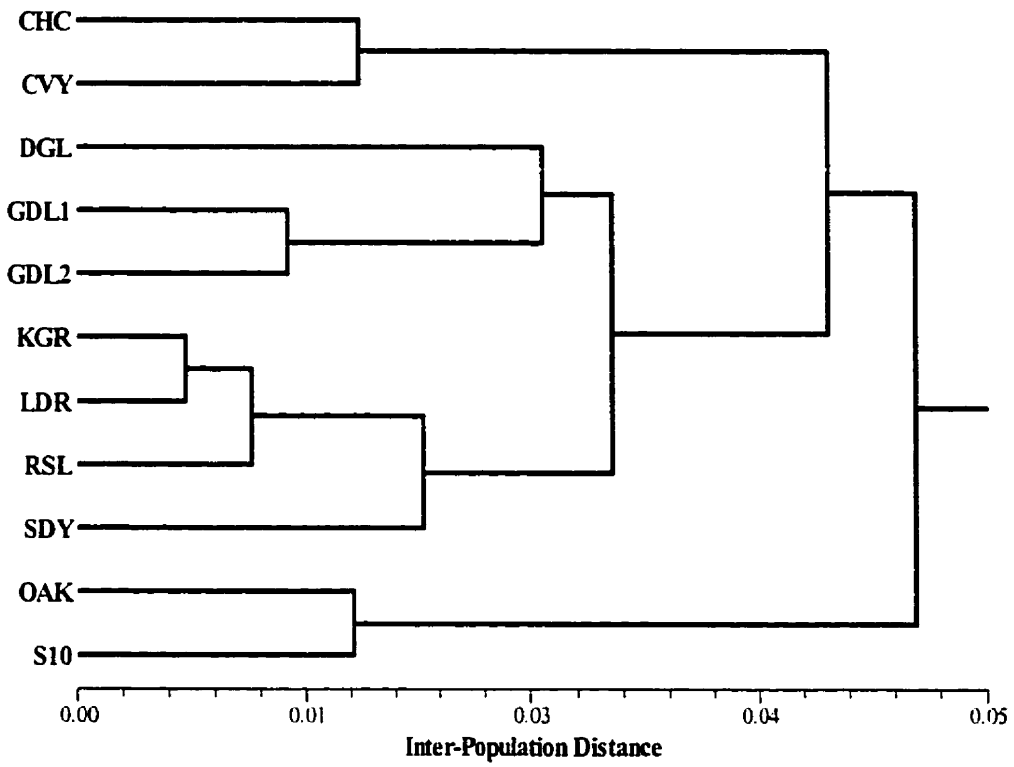


Figure 3. UPGMA Clustering of 11 blue grama plant collections from southern Manitoba, Canada based on 56 scored RAPD bands. (Site descriptions on page 48).

Table 2. RAPD variation and genetic distances of naturally occuring blue grama (Original) and their associated improved populations, selected for maintenance of genetic diversity (Ecovar) and selected without (Cultivar).

Population	Genetic variation within population†	Mean genetic variation per individual‡	Genetic distance between individuals within population		
			Maximum	Minimum	Mean
Original	247.73	0.238 a	0.789	0.130	0.621
Ecovar¶	221.10 *	0.237 ab	0.796	0.387	0.604
Cultivar§	220.42 *	0.233 b	0.806	0.316	0.601

† Values of within population sums of squares from AMOVA; * denotes population is different from the Original ($P < 0.002$).

‡ Mean genetic variation per individual was calculated according to Nienhuis et al. (1994).

¶ The Ecovar population was derived from 1 cycle of selection (selection intensity 20%) with equal representation of all plant collections comprising the Original.

§ The Cultivar population was derived from 1 cycle of mass selection (selection intensity 5%) from the Original.

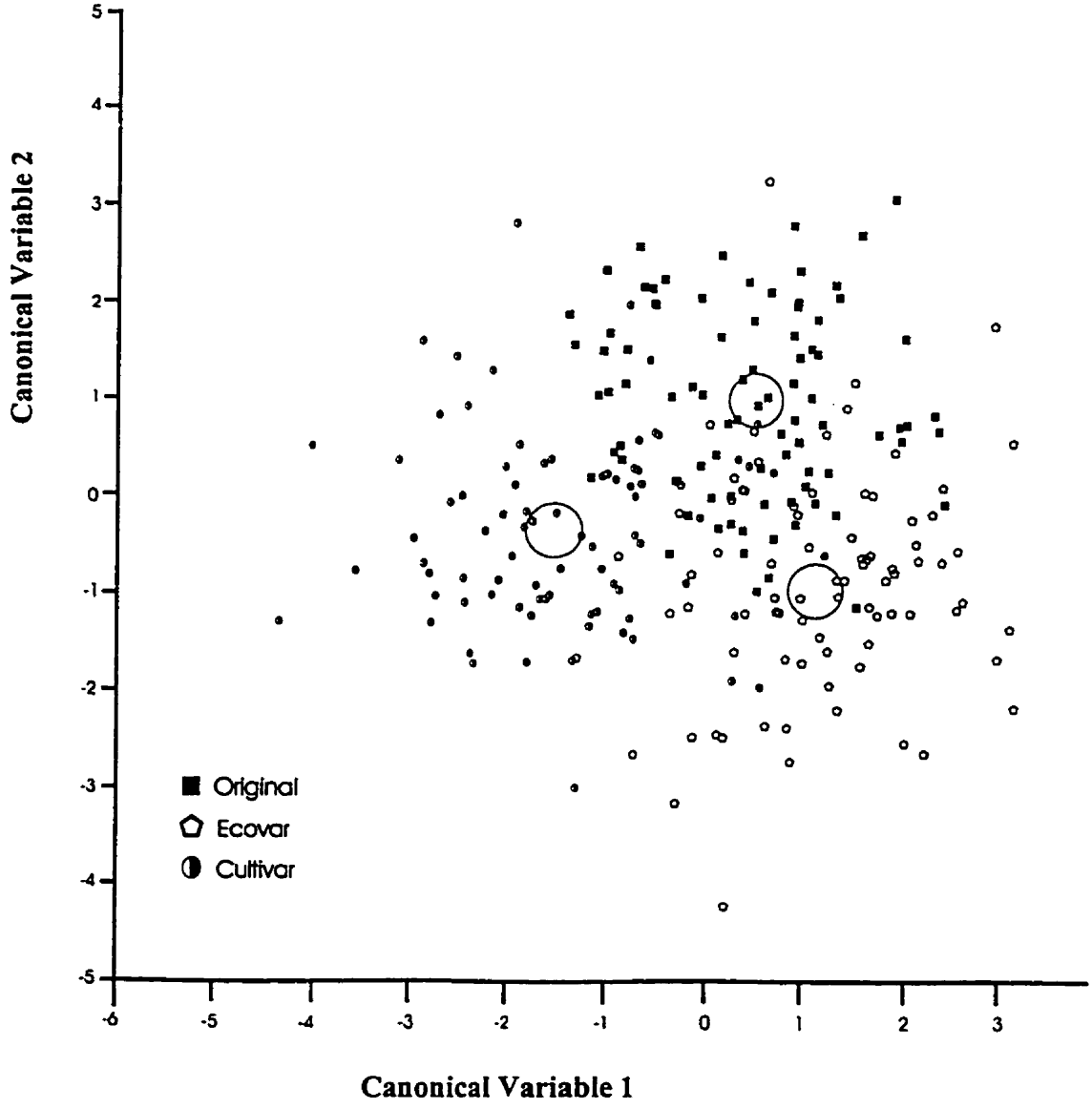


Figure 4. Scattergram of all individuals in the Original, Ecovar, and Cultivar blue grama populations with 95% confidence circles from canonical discriminant analysis based on 56 polymorphic RAPD bands.

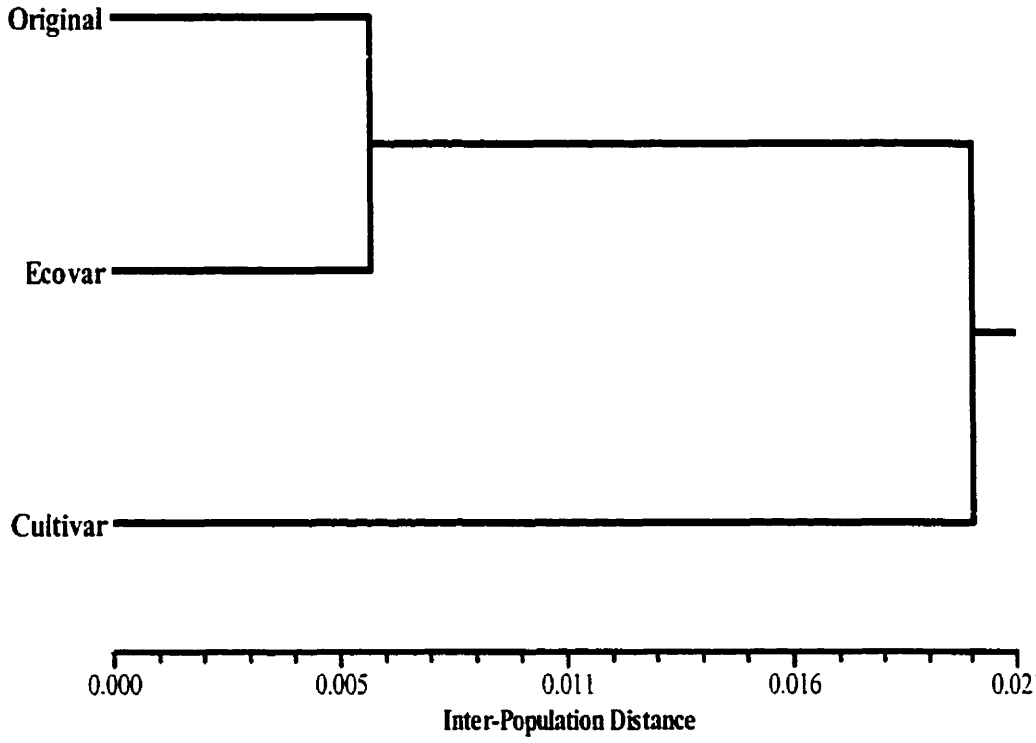


Figure 5. Clustering of Original, Ecovar, and Cultivar blue grama populations based on inter-population distances from AMOVA (Analysis of Molecular Variance) analysis with 56 polymorphic RAPD bands.

Table 3. Polymorphic RAPD bands having high discriminating power between blue grama Original, Ecovar and Cultivar populations.

Population discriminated	Primer number and scored band	RAPD band frequency			F-ratio†
		Original	Ecovar	Cultivar	
Original vs Ecovar	346, band c	0.45	0.07	0.17	18.57
	570, band f	0.46	0.20	0.22	12.33
Original vs Cultivar	336, band a	0.52	0.45	0.77	13.31
	336, band c	0.65	0.61	0.38	13.87
	346, band e	0.45	0.07	0.17	18.57
	353, band g	0.73	0.79	0.41	19.91
	388, band e	0.68	0.62	0.94	21.66
	389, band j	0.91	0.84	0.72	11.54
	564, band b	0.18	0.26	0.63	49.14
	570, band f	0.46	0.20	0.22	12.33
Ori vs. Eco vs. Cvr	346, band e	0.45	0.07	0.17	18.57
	353, band g	0.73	0.79	0.41	19.91
	388, band e	0.68	0.62	0.94	21.66
	564, band b	0.18	0.26	0.63	49.14

† F-ratios were obtained from canonical variates analysis.

GENERAL DISCUSSION

The present study is the first to characterize genetic variation in natural blue grama and little bluestem populations in Canada. It is also the first attempt to formulate a selection method for Ecovar development of native grasses, and to use the RAPD molecular technique to demonstrate genetic shifts that can occur as a consequence of selection in native grasses.

Based on morphological individual plant data, variation between plant collections of blue grama and little bluestem in Manitoba was found to be greater than variation within collections. The difference in agronomic performance between plant collections grown at a common nursery site gave a strong indication of ecotype differentiation between collections. The morphological differences observed between plant collections were therefore attributed to genetic differences.

High levels of variation between- and within- collections were found for almost all measured traits. Plant-harvested biomass showed variation that was subsequently reflected in variation in culm number, plant height, and seed yield. In general, the results from this study agreed with previous findings that northern populations of native grasses are made up of smaller, earlier-maturing plants compared to the larger and later-maturing plants from southern collections. Although the small plants of the northern collections also produced low seed yields, their reproductive effort appeared to emphasize successful caryopsis production, as indicated by their high Kernel Index. The variation found between and within plant collections showed that rapid Ecovar or cultivar development can be accomplished from selection both between and/or within collections.

The lack of interactions between plant collection and the evaluation environment further indicated genetic distinctiveness between plant collections. Although some plant collection (C) x environment (E) interactions were found, the variation due to plant collection was much greater than the interaction component, which showed that plant collections exhibited a degree of phenotypic plasticity but that the identity of each collection essentially remained distinguishable. The intrinsic genetic differences between plant collections were also demonstrated by evaluating over two years in a single environment. Although the perenniality of blue grama and little bluestem may buffer environmental differences between years, similar results to the C x E analysis provided further support for the relative distinctiveness of the plant collections.

An important component of this research was the development of an Ecovar selection protocol. Because very little was known about the species of interest, the fundamental information obtained from field measurements enabled the formulation of an approach to select for improved seed production while minimizing genetic shifts in blue grama and little bluestem. This approach was based on the need to balance between improved seed yield and maintaining genetic diversity. Selection criteria had direct bearing on the objective of the plant improvement program. However, selection criteria was the determining factor in producing genetic shifts due to correlated responses with indirectly selected traits. In order to minimize the loss of genetic diversity due to selection-induced genetic 'bottle-necking' from colloquial expression, the proposed selection method was coupled with appropriate pooling methods designed to make maximum use of the diverse genetic resources available.

This research sought to redress the current limitation of inadequate commercial seed quantities of native grasses in Canada. Therefore, the selection criteria evaluated for Ecovar development included seed yield per plant, Kernel Index, and Harvest Index. Kernel Index was the desired selection criterion because native grass species are planted on a pure live seed basis. However, the additional criterion of seed yield was included for blue grama, and Harvest Index was included for little bluestem to form the 'Combined Index' as a selection criterion for these species. The Combined Index was intended to improve total yield of viable seed because selection based on Kernel Index alone did not consider variation in total quantity of caryopses. A comparison of the selection criteria showed that Kernel Index was least likely to cause genetic shifts due to correlated responses with other traits. The Combined Index provided a compromise which allowed improvement of both Kernel Index and seed yield with minimal risks of genetic shifts in unselected traits.

To enhance genetic diversity and buffer potential genetic shifts from correlated responses, different pooling strategies were used to maximize genetic contribution from all plant collections to the Ecovar. The pooling strategy was designed to maximize genetic diversity in the Ecovar by providing equal representation of individuals from all original plant collections. In comparison, pooling using simple mass selection resulted in some plant collections being represented by only one individual. The unequal genetic pooling of the mass selection strategy increased the probability of a genetic shift occurring in the direction of the collections having the greatest representation of individuals. Such a genetic shift would be expected to occur during the initial intercrossing of parental selections, and would continue during stages of seed increase in

commercial fields. Interestingly, comparisons between the Ecovar selection methods indicated that selection criteria had a greater potential to cause genetic shifts than pooling method.

Equal pooling of plant collections presumably produced the least genetic shifts. However, successful blending of genetic diversity from individual selections ultimately depends on their ability to intercross. Although flowering date varied between plant collections, there was sufficient overlap between the flowering periods of individual plants to permit effective intercrossing. The range in flowering time suggested that intercrossing between individuals within the Ecovar population would occur in a continuous, sequential fashion from earlier to later flowering. Therefore, pollen exchange between individuals having widely separated flowering periods (i.e. extremely early and late) may not have occurred. Incomplete intercrossing between selections may be advantageous for maintaining genetic variation for flowering in an Ecovar population. Although the influence of flowering date was not considered in the present study, the selection method developed from this research provides a valuable foundation from which a more refined model can be derived, which may or may not consider variation in flowering.

The final evaluation of genetic diversity and determination of the impact of selection on genetic shifts was assessed using blue grama and the RAPD molecular technique. Progeny from intercrossed Ecovar and Cultivar selections were assayed along with a sample of individuals from each of the 11 original blue grama plant collections. Results from the RAPD analysis indicated that variation within the blue grama collections accounted for over 90% of the genetic diversity. These initial RAPD results

contradicted results from morphological measurements which indicated that a greater portion of genetic variation (>80%) was found between populations in comparison to within populations. However, both RAPD and morphological measurements showed that plant collections had similar levels of genetic variation.

Genetic assays at the DNA level with RAPDs have the advantage of being free of confounding environmental effects, but this molecular technique may be overly sensitive for use in the measurement/determination of genetic variation. The randomness of detecting nucleotide sequence variation and the practical limitation of sample size, in either number of individuals assayed and/or number of polymorphic markers, may not effectively detect biologically meaningful differences at the DNA level due to the prevalence of non-expressed DNA sequences otherwise known as 'junk-DNA'. The polyploid nature of blue grama may also contribute to high estimates of genetic variation. In this study, 56 polymorphic RAPD bands were scored with over 90 individuals from each of the Original, Ecovar and Cultivar populations.

Although the plant collections displayed similar levels of RAPD genetic variation, the Analysis of Molecular Variance (AMOVA) showed that the collections were genetically different. Maintenance of similar levels of genetic variation within each plant collection was indicated by similar values of mean genetic distance between individuals, which suggested a similar potential for wide crosses to occur within each collection. However, natural selection produced by habitat differences may also act independently on each collection to produce genetic distinctiveness that could be differentiated with the RAPD assay. This strengthened the position that the collections were differentiated based on morphological data despite the indication with RAPD

analysis that showed low variation between collections. This also suggested that phenotypic plant measurements may be more effective in characterizing genetic differences between plant collections of naturally occurring native grasses and other plant species than some DNA techniques because observable differences were attributable to heritable traits.

Cluster analysis of the plant collections using RAPD results illustrated the genetic relationship between collections. The clustering of GLD1 and GLD2, two collections from Goodlands, Manitoba, agreed with expected relatedness based on geographic origin. Unexpected groupings was also found, with the smaller and earlier flowering plants from the RSL (Russell) collection closely clustering with the KGR (Kruger) and LDR (Lauder) collections which contained larger and later flowering plants. Although the two collections from Goodlands (GDL1 and GDL2) were clustered together, the genetic distance between them was larger than that between KGR and LDR despite the larger geographic separation between the latter two collection sites. The Goodland collections were only approximately 3 km apart, with an elevational difference of 152 m. The contrast in genetic distances may have been attributable to the Goodlands collection sites having different levels of soil moisture compared to low soil moisture found at both KGR and LDR sites. This type of information may be useful for devising future collection expeditions of blue grama and other native grass species in efforts to obtain genetically diverse populations.

The effectiveness of Ecovar selection in maintaining the level of genetic diversity of the Original was determined with the RAPD results. This was facilitated by having a Cultivar population that was selected differently from the Ecovar population as a

comparison. The mass selection criteria used to develop the Cultivar population had a lower selection intensity than used in the Ecovar and did not require equal genetic representation from all the plant collections. High levels of RAPD variation were found for all three populations.

The Original population was found to have the highest level of genetic variation and the Ecovar and Cultivar had similar levels contrary to the expectation that the Cultivar would show lower levels of genetic variation compared to the Ecovar. This result may reflect the limited time for selection to act, as only one cycle of selection was made. The maintenance of genetic variation in the Ecovar and Cultivar indicated the potential for additional cycles of selection.

Although the Ecovar and Cultivar showed similar levels of genetic variation, the populations were found to differ with respect to the Original. Population comparisons with AMOVA (Analysis of Molecular Variance) indicated that the genetic variation due to population differences between the Cultivar and the Original was three times as large as that between the Ecovar and the Original. This showed that although similar levels of genetic variation were maintained in the Ecovar and Cultivar, larger genetic shifts occurred such that the Cultivar was more distant from the Original than the Ecovar.

Changes in RAPD band frequencies allowed determination of the specific genetic shifts that occurred in the Ecovar and Cultivar populations. For the Ecovar, only two RAPD bands were found to significantly change in frequency compared to eight bands observed in the Cultivar. The two bands in the Ecovar were in lower frequency compared to the Original, and probably reflected the loss of those alleles, which contributed to its distinction from the Original. In contrast, the Cultivar was found to

have both an increase as well as a decrease in frequency of certain RAPD bands. Five of the eight bands in the Cultivar were found to be lower in frequency compared to the Original, which reflected the possible greater loss of alleles due to selection than was observed in the Ecovar. Three of the eight RAPD bands in the Cultivar were higher in frequency than the Original, which could possibly be attributable to selection. Since the frequency of these bands increased, they could be linked with genes for the selected seed yield traits. The low proportion of scored bands that show exceptional ability to distinguish between the three populations testifies to the redudancy of RAPDs as the detection of variation in selectively neutral, non-expressed DNA sequences make up the majority of the genetic diversity measurement. However, the RAPD assay yielded four unique bands from four primers that were found to be able to distinguish between the three different, but genetically related, blue grama populations.

In conclusion, morphological and RAPD analyses showed that high genetic variation in naturally occurring populations of blue grama and little bluestem in southern Manitoba, Canada provides an opportunity for modification via selection. One cycle of mass selection with a higher selection intensity and unequal genetic representation from all original collections in the Cultivar maintained as much genetic diversity as the Ecovar method that used stratified selection with lower selection intensity and equal pooling. However, greater genetic shifts occurred in the Cultivar population, as observed by 1) a threefold magnitude genetic distance from the Original compared to the Ecovar, and 2) a greater number of RAPD bands that changed in frequency from the Original population. The changes in RAPD band frequencies may have reflected the loss and increase of specific alleles affected by selection.

Suggestions for Further Investigations

Although the results of this research demonstrated the efficiency of Ecovar selection and the resultant genetic shifts, additional investigations can be made. Because this was the first known analysis of genetic diversity and genetic shifts caused by selection in blue grama utilizing the RAPD molecular technique, it opens the possibility for comparison of genetic diversity between blue grama and other native grass populations from other sources in North America. The comparison of genetic diversity and determination of genetic relationships between collections more widely separated in latitude and longitude would enhance the scope of this study and contribute to the development of core collections of blue grama and little bluestem for Western Canada for future breeding work.

The present research provides a foundation for further investigations of genetic diversity and the effect of selection on genetic variation in blue grama and other native grasses. Additional comparison should be made between the Ecovar produced in this study and commercial cultivars from the USA. Before making definite conclusions, maintenance of genetic diversity in the Ecovar should continue to be assessed after additional cycles of selection, over the generations of seed increase, and following establishment at target planting sites.

REFERENCES

Aguilera, M.O. 1992. Intraspecific interactions in blue grama (*Bouteloua gracilis*) competition. Ph.D. Diss. Abst. Montana State University.

Anderson, K. and A.E. Aldous. 1938. Improvement of *Andropogon scoparius* Michx. by breeding and selection. J. Am. Soc. Agron. 30: 862-869.

Atkins, M.D., and J.E. Smith. 1967. Grass seed production and harvest in the Great Plains. USDA Farmers' Bulletin no. 2226.

Barbier, P. 1990. Genetic variation and ecotypic differentiation in the wild rice species *Oryza rufipogon*: II. Influence of the mating system and life history traits on the genetic structure of populations. Japanese J. Genetics 64: 273-286.

Beaver, J. and R. Macchiavelli. 1998. Breeding strategies for pyramiding genes for disease resistance. Annu. Rep. Bean Improv. Coop. Colorado State Univ., Fort Collins 41: 141-142.

Benedict, H.M. 1940. Effect of daylength and temperature on the flowering and growth of four species of grasses. J. Ag. Res. 61: 661-671.

Bennington, C.C. and J.B. McGraw. 1995. Natural selection and ecotypic differentiation in *Impatiens pallida*. Ecol. Monogr. 65:303-323.

Blake, A.K. 1939. Viability and germination of seeds and early life history of prairie plants. Ecol. Monogr. 5: 405-460.

Branson, L.R. 1941. An analysis of seed production of native Kansas grasses during the drought of 1939. Trans. Kansas Acad. Sci. 44:117-125.

Briske, D.D. and A.M. Wilson. 1977. Temperature effects on adventitious root development in blue grama seedlings. J. Range Manage. 30:276-280.

Briske, D.D. and A.M. Wilson. 1978. Moisture and temperature requirements for adventitious root development in blue grama (*Bouteloua gracilis*) seedlings. J. Range Manage. 31:174-178.

Brown, H.R. 1943. Growth and seed yields of native prairie plants in various habitats of the mixed-prairie. Trans. Kansas Acad. Sci. 46:87-99.

Brown, L. 1979. Grasses: An identification guide. Houghton Mifflin Co., Boston. pp. 7-10.

Burton, G.W. 1974. Recurrent restricted phenotypic selection increases forage yield of Pensacola bahiagrass. Crop Sci. 14: 831-835.

Carman, J.G. and D.D. Briske. 1984. Does long-term grazing create morphologic or genetic variation in little bluestem? Grazing Research in Texas 1980-1985. Texas Agric. Exper. Station Progress Report #4419.

Carpenter, J.A. and M.D. Casler. 1990. Divergent phenotypic selection response in smooth bromegrass for forage yield and nutritive value. Crop Sci. 30:17-22.

Carren, C.J., A.M. Wilson, R.L. Cuany, and G.L. Thor. 1987a. Caryopsis weight and planting depth of blue grama. I. Morphology, emergence, and seedling growth. J. Range Manage. 40: 207-211.

Carren, C.J., A.M. Wilson, and R.L. Cuany. 1987b. Caryopsis weight and planting depth of blue grama. II. Emergence in marginal soil moisture. J. Range Manage. 40: 212-216.

Chalmers, K.J., R. Waugh, J.L. Sprent, A.J. Simons, and W. Powell. 1992. Detection of genetic variation between and within populations of *Gliricidia sepium* and *Gliricidia maculata* using RAPD markers. Heredity. 69:465-472.

Chen, L.F.O., H.Y. Kuo, M.H. Chen, K.N. Lai, and S.C.G. Chen. 1997. Reproducibility of the differential amplification between leaf and root DNAs in soybean revealed by RAPD markers. Theor. Appl. Genet. 95: 1033-1043.

Church, G.L. 1929. Meiotic phenomena in certain Gramineae II. Paniceae and Andropogonae. Bot. Gaz. 88:63-84.

Coffin, D. P. and W. K. Lauenroth. 1992. Spatial variability in seed production of the perennial bunchgrass *Bouteloua gracilis* (Gramineae). Amer. J. Bot. 79: 347-353.

Conner, J. K. and A. Sterling. 1996. Selection for independence of floral and vegetative traits: evidence from correlation patterns in five species. Can. J. Bot. 74: 642-644.

Cooper, H.W. 1957. Some plant materials and improved techniques used in soil and water conservation in the Great Plains. J. Soil Water Conserv. 12: 163-168.

Coors, J. G. and M. C. Mardones. 1989. Twelve cycles of mass selection for prolificacy in maize. I. Direct and correlated responses. Crop Sci. 29: 262-266.

Cornelius, D.R. 1946. Comparison of some soil-conserving grasses. J. Am. Soc. Agron. 38: 682-689.

Cornelius, D.R. 1947. The effect of source of little bluestem seed on growth, adaptation, and use in revegetation seedings. J. Agric. Res. 74:133-143.

Coukos, C.J. 1944. Seed dormancy and germination in some native grasses. J.Am. Soc. Agron. 36: 337-345.

Crochemore, M.L., C. Huyghe, M.C. Kerlan, F. Durand and B. Julier. 1996. Partitioning and distribution of RAPD variation in a set of populations of *Medicago sativa* complex. Agronomie 16: 421-432.

Crowle, W.L. 1970. Revenue slender wheatgrass. Can. J. Plant Sci. 50: 748-749.

Dalgarin, M.C. and R.E. Wilson. 1975. Net productivity and ecological efficiency of *Andropogon scoparius* growing in an Ohio relict prairie. Ohio J. Sci. 75:194-197.

Deragon, J.M. and B.S. Landry. 1992. RAPD and other PCR-based analyses of plant genomes using DNA extracted from small leaf disks. PCR-Methods-and-Applications 3: 175-180.

Dewald, G.W. and S.M. Jalal. 1974. Meiotic behavior and fertility interrelationship in *Andropogon scoparius* and *A. gerardi*. Cytologia 39:215-223.

dos Santos, J.G., J. Nienhuis, P. Skroch, J. Tivang, and J.K. Slocum. 1994. Comparison of RAPD and RFLP genetic markers in determining genetic similarity among *Brassica oleracea* L. genotypes. Theor. Appl. Genet. 87:909-915.

Duebbert, H.F., E.T. Jacobson, K.F. Higgins, and E.B. Podoll. 1981. Establishment of seeded grasslands for wildlife habitat in the prairie pothole region. US Fish and Wildlife Service. Wildlife No. 234. Washington, D.C.

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

Falconer, D.S. and T.F.C Mackay. 1996. An Introduction to Quantitative Genetics 4th Ed. Longarm Ltd. Burnt Mill, Harlow, England.

Fehr, W.R. 1991. Principles of cultivar development. Vol.1: Theory and Techniques. pp.133-135. MacMillan Publishing Co.

Ferdinandez, Y.S.N. 1999. Characterization of meadow bromegrass X smooth bromegrass hybrid populations using morphological characteristics, quality parameters and RAPD markers. MSc. Thesis. Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK Canada.

Ferguson, M.E., L.D. Robertson, B.V. Ford-Lloyd, H.J. Newbury, and N. Maxted. 1998. Contrasting genetic variation amongst lentil landraces from different geographic origins. Euphytica 102: 265-273.

Frank, A.B., and L. Hofmann. 1989. Relationship among grazing management, growing degree days, and morphological development for native grasses on the northern Great Plains. J. Range Manage. 42: 199-202.

Fulbright, T.E., A.M. Wilson, and E.F. Redente. 1985. Green needlegrass and blue grama seedling growth in controlled environments. J. Range Manage. 38:410-414.

Fults, J.L. 1936. Blue grama grass for erosion control and range reseeding in the Great Plains and a method for obtaining seed in large lots. USDA No. 402.

Fults, J.L. 1942. Somatic chromosome complements in *Bouteloua*. Amer. J. Bot. 29:45-55.

Gaffney, F.B. and J.A. Dickerson. 1987. Species selection for revegetating sand and gravel mines in the Northeast. J. Soil Water Conserv. 42: 358-361.

Gidoni, D., M. Rom, T. Kunik, M. Zur, E. Izsak, S. Izhar, and N. Firon. 1994. Strawberry-cultivar identification using randomly amplified polymorphic DNA (RAPD) markers. Plant-Breed. 113:339-342.

Gillen, R.L., and A.L. Ewing. 1992. Leaf development of native bluestem grasses in relation to degree-day accumulation. J. Range Manage. 45: 200-204.

Gould, F.W. 1956. Chromosome counts and cytotaxonomic notes on grasses of the tribe Andropogoneae. Am. J. Bot. 43:395-404.

Gould, F.W. 1975. The Grasses of Texas. Texas A&M Univ. Press.

Greener Roadsides. 1997. Quarterly publication of the U.S. Federal Highway Administration. Publication No. FHW-PD-97-049.

Gunter, L.E., G.A. Tuskan, and S.D. Wullschleger. 1996. Diversity among populations of switchgrass based on RAPD markers. Crop Sci. 36: 1017-1022.

Hallauer, A. R. 1970. Genetic variability for yield after four cycles of reciprocal recurrent selections in maize. Crop Sci. 10: 482-485.

Halloran, G.M. and A.L. Pennell. 1982. Grain size and seedling growth of wheat at different ploidy levels *Triticum* spp. Ann. Bot. 49: 103-113.

Hamrick, J.L. and L.R. Holden. 1979. Influence of microhabitat heterogeneity on gene frequency distribution and gametic phase disequilibrium in *Avena barbata*. Evolution 33: 521-533.

Helms, T. C., A. R. Hallauer, and O. S. Smith. 1989. Genetic variability estimates in improved and nonimproved Iowa Stiff Stalk Synthetic maize populations. Crop Sci. 29: 959-962.

Heun, M. and T. Helentjaris. 1993. Inheritance of RAPDs in F1 hybrids of corn. Theor. Appl. Genet. 85:961-968.

Hitchcock, A.S. 1950. Manual of the grasses of the United States. 2nd.ed., Rev. by A. Chase. USDA Misc. Publ. No.200

Hubbard, F.T. 1917. *Andropogon scoparius* in the United States and Canada. Rhodora 19:100-105.

Huff, D.R. 1997. RAPD characterization of heterogeneous perennial ryegrass cultivars. Crop Sci. 37: 557-564.

Huff, D.R., J.A. Quinn, B. Higgins, and A.J. Palazzo. 1999. RAPD variation among native little bluestem (*Schizachyrium scoparium* (Michx.) Nash) populations from high and low fertlility in forest and grassland biomes. Crop Sci. In Press.

Huff, D.R., R. Peakall and P.E. Smouse. 1993. RAPD variation within and among natural populations of outcrossing buffalograss [*Buchloë dactyloides* (Nutt.) Engelm.]. Theor. Appl. Genet. 86: 927-934.

Hunter, A.W.S. 1934. A karyosystematic investigation in the Gramineae. Can. J. Res. 11:213-241.

Hyder, D.N., A.C. Everson, and R.E. Bement. 1971. Seedling morphology and seedling failures with blue grama. J. Range Manage. 24: 287-292.

Jacobon, E.T., D.B. Wark, R.G. Arnott, R.J. Haas, and D.A. Tober. 1994. Sculptured seeding: An ecological approach to revegetation. Restor. and Manage. Notes 12: 46-50.

Janick, J. 1986. Horticultural Science. 4th Ed. W.H. Freeman & Co. New York. pp.64-67.

Johnson, R.C. 1998. Genetic structure of regenerating populations of annual ryegrass. Crop Sci. 38: 851-857.

Jones, M.D. and L.C. Newell. 1946. Pollination cycles and pollen dispersal in relation to grass improvement. Nebr. Agric. Exp. Sta. Res. Bull. 148.

Kelly, J.D. and P.N. Miklas. 1998. The role of RAPD markers in breeding for disease resistance in common bean. Mol. Breed. 4:1-11.

Khan, S.M. and A.M. Wilson. 1984. Nonstructural carbohydrates and dehydration tolerance of blue grama (*Bouteloua gracilis)* seedlings. Agron. J. 76:637-642.

Kisha, T.J., C.H. Sneller, and B.W. Diers. 1997. Relationships between genetic distance among parents and genetic variance in populations of soybean. Crop Sci. 37:1317-1325.

Knapp, E.E. and K.J. Rice. 1994. Starting from seed: genetic issues in using native grasses for restoration. Restor. and Manage. Notes 12:40-45.

Knapp, E.E. and K.J. Rice. 1996. Genetic structure and gene flow in *Elymus glaucus* (blue wildrye): Implications for native grassland retsoration. Restor. Ecol. 4: 1-10.

Knapp, E.E. and P.G. Connors. 1999. Genetic consequences of a single-founder population bottleneck in *Trifolium amoenum* (Fabaceae). Am. J. Bot. 86:124-130.

Kneebone, W.R. 1957. Blue grama seed production studies. J. Range Manage. 10: 17-21.

Koppitz, H., H. Kühl, K. Hesse, and J.G. Kohl. 1997. Aspects of the importance of genetic diversity in *Phragmites australis* (Cav) Trin. Ex Steudel for development of reed stands. Bot. Acta. 110: 217-223.

Koppitz, H., H. Kühl, K. Hesse, and J.G. Kohl. 1997. Aspects of the importance of genetic diversity in *Phragmites australis* (Cav) Trin. Ex Steudel for development of reed stands. Bot Acta. 110: 217-223.

Lacou, V., K. Haurogne, N. Ellis, and C. Rameau. 1998. Genetic mapping in pea. 1. RAPD-based genetic linkage map of *Pisum sativum*. Theor. Appl. Genet. 97: 905-915.

LaGory, K.E., K. LaGory, and J.V. Perino. 1982. Response of big and little bluestem (*Andropogon*) seedlings to soil and moisture conditions *Andropogon gerardi*, *Andropogon scoparius*. Ohio J. Sci. 82:19-23.

Larsen, E.C. 1947. Photoperiodic responses on geographical strains of *Andropogon scoparius*. Bot. Gaz. 109: 132-149.

Laytin, C.R. and F.R. Ganders. 1984. The genetic consequences of contrasting breeding systems in *Plectritis* (Valerianceae). Evolution 38:1308-1325.

Loveless, M.D. and J.L. Hamrick. 1984. Ecological determinants of genetic structure in plant populations. Ann. Rev. Ecol. Syst. 15:65-95.

Majerus, M. 1975. Response of root and shoot growth of three grass species to decreases in soil water potential. J. Range Manage. 28: 473-476.

Marshall, D. R. and H. D. Brown. 1973. Stability of performance of mixtures and multilines. Euphytica. 22: 405-412.

McGinnies, W.J. 1984. Chemically thinning blue grama range for increased forage and seed production. J. Range Manage. 37:412-415.

McGinnies, W.J., W.A. Laycock, T. Tsuchiya, C.M. Yonker, and D.A. Edmunds. 1988. Variability within a native stand of blue grama. J. Range Manage. 5:391-395.

McMillan, C. 1956. Nature of the plant community.II. Variation in flowering behavior within populations of *Andropogon scoparius*. Am. J. Bot. 43: 429-436.

McMillan, C. 1959a. Nature of the plant community. V. Variation within the true prairie community-type. Amer. J. Bot. 46: 418-424.

McMillan, C. 1959b. The role of ecotypic variation in the distribution of the central grassland of North America. Ecol. Monogr. 29: 285-307.

McMillan, C. 1964. Ecotypic differentiation within four north american prairie grasses. I. Morphological variation within transplanted community fractions. Amer. J. Bot. 51: 1119-1128.

Millar, C.I. and W.J. Libby. 1989. Disneyland or Native Ecosystem: Genetics and Restorationists. Restoration and Management Notes. Vol. 7. No. 1: 18-24.

Miller, R.V. 1967. Ecotypic variation in *Andropogon scoparius* and *Bouteloua gracilis*. Ph.D. diss. Colorado State Univ., Fort Collins, Colorado.

Nagaoka,T. and Y. Ogihara. 1997. Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor. Appl. Genet. 94:597-602.

Nason, D.A., R.L., Cuany, and A.M. Wilson. 1987. Recurrent selection in blue grama. I. Seedling water uptake and shoot weight. Crop Sci. 27:847-851.

Nesbitt, K.A., B.M. Potts, R.E. Vaillancourt, A.K. West and J.B. Reid. 1995. Partitioning and distribution of RAPD variation in a forest tree species, *Eucalyptus globulus* (Myrtaceae). Heredity 74: 628-637.

Nevo, E., A. Beiles and T. Krugman. 1988a. Natural selection of allozyme polymorphisms: a microgeographical differentiation by edaphic, topographical, and temporal factors in wild emmer wheat (*Triticum dicoccoides*). Theor. Appl. Genet. 76:737-752.

Nevo, E., A. Beiles and T. Krugman. 1988b. Natural selection of allozyme polymorphisms: a microgeographical climatic differentiation in wild emmer wheat (*Triticum dicoccoides*). Theor. Appl. Genet. 75:529-538.

Nevo, E., A. Beiles, D. Kaplan and E.M. Golenberg. 1986. Natural selection of allozyme polymorphisms: a microsite test revealing ecological genetic differentiation in wild barley. Evolution 40:13-20.

Nevo, E., D. Zohary, A.H.D. Brown, and M. Haber. 1979. Genetic diversity and environmental associations of wild barley, *Hordeum spontaneum*, in Israel. Evolution 33:815-833.

Nielsen, E.L. 1939. Grass studies III. Additional somatic chromosome complements. Am.J.Bot. 26: 366-372.

Nienhuis, J., J. Tivang, and P. Skroch. 1994. Analysis of genetic relationships among genotypes based on molecular marker data. In: Analysis of Molecular Marker Data. Joint Plant Breeding Symposia Series, 5-6 August 1994. Corvallis, Oregon. pp. 8-14.

Olmsted, C.E. 1943. Growth and development in range grasses. III. Photoperiodic responses in the genus *Bouteloua*. Bot. Gaz. 105:165-181.

Parker, P.G., A.A. Snow, M.D. Schug, G.C. Booton, and P.A. Fuerst. 1998. What moleculars can tell us about populations: Choosing and using a molecular marker. Ecology 79: 361-382.

Peakall, R., P.E. Smouse and D.R. Huff. 1995. Evolutionary implications of allozyme and RAPD variation in diploid populations of dioecious buffalograss *Buchloë dactyloides*. Molec. Evol. 4: 135-147.

Pedreira, C.G.S. and R.H. Brown. 1996. Physiology, morphology, and growth of individuals of selected and unselected bahiagrass populations. Crop Sci. 36:138-142.

Phan, A.T. and S.R. Smith Jr. 1997. Genetic diversity as affected by selection methodology in native grass species. Proc. of the XVII International Grasslands Congress - Winnipeg, MB and Saskatoon, SK. 8-18 June 1997.

Phan, A.T. and S.R. Smith Jr. 2000. Seed yield variation in blue grama and little bluestem plant collections in southern Manitoba, Canada. Crop Sci. 40:555-561.

Poehlman, J.M. and D.A. Sleper. 1995. Breeding Field Crops. 4th Ed. Iowa State Univ. Press.

Raeber, J.G., and R.R. Kalton. 1956. Variation and inheritance of fertility and its components in *Bromus inermis*. Leyss. Agron. J. 48: 212-216.

Rajapakse, S., L.E. Belthoff, G. He, A.E. Estager, R. Scorza, I. Verde, R.E. Ballard, W.V. Baird, A. Callahan, R. Monet, and A.G. Abbott. 1995. Genetic linkage mapping in peach using morphological, RFLP, and RAPD markers. Theor. Appl. Genet. 90:503-510.

Raven, P.H., R.F. Evert, and S.E. Eichhorn. 1992. Biology of Plants. 5th Ed. Worth Publishers. New York. pp.158-159.

Rice, K., and S. Jain. 1985. Plant population genetics and evolution in disturbed environments. In: The ecology of natural disturbance and patch dynamics / edited by S.T.A.Pickett, P.S. White. Orlando, Fla. : Academic Press. pp. 287-303.

Riegel, A. 1940. A study of the variations in the growth of blue grama grass seed produced in various sections of the Great Plains region. Trans. Kansas Acad. Sci. 43:155-171.

Riegel, A. 1941. Life history and habits of blue grama. Trans. Kansas Acad. Sci. 44: 76-83.

Riegel, A. 1943. A source stufy of blue grama grass and the effect of different treatments on establishing stands of grass under field conditions at Hays, Kansas Trans. Kansas Acad. Sci. 46:103-109.

Rodgers, D.M., J.P. Murphy and K.J. Frey. 1983. Impact of plant breeding on the grain yield and genetic diversity of spring oats. Crop Sci. 23: 737-740.

Rohlf, F.L. 1997. NTSYS-pc: Numerical taxonomy and multivariate analysis system. Exter Publishers, Setauket, NY.

Roohi, R., D.A. Jameson and N. Nemati. 1991. The effect of light on adventitious root formation in blue grama. J. Range Manage. 44:184-185.

Roos, F.H. and J.A. Quinn. 1977. Phenology and reproductive allocation in *Andropogon scoparius* (Gramineae) populations in communities of different successional stages. Amer. J. Bot. 64: 535-540.

Rossetto, M., G. Jezierski, S.D. Hopper, and K.W. Dixon. 1999. Conservation genetics and clonality in two critically endangered eucalypts from highly endemic south-western Australia flora. Biol. Conserv. 88:321-331.

Russell, J.R., J.D. Fuller, M. Macaulay, B.G. Hatz, A. Jahoor, W. Powell, and R. Waugh. 1997. Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. Theor. Appl. Genet. 95:714-722.

Saiki, R.K., D.H. Gelfand, S. Stoffel, S.J. Scharf, R.Higuchi, G.T. Horn, K.B. Mullis, H.A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487-491.

SAS Institute. 1988. SAS/STAT User's Guide, 6.03 SAS Inst., Cary, NC.

Schwarz, A.G. and M.T. Reaney. 1989. Perennating structures and freezing tolerance of northern and southern populations of C4 grasses. Bot.Gaz. 150:239-246.

Sims, P.L., R.K. Lang'At, & D.N. Hyder. 1973. Developmental morphology of blue grama and sand bluestem. J. Range Manage. 26: 340-344.

Skroch, P. and J. Nienhuis. 1997. Impact of scoring error and reproducibility of RAPD data on RAPD based estimates of genetic distance. Theor. Appl. Genet. 91:1086-1091.

Smith, A.E and C.L. Leinweber. 1971. Relationship of carbohydrate trend and morphological development of little bluestem [*Andropogon scoparius*] tillers. Ecology 52: 1052-1057.

Smith Jr., S.R. and A.T. Phan. 1999. Maintaining genetic diversity in native grass breeding. VIth International Rangeland Congress Proc. 2:648-650.

Smoliak, S. and A. Johnson. 1980. Elbee northern wheatgrass. Can. J. Plant Sci. 60:1473-1475.

Smoliak, S. and A. Johnson. 1983. Walsh western wheatgrass. Can. J. Plant Sci. 63:759-761.

Snyder, L.A. and J.R. Harlan. 1953. A cytological study of *Bouteloua gracilis* from western Texas and eastern New Mexico. Amer. J. Bot. 70: 2303-2309.

Sokal, R.R. and C.D. Michener. 1958. A statistical method for evaluating systematic relationships. Univ. Kans. Sci. Bull. 38:1409-1438.

Staub, J., J. Bacher, and K. Poetter. 1996. Sources of potential errors in the application of random amplified polymorphic DNAs in cucumber. HortScience 31: 262-266.

Steinauer, E.M. and S.L. Collins. 1996. Prairie ecology. In: Prairie Conservation. F.B. Samson and F.L. Knoff Editors. Island Press, Washington, D.C.

Stoddart, L.A. and K.J. Wilkinson. 1938. Inducing germination in *Oryzopsis hymenoides* for range re-seeding. Agronomy J. 30: 763-768.

Stojsin, D. and L. W. Kannenberg. 1994. Genetic changes associated with different methods of recurrent selection in five maize populations: II. Indirectly selected traits. Crop Sci. 34: 1473-1479.

Stojsin, D. and L. W. Kannenberg. 1994. Genetic changes associated with different methods of recurrent selection in five maize populations: II. Indirectly selected traits. Crop Sci. 34: 1473-1479.

Stubbendieck, J., and D.F. Burzlaff. 1971. Nature of phytomer growth of blue grama. J. Range Manage. 24:154-156.

Stubbendieck, J., J.L. Launchbaugh, D.F. Burzlaff, and W.G. McCully. 1973. Stoloniferous blue grama. J. Range Manage. 26:230-231.

Thompson, J.A., R.L. Nelson, and L.O. Vodkin. 1998. Identification of diverse soybean germplasm using RAPD markers. Crop Sci. 38: 1348-1355.

Thompson, P.G., L.L. Hong, K. Ukoskit, and Z.Q. Zhu. 1997. Genetic linkage of randomly amplified polymorphic DNA (RAPD) markers in sweetpotato. J. Am. Soc. Hort. Sci. 122: 79-82.

Trupp, C.R., and A.E. Slinkard. 1965. Seedset rating as a measure of fertility in grasses. Crop Sci. 5: 599-600.

Tsuchiya, T., W. McGinnies, and A. Shahla. 1992. A chromosome study of blue grama (*Bouteloua gracilis*) in northern Colorado. Great Plains Res. 2:255-262.

Turesson, G. 1922. The genotypical response of the plant species to the habitat. Hereditas. 3: 211-350.

Van Auken, O.W., J.K. Bush, D.D. Diamond. 1994. Changes in growth of two C4 grasses (*Schizachyrium scoparium* and *Paspalum plicatulum*) in monoculture and mixture: influence of soil depth. Am. J. Bot. 81:15-20.

Van Der Sluijs, D.H. and D.N. Hyder. 1974. Growth and longevity of blue grama seedlings restricted to the seminal roots. J. Range Manage. 27: 117-119.

Vierling, R.A. and H.T. Nguyen. 1992. Use of RAPD markers to determine the genetic diversity of diploid, wheat genotypes. Theor. Appl. Genet. 84:835-838.

Vogel, K.P. and J.F. Pedersen. 1993. Breeding systems for cross-pollinated perennial grasses. Plant Breed. Rev. 11: 251-274.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kulper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucl. Acids Res. 23:4407-4414.

Waller, S.S., C.M. Britton, and J.D. Dodd. 1975. Soil fertility and production parameters of *Andropogon scoparius* tillers. J. Range Manage. 28:476-479.

Walton, M. 1990. Application of RFLP technology to applied plant breeding. Plant Biol. 11:335-346.

Weaver, J.E. and E. Zink. 1945. Extent and longevity of the seminal roots of certain grasses. Plant Physiol. 20: 359-379.

Welsh, J. and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucl. Acids Res. 18: 7213-7218.

Wendel, J.F. & N.F. Weeden. 1989. Visualization and interpretation of plant isozymes. In: Isozymes in Plant Biology. Edited by D.E. Soltis, and P.S. Soltis. Advances in Plant Sciences Series Vol.4. Dioscorides Press. Portland, Oregon.

Werner, B.K. and G.W. Burton. 1991. Recurrent restricted phenotypic selection for yield alters morphology and yield of Pensacola bahiagrass. Crop Sci. 31: 48-50.

Westman, A.L. and S. Kresovich. 1999. Simple sequence repeat (SSR) based polymorphism in *Brassica nigra* genebank accessions and weed populations. Euphytica 109: 85-92.

White, E.M. 1961. A possible relationship of little bluestem distribution to soils. J. Range Manage. 14: 243-247.

Williams J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18: 6531-6535.

Wilson, A.M. 1981. Air and soil temperature effects on elongation of adventitious roots in blue grama seedlings. Agron. J. 73:693-697.

Wilson, A.M. and D.D. Briske. 1979. Seminal and adventitious root growth of blue grama seedlings on the Central Plains. J. Range Manage. 32: 209-213.

Wilson, A.M. and J.A. Sarles. 1978. Quantification of growth drought tolerance and avoidance of blue grama [*Bouteloua gracilis*] seedlings. Agron. J. 70: 231-237.

Wilson, A.M., R.L. Cuany, J.G. Fraser, and W.R. Oaks. 1981. Relationships among components of seed yield in blue grama. Agron. J. 73:1058-1062.

Wuenscher, M.L. and G.C. Gerloff. 1971. Growth of *Andropogon scoparius* (little bluestem) in phosphorus deficient soils. New Phytol. 70: 1035-1042.

Zheng, Y.Q, D. Lindgren, O.Roswell, and J. Westin. 1997. Combining genetic gain and diversity by considering average coancestry in clonal selection of Norway spruce. Theor. Appl. Genet. 95:1312-1319.

A

B

Appendix 1. Blue grama chromosome staining from pollen-mother cells. **A)** Anaphase completion of division to form two daughter cells each with $x = 10$ and $2n = 20$. **B)** Metaphase chromosomes showing $2n = 28$, suggesting a basic chromosome number of $x = 7$.

Appendix 2. Seed germination of 11 blue grama plant collections in southern Manitoba grown at Winnipeg and harvested in 1994.

Blue grama	No. germinations	Mean	Std. Error
CHC	44	84.7	1.4
CVY	43	84.7	2.4
DGL	42	81.6	2.9
GDL1	41	87.5	2.5
GDL2	39	86.8	2.0
KGR	40	88.3	2.0
LDR	43	87.0	1.7
OAK	42	83.6	2.4
RSL	34	84.7	3.3
S10	41	85.5	2.6
SDY	45	86.8	1.7

Appendix 3. Ratings for dormancy, determinancy of culm growth, and leaf density in 11 blue grama plant collections in Winnipeg, 1994. Rating scheme used: 1 = low, 5 = high.

Collection	Mean dormancy rating†	No. Plants with non-determinate growth‡	Mean leaf density rating¶
CHC	3.13	5	4.18
CVY	2.75	23	4.32
DGL	2.58	21	4.27
GDL1	2.93	18	4.32
GDL2	2.88	22	4.05
KGR	2.18	22	4.28
LDR	3.33	14	4.51
OAK	3.49	25	4.40
RSL	2.13	29	3.42
S10	3.28	22	4.93
SDY	2.58	26	3.93

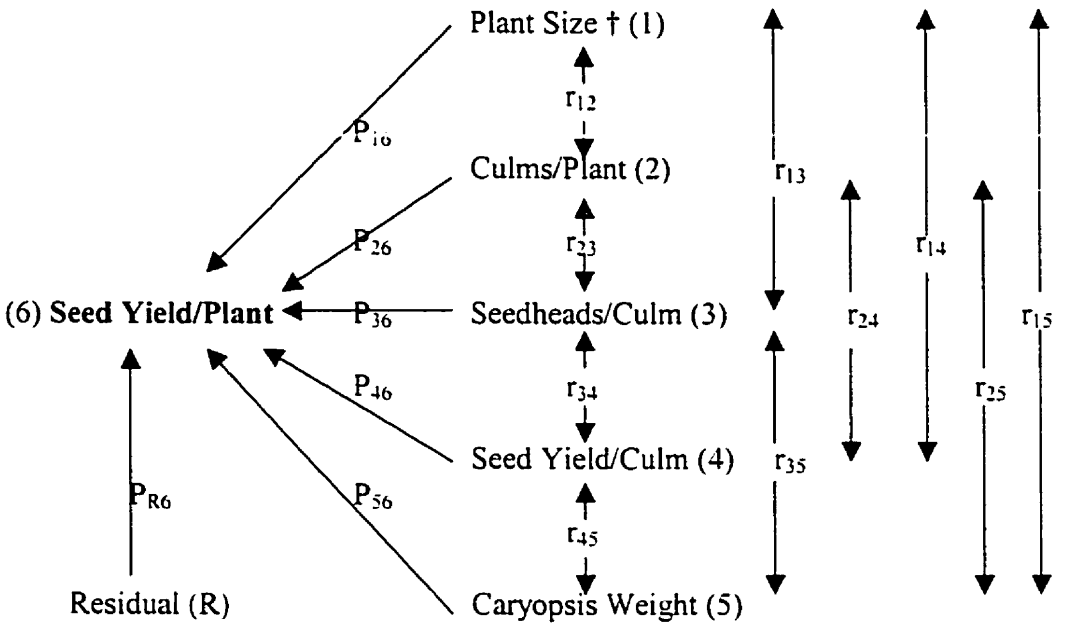
† Plant dormancy was scored based on appearance of new shoots

‡ Non-determinate growth was scored for plants producing reproductive culms continuously towards first frost in October.

¶ Scored during plant vegetative stage in June.

Appendix 4. Path coefficients for direct and indirect effects of several characters affecting seed yield in little bluestem

Character relationship to Seed Yield/Plant	Path Coefficient
Direct effect of Plant Size	0.385
indirect effect via Culms/Plant	0.360
indirect effect via Seedheads/Culm	0.005
indirect effect via Seedyield/Culm	0.194
indirect effect via Caryopsis Weight	0.000
Total Effect	0.944
Direct effect of Culms/Plant	0.467
indirect effect via Plant Size	0.297
indirect effect via Seedheads/Culm	-0.001
indirect effect via Seedyield/Culm	-0.011
indirect effect via Caryopsis Weight	0.000
Total Effect	0.752
Direct effect of Seedheads/Culm	0.013
indirect effect via Plant Size	0.139
indirect effect via Culms/Plant	-0.018
indirect effect via Seedyield/Culm	0.296
indirect effect via Caryopsis Weight	0.000
Total Effect	0.430
Direct effect of Seedyieid/Culm	0.403
indirect effect via Plant Size	0.185
indirect effect via Culms/Plant	-0.012
indirect effect via Seedheads/Culm	0.010
indirect effect via Caryopsis Weight	0.000
Total Effect	0.586
Direct effect of Caryopsis Weight	0.001
indirect effect via Plant Size	0.012
indirect effect via Culms/Plant	-0.030
indirect effect via Seedheads/Culm	0.001
indirect effect via Seedyield/Culm	0.048
Total Effect	0.032



Appendix 5. Path diagram of causal relationship between five correlated characters and seed yield of little bluestem. † Plant size = total plant harvested biomass.

Appendix 6. Proportion of total variation in little bluestem seed yield accounted for by 5 plant measurements as determined by step-wise regression

	Model	R^2
Seed Yield =	plant size†	0.892
Seed Yield =	plant size + culms/plant	0.893
Seed Yield =	plant size + culms/plant + seedheads/culm	0.912
Seed Yield =	plant size + culms/plant + seedheads/culm + seedyield/culm	0.954
Seed Yield =	plant size + culms/plant + seedheads/culm + seedyield/culm + caryopsis weight	0.955

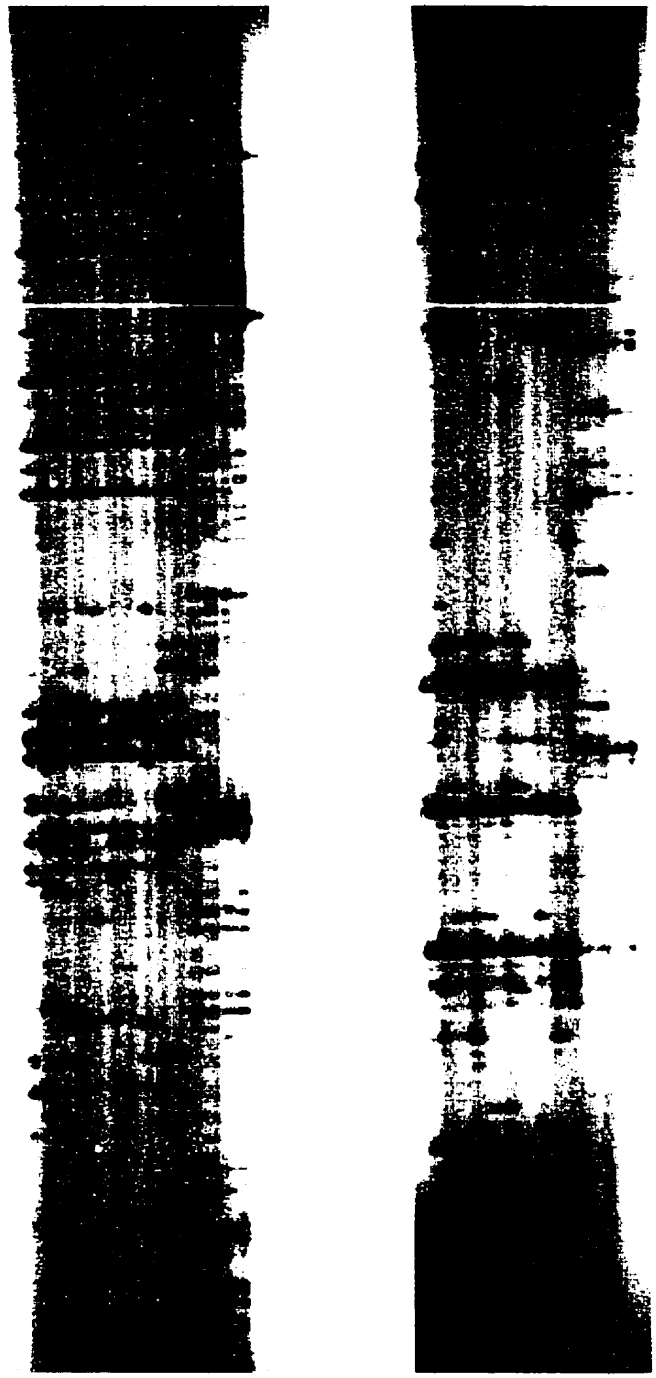
† Plant size = total plant harvested biomass.

Appendix 7. Primers yielding polymorphic RAPD bands in blue grama.

UBC primer	Scored band	Approx. molecular weight (base-pairs)
249	A	383
	B	482
	C	545
336	A	395
	B	422
	C	500
	D	533
346	A	390
	B	472
	C	543
	D	590
	E	779
	F	821
353	A	682
	B	745
	C	931
	D	991
	E	1037
	F	1136
	G	1200
	H	1260
388	A	452
	B	502
	C	563
	D	994
	E	1050
389	A	450
	B	516
	C	545
	D	590
	E	653
	F	695
	G	784
	H	825
	I	903
	J	1128
502	A	410
	B	530
	C	670
	D	1085

Appendix 7 continued. Primers yielding polymorphic RAPD bands in blue grama.

564	A	499
	B	611
	C	1011
	D	1330
	E	1500
570	A	452
	B	565
	C	613
	D	638
	E	684
	F	800
	G	1143
	H	1188
	I	1324
	J	1377
574	A	578
	B	671
	C	816
	D	979
580	A	647
	B	808
	C	942
	D	1056
600	A	639
	B	709
	C	807
	D	870
	E	973
	F	1506



Appendix 8. AFLP film (top and bottom portions) from assay of 10 blue grama plants from different collection sites in southern Manitoba (columns 1-10) using primers E-AAC and M-CCG with restriction enzymes EcoR3 and Mse10.